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**AN AVIAN ECOSYSTEM HEALTH INDICATOR:
THE REPRODUCTIVE EFFECTS INDUCED BY FEEDING
GREAT LAKES FISH TO WHITE LEGHORN LAYING HENS**

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AN AVIAN ECOSYSTEM HEALTH INDICATOR: THE REPRODUCTIVE EFFECTS INDUCED BY FEEDING GREAT LAKES FISH TO WHITE LEGHORN LAYING HENS

By

Cheryl Lynne Summer

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ABSTRACT

AN AVIAN ECOSYSTEM HEALTH INDICATOR:
THE REPRODUCTIVE EFFECTS INDUCED BY FEEDING GREAT LAKES
FISH TO WHITE LEGHORN LAYING HENS

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Carp from Saginaw Bay, Lake Huron, were incorporated into the diets of White Leghorn laying hens as a surrogate species to model the adverse reproductive effects observed in wild colonies of waterbirds on the Great Lakes. Three groups were fed diets containing 0, 3.4, and 34.5% carp, which resulted in total concentrations of PCBs of 0.50, 0.90, and 6.60 mg/kg, wet weight, respectively, for 8 weeks. Fatty Liver Hemorrhagic Syndrome (FLHS) occurred in hens of the control and low-dose diet groups, but the high-dose group hens were protected against FLHS. A dose- and time-dependent response to PCBs was observed in hatchability and mortality rates, organ weights, and occurrence of terata in embryos and chicks. Hatching rates averaged 85.3, 86.1, and 78.4% for the control, low-dose, and high-dose groups, respectively. Body, brain, liver, heart, spleen, and bursa weights, collected from 18-day embryos and hatched chicks, were significantly affected in both embryos and chicks. Deformities occurred in 17, 24, and 40% of the control, low-dose, and high-dose groups, respectively.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Mankind is engaged in a never ending quest for progress and the enrichment of life. The quest has led to the development of products which have made life easier, more productive, and which may prolong life itself. All of mankind's progress, in medicine, technology, science, and food production, has been made possible by the discovery of natural compounds and the creation of natural and synthetic compounds. Some of these have proven to be "miracle" compounds. Others, however, have proven to be pure poison.

It is either unfortunate or, perhaps, an unavoidable cost of progress, that man has created chemicals capable of making existence on this finite planet at the least difficult, and at the extreme, impossible. The group of organic compounds known as polychlorinated, diaromatic hydrocarbons (PCDAHs) are included in the caldron of toxic substances now poisoning us and our environment. These include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). PCDDs, PCDFs, and PCBs have a common structure of two chlorine-substituted benzene rings and, in general, share similar mechanisms of action and toxicological effects. Individually, however, they have quite different toxicities. This thesis is concerned, in particular, with the effects of PCBs.

PCBs have been extensively manufactured, distributed, and used industrially world-wide and have also become a global contaminant problem. Detectable concentrations of PCBs can be found in virtually every ecosystem compartment including fat samples from native mammals in the Arctic of Greenland (Bleavins 1980) and snow deposits of Antarctica

(Safe et al. 1987). Aquatic systems, however, are the primary areas of environmental contamination (Tillitt et al. 1991a).

Lipid solubility and resistance to natural degradation lead to the accumulation of PCBs in animal tissues and contribute to their increasing concentrations at higher levels in the food chain. Ultimately, humans are at the pinnacle of the food chain. PCBs are so persistent in animal tissues that if the present generation were able to stop any further exposure to them, and our children and grandchildren were not exogenously exposed, detectable levels of PCBs would be found in our descendants for the next 5 to 7 generations (Swain 1988, Ludwig 1990). Studies show that infants are exposed to PCBs via placental blood transfer and breast milk, and that several physical and functional parameters are significantly affected by pre- and post-natal exposure to PCBs (Jacobson et al. 1990). Researchers continue to explore the effects of PCBs on human health.

Wildlife species at the top of the food chain also demonstrate PCB-induced effects. In the Great Lakes basin, these species include fish-eating birds and mammals such as the colonial waterbirds (cormorants, gulls, and terns), bald eagle, osprey, great blue heron, mink, and otter. Decreased reproductive success, population declines, and high PCB concentrations have been noted in most of these species. Colonial waterbird population levels, reproductive trends, and contaminant concentrations have interested researchers for the past 30 years. In that period of time, populations were decimated by DDT-induced eggshell thinning, and have subsequently, recovered. More recently, the effects of PCBs have become the predominant focal point of research. It is, in fact, the decreased reproductive performance observed in colonial waterbirds that provided the impetus for this study.

The study described in this thesis was designed to determine the toxic potential of Great Lakes fish in the diet of an avian species, and in so doing, verify the relationship

between observations of reproductive anomalies in wild populations of Great Lakes colonial waterbirds and exposure to contaminants such as PCBs. For nearly 30 years, researchers have been trying to develop sound cause-effect linkages between contaminant exposure and reproductive anomalies. Great Lakes wildlife are exposed to a wide range of contaminants, but research has shown that it is the halogenated organic contaminants which are primarily responsible for the deleterious effects. Some members of the scientific and regulatory community are reluctant to accept the conclusions drawn by researchers in the field; rather, they argue that the effects are brought on by disease or by nutritional, genetic, or climatic factors. Thus, this study becomes a link in the overall chain of discovery and resolution of the question: Are the reproductive anomalies that occur in the waterbirds of the Great Lakes basin caused by exposure to contaminants in the food source? In an attempt to answer this question, the primary food source of the colonial waterbirds, i.e. Great Lakes fish, were collected from the environment and fed to laying hens in a controlled laboratory setting. Fish were used in the study, instead of pure technical mixtures of PCBs, to account for the changes in the relative concentrations of individual congeners due to environmental weathering, selective accumulation, and metabolism through the food chain. Throughout the trial, the hens and their progeny were examined for resulting toxic effects.

This study, conducted from June 14 to September 21, 1990, was supported by a cooperative agreement between the U.S. Fish and Wildlife Service and the Department of Animal Science, Michigan State University.

LITERATURE REVIEW

The Great Lakes

The Great Lakes, astounding glacial remnants of the last ice age and reservoirs for 20% of the world's freshwater supplies (Fox and Weseloh 1987), are an important resource for the inhabitants of their basin. Over 35 million Americans and Canadians live around the Great Lakes and use the 22,000 km^3 of water contained therein as a source for drinking water, a reservoir for chemical and biological waste disposal, recreational playgrounds, and waterways for transporting goods and raw materials. All of these activities occur in/on the single most important source of freshwater in the world (Hileman 1988).

The Great Lakes system is a relatively closed system and water retention times are high. In fact, less than 1% of the water volume flows out through the St. Lawrence River each year (Hileman 1988). Water entering Lake Superior will remain there for 191 years before it is flushed out, 99 years in Lake Michigan, 22 years in Lake Huron, 2.6 years in Lake Erie, and 6 years in Lake Ontario (Anonymous 1991).

Contaminants in the Great Lakes

Historically, the Great Lakes were thought to be vast enough to handle infinite inputs of contaminants and for nearly 200 years the Lakes and their connecting waters were treated as open sewers. Indiscriminate dumping polluted them with raw sewage, industrial wastes, and dredged polluted sediments. Municipal and agricultural runoff have also washed

pollutants and pesticides into the waters (Hileman 1988). The problem with the unrestricted dumping of pollutants into the Great Lakes, particularly of stable and organic compounds, is that the pollutants remain in the Lakes a very long time. Assuming contaminants remain suspended in the water column, this could be as long as 191 years which is the residence time of Lake Superior water. This assumption, however, is not valid because contaminants tend to adsorb to the suspended sediment particles and settle to the bottom thereby holding the contaminants in place even if the water was flushed from the Lakes more quickly. Additionally, the contaminants are only very slowly buried deep in the sediments where they are unavailable for exposure. Violent storms and bioturbation disturb the sediments, stir up the Lakes, and resuspend the contaminants so they are again available for uptake and when they resettle, they settle on the top of the sediment layer where benthic organisms are exposed to them. Therefore, the Lakes experience a continual input of new contaminants as well as a recycling of those already present.

The realization that the Great Lakes were being severely impacted by pollutants came slowly, but by the 1960s, concern about the health of the Lakes was in earnest. Rachael Carson (1962) helped initiate that concern with her book Silent Spring which brought the issue of contaminant poisoning to the attention of the public and started the process of scientific inquiry. In the 1960s and 1970s, monitoring programs revealed high levels of the organic pesticides DDT, dieldrin, hexachlorobenzene (HCB), and Mirex, and the industrial compounds, PCBs and dioxins, in fish, birds, sediments, water samples, precipitation, and humans in the lower Great Lakes (Hallett 1985).

In response to public outcry and the mounting scientific evidence of reproductive effects, restrictions and outright bans were placed on these chemicals during the 1970s (Bishop and Weseloh 1990, Fox and Peakall 1991). Since that time, concentrations of most

of the compounds have been declining throughout ecosystem compartments (Bishop and Weseloh 1990). Archived herring gull eggs gathered throughout the Lakes revealed marked decreases in contaminant concentrations from 1974-1990. Concentrations of PCBs in these eggs decreased from 180 ppm to 18 ppm, DDE decreased from 33 to 4.0 ppm, Mirex decreased from 7.4 to 0.68 ppm, dieldrin decreased from 0.90 to 0.32 ppm, and HCB decreased from 0.60 to 0.03 ppm (Weseloh et al. 1991).

It appears, however, that PCDAH contaminant concentrations have reached the lower asymptotic level of the first order exponential decay curve (Giesy 1991). Rates of decline in the 1980s were not as dramatic as in the 1970s (Bishop and Weseloh 1990). Contaminant levels have apparently reached steady state concentrations, and little further reduction is expected (Giesy 1991, Tillitt 1991). Furthermore, contaminant levels are equalizing throughout the Great Lakes Basin. For PCBs in particular, the trend is toward a more uniform distribution throughout the Great Lakes, though highly contaminated "hot spots" still occur in harbors and industrial stretches of rivers where inputs are concentrated. These heavily contaminated sediments, by the mechanism of sediment resuspension, will continue to be localized sources of contaminants (Hallett 1985).

PCBs

Production, Physical Properties, and Uses

Polychlorinated biphenyls (PCBs) are a class of synthetic organic compounds first synthesized in 1881, but they were not manufactured for industrial application until 1929 (Peakall and Lincer 1970, Cairns et al. 1986). The majority of PCB production had occurred in the United States, but France, Japan, Czechoslovakia, Russia, Germany, and Italy had also produced PCBs (Afghan 1985, Oliver et al. 1989). An estimated 1.2-1.5 million metric tons

of PCBs were manufactured globally (Hansen 1987, DeVoogt and Brinkman 1989). Uses of PCBs were restricted to closed systems (e.g. capacitors) in the early 1970s (Norstrom 1988), and U.S. production of PCBs was halted altogether in 1977 (Rodgers et al. 1988).

Industrial preparation of PCBs requires the substitution of chlorine atoms onto two covalently-bonded benzene rings. Ten possible sites of substitution allows for the formation of 209 congeners or structural variations (Cairns et al. 1986, Norstrom 1988), although, only about 100 congeners are common in industrial PCB mixtures (Huckins et al. 1988). PCBs were marketed as complex mixtures of congeners, and in the U.S., were sold exclusively by the Monsanto Company under the trade name "Aroclor" (hereby referred to as "A"). Specific mixtures are identified by a four digit number. For example, Aroclor 1242 (A1242) indicates a mixture of PCBs. The first two digits indicate the number of carbon atoms in the biphenyl group and the last two describe the percent chlorination by weight (Sawhney 1986).

The physical properties of PCBs lend to their unusual versatility in industrial applications. The physical states of these compounds vary with increasing chlorine content from a mobile, oily liquid phase, to white crystals and hard resins (Afghan 1985, Safe et al. 1987, DeVoogt and Brinkman 1989). PCBs are also characterized by their thermal stability, nonflammability, excellent electrical insulating properties, resistance to acids and bases, resistance to oxidation and reduction, compatibility with organic materials, lipid solubility, and resistance to chemical and biological degradation (Safe et al. 1987). PCBs have been popular for use in dielectric fluids, heat transfer fluids, plasticizers, protective coatings, hydraulic fluids, cardboard cartons, as "inert" ingredients in insecticides, as dust allayers in detergents, in carbonless carbon paper, microscope immersion oil, organic dilutents, flame

retardants, wax extenders, printing ink, lubricants, cutting oils, adhesives, and vacuum pumps (Bleavins 1980, Afghan 1985, Safe 1987).

PCBs in the Environment

Even though production of PCBs was banned in 1977 and their uses restricted, it is estimated that more PCBs remain in use today than have been released into the environment (Fox and Peakall 1991). It has been estimated that only about one third of the PCBs produced have reached the environment (Hansen 1987, Safe et al. 1987). Those PCBs that have reached the environment have done so by improper waste disposal techniques or accidental release (Borlakoglu and Walker 1989).

As discussed earlier, the most heavily PCB-contaminated areas are in harbors, bays, and rivers where industrial discharges occur. However, all five of the Great Lakes have measurable quantities of PCBs in the open water. Even non-industrial Lake Superior and inland Lake Siskiwit on Isle Royale are contaminated with PCBs. Lake Superior's drainage basin is in an area of low industrial, agricultural, and logging activity, and Lake Siskiwit is on a wilderness island, yet both have fish heavily contaminated with PCBs (Hallett 1988, Murphy 1988).

Clearly, then, other mechanisms are at work distributing PCBs to non-industrial areas. Evaporation from polluted bodies of water and atmospheric transport and deposition of contaminants into distant waters are the most important long range transport routes for pollutants (Rice 1985, Safe et al. 1987, Thomann et al. 1987, Hallett 1988). Atmospheric deposition accounts for the majority of the PCB contamination in Lake Superior (Norstrom 1988) and was estimated to introduce 85% of the PCB loading to Lake Siskiwit (Hallett 1988). Using median values reported by other researchers, Rice (1985) reported that

atmospheric loadings accounted for 76-98% of PCB loading to Lake Superior, 69-84% for Lake Michigan, 57-83% for Lake Huron, 45-67% for Lake Erie, and 43-63% for Lake Ontario.

Thus, a lake may accumulate PCBs via atmospheric deposition, tributary runoff, or direct industrial or municipal discharge. The PCBs cycle through the Lake's ecosystem; their movement dictated by their low solubility in water (Haque and Schmedding 1976, Oliver et al. 1989). They remain in the water column only until, adsorbed onto particulate matter, they settle out to the sediment layer. This type of particulate transport is the predominant mechanism of PCB movement in the aqueous environment (Haque and Schmedding 1976).

Deposition into the sediment layer does not insure that PCBs have been removed from the system. Sediment mixing due to natural weather-induced disturbances was discussed previously. Sediments are also disturbed, and as a consequence, PCBs are made reavailable by the feeding action of benthic organisms. By feeding below the sediment surface and excreting at the surface of the sediment layer, they effectively re-introduce "old" PCBs into the ecosystem (Eadie et al. 1988).

PCBs in the Food Chain

Colonial waterbirds are exposed to PCBs primarily by feeding on the aquatic food chain. Phytoplankton, zooplankton, and fish accumulate PCBs principally as a by-process of filtering the water for nutrients (Shaw and Connell 1986, Eadie et al. 1988, Hileman 1988). Because of the nonpolar, hydrophobic nature of PCBs, they tend to accumulate in the lipids of the biota (Hooper et al. 1990) and are biomagnified at every step of the food chain (Oliver et al. 1989). To illustrate this point, phytoplankton, the base of the food chain, accumulate PCBs as a consequence of water filtration. They are consumed by zooplankton

which take on the PCB body burden of phytoplankton in addition to their own. Forage fish eat vast quantities of zooplankton and, in turn, are eaten by top predator fish (Hileman 1988). At this point, the PCBs have been biomagnified approximately 12-fold from sediment levels to fish tissue concentrations (Coppock et al. 1990). Now the terrestrial food chain is introduced to the aquatic contaminants. Top predator species, such as colonial waterbirds, bald eagles, and humans, consume forage and predator fish and concomitantly, the body burdens they carry. Of the Great Lakes colonial waterbirds, the double-crested cormorant, Forster's tern, Caspian tern, and common tern are either predominantly or completely piscivorous. The herring gull and ring-billed gull are partially piscivorous, but are also scavengers (Harris 1988, Ludwig et al. 1988b). Fish to bird biomagnification factors of 15-30 have been recorded in these species (Coppock et al. 1990).

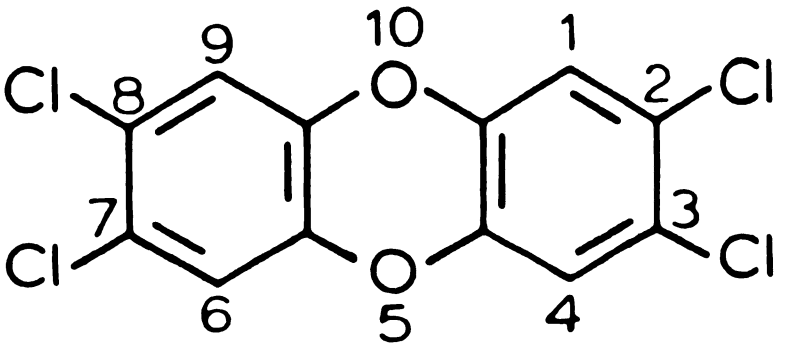
PCB concentrations are also magnified and transferred from the adult birds to their eggs. Avian eggs are good reservoirs for lipid soluble chemicals because of their high lipid content. On a whole egg basis, the egg is 7-10% lipids (Fox and Weseloh 1987), and the yolk is about 58% lipids (Kurita and Ludwig 1988). A biomagnification model by Hileman (1988) estimates PCB concentrations in herring gull eggs to be about 50,000 times greater than phytoplankton PCB concentrations. In 1978, DDE and PCB concentrations in Lake Ontario herring gull eggs were about 2.5×10^7 times greater than ambient water concentrations and about 20 times greater than salmon tissue PCB concentrations (Fox and Weseloh 1987). The average forage fish to waterbird egg biomagnification factor was estimated to be 23.3-23.7. Double- crested cormorants in Saginaw Bay (1989), however, had a biomagnification factor of 41 (Ludwig et al. 1991).

PCB Toxicity

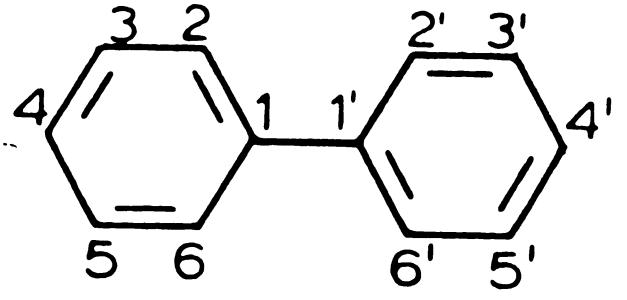
PCDAHs elicit similar toxic effects across all the phylogenetic lines and within the order *Aves* (Anonymous 1990, Tillitt et al. 1991a). Common toxic responses are altered biochemical activities, physical manifestations of toxicity, and reproductive effects of embryotoxicity and teratogenesis (Tillitt et al. 1992). These are induced through a common mechanism of action by binding of the PCDAH to the cytosolic Ah receptor and translocation of the PCDAH:receptor complex to the nucleus (Brunstrom and Reutergardh 1986, Safe 1987, Kannan et al. 1988, DeVoogt et al. 1990, Tillitt et al. 1991a) where it binds to DNA and alters gene expression (Denison et al. 1985).

PCB toxicity is often evaluated and reported as total PCB concentrations referenced to technical mixtures. While this may be satisfactory for reporting a measure of exposure, reporting total concentrations of PCBs as an indicator of toxicity or risk is inappropriate (Colborn 1989, DeVoogt et al. 1990, Tillitt et al. 1992). Doing so overlooks the different and wide ranging potencies of the congeners, the dissimilarity between technical and environmental mixtures of PCBs, and the complex interactions that occur in environmental PCBs that have been described as additive, synergistic, and/or antagonistic (Tillitt et al. 1991a, Tillitt et al. 1992). Thus, correlation between total PCBs and toxic effects is poor.

The congener specific toxicity should be considered when evaluating the toxic potential of environmental PCBs. Congener stereochemistry, the degree and orientation of chlorination, is the primary determinant of toxicity (Safe 1987, Kannan et al. 1988, Brunstrom 1990, DeVoogt et al. 1990). PCB congeners that are isostereomers to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), the most toxic PCDAH (Safe 1987), are also highly toxic. These include those congeners which can assume a coplanar configuration. Coplanar PCBs are those with no *ortho* chlorine substituents (Figure 1) (Brunstrom 1990,



2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin



Polychlorinated Biphenyl

Figure 1. The structure and chlorination sites of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs).

DeVoogt et al. 1990), thereby leaving the molecule free to rotate around its central phenyl-phenyl bond (DeVoogt et al. 1990). The most toxic coplanar PCBs are those with chlorine substituted at both *para* positions and two of the four *meta* positions. Of these, the greatest toxicity is found in the non-*ortho* coplanar congeners 3,4,3',4'-tetrachlorobiphenyl (IUPAC #77), 3,4,5,3',4'-pentachlorobiphenyl (IUPAC #126), and 3,4,5,3',4',5'-hexachlorobiphenyl (IUPAC #169) (Brunstrom and Andersson 1988, Anonymous 1990, Brunstrom 1990). These highly toxic coplanar PCBs are present in environmental mixtures in trace concentrations (DeVoogt et al. 1990).

Certain mono- and di-*ortho* PCBs are also highly toxic (Brunstrom and Reutergardh 1986, Anonymous 1990, Brunstrom 1990). While roughly three orders of magnitude less toxic than the most toxic non-*ortho* congeners (Brunstrom 1990), they are present in relatively high concentrations. Of the mono-*ortho* PCBs, 2,3,4,3',4'-pentachlorobiphenyl (IUPAC #105) contributes the most to overall toxicity and is found in high concentrations in Aroclor mixtures (Huckins et al. 1988, Smith et al. 1990). Factoring relative toxicity and concentration, it is evident the mono-*ortho* PCBs contribute significantly to the overall mixture toxicity (Huckins et al. 1988, Brunstrom 1990).

Effects of PCBs on Fish-Eating Colonial Waterbirds

The toxic effects experienced by different colonial waterbird species are a function of their sensitivity to and exposure to contaminants. PCBs have a low acute and sub-chronic toxicity in adult birds (Peakall 1986, Hallett 1988). Chronic effects may occur in the adults of some species, but the developing embryo has proven to be the most sensitive life-stage. Laboratory studies report LD_{50} values or other endpoints as a means of ranking relative sensitivities. LD_{50} values reported for the mallard, pheasant, bobwhite and Japanese quail for

six Aroclor mixtures reveal a decreasing sensitivity ranking of bobwhite quail, pheasant, mallard, and Japanese quail (Heath et al. 1972). Chickens are highly sensitive to PCBs (Peakall 1986), and were found to be more sensitive than other laboratory species and herring and black-headed gulls (Brunstrom 1989). It is much more difficult to rank the sensitivity of wild species. In many cases, they cannot be brought into the laboratory for study due to endangered or threatened status or a lack of successful techniques to raise them in captivity. Broadly speaking, researchers have determined that herring gulls are not very sensitive to PCBs. Herring gull data from the early 1970s showed that while these gulls had the greatest contaminant concentrations in their eggs, they had a zero incidence of abnormalities (Gilbertson et al. 1976). On the other end of the spectrum, double-crested cormorants (DCCO) are very sensitive (Kurita and Ludwig 1988). The other colonial species (Caspian, common, and Forster's terns and ring-billed gull) have sensitivities intermediate to the cormorant and the herring gull. Common tern data from the early 1970s showed that even while these terns had the lowest egg contaminant concentrations, they had the greatest incidence of abnormalities on Lakes Ontario and Erie (Gilbertson et al. 1976).

Those PCDAH isomers that are able to attach to the Ah receptor, namely 2,3,7,8-TCDD and its isostereomeric planar PCBs and PCDFs, elicit a common suite of toxic effects (Kubiak et al. 1989, Gilbertson et al. 1991). Clinical signs of PCDAH poisoning occur in mammals such as mink (Heaton 1992) and laboratory mice and rats (Neubert et al. 1973), reptiles such as the snapping turtle (Bishop et al. 1991), and numerous avian species including chickens, mallards, quail, and pheasants (Heath et al. 1972) and wild avian species. Characteristic symptoms associated with PCDAH poisoning include:

Embryo/Fetotoxicity: mortality and congenital anomalies - edema, bill, skull, and skeletal defects; soft tissue defects; unabsorbed yolk sacs (Colborn 1988, Ludwig et al. 1990) and growth retardation (Kubiak et al. 1989).

Weight loss (wasting syndrome): decreased hatchling body weights, increased liver:body weight ratios (Kubiak et al. 1989).

Immune suppression (Tillitt et al. 1991a).

Behavioral changes: feminization, lowered nest attentiveness, nest abandonment, and increased incubation times (Fox and Weseloh 1987, Kubiak et al. 1989).

Hepatotoxicity: aryl hydrocarbon hydroxylase (AHH) induction, porphyria, and vitamin A (retinol) depletion (Bishop and Weseloh 1990, Fox 1991).

Thymic atrophy and altered thyroid function (Gilbertson et al. 1991, Tillitt et al. 1991a).

Pinpointing the real causative factors of toxicity in wild populations is difficult because no single effect is diagnostic of PCDAH poisoning and these effects may be elicited by other compounds. Therefore, establishing the causative factor is a long-term process of careful experimentation and observation. Gilbertson and co-authors (1991) discuss the steps required for critical investigation and formulating conclusions about cause-effect relationships. Theirs is an intensive review of colonial waterbird case histories and anecdotal information which substantiates the linkage between reproductive impairment and PCDAH poisoning via the food source.

The impairment of colonial waterbird reproduction and the observed suite of toxic effects has been referred to as "chick edema disease" because of the similarities between wild bird symptoms and those described in the poultry industry. In 1957, PCB- and dioxin-contaminated feed killed millions of broilers in flocks in the eastern and midwestern U.S. Subsequent necropsies revealed that pericardial edema and ascites (fluid in the gut cavity) were common clinical signs (Firestone 1973, Gilbertson 1983, Gilbertson et al. 1991). When these effects were noted in the fish-eating waterbirds, researchers realized the parallels with

the broiler findings. Now the scenario is being described as Great Lakes Embryo Mortality, Edema, and Deformities Syndrome (GLEMEDS) (Gilbertson et al. 1991) to account for the embryo lethality and deformities that are also common to wild bird reproductive attempts.

The following is a brief synopsis of the overall trends found in the wild populations of Great Lakes colonial waterbirds. The reader is referred to the papers cited for detailed results and study information.

Herring Gull

The herring gull has long been used as a sentinel species for the long-term monitoring of contaminant-related biological effects. Researchers have been studying herring gull reproduction for the past 20 years, making it the most extensively studied species on the Great Lakes (Peakall 1988). It was chosen as an ideal sentinel species because it feeds at the highest trophic levels, resides year-round on the Great Lakes and remains fairly stationary on one lake, nests colonially, and its holarctic distribution allows for comparative studies (Peakall and Fox 1987, Weseloh et al. 1979).

The herring gull has made a dramatic rebound since the reproductive collapse it suffered in the late 1960s and early 1970s. In 1964, Keith found fledging rates of only 0.3-0.4 young/pair (Peakall 1988). Normal, maintenance level, reproductive success is considered to be about 1.0 young/pair fledged (Gilbertson 1983, Fox and Peakall 1991). Then in 1972, in eastern Lake Ontario, fledging was found to be only 0.10-0.21 chicks/pair, or about one chick fledged for every 10 nests (Peakall and Fox 1987, Gilbertson et al. 1991). The population made an incredible recovery over a period of three years from 1975 to 1977. This is demonstrated by data from Scotch Bonnet Island in Lake Ontario where hatching success

was only 2% in 1975, but increased to 37% in 1976, and 70% in 1977. Fledging success followed the same trend: 0.1 chicks/pair fledged in 1975, 0.4 and 1.0 chicks/pair fledged in 1976 and 1977, respectively (Gilbertson 1983).

The reproductive failures evident in the early 1970s were attributed to a low hatching success caused by embryonic mortality and egg loss (Peakall and Fox 1987, Peakall 1988), both of which were influenced by aberrant adult behavior. Decreased nest defense and nest abandonment contributed to egg loss, and abnormal incubation behavior and the subsequent cooling of the nests led to embryonic mortality (Gilman et al. 1977, Gilbertson 1983). In a 1975 egg exchange experiment, "dirty" adults (adults with elevated contaminant concentrations) were only able to hatch 7% of "clean" eggs, while "clean" adults were able to hatch 86% of "clean" eggs (Peakall and Fox 1987). Furthermore, in the mid-1970s, 38.5% of eggs disappeared from contaminated Scotch Bonnet Island colonies while only 2.2% and 10.1% of eggs disappeared from colonies in Lake Huron and Lake Superior (Peakall and Fox 1987). Therefore, aberrant adult behavior is clearly a factor in decreased hatching success.

Embryo mortality is also influenced by concentrations of contaminants found in the eggs. In the 1975 egg exchange experiment mentioned above, only 2% and 10% of "dirty" eggs hatched when incubated by "dirty" and "clean" adults respectively (Peakall and Fox 1987). Contaminant analysis of Scotch Bonnet Island eggs revealed PCB concentrations 10-100 times greater than in other colonies in North America and Europe (Gilbertson 1983).

Likewise, congenital anomalies in 1971-1975 Lake Ontario embryos were 100-200 times more prevalent than recorded background levels of one abnormality per 20,000 chicks (Peakall and Fox 1987). During this time, the deformity rates reached 0.07-6.8 per 1000 chicks (Peakall 1988). Abnormalities recorded include subcutaneous, pericardial, and peritoneal edema, growth retardation, hepatotoxicity (Fox and Weseloh 1987, Gilbertson

1989), shorter tarsal lengths, and unabsorbed yolk sacs (Gilbertson et al. 1991). Additionally, Gilman et al. (1977) observed cases of hydrocephaly and bill defects. A 1974 study comparing colonies in the Great Lakes and Alberta, Canada reported subcutaneous edema in 36% of Great Lakes chicks versus only 8% in Alberta colonies. Fifty percent of these edemas occurred in chicks that pipped and died while only 10% were found in live chicks. Great Lakes chicks also had a higher incidence of hydropericardium (Gilbertson 1983).

Certain biochemical parameters such as AHH induction, porphyrin concentrations, retinol concentrations, and thyroid goiter, have also been found to be useful indicators of contaminant exposure. Great Lakes chicks have increased induction of AHH activity, porphyrin concentrations, and exhibit retinol depletion (Gilbertson 1983, Peakall and Fox 1987, Peakall 1988, Gilbertson 1989). Induction of AHH activity is a general adaptive response to xenobiotic exposure (Fox and Weseloh 1987), but there is good correlation between AHH activity and concentrations of TCDD in eggs (Peakall and Fox 1987), as well as *in vivo* toxicity (Tillitt et al. 1991b). Increased porphyrin concentrations are a toxicant-induced derangement of heme biosynthesis and serve as a sensitive and specific biochemical endpoint (Fox and Weseloh 1987).

Ring-Billed Gull

The ring-billed gull has not been studied nearly as extensively as the herring gull. No detailed study looking for the occurrence of GLEMEDS has been done on this species (Gilbertson et al. 1991). It has been resident on the Great Lakes only since about 1900 when it was a rare visitor. Its population has irrupted following the alewife population explosion (Colborn 1988) and the population has grown about 10% annually over the last

30 years. In 1940, there were 20,000 ring-billed gulls, 141,000 in 1960, and 650,000 in 1984 (Fox 1982).

Contaminant-related population declines, behavioral changes, or altered reproductive success have not been noted for this species, but congenital anomalies have been observed. Background abnormality rates are estimated at less than 0.05/1000 chicks. During 1971 to 1975, the mean deformity rate on Lake Ontario was 0.35/1000 chicks with a maximum of 5.78/1000 chicks. Deformity rates declined, in the period from 1976 to 1980, for Lakes Michigan, Huron, and Ontario, but increased in Lake Superior (Fox 1982). Clinical signs of toxicity recorded from Lake Ontario colonies in the early 1970s included weak and emaciated birds, liver degeneration (fatty livers, necrosis, and hemorrhage) (Gilbertson 1989), one chick with a bill deformity, and 24 chicks with leg deformities which resulted in their inability to stand or walk erect (Gilbertson et al. 1976).

Common Tern

The common tern has also displayed impaired reproductive success on the Great Lakes in the past 20 years. It has, however, been battling both deteriorating habitat conditions and contaminated food resources. Nesting throughout the Great Lakes, except for northern Lake Superior, the population peaked at 16,000 pairs in the early 1960s. It then began to decrease, the most rapid decline being in the early 1970s, to only 5,000 pairs by the late 1970s. Declines continued to about 1984 and then apparently stabilized. Much of this decline has been attributed to rising lake levels which have forced the birds to abandon their favored gravelly island nesting sites. They are now nesting in areas that are subject to ring-billed gull predation and human disturbance (Weseloh et al. 1989, Anonymous 1991, Fox and Peakall 1991).

Environmental contaminants have also contributed to common tern population declines in the lower Great Lakes. The first observations of failed hatching and abnormalities were noted in Lake Ontario colonies in 1970 (Peakall 1988, Anonymous 1991). Studies throughout the 1970s have revealed the greatest anomaly rates observed in any species at any location to date. Only 0.2 deformities per 1000 chicks are normally expected (Peakall 1988), but from 1971 to 1973 the Lake Ontario colonies had 12.2 deformities per 1000 chicks (Gilbertson 1983). For the entire Great Lakes region, the mean deformity rate was 3.03/1000 chicks with a maximum of 10.7/1000 chicks (Peakall 1988). The incidence of anomalies quickly declined, just as in the herring gull and ring-billed gull, during the late 1970s. During the period of 1975 to 1980 the Lake Ontario rate dropped to less than 1.6 abnormalities per 1000 chicks (Gilbertson 1983). Common terns are still demonstrating contaminant-induced effects and deformed chicks are still being found in Saginaw Bay, Green Bay, and Lake Ontario (Fox and Peakall 1991).

Concentrations of contaminants in common tern eggs have also declined precipitously since the early 1970s. Eggs collected in 1981 from Lake Ontario had 80-90% less PCBs and DDE than eggs from 1969 to 1973 (Weseloh et al. 1989). Similar trends have been noted for Lake Erie eggs (Fox and Peakall 1991).

Caspian Tern

The Caspian tern, the largest of the terns, has been the subject of detailed investigation only since 1986. Prior to that, discussion of potential toxicological effects was only incidental. In 1972, the Lake Huron population was experiencing an 87% hatch rate and fledging 1.1 young/pair while the DCCO was completely failing in its reproductive attempts because of DDT-complex-induced eggshell thinning in the DCCO (Fox and Peakall

1991). In 1987, however, Caspian tern reproductive success in Lake Michigan declined, and the hatch averaged only 28% in Saginaw Bay, Lake Huron colonies. In fact, no young fledged that year in Saginaw Bay (Kurita et al. 1987, Kurita and Ludwig 1988). Other colonies offshore in Lake Huron hatched 58% of the eggs and fledged 0.79 young/pair (Kurita et al. 1987, Fox and Peakall 1991). Average hatching rates from 1986 to 1988 were 77% in northcentral Lake Michigan, 71% in Green Bay, and 51% in Saginaw Bay (Ludwig et al. 1988b).

Manifestations of GLEMEDS have been observed in Caspian terns in the Great Lakes Basin. Observations from 1962 to 1979 yielded a background deformity rate of only 0.103/1000 chicks (Ludwig et al. 1988b). During 1986 to 1988, deformities were observed at the rate of 3.17/1000 chicks in northcentral Lake Michigan, Green Bay, and Saginaw Bay. Lake Michigan and Saginaw Bay colonies had 21 and 105 times the background deformity rates, respectively (Ludwig et al. 1988b). The most frequent anomaly noted was an open gut cavity and unabsorbed yolk sac (Ludwig et al. 1988a, Gilbertson et al. 1991). Subcutaneous edema was also common (Kurita and Ludwig 1988). In 30 years of handling 15,385 live chicks, Ludwig and co-workers (1988a) have never encountered a crossed bill. Only Gilbertson et al. (1976) have reported a crossed bill Caspian tern in their 1972 survey of a Lake Ontario colony. Bill defects in dead Caspian tern chicks are more frequent, however, than bill defects in dead DCCO chicks (Ludwig et al. 1990). The overall incidence of abnormalities in Caspian terns dropped by about 30% in 1988. In Saginaw Bay, deformities in dead eggs dropped from 60% to 21%, but egg infertility, including early embryonic mortality, increased from 25% to 52%. Therefore, manifestations of developmental toxicity are still occurring in Great Lakes Caspian terns (Ludwig et al. 1990).

Recent reproductive failures in Caspian tern populations are significant because it is a migratory species and spends only six months on the Great Lakes. In the two to three weeks it is resident on the Great Lakes prior to nesting, it accumulates body burdens that are approximately 90% of those found in resident herring gulls (Struger and Weseloh 1985, Ludwig et al. 1988b). It is, however, completely piscivorous, unlike the omnivorous herring gull, and thus, is highly exposed to the contaminated aquatic food chain (Struger and Weseloh 1985).

Forster's Tern

The Forster's tern is another relatively uncommon and scattered nesting species on the Great Lakes. GLEMEDS, growth retardation, increased liver:body weight ratios, hepatotoxicity, and AHH induction have been observed in this species (Hoffman et al. 1987, Gilbertson 1989).

The primary toxicological investigations on this species occurred during 1983 in Green Bay and upriver, on inland Lake Poygan (Hoffman et al. 1987, Kubiak et al. 1989). Hoffman and co-workers collected eggs from both locations, artificially incubated them and found that Green Bay eggs hatched at a rate of only 52% of that of Lake Poygan eggs. Most of the mortality occurred late in incubation near hatch, and was often associated with edema. Chicks from Green Bay weighed 22% less and took 4.6 days longer to hatch than Lake Poygan chicks. Furthermore, the liver:body weight ratio was 26% greater and hepatic AHH activity was three times greater in Green Bay chicks than Lake Poygan chicks (Hoffman et al. 1987, Gilbertson et al. 1991).

In 1983, Kubiak and co-workers (1989) collected eggs from the same colonies that Hoffman studied. They monitored hatchability in the field, performed an egg exchange

experiment between the colonies and analyzed a sample of eggs for contaminants. Hatchability was 26% and 88% for the Green Bay and Lake Poygan colonies, respectively. The Green Bay colonies did not fledge any young while the Lake Poygan colonies fledged 55%. Hatching also took 8.25 days longer in the Green Bay colonies (Kubiak et al. 1989, Fox and Peakall 1991). Nest abandonment and egg disappearance occurred in the Green Bay colonies, but it was not noted in the Lake Poygan colonies (Kubiak et al. 1989, Fox and Peakall 1991). The egg exchange experiment revealed both intrinsic and extrinsic factors affected reproductive success in the Forster's tern. Results followed a pattern similar to that found in herring gulls whereby "clean" adults were more successful at hatching eggs than "dirty" adults. The contaminant analysis showed significant differences between locations for all PCDAHs screened for including 2,3,7,8-TCDD, PCDDs, PCDFs, mono-*ortho* PCBs, and total PCBs. Total PCBs were 22.2 and 4.5 ppm for Green Bay and Lake Poygan eggs and mono-*ortho* PCBs were 5,500 and 615 ppt (pg/g), respectively (Hoffman et al. 1987, Kubiak et al. 1989).

Double-Crested Cormorant

The history of the double-crested cormorant on the Great Lakes reflects the history of the contaminant problems on the Great Lakes. Cormorants emigrated to the Great Lakes early in this century and fared well until DDT/DDE-induced eggshell thinning led to a period of severe reproductive failures during the late 1950s, 1960s and early 1970s. Estimates indicate the population fell by 80% during that time (Ludwig 1984, Fox and Weseloh 1987, Peakall 1988, Anonymous 1991, Fox et al. 1991a,). With the decline of DDT/DDE concentrations, cormorants are currently experiencing a population explosion. The popu-

lation is now at the greatest level it has ever been on the Great Lakes and is continuing to grow (Ludwig 1984, Anonymous 1991, Fox et al. 1991a).

However, since concentrations of DDT/DDE decreased to below threshold levels for eggshell thinning, embryotoxic and teratogenic effects have become more prominent (Fox et al. 1991a, Tillitt et al. 1992). Virtually all GLEMEDS manifestations have been noted in cormorant chicks. The most commonly observed abnormalities include subcutaneous edema, hemorrhaging, enlarged yolk sac attachments, bill defects, exencephaly, and ancephaly (Kurita and Ludwig 1988). Clubbed feet, spinal abnormalities, abnormal appendages and feathering, ascites, gastroschisis, and eye deformities have also been noted (Kurita et al. 1987, Ludwig et al. 1988a).

Rates of deformities in Great Lakes cormorants are greater than those recorded in reference areas. Background deformity rates have been calculated to be 0.60/10,000 chicks in Alberta and Saskatchewan colonies in 1979 to 1987 (Tillitt et al. 1991a) versus 3.5/10,000 chicks in Lake Ontario colonies (Fox and Peakall 1991). Field studies in 1986 and 1987 revealed even greater deformity rates of 3.64 and 4.63 deformities per 1000 chicks for all of the Great Lakes (Kurita et al. 1987). Fox and co-workers (1991b) performed an exhaustive search of records of abnormalities in cormorants from 1979-1987 in an attempt to statistically analyze the long-term, basin-wide observations. Only data on bill defects offered a large enough sample size to analyze. They calculated a background bill deformity rate of 0.95/10,000 chicks. On the Great Lakes as a whole, the incidence was 22.4/10,000 chicks, but in the Green Bay region it was 52.1/10,000 (Fox et al. 1991b).

PCDAH concentrations have been analyzed in cormorant eggs from around the Great Lakes. H4IIE derived TCDD-Eqs in 1986 and 1987 DCCO eggs ranged from 35-344 pg TCDD-Eq/g (wet weight). Total PCBs were 0.05-14.84 μg/g (Tillitt et al. 1992). The

number of days of feeding on the Great Lakes that would produce LC_{100} concentrations of contaminants in eggs laid by resident cormorants has been calculated and complete egg lethality could be attained by only 4 months of feeding on Saginaw Bay, the most contaminated area (Anonymous 1990). Longer periods of feeding would be required to reach complete egg lethality on other areas of the Great Lakes (9.5, 15.4, and 107 months of feeding on Green Bay, northern Lake Michigan, and western Lake Superior, respectively).

White Leghorn Chicken-PCB Laboratory Studies

The toxic effects of commercial PCBs (Aroclors) on chickens are well documented (McCune et al. 1962, Flick et al. 1965, Scott et al. 1971, Briggs and Harris 1973, Britton and Huston 1973, Carlson and Duby 1973, Platonow and Reinhart 1973, Tumasonis et al. 1973, Cecil et al. 1974, Lillie et al. 1974, Harris et al. 1976) as are the toxic effects of individual congeners (Brunstrom and Darnerud 1983, Brunstrom and Reutergardh 1986, Brunstrom 1989, Brunstrom 1990, Brunstrom 1991). As in the colonial waterbirds, PCBs are not acutely toxic to adult hens (Peakall 1986). Flick et al. (1965) found low mortality in a study in which they fed day-old cockerel chicks 200 and 400 ppm PCBs for three weeks (0/24 chicks died with 200 ppm in the diet, 3/24 chicks died with 400 ppm in the diet).

The chicken embryo, however, is extremely sensitive to the toxic effects of PCBs. Early studies (Scott et al. 1971, Britton and Huston 1972, Platonow and Reinhart 1973, Cecil et al. 1974, Blazak and Marcum 1975) have shown that embryos are affected by lesser exposures than those required to elicit signs of toxicity in chicks or adult hens. Embryo mortality increased when eggs were injected with A1242 and A1254 to a final concentration of 5 ppm/egg (Carlson and Duby 1973), and hatchability was severely suppressed (only 5%

hatched) by injecting 10 mg/egg (McLaughlin et al. 1963). Feeding studies, incorporating PCBs into the diets of laying hens, have shown significant reproductive effects when hens were fed PCBs. Five ppm of A1254 fed to hens for 39 weeks decreased egg production during weeks 15-28 (Platonow and Reinhart 1973). Hatching rates were reduced when 10 and 20 ppm (A1232, A1242, A1248) were fed to hens for eight weeks (Harris et al. 1976). Twenty ppm of A1242 in the diet decreased the hatching rate to 50% of that of controls by the second week of feeding. Increasing the dose to 50 ppm in the diet for one week reduced the hatch of fertile eggs to only 14.8%, and no eggs hatched after two weeks of feeding (Briggs and Harris 1973). Ax and Hansen (1975) fed A1242 and A1254 to hens at 20 ppm for 10 weeks and found increased embryonic mortality in the eggs produced in the second week of feeding. Some PCB mixtures, however, are fairly innocuous. Jenkins et al. (1972) could not elicit any toxic effects from feeding hens 100 ppm of A1260. Similarly, feeding 20 ppm A1221 and A1268 to hens for nine weeks did not cause any adverse effects (Lillie et al. 1974). A comprehensive review of the early reproductive studies can be found in Roberts et al. (1978). Chicken studies in the past decade have focused primarily on identifying the most toxic congeners. Much of the work has been done by Brunstrom and co-workers in Sweden (refer to the listing above for details).

A suite of toxic effects in the embryos and hatchlings was common to all egg injection and feeding studies involving Aroclor mixtures or individual PCB congeners. Symptoms of chick edema disease (Gilbertson et al. 1991), embryo mortality, decreased hatchability (Roberts et al. 1978, Brunstrom and Darnerud 1983, Brunstrom 1988), teratogenesis (Peakall 1986, Brunstrom 1988, Brunstrom and Andersson 1988), and biochemical effects (Vos and Koeman 1970, Peakall 1986, Nikolaidis et al. 1988) were characteristic findings. Most of these toxic responses were also observed in this study and will be discussed later in this thesis.

STUDY INTRODUCTION AND OBJECTIVES

While a number of studies have examined the effects of commercial mixtures or individual congeners of PCBs on chickens, none have examined the birds' response to a naturally-contaminated environmental food resource. The situation on the Great Lakes offers an excellent opportunity for this type of study. Research has demonstrated reproductive failures in a variety of fish-eating wildlife species, and Great Lakes fish have been shown to be extremely toxic to mink in laboratory studies (Hornshaw et al. 1983, Heaton 1992). This study examined the effects of feeding Great Lakes fish to an avian laboratory species, the chicken (*Gallus domesticus*).

Great Lakes colonial waterbirds have exhibited adverse reproductive effects including embryolethality, teratogenesis, growth retardation, hepatotoxicity, and adult behavioral changes leading to nesting failure. These impairments to reproductive success are suspected of being caused by the consumption of contaminants in their primary food source, Great Lakes fish. Thus, the hypothesis of this study is: The same suite of toxic effects that are observed in fish-eating colonial waterbirds in the Great Lakes basin can be elicited and reproduced in the laboratory by feeding Great Lakes fish, naturally contaminated with PCDAHs, to laying hens.

The first objective of the study was to test an environmentally derived mixture of PCDAHs for reproductive impacts. It is important to stress that the contaminants of interest in this study were naturally occurring in the Great Lakes basin, have cycled through the

ecosystem, and have been exposed to natural weathering forces including photochemical, biochemical, and biological degradation. Reproductive impacts noted in other studies include changes in egg production levels, hatchability, mortality, and terata rates, as well as changes at the biochemical and cellular levels from exposure to commercial PCBs. Assuming the contaminants in Great Lakes fish were the cause of reproductive failures in colonial waterbirds, similar effects in the reproductive dynamics of the laying hen would be expected. The second objective was to assess the chicken as a model species for validating the observations noted in wild populations of fish-eating colonial waterbirds. Reproductive failures, the cause of which is still a matter of dispute, have been commonly observed in the waterbird nesting colonies on the Great Lakes. This study was conducted to ascertain if consumption of the waterbird's primary food resource, Great Lakes fish, would produce a similar suite of toxic effects in a controlled laboratory setting and in a sensitive, surrogate avian species.

The chicken was chosen as the species to study because other researchers have shown its "great" sensitivity to PCBs. Furthermore, it is easily maintained in captivity and protocols for artificial insemination and egg incubation are well defined. While it would be optimally beneficial to bring wild avian species into the laboratory for study, it is not practical at this time. Protocols for incubating wild bird eggs are not well defined, the age, health, and reproductive status of wild birds are difficult to assess, capture and captivity related stress may influence results, and some species are classified as endangered or threatened thereby making the collection of adults or eggs difficult if not impossible.

The chicken provides a sensitive model species to examine the effects of the food resource utilized by colonial waterbirds. Because the chicken is more sensitive to PCDAHs than any of the waterbird species, it is a useful avian model species for studying the effects

of these environmental contaminants. The protection of environmental health requires that the most sensitive species in the ecosystem be protected from deleterious factors or influences. With the appropriate understanding of relative sensitivities among species, a model or surrogate species, e.g. the chicken, can be a useful tool for determining the lower thresholds for toxicity.

Although small forage fish (e.g. alewife and smelt) are the staple dietary species of colonial waterbirds, carp were selected as a representative, naturally-contaminated food resource for this study because: 1) they were known to contain "great" concentrations of PCDAHs which permitted their incorporation into poultry rations at reasonably low levels, and 2) they tended to concentrate at the mouth of the Saginaw River in the late fall allowing for relatively easy collection of large quantities.

MATERIALS AND METHODS

Fish Acquisition and Preparation

The carp (*Cyprinus carpio*) was collected by electro-shocking in the mouth of the Saginaw River by personnel from the Michigan Department of Natural Resources and Michigan State University (MSU) in December 1988. The carp was transported to MSU and the whole fish were ground, blended into a homogeneous mixture, and stored frozen in sealed plastic bags at -5°C. Oceanfish scraps (cod, haddock, pollack, and flounder trimmings) were incorporated into the control and low-dose diets as a source of fish.

Before being incorporated into the diets, both the raw ground oceanfish and carp were cooked to dehydrate the fish, and to destroy the anti-thiamine enzyme, thiaminase. Thiaminase is present in certain species of raw fish, particularly so in carp, and can inactivate thiamine in both feed mixtures and in an animal's digestive tract. The presence of thiaminase in fish fed to foxes and mink can produce Chastek Paralysis, which is caused by a thiamine deficiency (Gnaedinger 1963). This disease, however, has not been reported in poultry. Thiaminase can be destroyed by cooking the fish for 6-7 minutes at 180°F (Gnaedinger and Krzeczkowski 1966). Three batches of fish were cooked at 180°F for approximately two hours in a 1000 lb-capacity feed mixer (Weiler and Company, Whitewater, WI - Model 1170M) that was fitted with a heat intake pipe and a portable diesel fuel heater. The fish was mixed continually throughout the cooking process to ensure that all of the fish was heated thoroughly and evenly. A total of 303 lbs of carp and 250 lbs of oceanfish were

cooked. At the end of the cooking process, the weight of the carp was reduced by 63% and the oceanfish by 25% and both were of a thick, yet fluid, consistency. Finely ground corn was thoroughly mixed into the fish to absorb remaining moisture thus producing a semi-dry fish meal which facilitated handling. The fish/corn mixture was then refrozen until incorporated with the other dietary ingredients into the experimental diets.

Diet Preparation

The treatment diets were formulated by Dr. D. Polin, MSU poultry nutrition specialist, based on the nutrient requirements of laying hens (National Research Council 1984). The diets were prepared using the equipment at the MSU Experimental Fur Farm which is part of the Poultry Science Teaching and Research Center. Samples of the dehydrated oceanfish and carp were submitted to Litchfield Analytical Services, Litchfield, Michigan for nutrient analysis. The control diet had 30.9% oceanfish, the low-dose diet contained 28.7% oceanfish and 3.4% carp, and the high-dose diet had 34.5% carp by dry weight. Appropriate quantities of oat hulls, isolated soy protein, soybean meal, alfalfa meal, ground corn, vitamin, mineral, and selenium premixes, limestone, salt, and Ethoxyquin were added to the dried fish/corn mixture. The mixed feed was returned to the freezer and stored frozen (0°F) until fed to the chickens. Samples of the mixed diets were submitted to Litchfield Analytical Services for nutrient analysis.

Acquisition and Acclimation of Study Hens

On April 6, 1990, 90 18-week old Babcock White Leghorn hens were purchased from Herbruck's Poultry Ranch, Clarksville, MI. Additionally, 16 adult roosters from the MSU breeding flock were allocated to the study. The birds were housed in four animal rooms at

the Poultry Science Teaching and Research Center. They were placed in individual cages in a three-tiered battery. The hens were housed in three separate rooms, one battery per room. The roosters were placed in similar cages in a separate room. Initially, all birds were fed a commercial layer mash *ad libitum*. Drinking water was available to the birds *ad libitum* throughout the study. Numbered leg bands were placed on each bird to ensure identification of individual birds.

A regimented lighting schedule was implemented to bring the hens into peak egg production. Each room was lighted by a 60 watt incandescent bulb for 10 hours each day, initially 7:00 am to 5:00 pm. At 20 weeks of age (4/18/90) the lighting period was increased to 12 hours per day (6:00 am to 6:00 pm). Beginning at 22 weeks of age (5/2/90), lighting was increased 15 minutes each week, alternating morning and evening time extensions, until the birds were 35 weeks old (8/15/90) at which time 15 3/4 hours of light were provided daily. This lighting regime was maintained throughout the remainder of the trial. The roosters were also exposed to the same lighting schedule.

Egg production was monitored daily for individual hens beginning May 8, 1990 to determine the time at which the hens reached peak production. Egg production was recorded on pre-printed trap-nest record forms. From the daily trap-nest records it was determined that peak production was achieved the week of June 13 (27 weeks of age).

Immediately prior to the beginning of the study, a preliminary feeding trial was conducted with eight birds for 12 days (June 7-18) to ensure that the hens would eat a diet containing a large proportion (30-35%) of fish product. Four hens were fed a commercial layer mash and four hens were fed a diet containing 30% oceanfish product and 70% commercial feed. On average, the hens fed the fish diet consumed more feed and gained

more weight than those fed the commercial feed. Egg production was comparable between the two groups.

Initially, the hens were artificially inseminated twice each week with pooled semen from the 16 roosters. The semen was collected by manual stimulation of the roosters and the hens were immediately inseminated with about 0.05 ml (approximately 50 million sperm) of the freshly collected semen. During the second week of the study, egg production fell severely and, in response to concern that handling stress might be impacting production, it was decided to inseminate the hens only once each week. No loss of fertility was recorded.

Immediately prior to the onset of the study, the 60 top-producing hens (each laying a minimum of six eggs/hen/week) were assigned to the study. Twenty hens were placed on each treatment diet. All hens were fed the control diet for two weeks to acclimate them to the high level of fish meal in the diet. Six hens were kept as replacements during the acclimation period in the event that some of the hens did not adjust to the high level of fish in the diet. The replacement hens were treated as if they were on the study. Five hens were later culled from the study and replaced during the acclimation period. After the two week acclimation period, the treatment diets were fed to the hens for eight weeks.

Data Collection - Adult Hen Feed Consumption, Body Weight, and Egg Production

Each hen was fed individually from its own feed reservoir. One week's supply of food was weighed and placed in sealed, plastic containers (one per hen) which were identified by the hen numbers. These were kept in the room with the hens, while the bulk feed supply was stored in a freezer. Feed was added daily from the individual plastic containers to the feeders attached to the cages to assure that the hens received feed *ad libitum*. Food consumption was measured weekly by collecting and weighing the uneaten

feed, and subtracting the weight of the orts from the initial quantity of feed provided for that week. The plastic containers were then refilled, weighed, and placed back into the respective rooms.

Hen body weights were recorded biweekly. The hens were removed from their cages and placed into a cone restraining device on a digital scale. Weights were recorded to the nearest gram and the hens returned to their cages.

Eggs were collected daily. A trap-nest record sheet was kept in each room and egg production was recorded daily. The date and hen number were marked on each egg with graphite pencil and the eggs were placed in a walk-in cooler maintained at 55-60°F. At the end of the week, the eggs were weighed, a sample retained from each diet group for composite chemistry, and the remainder placed in a Petersime incubator (Petersime Incubator Co., Gettysburg, Ohio - Model 5). During the incubation period, the incubator was maintained at 37.6°C and 90-92% humidity (wet bulb reading). The trays of eggs were automatically rotated at two hour intervals.

Data Collection - Fertility, Viability, Tissue Sample Collections

The eggs were handled three times during the incubation period, once to determine fertility and twice to determine viability. At day five of incubation, the eggs were candled to detect the presence of a vascular network indicating a fertile egg and the onset of embryogenesis. Non-fertile eggs were recorded and discarded. At day 11 of incubation, the eggs were again candled to determine the viability of the embryos. A heartbeat, and in some cases, movement of the embryo were visible with live embryos. Dead embryos were removed from the shell, the approximate age at time of death was determined by the progress of organogenesis, and the embryos were examined for gross pathologies. Estimation of age and

definition of pathologies were not possible on all embryos due to autolysis. A sample of eggs was taken from the incubator at day 11 of incubation. The individual eggs were wrapped in aluminum foil, and stored in liquid nitrogen for future vitamin A and porphyrin analyses. An embryo viability detector (EVD) provided by the U.S. Fish and Wildlife Service, East Lansing, MI, was used on day 18 to determine the viability of the late stage embryos. Candling was difficult late in incubation because the large embryo rendered the egg impermeable. The EVD works by converting any vibrations generated by a live embryo within an egg to audio signals that can be heard through earphones (Mineau and Pedrosa 1986). The EVD could have been used at day 11, but the candling technique was faster and more accurate at this stage of incubation. At day 18, any dead embryos were aged and examined for deformities. The live eggs were either transferred to hatching baskets, separated by hen, and returned to the incubator for hatching, or sacrificed for chemical analyses and histopathological examination. The latter eggs were carefully opened, the yolk sac detached, and the embryos quickly killed by decapitation. Each carcass (head and body) was weighed to the nearest tenth of a gram in a plastic weigh boat on an analytical balance. Each embryo was examined for gross deformities and the brain, heart, liver, spleen, and bursa were removed, trimmed, and weighed to the nearest tenth of a mg in an aluminum weigh boat on an analytical balance. The brain and a portion of the liver from each embryo were placed in a plastic bag, labeled with an identification number, and stored in liquid nitrogen for chemical analyses. A portion of the liver and the remainder of the tissues were placed in a 10% formalin solution and submitted for subsequent histopathological examination to Dr. J.A. Render, a certified veterinary pathologist, Department of Pathology, MSU.

Hatching ensued on the 21st day of incubation and all chicks were necropsied within 24 hours of hatching. The chicks were weighed live and then killed by decapitation. The brain, heart, liver, spleen, and bursa were removed, trimmed, and the weights recorded. Brain and liver tissues were collected from chicks from five different hens in each treatment group each week for chemical analyses. Any eggs that did not hatch were checked with the EVD to determine viability. Live eggs were returned to the incubator and dead eggs were opened and examined for deformities. The same procedures were carried out daily for all remaining unhatched eggs through the 25th day of incubation at which time incubation was terminated, the unhatched eggs were opened, and the embryos examined as previously described.

Hen Necropsy

At the termination of the trial, a 12-15 ml blood sample was collected from each hen by cardiac puncture and hematocrit values were determined. The remainder of the sample was centrifuged at 2,000 rpm for five minutes and the serum retained for future chemical analysis. The hens were killed by cervical dislocation, necropsied, and the brain, liver, and spleen weights were recorded to the nearest hundredth of a gram. A portion of the spleen and liver were preserved in 10% formalin for histopathology. The remainder of the tissues, including a sample of abdominal fat, were placed in plastic bags, labeled, and frozen in liquid nitrogen for chemical analyses.

Data Analysis

The data were analyzed using the statistical software, SAS (SAS Institute Inc., 1987). Significance of the main effects, time and treatment, was determined by a two-way analysis

of variance (ANOVA). Those parameters that had significant ($p \leq 0.05$) main effect interactions were then analyzed by a one-way ANOVA to determine the validity of the main effects. Where significant main effects occurred, the data were further analyzed by Tukey's all possible pairs test statistic. Levels of significance ranging from $p \leq 0.05$ to $p \leq 0.0001$ are reported in this thesis.

RESULTS

Major dietary components are presented in Table 1, and the nutrient analysis of the diets is provided in Table 2. Crude protein, fiber, and fat did not differ between the diets by more than four percent. Fat content increased with higher levels of carp substituted into the diet. Commercially prepared laying hen feeds provide about 14% crude protein, 4.5% crude fiber, 3% crude fat, (Purina Accu-Line Breeder 121 Ration, Purina Mills, Inc.) and about 1200 kcal of available energy. The treatment diets used in this study provided, on average, higher levels of crude protein (21%), fiber (6%), and fat (13.5%) (wet weight basis).

The raw carp, used in the study as the mechanism of PCB exposure, was analyzed by the Aquatic Toxicology Laboratory in the MSU Pesticide Research Center. Total PCB concentrations ranged from 6.19 to 10.36 mg/kg (8.40 $\pm$ 1.44 mg/kg mean $\pm$ S.E.) measured as A1248, A1254, A1260 (Heaton 1992). Samples of raw carp were also submitted to the U.S. Fish and Wildlife Service laboratory in Columbia, Missouri for congener specific analysis. One hundred five of the PCB congeners were summed and totaled 7.2 mg/kg (ppm), and the average TCDD-Eq concentration of the carp, summed from 17 AHH-active PCB congeners, totaled 535.76 pg/g (ppt). See Heaton (1992) for a description of the procedure used to analyze the carp.

Table 1. Major dietary components and contaminant concentrations of the control, low-dose, and high-dose diets fed to White Leghorn laying hens.

	Treatment Diet		
	Control	Low-Dose	High-Dose
Dietary Components (%)			
Carp	--	3.4	34.5
Oceanfish	30.9	28.7	--
Corn, #2 yellow	37.7	39.9	45.9
Oat hulls	6.7	6.1	11.1
Soybean meal (44%)	11.4	10.4	1.5
Isolated soy protein (90%)	3.0	2.1	--
Alfalfa meal (17%)	2.0	1.8	--
Vitamin premix	0.3	0.3	0.3
Mineral premix	0.3	0.3	0.3
Selenium premix	0.06	0.05	0.05
Limestone	7.5	6.8	6.3
Salt	0.06	0.05	--
Ethoxyquin (g/100 lbs. diet)[A]	5.67	5.67	5.67
Contaminant Conc.			
PCB concentration[B] (mg/kg)	0.50	0.90	6.60

[A] 90 % pure, Roche Animal Nutrition, Hoffman - LaRoche, Inc., Nutley, N.J.
[B] Total PCBs in the prepared treatment diets

Table 2. Nutrient analysis[^] of the adult hens' diets.

	Treatment Diet					
	Control		Low-Dose		High-Dose	
Dietary Component	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.
Fat (%)	11.50	12.89	13.55	15.28	15.45	16.87
Crude protein (%)	20.00	22.42	22.50	25.37	20.63	22.52
Crude fiber (%)	6.25	7.01	5.30	5.98	6.50	7.10
Calcium (%)	4.06	4.55	3.38	3.81	4.13	4.51
Phosphorus (%)	1.05	1.18	0.82	0.92	1.25	1.36
Potassium (%)	0.66	0.74	0.64	0.72	0.57	0.62
Magnesium (%)	0.14	0.15	0.13	0.15	0.12	0.14
Sodium (%)	0.236	0.265	0.197	0.222	0.13	0.14
Iron (ppm)	392.6	440.1	415.4	468.3	430.6	470.0
Manganese (ppm)	357.1	400.3	414.1	466.9	357.0	389.7
Copper (ppm)	38.4	43.0	59.4	67.0	45.8	50.0
Zinc (ppm)	304.8	341.6	406.5	458.3	469.1	512.1
Ash (%)	11.10	12.44	12.00	13.53	9.60	10.48

[^] Analysis provided by Litchfield Analytical Services, 535 Marshall St., P.O. Box 457, Litchfield, MI

During the first week of the study, the hens ate 102.9-106.5 g of food, an amount comparable to commercial hen food consumption (105 g/hen/day). A significant ($p \leq 0.0001$) time effect occurred, and the first week of feeding was statistically different than every other week. Average daily and weekly feed consumption rates are given in Table 3 and the corresponding PCB consumption values are given in Table 4. Consumption of feed fluctuated widely within each treatment group during the first half of the study, but decreased overall, and plateaued the last two weeks of the study so that the hens ate roughly 20% less at the end of the study than at the beginning. After stabilizing somewhat in the last half of the study, the high-dose group hens ate 1.7-8.5 g/hen/day more than the control hens and ate 0.1-1.5 g/hen/day more than the low-dose hens, but there were no statistical difference among the treatment groups. Conversely, biweekly weight gain averaged 53.4, 46.8, and 22.8 g for the control, low-dose, and high-dose hens, respectively (Table 5), and both time and treatment had highly significant ($p \leq 0.0001$) effects on body weight. Hens in each treatment group gained weight, on average, over the course of the trial. The high-dose hens, however, lost an average of 76.2 g immediately after being placed on the experimental diet and then resumed weight gain. More hens gained weight during each data period in the control and low-dose groups (79% and 81%, respectively) than in the high-dose group (63%). Thus, an inverse relationship existed in the second half of the study between the control and high-dose group hens with respect to feed consumption and body weight. While the high-dose hens ate more feed than the controls, they weighed less and fewer hens gained weight and did so at a slower rate. The low-dose hens had intermediate values for body weights and food consumption.

Table 3. Average daily and weekly hen food consumption by treatment group.

Week	Treatment Diet: Control[A] Avg. daily food cons. (g/hen/day±SE)	Control[A] Avg. weekly food cons. (g/hen/wk±SE)	Low-Dose[A] Avg. daily food cons. (g/hen/day±SE)	Low-Dose[A] Avg. weekly food cons. (g/hen/wk±SE)	High-Dose[A] Avg. daily food cons. (g/hen/day±SE)	High-Dose[A] Avg. weekly food cons. (g/hen/wk±SE)
1[B]	106.1 ± 3.4	742.5 ± 24.2	102.9 ± 3.2	720.3 ± 22.3	106.5 ± 2.5	745.7 ± 17.3
2[B]	100.9 ± 3.6	706.4 ± 25.2	92.6 ± 3.7	633.3 ± 19.3	90.1 ± 2.3	630.6 ± 16.1
3	90.7 ± 4.1	634.7 ± 28.8	85.5 ± 2.9	598.6 ± 20.2	75.9 ± 1.8	531.5 ± 12.4
4	83.6 ± 3.8	585.0 ± 26.2	89.7 ± 2.4	628.3 ± 17.0	80.0 ± 2.9	560.3 ± 20.0
5	92.4 ± 3.8	647.0 ± 26.2	94.8 ± 2.8	663.5 ± 19.2	87.7 ± 2.3	613.8 ± 16.0
6	90.5 ± 4.1	633.8 ± 28.6	92.1 ± 3.0	645.1 ± 20.7	92.2 ± 2.7	645.6 ± 18.9
7	85.7 ± 4.1	599.9 ± 28.5	89.7 ± 3.8	627.8 ± 26.5	91.2 ± 2.0	638.4 ± 13.8
8	81.9 ± 3.8	567.9 ± 30.1	90.0 ± 3.5	629.7 ± 24.5	90.4 ± 2.2	632.9 ± 15.4
9	82.3 ± 4.2[C]	566.6 ± 33.3	87.1 ± 2.2	609.6 ± 15.1	88.5 ± 2.3	619.8 ± 16.1
10	83.3 ± 4.2[D]	582.8 ± 29.7	87.4 ± 3.5	611.8 ± 24.3	88.5 ± 2.7	619.3 ± 19.0
Mean (wks 1-5)	94.7	663.1	93.1	648.8	88.0	616.4
Mean (wks 6-10)	84.7	590.2	89.3	624.8	90.2	631.2
Grand mean (wks 1-10)	89.7	626.7	91.2	636.8	89.1	623.8
Avg. total food cons. (g/hen)	6179.7		6382.7		6237.7	

[A] 20 hens per treatment diet
[B] All hens were fed the control diet for a two week acclimation period
[C] 19 hens on control diet
[D] 18 hens on control diet

Table 4. Calculated average daily and weekly hen PCB consumption by treatment group.

	Treatment Diet					
	Control[A]		Low-Dose[A]		High-Dose[A]	
Week	Avg. daily PCB cons. (mg/hen/day±SE)	Avg. weekly PCB cons. (mg/hen/wk±SE)	Avg. daily PCB cons. (mg/hen/day±SE)	Avg. weekly PCB cons. (mg/hen/wk±SE)	Avg. daily PCB cons. (mg/hen/day±SE)	Avg. weekly PCB cons. (mg/hen/wk±SE)
1[B]	0.0531 ± 0.0017	0.3713 ± 0.0121	0.0515 ± 0.0016	0.3601 ± 0.0112	0.0533 ± 0.0012	0.3729 ± 0.0087
2[B]	0.0505 ± 0.0018	0.3532 ± 0.0126	0.0463 ± 0.0018	0.3241 ± 0.0129	0.0451 ± 0.0012	0.3153 ± 0.0081
3	0.0454 ± 0.0021	0.3174 ± 0.0145	0.0770 ± 0.0026	0.5387 ± 0.0183	0.5011 ± 0.0118	3.5079 ± 0.0823
4	0.0418 ± 0.0019	0.2925 ± 0.0132	0.0808 ± 0.0022	0.5654 ± 0.0153	0.5282 ± 0.0190	3.6977 ± 0.1329
5	0.0462 ± 0.0019	0.3235 ± 0.0132	0.0853 ± 0.0025	0.5971 ± 0.0174	0.5788 ± 0.0152	4.0511 ± 0.1062
6	0.0453 ± 0.0021	0.3169 ± 0.0144	0.0830 ± 0.0027	0.5806 ± 0.0188	0.6086 ± 0.0180	4.2206 ± 0.1256
7	0.0429 ± 0.0020	0.2999 ± 0.0143	0.0807 ± 0.0034	0.5650 ± 0.0240	0.6019 ± 0.0131	4.2134 ± 0.0920
8	0.0410 ± 0.0019	0.2839 ± 0.0151	0.0810 ± 0.0032	0.5667 ± 0.0221	0.5966 ± 0.0146	4.1763 ± 0.1023
9	0.0412 ± 0.0021[C]	0.2833 ± 0.0167	0.0784 ± 0.0020	0.5486 ± 0.0137	0.5843 ± 0.0152	4.0904 ± 0.1067
10	0.0417 ± 0.0021[D]	0.2914 ± 0.0149	0.0787 ± 0.0031	0.5506 ± 0.0220	0.5839 ± 0.0180	4.0871 ± 0.1263
Mean (wks 1-5)	0.0474	0.3316	0.0682	0.4771	0.3413	2.3890
Mean (wks 6-10)	0.0424	0.2951	0.0804	0.5623	0.5951	4.1656
Grand mean (wks 1-10)	0.0449	0.3133	0.0743	0.5197	0.4682	3.2773
Avg. total food cons. (g/hen)	3.0899		5.1970		32.7780	

[A] 20 hens per treatment diet
[B] All hens were fed the control diet for a two week acclimation period
[C] 19 hens on control diet
[D] 18 hens on control diet

Table 5. Average hen body weight, the percentage of hens gaining or losing weight, and the average gain or loss of weight by treatment group.

	Treatment Diet								
	Control[A]			Low-Dose[A]			High-Dose[A]		
Week	Avg. body wt. (g/hen±SE)	%hens gain/loss[B]	Avg. gain/loss (g)	Avg. body wt. (g/hen±SE)	%hens gain/loss[B]	Avg. gain/loss (g)	Avg. (g/hen±SE)	%hens gain/loss[B]	Avg. gain/loss (g)
1[C]	1506±34.2	---	---	1468±28.1	---	---	1502±17.6	---	---
3	1613±35.4	95/5	+106.3	1556±27.8	95/5	+87.9	1604±27.9	95/5	+102.5
5	1630±37.3	60/40	+17.1	1561±30.4	50/50	+13.5	1528±29.8	10/90	-76.2
7	1711±45.4	90/10	+81.4	1625±34.3	95/5	+63.7	1583±29.7	85/15	+55.3
9	1734±47.8[D]	75/25	+35.8	1655±34.4	80/20	+29.8	1595±32.8	55/45	+11.8
10	1763±55.9[E]	75/25	+26.5	1694±35.2	85/15	+39.1	1611±35.7	70/30	+20.8
Mean		79/21	+53.4		81/19	+46.8		63/37	+22.8

[A] 20 hens per treatment group
[B] The percentage of hens experiencing a gain or loss of body weight
[C] All hens fed the control diet for a two week acclimation period
[D] 19 hens on control diet
[E] 18 hens on control diet

During the 14 days immediately prior to the study period the hens were laying 6.2 eggs/hen/week (89% egg production - Table 6). Egg production dropped immediately after the hens were placed on the fish diets, and differences were highly significant ($p \leq 0.0001$) with respect to treatment, but no significant difference occurred with respect to time. Egg production averaged 4.2, 5.2, and 5.8 eggs/hen/week over the entire study period for the control, low-dose, and high-dose hens respectively. During the last five weeks of the study, the control hens continued to lay fewer eggs (3.8 eggs/hen/week) while the high-dose hens returned to pre-trial production levels (6.3 eggs/hen/week). Production by the low-dose hens stabilized in the third week of the study and they laid about 5.2 eggs/hen/week through the remainder of the study. A greater number of control hens consistently laid fewer eggs than both low-dose or high-dose hens (see Table 7). Thirty percent of the control hens laid 0-3 eggs/hen/week during the first five weeks of the study. Comparably, only 15% and 13% of the low-dose and high-dose hens laid 0-3 eggs/hen/week during that same period. More hens in all groups, 41% of controls, 47% of low-dose, and 56% of high-dose hens, laid 6-7 eggs/hen/week during weeks 1-5. The divergence in egg production was more pronounced during the second half of the study when the proportion of hens laying 0-3 eggs/hen/week increased to 42% in the control group and 20% in the low-dose group, but decreased to only 1% in the high-dose group. Hens laying 6-7 eggs/hen/week decreased to 36% in the control group, and increased to 58% and 82% of the low- and high-dose group.

Significant differences ($p \leq 0.0001$) in weights of eggs were observed as a function of treatment and time (Table 6). At the beginning of the study, the mean weight of eggs from all treatment groups was 52 g which is comparable to eggs weighing 55-60 g from commercially producing hens, but quickly fell to 46 g during weeks two and three. Weights

Table 6. Average daily egg production and average egg weight of laying hens fed various concentrations of Saginaw Bay carp.

	Treatment Diet								
	Control[A]			Low-Dose[A]			High-Dose[A]		
Week	#eggs/wk±SE	Percent production[B]	Egg wt. (g/egg±SE)	#eggs/wk±SE	Percent production[B]	Egg wt. (g/egg±SE)	#egg/wk±SE	Percent production[B]	Egg wt. (g/egg±SE)
14-day pre-trial average	6.2±0.167	89	---	6.2±0.152	89	---	6.3±0.175	90	---
1[C]	5.3±0.252	76	52.6±0.46	4.9±0.271	70	51.6±0.49	4.8±0.267	69	51.8±0.59
2[C]	5.0±0.407	71	47.3±0.58	4.9±0.397	70	45.1±0.55	5.1±0.416	73	45.3±0.43
3	4.2±0.439	60	46.9±0.41	5.3±0.411	76	46.7±0.47	6.1±0.289	87	46.5±0.47
4	3.8±0.500	54	46.9±0.44	5.6±0.275	80	47.0±0.43	5.9±0.216	84	50.4±0.46
5	4.2±0.572	60	47.4±0.46	5.3±0.354	76	47.1±0.40	5.7±0.406	81	51.4±0.36
6	3.8±0.779	54	47.0±0.40	5.4±0.432	77	47.9±0.38	6.2±0.150	89	52.8±0.54
7	4.1±0.550	59	48.1±0.49	5.1±0.410	73	47.7±0.34	6.1±0.176	87	53.1±0.43
8	3.9±0.575	56	48.1±0.46	5.4±0.431	77	48.1±0.32	6.3±0.193	90	53.6±0.40
9	3.7±0.572	53	49.0±0.56	5.3±0.464	76	48.3±0.35	6.1±0.170	87	54.2±0.45
10	3.5±0.633	50	50.0±0.59	4.6±0.461	66	49.7±0.50	6.1±0.256	87	54.1±0.36
Mean (wks 1-5)	4.5	64	48.2	5.2	74	47.5	5.5	79	49.1
Mean (wks 6-10)	3.8	54	48.4	5.2	74	48.3	6.2	88	53.6
Grand mean (wks 1-10)	4.2	59	48.3	5.2	74	47.9	5.8	83	51.3

[A] 20 hens per treatment diet
[B] Percent production = (# eggs/wk) / (7 eggs/wk) where 7 eggs/wk = maximal egg production
[C] All hens fed the control diet for two week acclimation period

Table 7. The percentage of hens in each treatment group laying 0-3, 4-5, or 6-7 eggs/hen/week.

	Treatment Diet								
	Control[A]			Low-Dose[A]			High-Dose[A]		
	No. eggs/week			No. eggs/week			No. eggs/week		
Week	0-3	4-5	6-7	0-3	4-5	6-7	0-3	4-5	6-7
1[B]	10	30	60	10	60	30	10	65	25
2[B]	25	30	45	30	35	35	30	35	35
3	35	35	30	10	40	50	10	25	65
4	35	40	25	10	25	65	5	20	75
5	45	10	45	15	30	55	10	10	80
6	45	5	50	10	30	60	--	20	80
7	30	40	30	10	40	50	--	15	85
8	40	15	45	20	15	65	--	20	80
9	37	32	32[C]	25	10	65	--	15	85
10	50	22	28[D]	35	15	50	5	15	80
Mean (wks 1-5)	30	29	41	15	38	47	13	31	56
Mean (wks 6-10)	42	22	36	20	22	58	1	17	82
Grand mean (wks 1-10)	35	26	39	18	30	52	7	24	69

[A] 20 hens per treatment diet
[B] All hens fed the control diet for two week acclimation period
[C] 19 hens on control diet
[D] 18 hens on control diet

of eggs then increased through the remainder of the study with high-dose group eggs increasing the most to a final average weight of 54 g. During weeks 6-10 eggs from the control and low-dose groups were comparable in weight, but after week 4 the high-dose eggs were consistently heavier than the low-dose and control eggs by an average of 4.7g.

Fertility initially ranged from 93-98% (Table 8), but decreased in each treatment group by the second week. Fertility rates continued to be depressed throughout the course of the study in both the control and low-dose groups, but rebounded in the 7th week in the high-dose group hens to initial levels.

Mortality rates at both 11-days and 18-days of incubation did not follow any trends (Table 8). Generally, mortality levels at both 11- and 18-days of incubation were least in the high-dose group and greatest in the control group.

Full-term hatchability (Table 9) decreased and mortality rates increased (Table 10) with respect to time and treatment. At the beginning of the trial, 90.2-100% of the eggs hatched, but hatching decreased in all treatment groups during the second week of the study. While hatching rates in the control group stabilized throughout the remainder of the study, hatching rates increased in the second half of the study in the low-dose group and decreased in the high-dose group. A corresponding decrease in mortality was observed in the low-dose group and an increase in mortality was evident in the high-dose group. There was no delayed hatching effect in any treatment group and late-incubation mortality (23-25 days of incubation) did not increase significantly with respect to either time or treatment. Differences were also seen in the proportion of mortality that occurred during the early, mid, and late incubation stages as shown in Table 11. In the control and low-dose diet groups,

Table 8. Average rate of fertility for eggs incubated 5 days, and embryo mortality at day 11 and 18 of incubation.

	% Fertility			% Mortality			% Mortality		
	5-D incubation			11-D incubation			18-D incubation		
Week	CD[A]	LD[B]	HD[C]	CD	LD	HD	CD	LD	HD
1[D]	95	98	93	3.2	1.1	4.8	1.2	2.5	---
2[D]	86	71	82	5.1	9.5	6.4	5.9	11.8	7.3
3	68	76	89	7.8	6.9	3.0	16.3	3.3	2.3
4	67	72	91	9.1	6.9	5.0	13.5	3.3	1.1
5	72	79	83	9.4	1.3	3.4	2.4	2.8	---
6	71	71	87	2.2	5.6	2.0	7.1	3.3	2.2
7	77	70	93	---	1.4	2.8	5.7	6.2	---
8	69	70	96	3.9	4.3	0.9	2.2	6.7	3.9
9	68	74	92	---	2.7	2.0	4.9	3.1	1.1
10	77	68	94	2.3	8.8	1.0	7.5	---	3.1
Mean (wks 1-5)	78	79	88	6.9	5.1	4.5	7.9	4.7	2.1
Mean (wks 6-10)	72	71	92	1.7	4.6	1.7	5.5	3.9	2.1
Grand mean (wks 1-10)	75	75	90	4.3	4.8	3.1	6.7	4.3	2.1

[A] CD = Control diet (20 hens)
[B] LD = Low-Dose diet (20 hens)
[C] HD = High-Dose diet (20 hens)
[D] All hens fed the control diet for a two week acclimation period

Table 9. Average hatching rates at 21-22 and 23-25 days of incubation, and total hatching rates for eggs laid by hens fed various concentrations of Saginaw Bay carp.

	Treatment Diet								
	Control[A]			Low-Dose[A]			High-Dose[A]		
	% Hatching			% Hatching			% Hatching		
Week	Day 21-22	Day 23-25	Total	Day 21-22	Day 23-25	Total	Day 21-22	Day 23-25	Total
1[B]	98.7	1.3	100	91.8	2.7	94.5	88.8	1.4	90.2
2[B]	87.5	---	87.5	69.0	---	69.0	75.4	---	75.4
3	73.5	2.9	76.4	77.3	---	77.3	87.2	1.3	88.5
4	72.4	3.4	75.8	81.5	---	81.5	95.2	---	95.2
5	84.6	---	84.6	87.6	1.6	89.2	80.2	4.2	84.4
6	85.7	---	85.7	92.6	---	92.6	77.8	2.5	80.3
7	87.0	4.4	91.4	87.9	---	87.9	65.9	---	65.9
8	80.9	4.8	85.7	90.7	---	90.7	69.6	2.2	71.8
9	75.0	2.8	77.8	91.7	1.7	93.4	55.4	2.4	57.8
10	88.3	---	88.3	85.2	---	85.2	73.9	1.1	75.0
Mean (wks 1-5)	83.3	1.5	84.9	81.4	0.9	82.3	85.4	1.4	86.7
Mean (wks 6-10)	83.4	2.4	85.8	89.6	0.3	90.0	68.5	1.6	70.2
Grand mean (wks 1-10)	83.4	2.0	85.3	85.5	0.6	86.1	76.9	1.5	78.4

[A] 20 hens per treatment diet

[B] All hens fed the control diet for a two week acclimation period

Table 10. Average mortality rates at 21-22 and 23-25 days of incubation, and total mortality rates for eggs laid by hens fed various concentrations of Saginaw Bay carp.

	Treatment Diet								
	Control[A]			Low-Dose[A]			High-Dose[A]		
	% Mortality			% Mortality			% Mortality		
Week	Day 21-22	Day 23-25	Total	Day 21-22	Day 23-25	Total	Day 21-22	Day 23-25	Total
1[B]	---	---	---	4.1	1.4	5.5	9.8	---	9.8
2[B]	7.1	5.4	12.5	26.2	4.8	31.0	21.3	3.2	24.5
3	14.7	8.8	23.5	13.2	9.5	22.7	6.4	5.1	11.5
4	17.2	6.9	24.1	7.5	11.2	18.7	3.7	1.2	4.9
5	10.3	5.1	15.4	7.9	3.1	11.0	8.4	7.0	15.4
6	11.4	2.9	14.3	7.4	---	7.4	11.1	8.7	19.8
7	4.4	4.4	8.8	8.7	3.5	12.2	27.3	6.9	34.2
8	9.5	4.8	14.3	7.4	1.9	9.3	26.1	2.2	28.3
9	13.9	8.4	22.3	3.3	3.4	6.7	25.3	16.8	42.1
10	12.7	2.1	14.8	12.7	2.1	14.8	19.3	5.7	25.0
Mean (wks 1-5)	9.9	5.2	15.1	11.8	6.0	17.8	9.9	3.3	13.2
Mean (wks 6-10)	10.4	4.5	14.9	7.9	2.2	10.8	21.8	8.1	29.9
Grand mean (wks 1-10)	10.2	4.8	15.0	9.8	4.1	14.3	15.8	5.7	21.6

[A] 20 hens per treatment diet

[B] All hens fed the control diet for a two week acclimation period

Table 11. The proportion of embryo and chick mortality occurring during early (day 11), mid- (day 18), and late (days 21-25) stages of incubation.

	Treatment Diet								
	Control[A]			Low-Dose[A]			High-Dose[A]		
	% Mortality			% Mortality			% Mortality		
	Day 11	Day 18	Day 21-25	Day 11	Day 18	Day 21-25	Day 11	Day 18	Day 21-2
Weeks 1-5	30.3	27.3	42.4	23.1	18.0	59.0	27.0	10.8	62.2
Weeks 6-10	9.3	27.9	62.8	27.8	22.2	50.0	6.1	6.8	87.2
Weeks 1-10	22.0	27.5	50.5	25.0	17.7	55.3	13.1	8.1	78.8

[A] 20 hens per treatment group

mortality was split almost evenly between the early and mid stages (day 11 and 18 of incubation) and late stage incubation (days 21-25). Late stage mortality, however, was predominant (whole study average = 79%) in the high-dose group, and particularly so during the second half of the study (87%).

Tissue samples including the heart, spleen, bursa, and liver were collected from 139 18-day embryos for histopathological examination. Nonspecific or background lesions were observed in certain individual tissues, but no distinct or constant lesions were confined to a certain treatment group.

Statistical analysis of the body, brain, liver, heart, spleen, and bursa weights from 18-day embryos revealed significant effects in liver, spleen, and bursa weights as determined by a two-way ANOVA and Tukey's tests of both treatment and time main effects.

Highly significant ($p \leq 0.0001$) treatment and time effects were evident in the embryo liver weights (Table 12), and liver weights increased in all groups over time. However, the gain was greater in the high-dose group (+19%) than in the control (+2%) and low-dose (+1%) groups. The high-dose group livers always weighed more, on average, than those of the low-dose and control groups and during the second half of the study, the high-dose group embryo livers weighed 23-28% more than the control or low-dose group livers.

Spleen weights from the 18-day embryos remained constant in the control group, but decreased by about 7.5% during the second half of the study in both the low-dose and high-dose embryos (Table 12). A significant time effect ($p \leq 0.05$) was evident. The differences among treatment groups was variable. During weeks 1-5, the high-dose spleens weighed 9% more than those of either the control or low-dose groups. However, during the second half of the study, spleens from both the control and high-dose groups weighed 9% more than those from low-dose group.

Table 12. The average liver, spleen, and bursa weights from 18- day embryos, the percent difference among treatments, and the percent change over time within the control diet (CD), low-dose diet (LD), and high-dose diet (HD) groups.

18-day Embryo Livers

Mean liver weights (g)

Week	CD n	CD g	LD n	LD g	HD n	HD g	% Treatment differences			% change within treatments over time
wks 1-5	20	0.4307	24	0.4486	27	0.5094	HD=18%>CD	HD=14%>LD	LD=4%>CD	CD= +2%
wks 6-10	17	0.4409	11	0.4540	29	0.6066	HD=38%>CD	HD=33%>LD	LD=3%>CD	LD= +1%
wks 1-10	37	0.4358	35	0.4513	56	0.5580	HD=28%>CD	HD=23%>LD	LD=3%>CD	HD= +19%

18-Day Embryo Spleens

Mean spleen weights (g)

Week	CD n	CD g	LD n	LD g	HD n	HD g	% Treatment differences			% change within treatments over time
wks 1-5	19	0.0069	24	0.0069	26	0.0075	HD=9%>CD	HD=9%>LD	LD=CD	CD= ±0%
wks 6-10	16	0.0070	10	0.0064	29	0.0070	HD=CD	HD=9%>LD	LD=9%<CD	LD= -8%
wks 1-10	35	0.0070	34	0.0066	55	0.0070	HD=3%>CD	HD=9%>LD	LD=6%<CD	HD= -7%

Table 12 (cont'd).

18-Day Embryo Bursae										
	Mean bursa weights (g)									
	CD		LD		HD					% change within treatments over time
Week	n	g	n	g	n	g	% Treatment differences			
wks 1-5	20	0.0267	24	0.0256	27	0.0275	HD=3%>CD	HD=7%>LD	LD=4%<CD	CD= +20%
wks 6-10	17	0.0320	11	0.0292	27	0.0209	HD=53%<CD	HD=40%<LD	LD=10%<CD	LD= +14%
wks 1-10	37	0.0294	35	0.0274	54	0.0242	HD=21%<CD	HD=13%<LD	LD=7%<CD	HD= -24%

Bursa weights of the 18-day embryos increased significantly ($p \leq 0.001$) in the control and low-dose groups, and decreased significantly ($p \leq 0.001$) in the high-dose group with respect to time. On average, bursa weights increased in both the control (+20%) and low-dose (+14%) groups, but decreased by 24% in the high-dose group between the first and second halves of the study (Table 12). Bursa weights of the high-dose group were greatest during weeks 1-5, but during weeks 6-10, the control group bursa weights were 53% greater and the low-dose group bursa weights were 40% greater than the bursa weights in the high-dose group.

A total of 1,479 chicks hatched and the brain, liver, heart, spleen, and bursa were collected from each and weighed. Subsequent statistical analysis, comparable to that applied to organs taken from 18-day embryos, shows significant effects in body weights and brain, liver, heart, and bursa weights.

Body weights of chicks varied significantly ($p \leq 0.0001$) as a function of both time and treatment. An interaction between the main effects confounded the interpretation, but Tukey's test showed significant ($p \leq 0.0001$) time and treatment differences consistently during weeks 4-10, thus reinforcing the conclusions of the two-way ANOVA. The average body weights for the first and second halves of the study, shown in Table 13, indicate slight increases of 1% and 2% for the control and low-dose chicks and a 9% increase with time in the high-dose group. The high-dose chicks always weighed the most, and were about 9.5% greater than the control and low-dose chicks during the second half of the study.

Significant ($p \leq 0.01$) increases with respect to time and treatment were evident in the analysis of chick brain weights. No interaction of the main effects occurred. Brain weights increased from the first to the second half of the study in all of the treatment groups, though the increases were small (1-2%). Likewise, the high-dose group chicks always had the

greatest brain weights (Table 13), but they were only 2-3% heavier than those found in the control and low-dose groups.

Liver weights of chicks increased significantly ($p \leq 0.0001$) with respect to time and treatment. A main effect interaction occurred, but the one-way analysis showed support for time and treatment differences from week 4 to week 10. The liver weights increased in all groups over the course of the study. The weights of the livers in the high-dose group increased by 32% over time, whereas the control and low-dose liver weights increased by 3-5% (Table 13). The high-dose group chicks always had the heaviest livers, and this was particularly evident during the second half of the study when high-dose chick liver weights were 44% greater than those reported in the low-dose chicks, and 46% greater than the control liver weights.

Decreases in heart weights, ranging from 1-3.5% and significant ($p \leq 0.001$) with respect to time, were evident in all treatment groups. The high-dose group chicks had the largest hearts (5-8% larger than the control or low-dose chick heart weights) throughout the study and had the smallest decreases in heart weights over time (Table 13).

Bursa weights of the chicks showed a strong time effect ($p \leq 0.0001$) and a weaker treatment effect ($p \leq 0.05$). A main effect interaction was also significant, but the conclusion of significant main effects was reinforced by the one-way analysis and Tukey's test. The weights, averaged for the first and second halves of the study and shown in Table 13, show a 3% increase in control bursa weights, and a 5% and 16% decrease in bursa weights in the low-dose and high-dose groups, respectively. Initially, the high-dose chicks had the greatest bursa weights, but by the second half of the study both the control and low-dose chicks had bursa weights about 4% greater than those in the high-dose group.

Table 13. The average body, brain, liver, heart, and bursa weights from hatched chicks, the percent difference among treatments, and the percent change over time within the control diet (CD), low-dose diet (LD), and high-dose diet (HD) groups.

Hatched Chick Bodies

Mean body weights (g)

Week	CD n	CD g	LD n	LD g	HD n	HD g	% Treatment differences			% change within treatments over time
wks 1-5	206	34.21	239	33.94	315	34.65	HD=1%>CD	HD=2%>LD	LD=1%<CD	CD= +1%
wks 6-10	164	34.49	247	34.75	304	37.81	HD=10%>CD	HD=9%>LD	LD=1%>CD	LD= +2%
wks 1-10	370	34.35	486	34.34	619	36.23	HD=5%>CD	HD=5%>LD	LD=CD	HD= +9%

Hatched Chick Brains

Mean brain weights (g)

Week	CD n	CD g	LD n	LD g	HD n	HD g	% Treatment differences			% change within treatments over time
wks 1-5	206	0.8177	239	0.8182	315	0.8325	HD=2%>CD	HD=2%>LD	LD=CD	CD= +2%
wks 6-10	165	0.8302	247	0.8273	304	0.8523	HD=3%>CD	HD=3%>LD	LD=CD	LD= +1%
wks 1-10	371	0.8240	486	0.8228	619	0.8424	HD=2%>CD	HD=2%>LD	LD=CD	HD= +2%

Table 13 (cont'd).

Hatched Chick Livers										
Mean liver weights (g)										
	CD		LD		HD					% change within treatments over time
Week	n	g	n	g	n	g	% Treatment differences			
wks 1-5	206	0.6898	239	0.6910	315	0.7926	HD=15%>CD	HD=15%>LD	LD=CD	CD= +3%
wks 6-10	165	0.7138	247	0.7262	304	1.0437	HD=46%>CD	HD=44%>LD	LD=2%>CD	LD= +5%
wks 1-10	371	0.7018	486	0.7086	619	0.9182	HD=31%>CD	HD=30%>LD	LD=1%>CD	HD= +32%
Hatched Chick Hearts										
Mean heart weights (g)										
	CD		LD		HD					% change within treatments over time
Week	n	g	n	g	n	g	% Treatment differences			
wks 1-5	206	0.2157	238	0.2124	315	0.2233	HD=4%>CD	HD=5%>LD	LD=1%<CD	CD= -2%
wks 6-10	165	0.2112	247	0.2051	304	0.2215	HD=5%>CD	HD=8%>LD	LD=3%<CD	LD= -4%
wks 1-10	371	0.2134	485	0.2088	619	0.2224	HD=4%>CD	HD=6%>LD	LD=2%<CD	HD= -1%

Table 13 (cont'd).

Hatched Chick Bursae										
	Mean bursa weights (g)									
	CD		LD		HD					% change within treatments over time
Week	n	g	n	g	n	g	% Treatment differences			
wks 1-5	206	0.0359	239	0.0372	315	0.0383	HD=7%>CD	HD=3%>LD	LD=4%>CD	CD= +3%
wks 6-10	165	0.0369	247	0.0354	303	0.0323	HD=14%<CD	HD=10%<LD	LD=4%<CD	LD= -5%
wks 1-10	371	0.0364	486	0.0363	618	0.0353	HD=3%<CD	HD=3%<LD	LD=CD	HD= -16%

Of the 2,081 embryos/chicks (Table 14) examined for abnormalities, 29% (607) displayed teratogenic effects. Sixty percent of the deformities (terata) occurred in high-dose group embryos and chicks, while the low-dose and control groups had 25% and 15% of the terata, respectively. Furthermore, the majority of abnormalities (82-93%) occurred in the last half of the study.

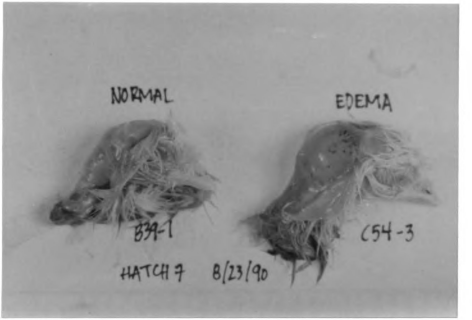
Common terata observed in the embryos and chicks included edema in the regions of the head, neck, abdomen, and back. Hemorrhages were also common in the head and neck musculature, and edematous brain tissue and microcrania were noted on occasion. Deformities of the beak and limbs were also noted as were eye and yolk sac anomalies. Grouped into six categories and tabulated across all treatment groups for the entire study, head/neck edema was the most prevalent teratogenic effect (64%), followed by abdominal edema (15%), foot/leg deformities (14%), miscellaneous abnormalities (2.9%), skull/brain malformations (2.6%), and yolk sac anomalies (1.6%). The teratogenic effects observed in this study are described below in order of decreasing prevalence.

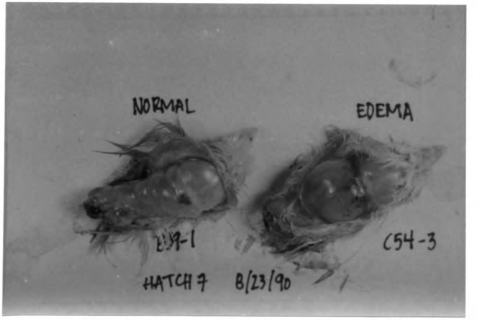
1. Head and Neck Edema and Hemorrhage - This included intramuscular and/or subcutaneous edema and hemorrhage (Figure 2) of the muscle mass on the back of the neck, immediately below the skull, known as the "pipping muscle." It also included edema and hemorrhage of the cranial region. Intramuscular edema was characterized by fluid infiltration into the muscle tissue causing the affected muscle tissue to enlarge and appear yellowish in color compared to non-edematous muscle tissue. Subcutaneous edema (Figure 3) was identified by the presence of a gelatinous mass of yellowish fluid on the surface of the muscle tissue immediately below the skin. The severity of all edema and hemorrhaging was classed as "mild," "moderate," or "severe."

Table 14. The numbers of embryos and chicks examined for deformities.

	Treatment Diet			
	Control	Low-Dose	High-Dose	Total
No. embryos dead at day 11	24	33	29	86
No. embryos dead at day 18	30	26	18	74
No. of embryos taken for histopathology	38	37	64	139
No. hatched chicks	373	486	620	1479
No. full-term dead chicks	55	73	175	303
Total no. embryos/chicks examined for deformities	520	655	906	2081

Figure 2. A lateral view (top photo) and dorsal view (bottom photo) of a normal chick (left) and of a chick with a moderate subcutaneous and intramuscular head and neck edema and hemorrhage (right).


NORMAL
EDEMA
B39-1
C54-3
HATCH 7 8/23/90


NORMAL
EDEMA
B39-1
C54-3
HATCH 7 8/23/90

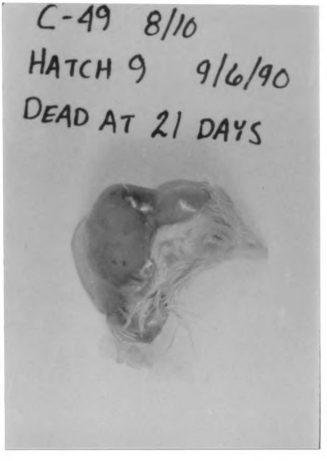


Figure 3. A chick with "severe" subcutaneous and intramuscular head and neck edema and hemorrhage.

Head and neck edema/hemorrhage was the most prevalent teratogenic effect in all treatment groups, particularly so during the second half of the study (Table 15), and accounted for 51.7%, 52.3% and 70.0% of all the terata in the control, low-dose, and high-dose groups, respectively. This was also the most prevalent anomaly in both dead and live chicks (Table 16), but it described a greater proportion of the terata in dead chicks (56-78%) than in live chicks (48-67%) (Table 15). Eighteen-day embryos were the only group that showed a greater occurrence of other types of terata.

2. Abdominal Edema and Hemorrhage - Edema in the abdominal region was either intramuscular or subcutaneous. It was characterized and classified as described above. Additionally, however, this included the occasional incidence of a blister of clear fluid on the lower back (Figures 4 and 5) which was described as a "blister edema".

Abdominal edema and hemorrhage, the second most frequent class of terata overall, occurred primarily during the second half of the study and accounted for 20.1%, 24.4%, and 10.7% of the terata in the control, low-dose, and high-dose groups, respectively (Table 15). Most of the abdominal terata were found in live chicks (weeks 6-10) in the control and low-dose groups, but accounted for the greatest proportion of terata in high-dose group 18-day embryos (Table 15). "Blister edemas" were seen in each dose group, including a live chick in the control group (3.7% of abdominal terata), two live chicks in the low-dose group (3.3% of abdominal terata), five high-dose 18-day embryos (83.3% of abdominal terata), 10

Table 15. Incidence of the six major classes of terata in 18-day embryos, dead embryos/chicks, and hatched chicks from hens fed various concentrations of Saginaw Bay carp.

Control Diet							
Terata	18-day embryos wks 1-5	18-day embryos wks 6-10	Dead wks 1-5	Dead wks 6-10	Hatched chicks wks 1-5	Hatched chicks wks 6-10	Total
Head/neck edema/hem.	--	--	1	30 (70%)	--	46 (53%)	77 (51.7%)
Abdominal edema/hem.	--	--	2	--	1	27 (31%)	30 (20.1%)
Foot/leg deformities	--	--	6	3 (7%)	6	13 (15%)	28 (18.8%)
Misc.	--	--	2	3 (7%)	--	1 (1%)	6 (4.0%)
Skull/brain deformities	--	--	--	4 (9%)	1	--	5 (3.4%)
Yolk sac deformities	--	--	--	3 (7%)	--	--	3 (2.0%)
Total	--	--	11	43 (100%)	8	87 (100%)	149 (100%)

Table 15. (cont'd)

	Low Dose						
Terata	18-day embryos wks 1-5	18-day embryos wks 6-10	Dead wks 1-5	Dead wks 6-10	Hatched chicks wks 1-5	Hatched chicks wks 6-10	Total
Head/neck edema/hem.	--	1 (100%)	1	40 (71.4%)	--	93 (51%)	135 (52.3%)
Abdominal edema/hem.	--	--	2	1 (1.8%)	--	60 (33%)	63 (24.4%)
Foot/leg deformities	--	--	7	8 (14.3%)	3	26 (14%)	44 (17.0%)
Misc.	--	--	2	3 (5.4%)	--	2 (1%)	7 (2.7%)
Skull/brain deformities	--	--	2	2 (3.6%)	--	--	4 (1.6%)
Yolk sac deformities	--	--	3	2 (3.6%)	--	--	5 (1.9%)
Total	--	1 (100%)	17	56 (100%)	3	181 (100%)	258 (100%)

Table 15. (cont'd)

	High Dose						
Terata	18-day embryos wks 1-5	18-day embryos wks 6-10	Dead wks 1-5	Dead wks 6-10	Hatched chicks wks 1-5	Hatched chicks wks 6-10	Total
Head/neck edema/hem.	--	4 (24%)	3	247 (80%)	3	307 (68%)	564 (70.0%)
Abdominal edema/hem.	--	6 (35%)	1	18 (6%)	4	57 (13%)	86 (10.7%)
Foot/leg deformities	--	1 (6%)	2	29 (9%)	6	59 (13%)	97 (12.0%)
Misc.	--	--	1	4 (1%)	3	15 (3%)	23 (2.9%)
Skull/brain deformities	--	6 (35%)	1	5 (2%)	--	11 (2%)	23 (2.9%)
Yolk sac deformities	--	--	6	5 (2%)	--	1 (<1%)	12 (1.5%)
Total	--	17 (100%)	14	308 (100%)	16	450 (100%)	805 (100%)

Table

Head

Abdo

Foot

Misc

Skul

Yolk

Tota

Table 16. The number of incidences of the six major classes of terata in all 18-day embryos, dead embryos/chicks, and hatched chicks.

Terata	18-day embryos	Dead embryos/ chicks	Hatched chicks	Total
Head/neck edema/hem.	5	322	449	776
Abdominal edema/hem.	6	24	149	179
Foot/leg deformities	1	55	113	169
Misc.	--	15	21	36
Skull/brain deformities	6	14	12	32
Yolk sac deformities	--	19	1	20
Totals	18	449	745	1212

Figure 4. A hatched chick with a blister of clear fluid ("blister edema") on the lower back.

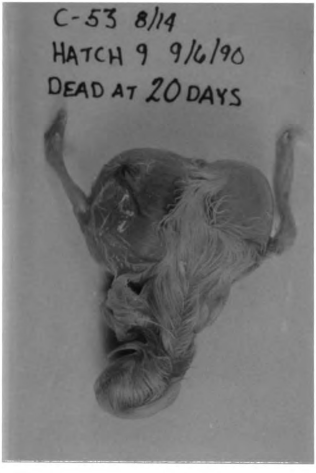


Figure 5. A chick that died at 20-days of incubation with a severe "blister edema" over the entire lower back region.

dead chicks in the high-dose group (55.6% of abdominal terata), and four live chicks in the high-dose group (7.0% of abdominal terata).

3. Foot and Leg Deformities - Limb deformities consisted of curled, clenched, or splayed toe/s and feet that rotated inward or were clubbed. In some cases, the legs were splayed so that the chick was unable to stand erect with its legs directly underneath its body (Figure 6). Occasionally, bilaterally malformed femurs (femurs with a 90° angle beginning in the center of the length of the bone - Figure 7) were observed. Every incidence of femoral bone deformity occurred in dead chicks in the high-dose group.

Foot and leg deformities accounted for roughly 12-19% of the terata that occurred in the three treatment groups and were more prevalent in live chicks than in dead chicks (Table 16). In all groups, a time-dependent trend was evident with more foot/leg deformities occurring during the second half of the study (Table 15).

4. Miscellaneous Deformities - This category included those observations of deformities that were occasional in nature. Eye deformities consisted of embryos and chicks that had one or both eyes missing, enlarged, or reduced in size. Beak abnormalities included missing, shortened, or crossed beaks. Incomplete feathering, weak chicks, fluid-filled yolk sacs, and liver hemorrhages were incidental. Chicks described as weakened were unable to raise their heads or straighten their bodies from the curled, embryonic position (Figure 8). These occasional teratogenic effects occurred in each treatment group and accounted for about 3-4% of the terata in each group.

Figure 6. Foot and leg deformities included clubbed feet (top photo) and chicks that were unable to stand erect (bottom photo).


Hatch 9
/90


B25 Hatch 9
9/7/90

Figure 7. A normal leg from a control group chick (left) and a leg with a femoral bone deformity from a high-dose group chick (right).

Figure 8. A "weakened condition" chick that hatched from the high-dose group and remained in a curled, embryonic position.

5. Skull and Brain Deformities - In some individuals the top of the skull covering the brain was either missing or soft. When the top of the skull was missing, the brain was exposed (Figure 9). Malformed brain cases that were too small, to a varying degree (Figure 10), to accommodate the brain (microcrania) were observed on occasion. Every incidence of a skull deformity was paired with edematous brain tissue, but edematous brain tissue also occurred in other embryos and chicks with normal skulls. Occasional hemorrhaging of the brain tissue was also included in this category.

The greatest proportion of skull and brain abnormalities occurred in 18-day embryos (Table 16), all of which were in the high-dose group. These types of deformities were also observed in dead chicks in the low-dose group and high-dose group chicks (dead and live) (Table 15).

6. Yolk Sac Deformities - Unabsorbed yolk sacs in fully incubated chicks were noted, as were edematous or missing sphincters (the tissue which normally closes the gut wall after yolk sac absorption) which resulted in incomplete abdominal closure. These abnormalities were infrequent and were most common in dead chicks (Table 16).

A time- and dose-dependent increase in the incidence of terata occurred during the study. Over the course of the entire study, terata occurred in 17% of the control, 24% of the low-dose, and 40% of the high-dose group embryos and chicks.

Figure 9. A chick with microcrania and an exposed brain.

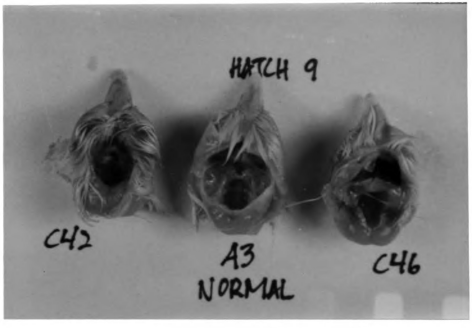


Figure 10. A chick with a normal brain case (center) flanked by chicks with varying degrees of microcrania (left and right).

A shift from single to multiple occurrences of terata was evident and followed a time- and dose-dependent pattern. Of all the embryos/chicks that had deformities during the first half of the study (Table 17), the proportion of embryos/chicks with a single deformity ranged from 73-85% in all treatment groups. Cases of single deformities decreased in the last half of the trial and individuals with multiple deformities (ranging from 2-7 separately identifiable deformities per individual) became more prevalent and were found in 49% of the control, 45% of the low-dose, and 68% of the high-dose embryos/chicks that had deformities.

The average body, liver, and spleen weights were least, while the hematocrit values were greatest in the high-dose group hens (Table 18). During the study, two control group hens died, the first during week eight and the second during week nine. Subsequent necropsy revealed severe hemorrhaging from the livers of both hens. Necropsy of the remaining hens at the end of the study revealed that 14 control hens (70%), 15 low-dose group hens (75%), and three high-dose group hens (15%) displayed clinical signs (necrotic, friable, tumorous, and hemorrhaged livers) and histopathologic lesions indicative of fatty liver hemorrhagic syndrome (FLHS). Liver weights of the control hens were highly variable (range = 90 g) while the high-dose hen's liver weights were within a 37 g range. On average, the control livers weighed 12 and 19 g more than the livers of the low-dose and high-dose groups, respectively.

Table 17. The number of embryos and chicks with either a single or multiple incidence of terata from hens fed Saginaw Bay carp.

	Treatment Diet		
	Control	Low-Dose	High-Dose
Total no. embryos/chicks examined for deformities	520	655	906
Weeks 1-5			
No. embryos/chicks with a single deformity	13 (81%)	11 (73%)	22 (85%)
No. embryos/chicks with multiple deformities	3 (19%)	4 (27%)	4 (15%)
Total	16 (100%)	15 (100%)	26 (100%)
Weeks 6-10			
No. embryos/chicks with a single deformity	38 (51%)	76 (55%)	107 (32%)
No. embryos/chicks with multiple deformities	36 (49%)	63 (45%)	230 (68%)
Total	74 (100%)	139 (100%)	337 (100%)
Grand Totals (Weeks 1-10)			
No. embryos/chicks with a single deformity	51 (57%)	87 (56%)	129 (36%)
No. embryos/chicks with multiple deformities	39 (43%)	67 (44%)	234 (64%)
Total	90 (100%)	154 (100%)	363 (100%)
Overall deformity rate	17%	24%	40%

Table 18. Organ weights, hematocrit values (hcts), and incidence of FLHS^ for laying hens fed various concentrations of Saginaw Bay carp.

	Organ Weight (g) ± SE					
Treatment Diet	Body	Brain	Liver	Spleen	Hcts %RBC ± SE	#Hens with FLHS (%)
Control	1763.22±55.91	3.2328±0.0529	63.35±5.76	1.2223±0.0643	27.90±0.95	14(70%)
Low-Dose	1693.75±35.25	3.1322±0.0358	51.31±2.80	1.1568±0.0818	26.19±0.85	15(75%)
High-Dose	1610.75±35.68	3.1807±0.0381	44.61±1.71	1.0530±0.0805	32.15±0.75	3(15%)

^ FLHS = Fatty Liver Hemorrhagic Syndrome

DISCUSSION

The signs of FLHS observed in this study, particularly in the control and low-dose diet groups, closely resembled those described by other researchers and the pictorial description given by Wolford et al. (1971). Couch (1956) was the first to characterize the syndrome and reported that hens afflicted with FLHS appeared healthy but had excess abdominal fat, fatty livers, liver capillary hemorrhages, liver hematomas, and increased mortality. A 25-30% increase in body weight and a 33% drop in egg production was also described as typical. Ringer and Sheppard (1963) further noted that immediately prior to death the comb, face, and wattles were pale and the comb was cool to the touch. They did not, however, note a decrease in egg production in their study hens.

Physical characteristics, similar to those listed above, were evident in the laying hens in this study. FLHS hens were visually identified by the condition of the liver at necropsy. The severity of the syndrome ranged from livers that were only slightly hemorrhagic and mahogany in color to livers that were yellow-brown (Tudor 1967, Wolford et al. 1971), extremely friable (to the point that the liver could not be removed from the hen in one piece, but rather in many small fragments), tumorous (Figure 11), and hemorrhagic. All hens appeared to be in good physical condition prior to necropsy as expected according to Couch (1956).

The two hens in the control group that died during the study had severe liver hemorrhaging into the abdominal cavity. According to Wolford et al. (1971), FLHS-

Figure 11. A lateral (top photo) and ventral (bottom photo) view of a tumorous liver in a hen with Fatty Liver Hemorrhagic Syndrome.

associated mortality results from the rupture of the liver capsule (Capsule of Glisson) and the subsequent hemorrhage of the liver into the abdominal cavity. Histopathologic examination of the two hens showed hepatic necrosis and hemorrhage, moderate heterophilic infiltration, capsular fibrosis, and diffuse vacuolar change. Tudor (1967) reported that liver hemorrhaging and the decreased blood flow leads to parenchymatosis degeneration and fibrosis of the hepatic tissue.

FLHS is not uncommon in commercial laying operations and most frequently occurs in hens that are actively producing eggs (Wolford et al. 1971, Wolford and Polin 1972, Solarzano 1990). The syndrome may also be influenced by environmental temperature, as FLHS mortality increases during the summer months (Schexnailder and Griffith 1973, Lee et al. 1975), and the high energy diet that laying hens are typically fed (Lee et al. 1975, Solarzano 1990). Laying hens have a high level of fat in the liver compared to non-laying hens (Griffith et al. 1969), and therefore, hepatic fat infiltration should not solely be considered diagnostic. It is not the accumulation of fat that is fatal, but the excessive liver lipid accumulation leads to the terminal stages of FLHS - liver hemorrhaging, rupture of the liver capsule, and the subsequent hemorrhaging into the abdominal cavity (Wolford et al. 1971).

The prevalence of FLHS in the control (70%) and low-dose diet (75%) groups at first seemed remarkable when compared to only a 15% occurrence in the high-dose group. A similar pattern was noted by Hurley and co-workers (1976) when they fed two groups of hens either a commercial ration or a commercial ration with the addition of 2,4,5,3',4'-PCB. The livers of the treated chickens were visibly less fatty than those of the control hens, but one treated hen had an abnormal mass in the left lobe of the liver. Hansen (1975) concluded that PCB-related microsomal induction may offer some protection against FLHS in confined

laying hens by increasing the rates of steroid and fatty acid oxidation. A1254 was specifically found to offer increased protection against FLHS, but hens fed A1242, which has the potential to lower the rate of lipid metabolism, had more severe FLHS (Hansen 1975). Thus, it would appear that the high-dose group hens in this study were experiencing a PCB-related protection against FLHS.

The unusual food consumption, body weight, and egg production trends observed in this study may be explained, at least in part, by the occurrence of FLHS. The control hens consistently weighed the most throughout the study yet ate the least amount of feed over the last half of the study. Part of the weight differential may be attributed to the manifestations of FLHS in the control group including larger livers (on average), presumably due to hepatic fat infiltration, and potentially excess abdominal fat (this was not quantified, however). Livers of the control hens were on average 12.0 g and 18.7 g larger than the livers of the low-dose and high-dose hens, respectively. A higher energy demand in the high-dose group, leading to greater food consumption and lesser body weights, may have occurred due to the greater rates of egg production. Furthermore, the 30% decline in the egg production of the control group is not unexpected in light of FLHS and the observations of Couch (1956). The initial decrease in egg production in all groups was probably a result of the sudden and drastic change in the hens' diet with the onset of the study. The hens in the low-dose and high-dose groups quickly acclimated to the diets and began laying more eggs. The high-dose hens, however, made the quickest and most complete recovery and by the end of the study had returned to pre-trial egg production rates. The low-dose hens never returned to pre-trial egg production levels, but due to the high occurrence of FLHS in the low-dose group, this is not unexpected.

In more traditional PCB-chicken feeding studies, i.e. studies without the FLHS factor, food consumption and body weight were usually not significantly affected by exposure to PCBs (Scott et al. 1971, Britton and Huston, 1973, Stendell 1976). Lillie et al. (1974) found that 2 ppm and 20 ppm of A1242, A1248, A1254 and 20 ppm of A1221, A1232, A1268, and A5442 had no effect on body weight after nine weeks of treatment, but 20 ppm of A1242, A1248, and A1254 significantly reduced food consumption. Hens in the present study experienced about a 20% decline in food consumption over time, but the differences among treatment groups were not significant. Body weights differed, however, by 9% and were influenced by the effects of both time and treatment.

Egg production is generally decreased by exposure to PCBs and significant decreases in egg production have been reported. Twenty ppm of A1232, A1242, A1248, and A1254 caused a significant drop in egg production when fed to hens (Lillie et al. 1974), Ten ppm of A1248 fed to hens for eight weeks decreased egg production by 10% (Scott et al. 1971), and A1254 fed at 5 ppm decreased egg production during weeks 15-28 of Platonow and Reinhart's (1973) study. No egg production effects were observed, however, when 5, 10, 20, 40, and 80 ppm of A1242 were fed to hens for six weeks (Britton and Huston, 1973). Similarly, Briggs and Harris (1973) did not find significant changes in egg production when they fed 20 and 50 ppm A1242 to hens for two weeks. In the present study, however, a significant decrease in egg production was observed in the control diet group in relation to both the low-dose and high-dose diet groups. Two factors contributed to the decreases observed in egg production. Firstly, the sudden dietary change probably led to the immediate drop in egg production in all groups. The consistently lower level of egg production by the control and low-dose group hens, however, may be attributed to FLHS.

The high-dose group hens, though, did not have a long-term treatment induced change in egg production levels.

A similar explanation may be given for the trends observed in egg weights. Decreased egg weights were observed throughout the study in both the control and low-dose diet groups, the two groups most afflicted with FLHS. Decreased egg weights in the high-dose group were immediate, but only temporary. The smaller eggs in the control and low-dose groups, however, may confound the interpretation of the organ weight effects. Those weights that had only slightly significant treatment differences, 18-day embryo body weights, chick brain weights, and chick heart weights, may not have been significant if the difference in egg weights did not exist, and the levels of significance may have been reduced in the 18-day embryo and chick liver weights. Similarly, those organ weights that had higher levels of significance coupled with decreases in the low-dose and high-dose groups, namely 18-day embryo spleen and bursa weights, and chick bursa weights, may have shown even greater levels of significance had egg weights been unaffected. Organ weights normalized to either body or brain weights were not reported in the present study due to the occurrence of significant time or treatment effects found in both body and brain weights.

Organs, including the liver, spleen, bursa, and thymus, have been reported to be sensitive to PCBs. In chicks fed 400 ppm A1260 for 60 days (Vos and Koeman 1970) or five different hexaclorobiphenyls (McKinney et al. 1976), liver weights were significantly greater and spleen weights were significantly less than those of controls. When 3,4,3',4'-TCB was injected into chicken eggs at day nine of incubation, Rifkind and Muschick (1983) noted that the weights of the livers and spleens from the 18-day embryos had significantly increased to 117% and 122% of controls. Thymus weights were 79% of controls, and the bursa weights were 90% of controls but were not significantly different from controls. Weights of the

spleen and bursa were also less in chicks produced by hens fed various levels of A1232, A1242, and A1248 (Harris et al. 1976).

Some investigations of the effect of TCDD-like congeners on the bursa of Fabricius in chicks and the potential immune system effects have been conducted. In laboratory animals, one of the most pronounced toxic effects of TCDD is thymic atrophy and the depletion of cells in other lymphoid organs such as the spleen and lymph nodes (Nikolaidis et al. 1988). The bursa is the site of B-cell development and differentiation in the chick and is responsible for establishing competency in immunoglobulin synthesis and producing the antibody response (Harris et al. 1976, Nikolaidis et al. 1988, 1989). Harris et al. (1976) suspected reduced antibody production when bursa weights were reduced in chicks produced by hens fed PCBs, but when challenged with *Brucella abortus*, antibody production proved to be normal. Other studies that reported lesser bursa weights from feeding chicks organochlorine pesticides (DDT and Mirex) also failed to show immunological suppression when challenged with bovine serum albumin, sheep red blood cells (Glick 1974), or heat-killed *Salmonella pullorum* (Latimer and Siegel 1974). Significant decreases in the number of lymphoid cells and bursal follicles were found in 19-day embryos when 300 μg 3,4,3',4'-TCB/kg egg was injected into chicken eggs at day 13 of incubation. The bursae were almost devoid of lymphoid cells. Furthermore, the bursae from embryos treated with lesser concentrations of 3,4,3'4'-TCB had fewer follicles and the follicles contained fewer lymphoid cells compared to the control bursae (Nikolaidis et al. 1988, 1989). In the present study, bursa weights were reduced significantly in both 18-day embryos and in chicks. No assessment of potential immune suppression was conducted, however.

No treatment-induced decrease in egg fertility was observed in the high-dose group. This is in agreement with the findings of other studies where chickens were fed diets

containing PCBs (Briggs and Harris 1973, Tumasonis et al. 1973, Ax and Hansen, 1975), However, both the control and low-dose groups experienced lower fertility rates, probably as a result of FLHS-induced physiological changes occurring in the hens. Likewise, the higher early stage embryo mortality evident in the control and low-dose groups was part of the unanticipated results in this study and was thought to be FLHS-related.

PCB-related embryo lethality, however, was clearly apparent in this study. Hatching averaged 95% for all groups during the first week of the trial and decreased in all treatment groups as the study progressed. The decreases, however, were only 5-9% in the control and low-dose groups, but hatching in the high-dose group was nearly 25% lower by the second half of the study. The declines in the control and low-dose group hatching rates did not continue into the second half of the study indicating that their declines may have been a result of hen response to the change in diets. Similarly, the lesser hatching rates in the high-dose group during the first part of the study may also be attributed to the hens' response to the change in diet, but the drastic drop in hatching rates during the second half of the study was clearly treatment induced. Interpretation of the impact of treatment on the low-dose group was difficult, because while some recovery in hatching rates occurred during the second half of the study, the recovery was not complete. Therefore, a question remains as to whether the treatment induced the slight depression of hatching rates or whether the lower rates were related to some other factor, perhaps FLHS.

Marked declines in the rate of hatchability have been noted in many other studies, and hatchability is considered to be more sensitive to PCB-induced effects than is egg production (Peakall 1986). In addition to those studies previously noted, 20 ppm of A1242 in the diet reduced hatchability by 50% by the second week of feeding in a study by Briggs and Harris (1973), and 20 ppm of A1232, A1242, A1248, and A1254 in the diet for nine

weeks decreased hatching rates to 44.5, 11.0, 1.8,and 69.0%, respectively, in studies conducted by Lillie et al. (1974). Britton and Huston (1973) found that 2.4 ppm A1242 in the egg yolk reduced hatchability. Fifteen ppm A1254 in the egg yolks also decreased hatching, but at egg yolk concentrations less than 5 ppm A1254, normal hatching rates returned (Platonow and Reinhart 1973). Reduced hatchability was noted when chickens were fed diets containing A1232, A1242, A1248 at 10 ppm for six weeks, but not at dietary concentrations of 5 ppm (Britton and Huston 1972). This reduced hatchability was associated with concentrations greater than 3 ppm in the yolk (about 1 ppm whole egg concentration) of eggs from hens fed diets containing 10 ppm A1242 (Britton and Huston 1972), and with a whole egg concentration of about 5 ppm in eggs from hens fed diets containing 50 ppm A1254 (Platonow and Reinhart 1973). Scott et al. (1971) found that hatching rates were reduced to 50% by feeding 10 ppm A1248 and to 2.4% by feeding 20 ppm A1248 for eight weeks. Feeding 0.5 or 1.0 ppm A1248 did not affect hatching rates. The diets in this study exposed the hens to lower levels of PCBs than those listed above, and yet significant reductions in hatching occurred. Noticeable reductions in high-dose group hatchability started during the 7th week of the trial (5th week of exposure) when the hens had consumed about 20.4 mg PCBs.

As with other studies (Scott et al. 1971, Lillie et al. 1974, Ax and Hansen 1975, Harris et al. 1976), most of the mortality in this study, and particularly in the high-dose group, occurred in the late stages of incubation (days 21-25). Briggs and Harris (1973) found that the greatest proportion of mortality occurred during the mid-stages of incubation (days 7-15) when they fed A1242 to hens. The timing of mortality may be influenced by the timing and intensity of exposure in relation to organogenesis. In Carlson and Duby's (1973) egg injection study, embryo mortality occurred at early developmental stages, but if injection

occurred after organogenesis was complete (day nine) there was no increase in embryolethality. A similar pattern was seen when hens were exposed to 50 ppm A1254 (in drinking water) for a period of six weeks. As PCB concentrations in the egg yolks increased, mortality occurred at progressively earlier stages of incubation (Tumasonis et al. 1973). The low-dose group of this study did have an increasing percentage of mortality occurring earlier in incubation as time progressed, but this was not true for the high-dose group. Late-stage mortality was more predominant in the high-dose group during the second half of the study than in the first half, but decreased in the low-dose group.

In a study of the effects of A1254 on the levels of gluconeogenic enzymes in embryos and chicks, Srebocan et al. (1977) concluded that A1254-induced alterations of the energy utilization mechanism might be responsible for embryo mortality at time of pipping. In one experiment, they injected chicken eggs on day zero of incubation with A1254 to a give a final concentration of 0.05, 0.1, 0.5, or 5 ppm and subsequently recorded moderate decreases of phosphoenol-pyruvate carboxykinase and fructose-1,6-diphosphatase (activities reduced to 80% of control activity), but not of glucose-6-phosphatase in 14-day embryos. In a second experiment, A1254 was fed to growing chicks for 14 days over a dietary range of 0.1-200 ppm, which resulted in severely reduced activities of fructose-1,6-diphosphatase and glucose-6-phosphatase, but not of phosphoenolpyruvate carboxykinase. The pattern of enzymatic change observed, i.e. more severe decreases in gluconeogenic enzymes in the chicks than in the embryos, does not in itself support the conventional thinking that the embryonic stage is more sensitive to the effects of PCBs. The authors speculated, however, that the energy demand is more critical during the earlier life stages and much less so at later stages, thereby making the embryo sensitive to even a moderate change in gluconeogenic enzyme activities, especially at the critical time of pipping.

Harris et al. (1976) and Scott et al. (1971) noted that many of the chicks that died late in incubation had pipped the shell, but failed to hatch. Roughly 25-33% of the embryos that died late in incubation in this study had pipped, and the remainder had fully developed but had not pipped the shell. Of those that had pipped the shell, 17%, 29%, and 30% of the control, low-dose, and high-dose group embryos, respectively, were seen to have some type of teratogenic effect, and this proportion increased between the first and second halves of the study in all groups. An even greater incidence of abnormalities, 48%, 59%, and 66% in the control, low-dose, and high-dose groups, respectively, occurred in those embryos that died without pipping the shell. These increased over time, and during the second half of the trial, 64% of the controls, 74% of the low-dose, and 96% of the high-dose embryos that died without pipping had some type of teratogenic effect.

Most of the teratogenic effects observed in this study were similar to those reported by other researchers in both laboratory studies and wild flocks birds. Edema and hemorrhaging of the head/neck region was the most predominant terata observed in this study, and also in experiments reported by Cecil et al. (1974) and Lillie et al. (1974). Subcutaneous edema was a highly predominant abnormality observed in populations of the DCCO and Caspian tern (Kurita and Ludwig 1988), and references of edema occurring in herring gulls can be found in Fox and Weseloh (1987), Gilbertson (1989), and Gilbertson et al. (1991). The edema in this study was noted only in the later stages of incubation (18-25 days) as was the case in naturally-incubated DCCO eggs (Kurita and Ludwig 1988). Furthermore, head/neck edema was found in over half of the dead embryos and chicks of all groups (57.4%, 56.2%, and 77.6% in the control, low-dose, and high-dose groups, respectively). If the edema is severe enough, it may restrict the ability of the chicks to pip the shell, or it may hinder the action of the pipping muscle so that the chick is weakened to

the point that it cannot complete the hatching process. Ludwig et al. (1990) noted DCCO chicks with subcutaneous edema so severe that the head and neck region was swollen to 2-3 times the normal size "as though injected with serous fluid." They too speculated that this condition impaired the ability of the chick to raise its head and pip the shell.

The abdominal edema that occurred in this study was always subcutaneous in nature. Generally speaking, it occurred over the breast area, but sometimes extended down to the inguinal region. In wild DCCO embryos, edema within the gut cavity and around the organs (termed "ascites") (Kurita et al. 1987) was observed as was subcutaneous edema around the leg junction to the body (Ludwig et al. 1990). Ascites was not observed in the embryos or chicks in the present study.

Photos of chicks with "blister edemas" or "edematous cysts on [the] rump" have been published by Brunstrom and Darnerud (1983) and Lillie et al. (1974). This form of terata was observed in both dead and live chicks in this study. If not too severe, the blister edema did not appear to be debilitating to the chick. However, very large edematous cysts were observed in certain dead embryos, the largest of which contained 7.2 g of fluid.

Hydropericardium has been frequently reported in the literature in studies with chickens and wild birds. In feeding studies where A1242, paint fractions (McCune et al. 1962), or 20 ppm A1254 (Bayer and Bird 1974) were fed to chicks, and in egg injection studies with 3,4,3',4'-TCB (Brunstrom 1988, 1989, 1990), edematous infusion into the pericardial sac was not uncommon. Five μg 3,4,3',4'-TCB/kg injected into the egg caused hydropericardium in 34% of live embryos and when 20 μg/kg was injected, 67% of the embryos had pericardial edema (Brunstrom 1988). On two or three occasions during the course of this trial, chicks were suspected to have some degree of pericardial edema. The

magnitude of the edema, however, was such that positive identification was not possible and attempts to draw off the fluid were unsuccessful.

Other teratogenic effects common to both laboratory chicken studies and surveys of wild bird populations are deformities manifested in the legs and feet. Chicks that hatched with short, bowed legs and crooked, clenched toes were reported by Tumasonis et al. (1973). Chicks with rotated ankles were reported by Lillie et al. (1974) and Cecil et al. (1974). Clubbed feet, a condition characterized by the inward rotation of the tarsometatarsus or femur (Ludwig et al. 1990), was reported to be common in Caspian terns and infrequent in DCCO (Kurita et al. 1987).

In addition to these abnormal leg conditions, some chicks in this study had seemingly normal legs, yet they were unable to stand erect. Gilbertson (1976) described a similar condition in ring-billed gulls observed during 1972 and 1973. The gulls, like the chicks, were unable to stand or walk properly and resorted to the use of their wings to move themselves about on the ground. This condition, termed "perosis" in poultry and generally attributed to a manganese deficiency, occurs when the birds have no support at the tibiotarsal tarsometatarsal joint. The lack of support arises when the gastrocnemius tendon and the hypotarsal sesamoid of both legs slip from the condyles on the cartilaginous articular surface on the tibiotarsus.

The bilaterally malformed femurs that occurred in dead embryos of the high-dose group have not, to my knowledge, previously been described in the literature. They first occurred during the 4th week of the hens' exposure to the treatment diets. The early stages of this malformation were seen in one 18-day embryo. The rest of the chicks survived until the 20-25th day of incubation, though all eventually died in the shell without pipping. The physiological basis for this deformity is unknown.

Deformities related to the incomplete absorption of the yolk sac were relatively uncommon in this study. Only one chick hatched with an unabsorbed yolk sac and the remaining occurrences were seen in dead chicks. In some cases, the organs were outside of the body wall. Most of the chicks with this condition were in the treatment diet groups, however, it was also found in three dead chicks in the control group. Lillie et al. (1974) reported cases of unabsorbed yolk sacs in chicks originating from PCB-fed hens. This type of anomaly has been observed "very frequently" in Caspian terns (Kurita et al. 1987), and has also been observed in DCCO, herring and ring-billed gulls, common terns, and red-breasted mergansers (Ludwig et al. 1990). The syndrome, termed gastroschisis ("split-belly"), described by Ludwig et al. (1990) included enlarged yolk sac attachments, organs that remained outside of the body, and a thin body wall.

The remainder of the deformities that were observed in this study, namely deformities of the eye, beak, skull/brain, and abnormal feathering patterns, were rarely observed. However, they are mentioned by other researchers including McLaughlin et al. (1963), Carlson and Duby (1973), Platonow and Reinhart (1973), Lillie et al. (1974), Brunstrom and Darnerud (1983), Brunstrom (1988, 1989, 1990), Kubiak et al. (1989), Ludwig et al. (1990), and Fox et al. (1991b).

The expected rate of occurrence, per 1000 chicks, for the six major categories of teratogenic effects observed in this study are shown in Table 19. The expected rates for the control, low-dose, and high-dose diet groups, based on the number of terata observed in each group, are presented. Additionally, calculations for two other potential reference groups are presented in an attempt to define a "control" group that was minimally affected by FLHS. The first alternative used the first five weeks of the study in the control group because the unexpected, corresponding increases in terata and decline in hen production values did not

begin until weeks four and five in that group. The second alternative used the values from the first two weeks of the study for all diet groups because all groups received the control feed during that time as an acclimation diet. Expected terata rates are less in both alternative "control" groups than those calculated using the actual control group values. The two alternative "control" rates probably more closely approximate real world terata rates, but sporadic occurrences of foot/leg and yolk sac deformities still cause rather high expected rates in those categories.

Expected rates for the more serious (severe head/neck edema and hemorrhage) and the more unusual ("blister edema" and femoral bone deformity) are also presented in Table 19. The ratio of head/neck edema:severe head/neck edema is much smaller in the high-dose group (4.6:1) than in the low-dose group (18.7:1) or control (14.8:1) group indicating that if a high-dose chick has head/neck edema, it is 3-4 times more likely to have a severe edema than either the control or low-dose group chicks. The ratios for abdominal edemas:"blister edema" were even larger (4.5:1, 32:1, and 42.5:1 for the high-dose, low-dose, and control groups, respectively). The increased likelihood of "blister edemas" in the high-dose group probably is not as critical as the increased likelihood of severe head/neck edema because of its interference with hatching.

Also critical to the survival probabilities of the chicks is the shift from single occurrences of terata to multiple deformities occurring in individual chicks. In the control and low-dose groups the trend shifted, between the first and second halves of the study, from about 70-80% single terata to 50-55% single terata. An even greater increase (53%) in multiple terata occurred during the second half of the study in the high-dose group. Chicks that are afflicted with only one terata are, intuitively speaking, more likely to have better survival probabilities than those chicks that are affected with multiple (up to 7 terata/

individual in this study) deformities. This was not examined in this study as the chicks were sacrificed within 24 hours of hatching. In the stressor-filled natural environment, however, chicks that are weakened by the extra effort to hatch with edema of the pipping muscle, crippled by clubbed or otherwise deformed feet, or left unable to forage due to shortened or twisted beaks have virtually no chance of survival.

Table 19. The expected rate of occurrence (per 1000 chicks) of terata observed in chicks from hens fed various concentrations of Saginaw Bay carp.

	Treatment Diet			Alternative Reference Controls	
Terata	Control	Low-Dose	High-Dose	Wks 1-5 of control diet group	Wks 1-2 of all diet groups
Head/neck edema/hem.	148	206	623	3	2
Severe head/neck edema/hem.	10	11	136	--	--
Abdominal edema/hem.	58	96	95	10	2
Blister edema	2	3	21	--	--
Foot/leg deformities	54	67	107	41	7
Femoral bone deformities	--	--	14	--	--
Misc.	12	11	25	7	--
Skull/brain deformities	10	6	25	3	4
Yolk sac deformities	6	8	13	--	18

SUMMARY

This study has shown that the primary food source of the colonial waterbirds, i.e. Great Lakes fish, is indeed capable of causing adverse reproductive effects in a model avian species, the chicken. Though not acutely toxic to the adult laying hens, a dose- and time-dependent response was seen in the embryos and chicks. Toxicity was manifested by increased mortality and decreased hatching rates as the percentage of carp in the diet increased. Furthermore, embryos and chicks displayed various terata including 1) Head and Neck Edema and Hemorrhage, 2) Abdominal Edema and Hemorrhage, 3) Foot and Leg Deformities, 4) Miscellaneous Deformities, 5) Skull and Brain Deformities, and 6) Yolk Sac Deformities. Increasing concentrations of carp also significantly affected various organ weights in 18-day embryos and hatched chicks. At 18-days of incubation, weights of the embryo's livers were proportional to while weights of the spleens and bursae were inversely proportional to the concentration of PCBs in the diets. After three additional days of incubation, significant effects in body, brain, liver, heart, and bursa weights were observed in hatched chicks.

RECOMMENDATIONS

This study has laid the groundwork for further investigation into contaminant-related reproductive failures in colonial waterbirds by eliciting toxic effects in a model laboratory avian species that resemble those observed in the wild. Suggestions for future investigation include: 1) an egg injection study whereby an extract of carp is injected into chicken eggs to compare the results with the effects from feeding the carp to laying hens, 2) an egg injection study whereby fractions from an extract of carp are injected into chicken eggs to isolate the contaminants which elicit GLEMEDS-like toxic effects in the chicken, and 3) an egg injection study whereby an extract of carp is injected into the eggs of a wild bird species to compare the results of injecting carp extract into eggs of a wild bird species with the effects of injecting carp extract into chicken eggs.

APPENDIX

APPENDIX A

Scientific Names of Species

Double-Crested Cormorant	(*Phalacrocorax auritus*)
Herring Gull	(*Larus argentatus*)
Ring-billed Gull	(*Larus delawarensis*)
Black-headed Gull	(*Larus ridibundus*)
Common Tern	(*Sterna hirundo*)
Caspian Tern	(*Sterna caspia*)
Forster's Tern	(*Sterna forsteri*)
Bald Eagle	(*Haliaetus leucocephalus*)
Osprey	(*Pandion haliaetus*)
Great Blue Heron	(*Ardea herodias*)
Mink	(*Mustela vison*)
River Otter	(*Lutra canadensis*)
Mallard	(*Anas playtyrhynchos*)
Pheasant	(*Phasianus colchicus*)
Bobwhite Quail	(*Colinus virginianus*)
Japanese Quail	(*Coturnix japonica*)
Snapping Turtle	(*Chelydra S. serpentina*)
Smelt	(*Osmerus mordax*)
Alewife	(*Alosa pseudoharengus*)

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