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EGG INJECTION STUDIES TO DETERMINE THE EFFECTS OF POLYHALOGENATED ENVIRNONMENTAL CONTAMINANTS ON AVIAN SPECIES

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DEBRA CURTIS POWELL

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Steven J. Bursian

Dr. Steven J. Bursian
Major professor

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EGG INJECTION STUDIES TO DETERMINE THE EFFECTS OF POLYHALOGENATED ENVIRONMENTAL CONTAMINANTS ON AVIAN SPECIES

By

Debra Curtis Powell

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ABSTRACT

EGG INJECTION STUDIES TO DETERMINE THE EFFECTS OF POLYHALOGENATED ENVIRONMENTAL CONTAMINANTS ON AVIAN SPECIES

By

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Great Lakes waterbird populations have experienced less than expected egg hatchability and greater than expected developmental abnormalities. Such deleterious effects have been attributed to polyhalogenated diaromatic hydrocarbons such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). PCBs are of primary concern since they are present in significant quantities in the environment. Specific PCB congeners, 3,3',4,4',5-pentachlorobiphenyl (IUPAC #126), 3,3',4,4'-tetrachlorobiphenyl (IUPAC #77), and 2,3,3',4,4'-pentachlorobiphenyl (IUPAC #105), were injected (singly and in combination) into the yolks of White Leghorn chicken (*Gallus domesticus*) eggs prior to incubation. In a subsequent experiment, congener 126 was injected into chicken and double-crested cormorant (*Phalacrocorax auritus*) eggs prior to incubation. Finally, chicken eggs were injected with one of four concentrations (0.001, 0.01, 0.1, and 1.0 egg equivalent) of an extract derived from double-crested cormorant eggs collected from Green Bay, WI. PCB-induced increases in mortality were observed in all experiments. Incidences of abnormalities, body weights, and relative weights of the brain, bursa, heart, liver, and spleen were also significantly affected.

To my husband, Jon
and
my parents, David and RuthAnn Curtis

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LIST OF ABBREVIATIONS

AHH	Aryl hydrocarbon hydroxylase
Ah-r	Ah receptor
EROD	Ethoxyresorufin *O*-deethylase
EVD	Embryo viability detector
FWS	U.S. Fish and Wildlife Service
LOAEL	Lowest observable adverse effect level
MSU	Michigan State University
NOAEL	No observable adverse effect level
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzo furan
PHDH	Polyhalogenated diaromatic hydrocarbon
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TCDD-EQ	TCDD-equivalents (H4IIE bioassay derived)
TEF	Toxic equivalency factor
TEQ	TCDD toxic equivalents (chemical analysis)
WQC	Water quality criteria

GENERAL INTRODUCTION

The Great Lakes have been contaminated as a result of industrial activities throughout the region. These contaminants are threatening many wildlife species that reside in the Great Lakes basin. In particular, fish-eating colonial waterbirds, such as the double-crested cormorant (*Phalacrocorax auritus*), have experienced less than expected hatchability of eggs with an accompanying greater than expected incidence of developmental abnormalities in both hatchlings and unhatched embryos (Fox et al., 1991b). Such deleterious effects have been primarily attributed to polyhalogenated diaromatic hydrocarbons (PHDHs) such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). PCBs are of primary concern among these toxicants since they have been shown to exist in significant quantities in the environment (Fox et al., 1991a; Tillitt et al., 1992). Conclusions about the cause/effect relationship between exposure to chemicals such as PCBs and the occurrence of deleterious effects have, thus far, been based on field observations of aquatic birds living near industrial areas. A controlled laboratory study has been needed to substantiate the link between contaminant exposure and effects observed in the field. Thus, the hypothesis tested by this project is that environmentally-derived contaminants are responsible for the decline in egg hatchability and increase in

teratogenic effects observed in colonial fish-eating waterbirds.

The first objective of this project was to determine the effects of 3,3',4,4'-tetrachlorobiphenyl (IUPAC #77), 3,3',4,4',5-pentachlorobiphenyl (IUPAC #126), and 2,3,3',4,4'-pentachlorobiphenyl (IUPAC #105) on developing White Leghorn chicken (*Gallus domesticus*) embryos. These PCB congeners are considered to be among the most toxic congeners, and they constitute a significant proportion of the PCB congeners found in the environment (Yamashita et al., 1993).

The second objective involved determining the difference in species sensitivity between the White Leghorn chicken and the double-crested cormorant to the most toxic of these PCB congeners, congener 126. The chicken has been widely used in laboratory toxicity studies and is an excellent species for egg injection studies since successful incubation and hatching conditions have been established (McLaughlin et al., 1963; Brunström, 1989, 1990). It would be ideal if the chicken could serve as an animal model for wild avian species like the cormorant in PHDH toxicity studies.

The third objective was to determine the effects of environmentally-derived contaminants on the chicken embryo. Environmentally-derived contaminants were extracted from cormorant eggs collected in 1988 from Green Bay, Wisconsin. Because these contaminants have undergone weathering in the environment and metabolism in the food chain, the mixture injected into the eggs was a more accurate representation of what the animals encounter in the field.

BACKGROUND AND LITERATURE REVIEW

Great Lakes Contamination

The Great Lakes basin has become polluted by industrial activity for over a century. Despite cleanup efforts in recent decades, the Great Lakes still face the problem of persistent toxic contaminants. Large populations, heavy industry, and the fact that the Great Lakes have a slow flushing rate for contaminants make this region particularly susceptible to these persistent contaminants. For example, it takes 182, 106, and 21 years for water entering Lakes Superior, Michigan, and Huron, respectively, to be flushed out again. The primary concern with these contaminants is their effect on aquatic organisms, since some of these compounds bioaccumulate. Phytoplankton, in the process of filtering for nutrients, scavenge persistent contaminants. They in turn are eaten by zooplankton which are preyed upon by fish which in turn are consumed by piscivorous birds and mammals (Hileman, 1988).

In areas of heavy pollution, there has been a high incidence of tumors, goiters, lack of secondary sex characteristics, and high embryo mortality in fish which are believed to be a result of contamination in the Great Lakes (Hileman, 1988). There is evidence that contaminated fish from the Great Lakes have deleterious effects on piscivorous mammals and birds. Mink (*Mustela vison*) experienced reproductive failure and/or high kit mortality when fed fish from the Great Lakes (Aulerich et al., 1971). Fish-eating waterbirds residing in the Great Lakes have also experienced reproductive failure in addition to teratogenic effects (Gilbertson, 1974; Weseloh et al., 1983; Fox et al., 1991a).

Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are a group of chlorinated aromatic organic compounds which are of major concern due to their potential toxicity and also their wide distribution and persistence in the environment (Tanabe, 1988). PCBs are one group of compounds that are still found in greater concentrations than the concentrations specified in water quality agreements established between the United States and Canada in the 1970s. In 1986, the concentration of total PCBs in the Great Lakes ranged from 0.337 to 1.8 ng/L. These concentrations are much greater than the water quality criteria (WQC) for six of the major wildlife species studied in the Great Lakes (Ludwig et al., 1993). A WQC for total PCBs of 0.001 ng/L has been suggested to protect the most sensitive of the wildlife species affected by PCBs (Ludwig et al., 1993). Concentrations of PCBs and DDE in the eggs of piscivorous colonial waterbirds in Canada indicated that the Great Lakes basin has the greatest concentration of PCBs and DDE in the country (Vermeer and Peakall, 1977).

PCBs, commercially introduced in 1929, were widely used in industrial products. Commercial mixtures of PCBs, such as the Aroclor® mixtures sold by Monsanto Chemical Company, were prepared by the chlorination of biphenyls and sold by the amount of chlorine they contained. For example, Aroclor® 1260 contained 60% chlorine by weight (Safe, 1991).

PCBs are chemically stable. If they contain more than four chlorine atoms they are not affected by boiling with sodium hydroxide or nitric acid and are nonflammable

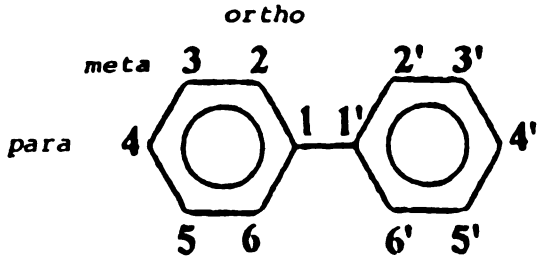
(Reynolds, 1969). They also have little water solubility (Huckins et al., 1988) and are good electrical insulators. Due to these properties, they were used for a variety of purposes, most notably as heat transfer fluids in capacitors and transformers, and as flame retardants. In 1966, they were first identified in the environment. They are quite possibly one of the largest groups of environmental pollutants due to widespread use and careless disposal methods (Richardson et al., 1982). U.S. production of PCBs ended in 1977 (Safe, 1991).

Environmental contamination by PCBs occurs through a variety of means. Some primary routes of contamination are leaks or spills from landfills, storage facilities, or equipment utilizing PCBs, and incinerator or engine emissions (Richardson et al., 1982). It has also been suggested that PCBs may have been used in pesticides to increase their kill life because PCBs can hold volatile ingredients (Reynolds, 1969). If this was done, it represents a significant source of the PCBs found in the environment. Once in the environment, PCBs can be transported via the air or the water. In water, PCBs are primarily adsorbed to particles since they are insoluble in water (Haque and Schmedding, 1976).

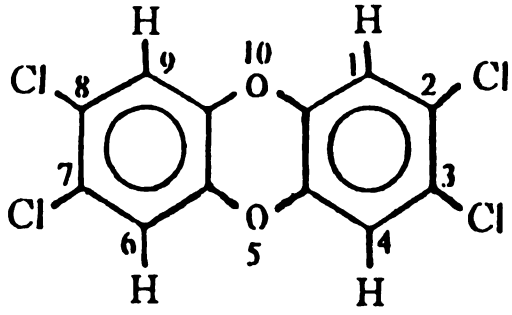
Studies of PCBs are more complicated now that it has been discovered that different congeners vary in toxicity and in their uptake and elimination by organisms. Over 200 chlorobiphenyls can theoretically exist by changing the number and location of chlorine atoms on the biphenyl molecule. Approximately 100 of these can be found in PCB technical preparations (Albro et al., 1981). However, after weathering in the environment and biochemical alterations by organisms, the commercial mixtures first

released in the environment are rarely reflected in the environmental mixtures to which humans and wildlife are now exposed. Thus, the individual congeners have increasingly become the focus of research rather than the commercial PCB mixtures. Coplanar PCBs are the most toxic of the PCBs. They are substituted in both para positions and two or more of the meta positions, and are structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic of the man-made chemicals (Figure 1) (Safe, 1991). TCDD is an unwanted by-product of chemical manufacturing and industrial processes. TCDD binds to a cytosolic receptor called the Ah receptor (Ah-r), and it is this binding event that is thought to elicit the effects associated with TCDD and structurally similar "dioxin-like" compounds. The compound and receptor form a ligand complex that can interact with specific areas of the genome and lead to the induction of gene transcription (Safe, 1991).

Three of these coplanar PCBs in order of decreasing toxicity are 3,3',4,4',5-pentachlorobiphenyl (IUPAC# 126), 3,3',4,4'-tetrachlorobiphenyl (IUPAC# 77), and 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC# 169) (Figure 2). All have been shown to be present in significant quantities in commercial PCB preparations (Tanabe et al., 1987). Each has been found in humans, marine and terrestrial mammals and birds, and marine and freshwater fish (Tanabe, 1988). When a variety of animal species were tested for residual concentrations of these PCBs, congener 77 was found in the greatest concentration followed by congeners 126 and 169 (Tanabe et al., 1987).

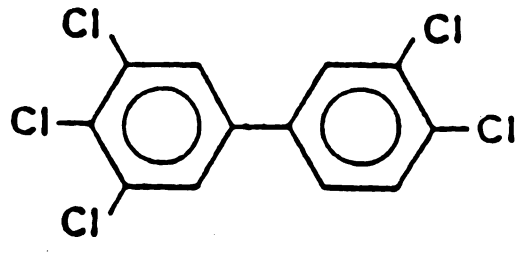


Polychlorinated Biphenyl

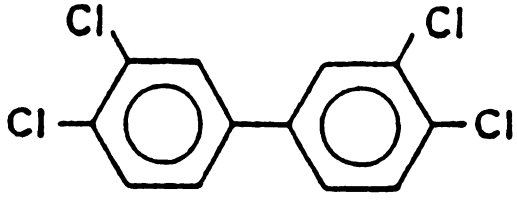


2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Figure 1. The structures of PCBs and TCDD.



3,3',4,4',5-pentachlorobiphenyl



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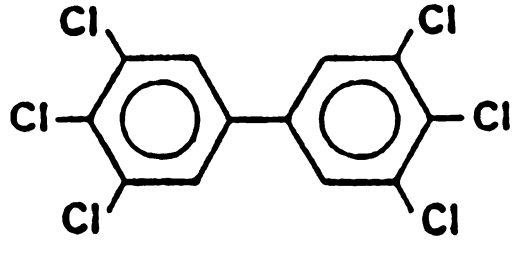

3,3',4,4',5,5'-hexachlorobiphenyl

Figure 2. The structure of three coplanar PCBs.

Mono-*ortho* PCB congeners, chlorinated in one of the *ortho* positions in addition to the para and meta positions required of coplanar congeners, can also bind to the Ah-r. They are often present in greater environmental concentrations than non-*ortho* congeners (Brunström, 1990). Congener 105 (2,3,3',4,4'-pentachlorobiphenyl), while less toxic than the coplanar congeners, may be responsible for a significant portion of the toxicity attributed to PCBs because of its concentration in the environment (Kubiak et al., 1989; Smith et al., 1990; Yamashita et al., 1993).

In accessing complex environmental mixtures, toxic equivalency factors (TEFs) have been developed to evaluate these mixtures based on a common measure of toxicity. The intent is to compare the toxicity of a particular compound or mixture of compounds to a standard compound, which is usually TCDD. The test compound or mixture must have a similar mode of action as the standard, thus TEFs can be derived for dioxin-like compounds when the standard is TCDD. The standard is given a TEF of 1.0 and all other compounds generally have TEFs less than 1. To calculate the TEF of a compound, the endpoint or measured response must be known or determined for TCDD and then determined for the test compound. The TEF for the compound can then be obtained by dividing the dose which results in the response at a specified magnitude for TCDD by the dose for the same response for the test compound. If a variety of endpoints are measured, one can obtain a range of TEFs for a particular compound (EPA, 1991). The toxicity of a mixture of compounds can be expressed in toxic equivalents (TEQs) by multiplying the TEF of each compound in the mixture by the measured concentration of each compound in the total mixture (Safe, 1991; Bosveld et al., 1992). The toxicity of environmental

extracts can also be tested using the H4IIE bioassay. TCDD toxic equivalents (TCDD-EQs) are determined by comparing the hepatic enzyme induction in extract-treated cells with TCDD-treated cells (Tillitt et al., 1991).

Samples from double-crested cormorant (*Phalacrocorax auritus*) eggs indicate that PCB congeners 126, 77, and 105 account for over 90% of the TEQs associated with the egg (Yamashita et al., 1993). When fish (salmon from Lake Michigan) were examined, congeners 126, 77, 105, and 118 (2,3',4,4',5-pentachlorobiphenyl) accounted for 92% of the TEQs in the fish (Williams et al., 1992).

PCBs are known to cause a variety of effects that include body weight loss, atrophy of the thymus, liver injury, skin cell disorders (eg. chloracne in humans), reproductive dysfunction, depressed immune response, and teratogenic effects. In addition, some PCBs are believed to be carcinogenic and possibly mutagenic in some species (Safe, 1989). Some studies (Jacobson et al., 1993) have also shown intellectual deficits in children as a result of in utero and/or lactational exposure. Laboratory studies with PCBs have demonstrated many toxic effects in avian species. Liver lesions, hydropericardium, shortened beak, microphthalmia, and subcutaneous edema have been observed in PCB-exposed chick embryos (Brunström, 1988). Dioxin-like compounds have also been shown to decrease the number of B-cells in the bursa of Fabricius (Andersson et al., 1991). Besides the previously mentioned effects, dioxin-like compounds are known to be strong inducers of cytochrome P-450 1A1 and 1A2 (Brunström, 1991; Sanderson et al, 1994) as indicated by increased hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity. There have been significant positive correlations between TCDD concentrations in the

environment and hepatic EROD activity in blue heron chicks (Bellward et al., 1990).

There are differences in sensitivity to the toxic effects of PCBs among species. Of the avian species, the White Leghorn chicken (*Gallus domesticus*) is one of the most sensitive. Turkeys (*Meleagris gallopavo*) are approximately 50 times less sensitive to congener 77 than chickens when this congener is injected into the yolk (Brunström and Lund, 1988). Pheasant (*Phasianus colchicus*), goldeneye (*Bucephala clangula*), black-headed gull (*Larus ridibundus*), mallard (*Anas platyrhynchos*), goose (*Anser anser*), and herring gull (*Laurus argentatus*) embryos are also much less sensitive to congener 77 than domestic chicken embryos (Brunström and Reutergardh, 1986; Brunström, 1988). These species differences may be due to differences in the concentration of or affinity for the Ah-r or differences in the metabolism of PCBs. Another explanation for such differences in toxic effects could be the greater genetic variation in wild species when compared to domestic species. (Brunström and Reutergardh, 1986).

Nonetheless, the domestic chicken is often used as a laboratory model for the toxic effects of compounds to which wild avian species are exposed. The chicken was used as a model species for colonial waterbirds in a study where carp from Saginaw Bay was fed to adult hens. Eggs laid by these hens had a dose-dependent increase in the incidence of teratogenic effects similar to the effects seen in colonial waterbird populations (Summer, 1992).

Waterbird Effects

As indicated previously, some populations of fish-eating waterbirds in the Great Lakes have experienced increased embryo mortality, changes in reproductive behavior, increased incidence of abnormalities (including edema), histopathological anomalies, suppression of the immune system, and changes in biochemical homeostasis. These avian populations have been located primarily in areas where contamination is greatest (Tillitt et al., 1991). GLEMEDS, Great Lakes embryo mortality, edema, and deformities syndrome, is an acronym often used to refer to the effects observed in these waterbird populations (Gilbertson et al., 1991). The developmental abnormalities include club feet, missing eyes, defective feathering, and bill deformities (Yamashita et al., 1993). Other effects include enlargement, necrosis, and porphyria of the liver, as well as subcutaneous, peritoneal, and pericardial edema. These symptoms are also characteristic of a poultry condition called chick edema disease which is caused by organochlorine compounds that are structurally similar to TCDD. Clinical signs of this disease include reduction in body weight gains, unsteady gait, labored breathing, ruffled feathers, and increased morbidity. Observed lesions include hydropericardium, subcutaneous and peritoneal edema, swollen and pale kidney, and swollen liver (Gilbertson et al., 1991).

Colonial fish-eating birds are often used as indicators of environmental contamination. Significant amounts of environmental contaminants like PCBs can be found in these birds since they bioaccumulate, and their diet consists almost entirely of fish and other food items which bioconcentrate contaminants. As an illustration, PCBs can be

bioconcentrated in the eggs of these birds by as much as 2.5 x 10^7 times the concentration of PCBs in the water (Fox et al., 1991b).

In assessing the effects of environmental contamination on colonial waterbirds, reproductive outcome (the production of viable offspring) is the primary monitoring method. Of the species of colonial fish-eating waterbirds, herring gulls, Forster's terns (*Sterna forsteri*), and double-crested cormorants have been the most studied species (Fox et al., 1991b).

Herring Gulls (*Larus argentatus*)

Studies of herring gulls in the Great Lakes have provided information on contamination trends (Gilman et al., 1977; Bishop and Weseloh, 1990). Lake Ontario and Lake Michigan herring gulls are the most contaminated with TCDD and PCBs while Lake Superior herring gulls are the least contaminated (Bishop and Weseloh, 1990). An association was found between residual concentrations of contaminants in the eggs and embryo lethality. The compounds identified as the primary contaminants in Lake Ontario eggs were PCBs and mirex (Gilman et al., 1977).

A study with extracts of organochlorine compounds from herring gull eggs injected into uncontaminated gull eggs produced no increase in mortality over controls (Gilman et al., 1978). That study suggested either that organochlorine compounds in eggs are not the direct cause of the high embryo mortality observed in Lake Ontario or that the effect of contaminants may occur prior to laying of the egg in the form of alterations in genetics or structure of the egg.

Studies on gulls from Lake Ontario have shown that abnormal behavior on the part of the adult can contribute significantly to egg loss and subsequent low reproductive outcome. Egg exchange experiments were performed between colonies on Prince Edward Island and Lake Ontario which indicated that adults in Lake Ontario colonies incubating eggs from Prince Edward Island had no better success than Lake Ontario gulls incubating their own eggs. These birds showed an obvious lack of nest defense and attentiveness (Fox et al., 1978). Inattentiveness leads to significant cooling and overheating of eggs which results in increased egg lethality. Nests in Lake Ontario were unattended longer than nests in less contaminated areas (Bishop and Weseloh, 1990).

Forster's Terns (*Sterna forsteri*)

In a 1983 study, Forster's tern eggs were collected from Green Bay, WI, a Lake Michigan colony. Birds hatched from this contaminated region had relative liver weights (% body weight) that were, on average, 26% greater than relative liver weights of birds from an uncontaminated area. PCB congeners 105 and 126 accounted for over 90% of the total PCB concentration. From this study, contamination was strongly suggested as being the causal factor in decreased reproductive outcomes (Kubiak et al., 1989).

Double-Crested Cormorants (*Phalacrocorax auritus*)

It has been suggested that cormorants are more sensitive to some contaminants than other species (Fox et al., 1991c). This species is long-lived and entirely piscivorous, yellow perch being a major prey item (Weseloh et al., 1983). It is also an abundant

species which breeds throughout the Great Lakes region. Thus, it has been extensively studied as a bioindicator for contamination (Fox et al., 1991c).

These birds experienced a population decline in the Lake Huron region during the 1960s (Ludwig, 1984). Their colonies were still small in 1972, and they were unable to reproduce successfully due to egg loss and breakage (Weseloh et al., 1983). This was primarily attributed to p,p'-DDE which caused eggshells to become thinner (Peakall, 1988). Egg concentrations of DDE were 14.5 $\pm$ 5.6 ppm and PCB concentrations were 23.8 $\pm$ 9.6 ppm. The cormorant populations began to recover in the late 1970s and their numbers are elevated over those reported in the early 1970s (Scharf and Shugart, 1981; Weseloh et al., 1983; Ludwig, 1984).

Cormorant chicks from the upper Great Lakes region were studied from 1986-1989 and were found to have the following developmental abnormalities: crossbill, club foot, hip dysplasia, dwarf appendages, subcutaneous edema, unabsorbed yolk-sac (gastroschisis), and deformed as well as diseased eyes. These abnormalities have been correlated with concentrations of PCBs in cormorant eggs (Tillitt et al., 1991). Since these birds have been increasing in abundance and distribution, neither the quantity of food nor its nutritional quality are responsible for the previously described malformations in chicks. There is also no evidence for the presence of infectious disease. Evidence in support of a chemical contaminant as the causative agent includes reduced survival and growth of embryos, elevated hepatic microsomal aryl hydrocarbon hydroxylase (AHH) activity, congenital abnormalities, and increased liver to body weight ratios. The induction of AHH activity indicates that polyhalogenated diaromatic hydrocarbons (PHDHs) may be the

chemical agent responsible (Fox et al., 1991a).

The bioaccumulation factor for PCBs from fish to cormorants is approximately 53 (Weseloh et al., 1983). The bioaccumulation factor is the concentration of PCBs in the predator (cormorant) divided by the concentration of PCBs in the prey (fish). Fat in cormorants was found to have concentrations of PCBs 10-100 times greater than marine fishes and 100-1,000 greater than freshwater fishes (Scharenberg, 1991). Of the chemicals analyzed in cormorant egg samples, PCBs (3.6-7.3 μg/g) and p,p'-DDE (2.3-6.3 μg/g) were found in the highest concentrations (Yamashita et al., 1993). According to a study done in the late 1980s, there was a significant correlation between cormorant egg mortality and PCB concentrations in eggs taken from the same colony (Tillitt et al., 1991). Cormorant eggs from Green Bay, WI have some of the greatest concentrations of PCBs and other contaminants found in the Great Lakes region (Yamashita et al., 1993). PCBs in eggs from these birds were also found to be similar to congeners comprising Aroclor® 1254 (Stalling et al., 1985). A significant, positive regression has been found between toxic equivalents in cormorant chicks from different locations in Canada and the induction of liver enzymes (Sanderson et al., 1994). This was supported by similar research in the Netherlands (Van den Berg et al., 1994)

In a 1973 study, contaminant concentrations were determined in cormorants from South Dakota (Greichus et al., 1973). Average PCB concentrations in an adult cormorant body, cormorant egg, and cormorant nestling were 4.6 ppm, 5.9 ppm, and 0.39 ppm, respectively. Concentrations of PCBs in cormorants were 60 fold greater than those in fishes. The PCB concentrations in eggs of cormorants also appeared to reflect the PCB

concentrations in the adult bodies.

Egg Injections and Incubation

The avian embryo is exposed to toxins present in the egg from fertilization through hatching and even beyond since the yolk is not entirely absorbed until several days after hatching. Thus, the chicken egg is a practical way of observing toxicological effects (Fox et al., 1991b). The injection of substances into an egg is a fairly inexpensive and simple way to study toxicity. According to McLaughlin and associates (1963), the earliest published work in this area appeared in the 1890s by Fere. Fere's interest was primarily the teratogenic effects produced by chemicals. He injected test substances prior to incubation and opened the eggs after a few days of incubation.

The toxicity of several test substances has been examined by injecting them into the yolks of chicken eggs prior to incubation (McLaughlin et al., 1963). The injection method consisted of what can be called a "yolk shake". An egg was given a quick shake prior to injection to free the germinal disk such that it was able to float freely which reduced the risk of injury to the embryo during injection. The needle was inserted perpendicular to the long axis of the egg. Injections were done in volumes of up to 0.10 ml. Of the substances injected, the ones with no or little toxicity were water, propylene glycol, corn oil, peanut oil, isotonic saline solution, and isotonic glucose solution. Since eggs injected with these solutions had near normal rates of hatching (95%, 95%, 90%, 90%, 90%, and 90%, respectively), these chemicals can be considered to be potential vehicles for testing

non-liquid substances.

Recent work involving egg injections has focused on this technique as an alternative to the long and costly mammalian studies required for toxicity testing. Hashizume and associates (1992) examined differences in toxicity among chemicals depending on whether the site of injection was the air cell or yolk. Injection of distilled water and physiological saline produced greater mortality when injected into the yolk as compared to air cell (18.4% and 16.0% vs. 10.2% and 11.7%). However, sesame oil produced greater mortality (26.3%) when injected into the air cell than it did when injected into the yolk (13.3%). Some differences in malformations were noticed between the two injection sites, but, as with mortality, the differences varied with the substance being tested. They also noted greater mortality the earlier the time of administration.

A considerable amount of research involving the injection of PCBs into chicken eggs has been done by Brunström and associates. The primary obstacle to successful injection studies is finding a proper vehicle (Brunström and Orberg, 1982). One objective of their research was to devise a vehicle which could be used for lipophilic substances such as PCBs. After much experimentation, an emulsion of lecithin, peanut oil, and water was chosen as the vehicle. They examined how well a lipophilic substance in this vehicle would be taken up by the embryo. This was accomplished by using ^{14}C-labelled 2,2',4,5'-tetrachlorobiphenyl and assessing distribution by autoradiography (Brunström and Darnerud, 1983). The autoradiographs indicated a continual uptake of the injected PCBs by the embryo. Thus, in this experiment, the emulsion vehicle provided early uptake of the test substance. The injection procedure as well as the vehicle were apparently harmless

to the embryo.

The relative toxicity of PCB congeners 126, 77, and 169 has been determined in chickens when injected into the yolk on day 4 of incubation (Brunström and Andersson, 1988). The most toxic of the three, congener 126, is approximately 50 times more potent than the least toxic of the three, congener 169, based on mortality. All three were shown to induce EROD activity and produced abnormalities such as subcutaneous edema, hydropericardium, microphthalmia, and shorter upper beaks. In addition to the above coplanar PCB congeners, Brunström (1990) has demonstrated the toxicity of several mono-*ortho* PCB congeners in chickens. He found that PCB congeners with an *ortho* chlorine adjacent to a meta chlorine were more toxic than congeners with an *ortho* chlorine adjacent to a meta hydrogen (Figure 1). These congeners also induced EROD in a dose-dependent fashion suggesting that their toxicity is mediated via the Ah-r. Similar abnormalities were also caused by these congeners.

The toxic effects of congener 77 have been compared in chickens and turkeys (Brunström and Lund, 1988). Mortality of both species was proportional to dose, but turkeys were about 50 times less sensitive than chickens. Brunström (1990) noted that while non-*ortho* PCB congeners are extremely toxic in chick embryos, they seem to be less toxic in embryos of many other avian species (Brunström et al., 1990). Brunström (1989) suggests that it is difficult to predict the effects of such compounds on reproduction in wild avian embryos given the great differences between species.

While injections into the chicken egg are done routinely, injections into wild avian eggs, particularly those of the cormorant, have been limited. Incubation techniques have

been a complicating factor in the success of such experiments. Larson (1991) injected "undeveloped" cormorant eggs from Bachelor's Island in Lake Winnipegosis (Manitoba, Canada) with TCDD and PCB congener 126. Analysis of 10 uninjected eggs revealed a total PCB concentration of 910 ppb. The same three doses (1.84 pg/g, 184 pg/g, and 18,400 pg/g) were used for both TCDD and PCB congener 126. The vehicle used was 1,4-dioxane. Chicken eggs were also injected with the same dose of TCDD and congener 126 (15,100 pg/g). All chicken eggs died within a week of injection. The hatching rate of chicken eggs injected with the vehicle alone was 66.7%. Cormorant egg hatchability was poor in all groups. Thus, Larson based analysis on embryos alive on days 7 and 23. Cormorant mortality at any dose of congener 126 or TCDD was not significantly greater than the vehicle on either day. Larson's results suggested that the NOAELs (no observable adverse effect level) were the greatest doses of both TCDD and congener 126 (18,400 pg/g).

There has been limited success in the artificial incubation of cormorant eggs from day 0. The incubation conditions for cormorant eggs in Larson's study (1991) were 37.5°C and 65.5% relative humidity. The relative humidity was raised to 67% on day 26 of incubation. Eggs were placed with their blunt end up and automatically rotated six times a day. These conditions did not afford proper development of the aircell and as a result only 3% of the cormorant eggs hatched. Unincubated cormorant (*Phalacrocorax carbo*) eggs from the Netherlands incubated at 37.5°C with 50% relative humidity for 24 to 25 days and 80% relative humidity for the last one to two days resulted in less than half the eggs hatching (Van den Berg et al., 1994). A more successful incubation procedure

for the double-crested cormorant (*Phalacrocorax auritus*) was developed by Sotherland (unpublished data). The incubation temperature used was 37.2°C (99°F). Eggs were placed horizontally and rotated automatically every two hours in addition to being rotated by hand twice a day. Pipped eggs were transferred to a hatching room which was maintained at 37°C and 70% relative humidity. Sotherland also noted that hatching success was greater if the eggs had been incubated for about a week by the parents. This has been supported by Sanderson et al. (1994), who obtained 77% hatchability of cormorant eggs naturally incubated for two weeks by the parents prior to artifical incubation (35.4°C, 46% relative humidity). However, natural incubation is not practical for egg injection studies if one wants to determine the effects of compounds which would be present from fertilization through hatch.

REFERENCES

Albro, P.W., J.T. Corbett, and J.L. Schroeder. 1981. Quantitative characterization of polychlorinated biphenyl mixtures (Aroclors 1248, 1254, and 1260) by gas chromatography using capillary columns. J. Chromatogr., 205:103-111.

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

Andersson, L., E. Nikolaidis, B. Brunström, A. Bergman, and L. Denker. 1991. Effects of polychlorinated biphenyls with Ah receptor affinity on lymphoid development in thymus and the bursa of fabricius of chick embryos in ovo and in mouse thymus analgen in vitro. Toxicol. Appl. Pharmacol., 107:183-188.

Bellward, G.D., R.J. Norstrom, P.E. Whitehead, J.E. Elliott, S.M. Bandiera, C. Dworschak, T. Chang, B. Cadario, L.E. Hart, and K.M. Cheng. 1990. Comparison of polychlorinated dibenzodioxin and dibenzofuran levels with hepatic mixed-function oxidase induction in great blue herons. J. Toxicol. Environ. Health, 30:33-52.

Bishop, C., and D.V. Weseloh. 1990. A state of the environment fact sheet. Contaminants in herring gull eggs from the Great Lakes. Environment Canada SOE Fact Sheet 90-2. Toronto, Ontario, 12 pp.

Bosveld, B., M. van den Berg, and R.M.C. Theelen. 1992. Assessment of the EROD inducing potency of eleven 2.3,7,8-substituted PCDD/Fs and three coplanar PCBs in the chick embryo. Chemosphere, 25:911-916.

Brunström, B. 1988. Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. Poultry Sci., 67:52-57.

Brunström, B. 1989. Toxicity of coplanar polychlorinated biphenyls in avian embryos. Chemosphere, 19:765-768.

Brunström, B. 1990. Mono-*ortho*-chlorobiphenyls: toxicity and induction of 7-ethoxyresorufin O-deethylase (EROD) activity in chick embryos. Arch. Toxicol., 63:188-192.

Brunström, B. 1991. Toxicity and EROD-inducing potency of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in avian embryos. Comp. Biochem. Physiol., 100:241-243.

Brunström, B. and L. Andersson. 1988. Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated biphenyls (PCBs) chick embryos. Arch. Toxicol., 62:263-266.

Brunström, B., D. Browman, and C. Näf. 1990. Embryotoxicity of polycyclic aromatic hydrocarbons (PAHs) in three domestic avian species, and of PAHs and coplanar polychlorinated biphenyls (PCBs) in the common eider. Environ. Pollut., 67:133-143.

Brunström, B. and P.O. Darnerud. 1983. Toxicity and distribution in chick embryos of 3,3',4,4'-tetrachlorobiphenyl injected into eggs. Toxicology, 27:103-110.

Brunström, B. and J. Lund. 1988. Differences between chick and turkey embryos in sensitivity to 3,3',4,4'-tetrachlorobiphenyl and in concentration /affinity of the hepatic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Comp. Biochem. Physiol., 91:507-512.

Brunström, B. and J. Orberg. 1982. A method for studying embryotoxicity of lipophilic substances experimentally introduced into hens' eggs. Ambio, 11:209-211.

Brunström, B. and L. Reutergardh. 1986. Differences in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3',4,4'-tetrachlorobiphenyl, injected into the egg. Environ. Pollut., 42:37-45.

EPA Workshop report on toxicity equivalency factors for polychlorinated biphenyl congeners, Risk Assessment Forum. United States Environmental Protection Agency. 1991. EPA/625/3-91/020.

Fox, G.A., A.P. Gilman, D.B. Peakall, and F.W. Anderka. 1978. Behavioral abnormalities of nestling Lake Ontario herring gulls. J. Wildl. Manage., 42:477-483.

Fox, G.A., B. Collins, E. Hayakawa, D.V. Weseloh, J.P. Ludwig, T.J. Kubiak, and T.C. Erdman. 1991a. Reproductive outcomes in colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains. J. Great Lakes Res., 17:153-167.

Fox, G.A., M. Gilbertson, A.P. Gilman, and T.J. Kubiak. 1991b. A rationale for the use of colonial fish-eating birds to monitor the presence of developmental toxicants in Great Lakes fish. J. Great Lakes Res., 17:151-152.

Fox, G.A., D.V. Weseloh, T.J. Kubiak, and T.C. Erdman. 1991c. Reproductive outcomes in colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains. J. Great Lakes Res., 17:153-157.

Gilbertson, M. 1974. Pollutants in breeding herring gulls in the lower Great Lakes. Can. Field-Nat., 88:273-280.

Gilbertson, M., T. Kubiak, J. Ludwig, and G.A. Fox. 1991. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J. Toxicol. Environ. Health, 33:455-520.

Gilman, A.P., D.J. Hallett, G.A. Fox, L.J. Allan, W.J. Learning, and D.B. Peakall. 1978. Effects of injected organochlorines on naturally incubated herring gull eggs. J. Wildl. Manage., 42:484-493.

Gilman, A.P., G.A. Fox, D.B. Peakall, S.M. Teeple, T.R. Carroll, G.T. Haymes. 1977. Reproductive parameters and egg contaminant levels of Great Lake herring gulls. J. Wildl. Manage., 41:458-468.

Greichus, Y.A., A. Greichus, and R.J. Emerick. 1973. Insecticides, polychlorinated biphenyls, and mercury in wild cormorants, pelicans, their eggs, food, and environment. Bull. Environ. Contam. Toxicol., 9:321-328.

Haque, R. and D. Schmedding. 1976. Studies on the adsorption of selected polychlorinated biphenyl isomers on several surfaces. J. Environ. Sci. Health, B11(2):129-137.

Hashizume, R., A. Noda, M. Itoh, Y. Yamamoto, S. Masui, M. Oka, T. Nakamura. 1992. Studies on teratological testing using chicken embryos-effects of solvents, injection sites, and the age of the embryo. Exp. Anim., 41:349-356.

Hileman, B. 1988. The Great Lakes cleanup effort. Chem. Engineering News, pp. 22-39.

Huckins, J.N., T.R. Schwartz, J.D. Petty, and L.M. Smith. 1988. Determination, fate, and potential significance of PCBs in fish and sediment samples with emphasis in selected AHH-inducing congeners. Chemosphere, 17:1995-2016.

Jacobson, J.L. and S.W. Jacobson. 1993. A 4-year follow up study of children born to consumers of Lake Michigan fish. J. Great Lakes Res., 19:776-783.

Kubiak, T.J., H.J. Harris, L.M. Smith, T.R. Schwartz, D.L. Stalling, J.A. Trick, L. Sileo, D.E. Docherty, and T.C. Erdman. 1989. Microcontaminants and reproductive impairment of the Forster's Tern on Green Bay, Lake Michigan-1983. Arch. Environ. Contam. Toxicol., 18:706-727.

Larson, J.M. 1991. Reproductive and developmental anomalies in double-crested cormorants (*Phalacrocorax auritus*) on Lake Michigan: an environmental contaminants study. M.S. Thesis, University of Wisconsin, Madison, WI, 120 pp.

Ludwig, J.P. 1984. Decline, resurgence and population dynamics of Michigan and Great Lakes double-crested cormorants. Jack-Pine Warbler, 62:91-102.

Ludwig, J.P., J.P. Giesy, C.L. Summer, W. Bowerman, R. Aulerich, S. Bursian, H.J. Auman, P.D. Jones, L.L. Williams, D.E. Tillitt, and M. Gilbertson. 1993. A comparison of water quality criteria for the Great Lakes based on human and wildlife health. J. Great Lakes Res., 19:789-807.

McLaughlin, J., J.P. Marliac, M.J. Verrett, M.K. Mutchler, and O.G. Fitzhugh. 1963. The injection of chemicals into the yolk sac of fertile eggs prior to incubation as a toxicity test. Toxicol. Appl. Pharm., 5:760-771.

Peakall, D.B. 1988. Known effects of pollutants on fish-eating birds in the Great Lakes of North America. in: Toxic Contamination in Large Lakes. Vol. I. Chronic Effects of Toxic Contaminants in Large Lakes. ed. N.W. Schmidtke. Proceedings of the World Conference on Large Lakes. May 18-21, 1986. Mackinac Island, MI, pp. 39-54.

Reynolds, L.M. 1969. Polychlorinated biphenyls (PCBs) and their interference with pesticide residue analysis. Bull. Environ. Contam. Toxicol., 4:128-143.

Richardson, B.J. and J.S. Wald. 1982. Polychlorinated biphenyls (PCBs): An Australian viewpoint on a global problem. Search, 13:17-25.

Safe, S. 1991. Polychlorinated dibenzo-*p*-dioxins and related compounds: sources, environmental distribution and risk assessment. Environ. Carcinog. & Ecotox. Rev., C9(2):261-302.

Safe, S. Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, 2nd Edition, edited by R.D.Kimbrough and A.A. Jensen, p. 131. Elsevier-North Holland, Amsterdam, 1989.

Sanderson, J.T., R.J. Norstrom, J.E. Elliott, L.E. Hart, K.M. Cheng, and G.D. Bellward. 1994. Biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in double-crested cormorant chicks (*Phalacrocorax auritus*). J. Toxicol. Environ. Health, 41:247-265.

Scharenberg, W. 1991. Cormorants (*Phalacrocorax carbo sinensis*) as bioindicators for polychlorinated biphenyls. Arch. Environ. Contam. Toxicol., 21:536-540.

Scharf, W.C. and G.W. Shugart. 1981. Recent increases in double-crested cormorants in the United States Great Lakes. Am. Birds, 35:910-911.

Smith, L.M., T.R. Schwartz, and K. Feltz. 1990. Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota. The question of their relative toxicological significance. Chemosphere, 21:1063-1085.

Stalling, D.L., R.J. Norstrom, L.M. Smith, and M. Simon. 1985. Patterns of PCDD, PCDF, and PCB contamination in Great Lakes fish and birds and their characterization by principal components of analysis. Chemosphere, 14:627-643.

Summer, C.L. 1992. An avian ecosystem health indicator: the reproductive effects induced by feeding Great Lakes fish to White Leghorn laying hens. M.S. Thesis. Dept. Animal Science, Michigan State University, East Lansing, MI, 117 pp.

Tanabe, S., N. Kannan, An. Subramanian, S. Watanabe, and R. Tatsukawa. 1987. Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. Environ. Pollut., 47:147-163.

Tanabe S. 1988. PCB problem in future: foresight from current knowledge. Environ. Pollut., 50:5-28.

Tillitt, D.E., G.T. Ankley, D.A. Verbrugge, J.P. Giesy, J.P. Ludwig, and T.J. Kubiak. 1991. H4IIE rat hepatoma cell bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in colonial fish-eating waterbird eggs from the Great Lakes. Arch. Environ. Contam. Toxicol., 21:91-101.

Tillitt, D.E., G.T. Ankley, J.P. Giesy, J.P. Ludwig, H. Kurita-Matsuba, D.V. Weseloh, P.S. Ross, C.A. Bishop, L. Sileo, K.L. Stromborg, J. Larson, and T.J. Kubiak. 1992. Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes. Environ. Toxicol. Chem., 11:1281-1288.

Van den Berg, M., B.L.H.J. Craane, T. Sinnige, S. van Mourik, S. Dirksen, T. Boudewijn, M. van der Gaag, I.J. Lutke-Schipholt, B. Spenkelink, and A. Brouwer. 1994. Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant after in ovo exposure. Environ. Toxicol. Chem., 13:803-816.

Vermeer, K. and D.B. Peakall. 1977. Toxic chemicals in Canadian fish-eating birds. Mar. Pollut. Bull., 8:205-210.

Weseloh, D.V., S.M. Teeple, and M. Gilbertson. 1983. Double-crested cormorants of the Great Lakes: egg-laying parameters, reproductive failure, and contaminant residues in eggs, Lake Huron 1972-1973. Can. J. Zool., 61:427-436.

Williams, L.L., J.P. Giesy, N. DeGaian, D.A. Verbrugge, D.E. Tillitt, and G.T. Ankley. 1992. Prediction of concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents from total concentrations of polychlorinated biphenyls in fish fillets. Environ. Sci. Technol., 26:1151-1159.

Yamashita, N., S. Tanabe, J.P. Ludwig, H. Kurita, M.E. Ludwig, and R. Tatsukawa. 1993. Embryonic abnormalities and organochlorine contamination in Double-Crested Cormorants (*Phalacrocorax auritus*) and Caspian Terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ. Pollut., 79:163-173.

CHAPTER 1

THE EFFECTS OF 3,3',4,4'-TETRACHLOROBIPHENYL, 2,3,3',4,4'-PENTACHLOROBIPHENYL, AND 3,3',4,4',5-PENTACHLOROBIPHENYL ON DEVELOPING CHICKEN EMBRYOS WHEN INJECTED PRIOR TO INCUBATION

ABSTRACT

Great Lakes waterbird populations have experienced less than expected hatchability of eggs and a greater than expected incidence of developmental abnormalities. Such deleterious effects have been attributed to polyhalogenated hydrocarbons such as polychlorinated biphenyls (PCBs). PCBs are of primary concern since they are present in significant quantities in the environment. Specific PCB congeners, 3,3',4,4',5-pentachlorobiphenyl (IUPAC #126), 3,3',4,4'-tetrachlorobiphenyl (IUPAC #77), and 2,3,3',4,4'-pentachlorobiphenyl (IUPAC #105), were injected (singly or in combination) into the yolks of White Leghorn chicken (*Gallus domesticus*) eggs prior to incubation. Teratogenicity was assessed in dead embryos and in hatchlings. Hatchlings were raised for three weeks to assess body weight gain and mortality. At the end of the three week period, chicks were subjected to necropsy and the brain, bursa, heart, liver, spleen, and testes were removed and weighed. All three congeners caused increased mortality with approximately 50% mortality occurring at 0.6, 4.9, and 5,592 μg/kg egg for congeners 126, 77, and 105, respectively. All three congeners also produced significantly more abnormalities than the vehicle. Chicks from PCB-injected eggs had lower body weights at the second and third weeks of age. Congener 126 caused lower relative bursa weights, congener 77 caused greater relative spleen weights and lower relative liver weights, and all three congeners caused relalive heart weights to be greater when compared to control.

INTRODUCTION

Years of industrial activity in the Great Lakes region have led to environmental contamination which is threatening many wildlife species that reside in the Great Lakes basin (Peakall, 1988). Fish-eating colonial waterbirds, such as double-crested cormorants (*Phalacrocorax auritus*), have experienced reduced hatchability of eggs with a concomitant greater rate of developmental abnormalities in both hatched and unhatched chicks (Weseloh et al., 1983). Such deleterious effects have been primarily attributed to aromatic hydrocarbons such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polyhalogenated dibenzofurans (PCDFs). PCBs are of primary concern among these industrial contaminants as they have been shown to exist in significant quantities in the environment (Tillitt et al., 1991; Yamashita et al., 1993; Jones et al., 1994).

The non-*ortho* PCB congeners, 3,3',4,4',5-pentachlorobiphenyl (IUPAC #126) and 3,3',4,4'-tetrachlorobiphenyl (IUPAC #77), have been shown to cause abnormalities and lethality when injected into chicken eggs (Brunström and Darnerud, 1983; Brunström, 1988; Brunström and Andersson, 1988; Brunström and Lund, 1988; Brunström, 1991). These compounds are structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and can thus bind to the Ah-receptor (Ah-r) (Brunström and Andersson, 1988; Brunström and Lund, 1988; Brunström, 1991). The toxicity of these compounds observed in chick embryos is thought to be mediated through this Ah-r (Brunström, 1990). Mono-*ortho* congeners, such as 2,3,3',4,4'-pentachlorobiphenyl (IUPAC #105), can also bind to the

Ah-r and are present in greater concentrations in some commercial preparations of PCBs than non-*ortho* PCBs (Brunström, 1990). Congener 105 together with congener 126 accounted for more than 90% of the toxic equivalents (TEQs) in Forster's tern eggs collected at Green Bay, WI in 1983 (Kubiak et al., 1989).

The objective of the present study was to determine the effects of three environmentally relevant PCB congeners (Kubiak et al., 1989; Yamashita et al., 1993) when injected into the yolks of White Leghorn chicken (*Gallus domesticus*) eggs prior to incubation. Yolk injection was chosen since the majority of environmental contaminants are lipophilic and hence will be deposited into the yolk. Injection on day 0 of incubation was significant since wild avian embryos are exposed to contaminants from the time of fertilization, and laboratory exposure just prior to incubation ensures that the potential adverse effects induced by contaminants during early development will be included in the analysis of toxicity of the contaminant tested.

MATERIALS AND METHODS

Doses of PCB congeners 126, 77, and 105 were chosen to bracket the smallest and greatest concentrations detected in cormorant eggs collected from various sites in the Great Lakes (Yamashita et al., 1993) as well as previously reported LD_{50}s for seven-day-old chicken embryos injected on day 4 (Brunström and Andersson, 1988). These three congeners were also injected in all possible combinations at the smallest environmental concentration detected in cormorant eggs collected from various Great Lakes sites

(Yamashita et al., 1993).

Doses and Design: The PCB congeners were purchased from AccuStandard (New Haven, CT). Doses for congener 126 were 0.1, 0.3, 0.9, 2.7, or 8.1 μg/kg egg. Congener 77 was injected at doses of 1.0, 3.0, 9.0, 27.0, or 81.0 μg/kg egg and congener 105 was injected at doses of 100, 300, 900, 2700, or 8100 μg/kg egg. The doses chosen for the congener combinations were the lowest environmental concentrations detected in cormorant eggs collected from five colonies around the Great Lakes. Eggs from Tahquamenon Island in Lake Superior had the lowest concentrations of these congeners; 0.80, 0.98, and 110 μg/kg egg for congeners 126, 77, and 105, respectively (Yamashita *et al.*, 1993). There were four congener combinations: 105 plus 77, 105 plus 126, 77 plus 126, and 105 plus 77 plus 126. There were 20 eggs/treatment group per replicate and three replications of each congener and combination of congeners. Due to factors unrelated to the experiment, a fourth replicate was added to increase the sample size of chicks exposed to congener 77. In addition to the dose groups, there were non-injected and vehicle control groups in each replicate.

Vehicle: The injection vehicle was an emulsion of lecithin from egg yolk (Merck: Darmstedt, Germany), peanut oil, and water. The lecithin was initially dissolved in dichloromethane (Brunström and Darnerud, 1983). The lecithin and peanut oil were combined (1:10, w/v) and then placed in a waterbath (60°C) for approximately one hour to allow the dichloromethane to evaporate. The lecithin/peanut oil (L/PO) solution was autoclaved for 30 min and then mixed with sterile distilled water (2:3, v/v) as described by Brunström and Orberg (1982).

Dose Solutions: Dosing solutions were prepared from an initial stock solution of PCB congener in L/PO. Congeners 126 and 77 (5 mg) readily dissolved in L/PO (10 ml) following sonication for 10 min. Congener 105 was first dissolved in dichloromethane since it was uncertain if 202 mg could be dissolved in 10 ml L/PO. Using a nitrogen evaporator, dichloromethane was then evaporated. Appropriate proportions of stock solution and L/PO were combined to provide two ml total L/PO solution. Three ml sterile distilled water were then added as previously described. New dosing solutions were prepared for each replicate.

Egg preparation: Eggs from White Leghorn chickens were obtained from the Michigan State University (MSU) Poultry Science Teaching and Research Center. All eggs were candled to locate the air cell and then weighed to determine the injection volume (1 μl/g egg). After eggs were weighed and labeled, they were positioned horizontally and placed in a cooler (58°F) until injection on the following day. Eggs were laid horizontally overnight to allow the germ spot to float away from the site of injection thus reducing potential injury during injection.

Injections: The injections were made prior to incubation (day 0) and the site of injection was the yolk sac. Eggs were removed from the cooler and left horizontal until after being injected. A 100μl syringe (22S gauge) (Hamilton Company, Reno, NV) was used for the injections. It was sterilized with 70% alcohol prior to insertion into the vial and before injection into the egg. The needle length was marked at approximately 29 mm to ensure injection into the yolk (this length was chosen after a series of injections of a colored solution into the yolks of infertile eggs). The blunt end of the egg was surface

sterilized with 70% alcohol before a small hole was made in the shell over the air cell with a sterile pin. After horizontal insertion of the needle, the solution was slowly injected, and the needle was left in for a moment following injection. Upon removal of the needle, the hole was sealed with melted paraffin. Following injections, all eggs were placed in the incubator.

Incubation: Eggs were incubated in a Petersime (Gettysburg, OH) poultry incubator for up to 24 days with their blunt end up. Conditions in the incubator were standard for commercial operations, 37.5-37.7°C (99.5-99.75°F) with wet bulb readings of 85-87°F to yield 65% relative humidity. Eggs were automatically rotated every two hours. Embryo viability was determined by candling on days 4 and 11. On day 18, viability was assessed with an embryo viability detector (EVD) which was provided by the U.S. Fish and Wildlife Service (FWS). The EVD detects vibrations within the egg and changes these vibrations to sound waves which can be heard in headphones attached to the EVD (Mineau and Pedrosa, 1986). Any non-viable eggs on days 11 or 18 were opened to estimate the stage of development and to determine the presence of any anomalies. Viable eggs were then transferred to hatching baskets in a second incubator with higher humidity (70-75%) than the incubator used for incubation prior to day 18.

Post-hatch: Upon hatching, each chick was weighed and uniquely identified with a wing band. Chicks were also examined for abnormalities. Once chicks were sufficiently dry, they were moved to a floor pen with pine and spruce wood chips (Pestell, Agri-Products, New Hamburg, Ontario, Canada) and acclimated to food and water by dipping their beaks in water and feed (Purina Chick Starter) which were both provided *ad libitum*.

All chicks were raised for a period of 21 days with body weights being recorded on a weekly basis. In studies involving herring gulls, a three-week survival period was used to estimate reproductive success since mortality of chicks was observed during the first three weeks after hatch (Gilman et al., 1977). Any chick which was determined incapable of acquiring food, water, or warmth on its own was euthanized.

Necropsies: At the end of the three weeks, chicks were killed by cervical dislocation. The organs taken for weighing included the brain, bursa, heart, liver, spleen, and testes.

Histopathology: The bursa, liver, spleen, and testes from five (where possible) three-week-old chicks per dose were saved in 10% formalin. Organs from the greatest dose group of each congener were examined by a veterinary pathologist.

Data Analysis: All comparisons were made with respect to the vehicle control. Due to the relatively low rate of hatching in the vehicle control group, comparisons with the non-injected controls alone or in combination with vehicle controls was not considered to be appropriate. Mortality was evaluated using a 2x2 contingency table and Bonferroni Chi-Square ("infertiles" were considered early deads since it was often difficult to distinguish between the two). The same analysis was used to test the occurrence of abnormalities. LD_{50} values were obtained using probit analysis. Analysis of body weight was conducted using univariate analysis of variance for split-plot repeated measures (Gill, 1986) followed by Dunnett's test for comparisons with control. Organ weights and differences between left and right testes were analyzed using a one-way ANOVA and Dunnett's test. Analyses of variance were conducted with the statistical software SAS

(SAS Institute Inc., 1992). The level of statistical significance was 0.05 unless otherwise stated.

RESULTS

Mortality: The vehicle control group had statistically greater mortality than the non-injected control group. Despite great mortality in the vehicle control group, all three congeners (singly or in combination) produced significantly greater mortality than the vehicle control at the greater doses (Tables 1-4). The LD_{50}s (95% confidence limits) (Table 5) were 0.6 μg/kg egg (0.4 - 0.7) for congener 126, 4.9 μg/kg egg (3.6 - 6.1) for congener 77, and 5,592 μg/kg egg (2,256 - 7,637) for congener 105. Injection of combinations containing congener 126 produced significantly greater mortality compared to the vehicle. There was no statistically significant mortality post-hatch induced by any of the congeners or combinations. Chicks that died after transfer to floor pens were usually those that hatched late or were weak at hatch. These chicks occurred in both the vehicle control and treated groups.

Teratogenicity: The types of morphological abnormalities observed included edema; skull and brain abnormalities; crossed, missing, and shortened beaks; curled toes; leg and hip abnormalities; and missing or small eyes. Congener 126 (Table 6) produced significantly more abnormalities at 0.9 μg/kg egg (22%) when compared to the vehicle control group (7%). The two greatest doses (2.7 and 8.1 μg/kg egg) resulted in such high mortality that statistical assessment of abnormalities was not possible. The predominant

types of abnormalities observed in the 0.9 μg/kg egg dose group were miscellaneous abnormalities which included unusually small embryos, embryos with abnormal head/jaw area, or embryos with abnormal organs. Congener 77 (Table 7) produced significantly more abnormalities at 27 μg/kg egg (15%) than the vehicle control group (4%). Ninety percent of the eggs injected with 81 μg/kg egg died by day 11, thus, the incidence of abnormalities in surviving embryos was not statistically significant. The predominant abnormalities which occurred in the 27 μg/kg egg group were beak deformities such as crossed or shortened upper or lower beaks. Congener 105 (Table 8) caused greater abnormalities relative to the vehicle control group (5%) only at the greatest dose, 8100 μg/kg egg (22%). The predominant abnormalities included unusually small embryos, embryos with abnormal head/jaw area, or embryos with abnormal organs. None of the combinations (Table 9) of congeners produced any significant differences in the occurrence of abnormalities. Edema was the predominant type of abnormality observed in embryos receiving the combination of all three congeners.

Body Weights: The greater doses of all three of the congeners tested produced significantly lower chick body weights than the vehicle control (Tables 10-13). Congener 126 caused reduced body weights beginning at two weeks of age at doses of 0.9 and 2.7 μg/kg egg. Chicks hatching from eggs injected with 3 μg/kg egg of congener 77 were significantly lighter than vehicle controls at three weeks of age while chicks dosed with 9 μg/kg egg were significantly lighter than controls beginning at two weeks of age. A dose of 300 μg/kg egg of congener 105 caused lower body weights at two weeks of age, 900 and 2700 μg/kg egg produced significantly lower body weights at three weeks of age, and

8100 μg/kg egg resulted in lower body weights after only one week post hatch. Congener 126 in combination with congener 105 and/or congener 77 resulted in lower body weights beginning at two weeks.

Organ Weights: All organ weights are expressed as actual and relative weights. Since body weights were affected, statements of significance are in reference to relative organ weights (% body weight). Congener 126 caused an increase in brain (Table 14) weight and a decrease in weights of the bursa of Fabricius (Table 15) at the 0.9 μg/kg egg dose. This dose also caused an increase in heart (Table 16) weights when compared to the vehicle control group. Congener 77 caused an increase in brain (Table 20) weight at 9.0 μg/kg egg. This congener caused an increase in heart (Table 22) weight and a decrease in liver (Table 23) weight at a dose of 3.0 μg/kg egg. Spleen (Table 24) weights were greater in the 9.0 μg/kg egg dose group than in the vehicle control. Congener 105 caused an increase in brain (Table 26) and heart (Table 28) weights at 8100 μg/kg egg. There were no significant differences in any of the lower dose groups. Only the combination of congeners 105, 77, and 126 caused an increase in brain (Table 32) weight and a decrease in the weight of the bursa of Fabricius (Table 33). Relative heart weight (Table 34) was increased by the combination of congeners 77 and 126, as well as the combination of congeners 105, 77, and 126. The combination of 105 and 126 was the only combination treatment to affect the liver (Table 35) which was decreased in weight.

Histopathology: There were no significant histological lesions associated with PCB treatment in any of the tissues examined.

Table 1. Effect of congener 126 injected into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability.

Dose μg/kg egg	Dose nmol/kg egg	# dead/ # eggs	% Mortality
Non-injected	0	7/60	11.7
0[a]	0	22/59	37.3
0.1	0.3	12/60	20.0
0.3	0.9	23/60	38.3
0.9	2.8	50/60[b]	83.3
2.7	8.3	59/60[b]	98.3
8.1	24.8	60/60[b]	100

[a] Vehicle (lecithin/peanut oil and water emulsion)
[b] Significantly different from control at $p<0.05$

Table 2. Effect of congener 77 injected into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability.

Dose μg/kg egg	Dose nmol/kg egg	# dead/ # eggs	% Mortality
Non-injected	0	13/80	16.3
0[a]	0	32/80	40.0
1.0	3.4	21/80	26.3
3.0	10.3	29/80	36.3
9.0	30.8	54/80[b]	67.5
27.0	92.5	80/80[b]	100
81.0	277.4	80/80[b]	100

[a] Vehicle (lecithin/peanut oil and water emulsion)
[b] Significantly different from control at $p<0.05$

Table 3. Effect of congener 105 injected into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability.

Dose μg/kg egg	Dose nmol/kg egg	# dead/ # eggs	% Mortality
Non-injected	0	9/60	15.0
0[a]	0	24/60	40.0
100	306	28/60	46.7
300	919	25/60	41.7
900	2,757	31/58	53.5
2700	8,271	31/60	51.7
8100	24,813	50/59[b]	84.8

[a] Vehicle (lecithin/peanut oil and water emulsion)
[b] Significantly different from control at $p < 0.05$

Table 4. Effect of congener combinations injected into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability.

Combination[a]	Total (PCBs) nmol/kg egg	# dead/ # eggs	% Mortality
Non-injected	0	8/58	13.8
0[b]	0	31/60	51.7
105/77	340.4	25/60	41.7
105/126	339.5	47/59[c]	79.7
77/126	5.9	50/60[c]	83.3
105/77/126	342.9	55/60[c]	91.7

[a] Doses in μg(nmol)kg egg for congeners 126, 77, and 105 are 0.80 (2.5), 0.98 (3.4), and 110 (337), respectively
[b] Vehicle (lecithin/peanut oil and water emulsion)
[c] Significantly different from control at $p < 0.05$

Table 5. LD_{50}s of congeners 126, 77, and 105 injected into the yolks of White Leghorn chicken eggs on day 0 of incubation.

Congener	LD_{50} μg(nmol)/kg egg	95% Confidence Limits	
		Lower	Upper
126	0.6 (1.8)	0.4 (1.2)	0.7 (2.3)
77	4.9 (16.8)	3.6 (12.4)	6.1 (21.0)
105	5,592 (17,130)	2,256 (6,912)	7,637 (23,394)

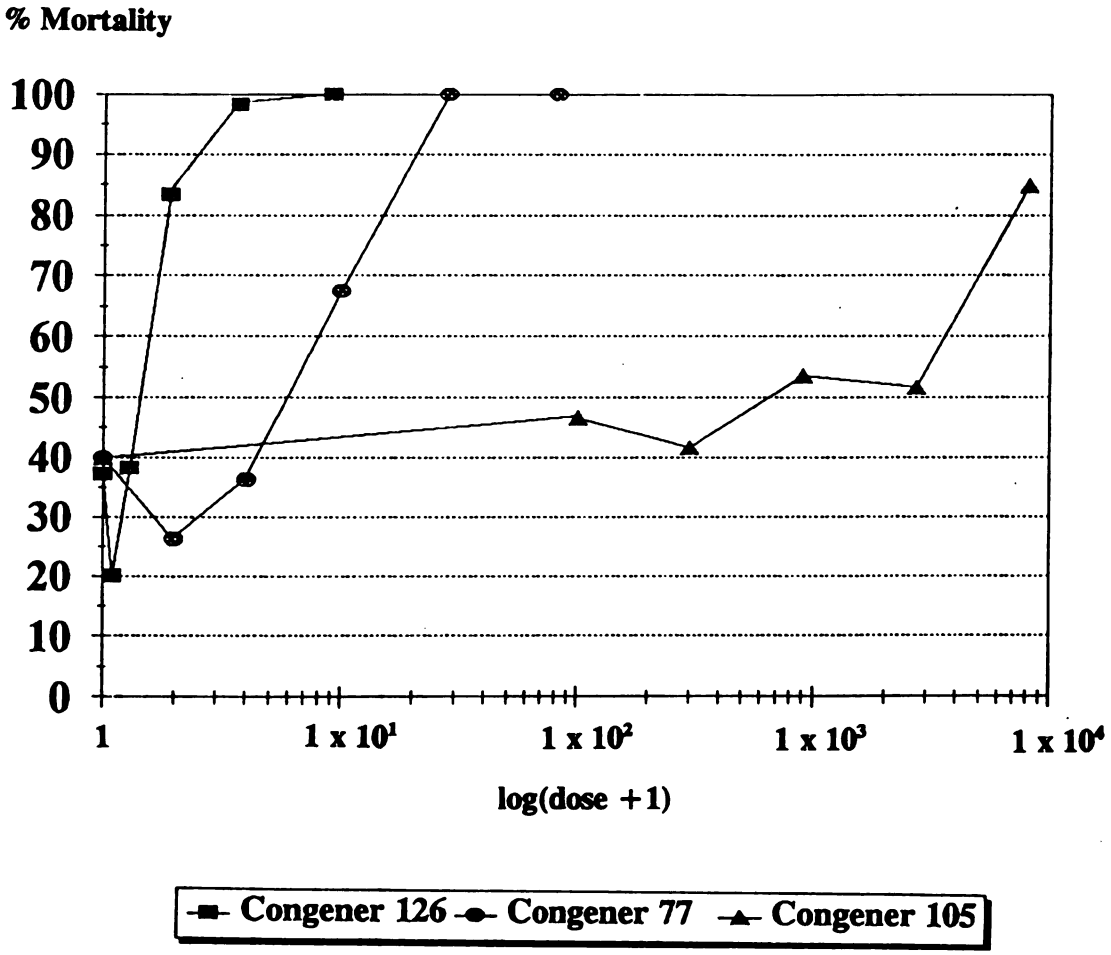

Figure 3. Effect of PCB congeners 126, 77, and 105 on mortality. One was added to each dose to allow inclusion of the vehicle control on a log scale. For example, vehicle control (0 μg/kg egg) = 1 and the highest PCB congener dose (8100 μg congener 105/kg egg) = 8101.

Table 6. Abnormalities caused by congener 126 in White Leghorn chicken embryos.

	NI[a]	VC	0.1	0.3	0.9	2.7	8.1
#Dead by Day 11	6/60	9/59	7/60	10/60	11/60	42/60	59/60
#Abnormal Embryos/#Eggs	0/60	4/59	5/60	8/60	13/60[b]	12/60	0/60
Total Abnormalities	0	5	6	8	14	15	0
% Abnormalities by Type							
Head/Neck Edema	-	-	-	13%	14%	7%	-
Blister Edema	-	-	-	38%	21%	-	-
Skull/Brain	-	-	17%	-	-	-	-
Beak							
-Crossed	-	-	17%	13%	-	13%	-
-Missing	-	20%	-	-	-	13%	-
-Short upper/lower	-	20%	-	38%	14%	13%	-
Small/Missing Eye(s)	-	20%	17%	-	-	20%	-
Misc.[c]	-	-	-	-	36%	33%	-

[a] NI = non-injected, VC = vehicle control, doses expressed as μg PCB congener/kg egg
[b] Statistically different from vehicle control at $p<0.10$
[c] Unusually tiny embryos and embryos with questionable looking heads/jaws and abnormal organs

Table 7. Abnormalities caused by congener 77 in White Leghorn chicken embryos.

	NI[a]	VC	1	3	9	27	81
#Dead by Day 11	5/80	18/80	12/80	17/80	14/80	56/80	76/80
#Abnormal Embryos/#Eggs	3/80	3/80	3/80	8/80	10/80	12/80[b]	3/60
Total	3	5	4	10	10	12	4
% Abnormalities by Type							
Head/Neck Edema	-	20%	-	10%	40%	-	-
Blister Edema	-	-	-	-	10%	-	-
Skull/Brain	33%	-	-	10%	-	-	-
Beak							
-Crossed	-	-	-	-	-	8%	-
-Missing	-	-	-	10%	-	-	-
-Short upper/lower	-	20%	50%	20%	30%	33%	75%
Small/Missing Eye(s)	-	-	25%	10%	-	25%	-
Curved/Curled Toes	-	-	-	20%	-	-	25%
Leg/Hip Abnormalities	-	40%	-	10%	-	-	-
Wing Abnormalities	-	20%	-	-	-	-	-
Misc.[c]	67%	-	25%	10%	20%	33%	-

[a] NI = non-injected, VC = vehicle control, doses expressed as μg PCB congener/kg egg
[b] Statistically different from vehicle control at $p<0.10$
[c] Unusually tiny embryos, abnormal head, neck, or jaw, and abnormal organs

Table 8. Abnormalities caused by congener 105 in White Leghorn chicken embryos.

	NI[a]	VC	100	300	900	2700	8100
#Dead by Day 11	3/60	17/60	9/60	14/60	16/58	13/60	13/59
#Abnormal Embryos/#Eggs	2/60	3/60	8/60	8/60	4/58	10/60	13/59[b]
Total Abnormalities	3	5	11	9	5	11	13
% Abnormalities by Type							
Head/Neck Edema	-	-	-	22%	20%	9%	23%
Blister Edema	33%	20%	9%	-	-	36%	15%
Skull/Brain	-	20%	9%	10%	-	-	-
Beak							
-Crossed	-	20%	18%	11%	20%	-	-
-Missing	-	-	-	-	-	-	-
-Short upper/lower	-	-	-	11%	20%	9%	8%
Small/Missing Eye(s)	-	20%	18%	11%	20%	-	-
Curved/Curled Toes	33%	-	9%	11%	-	9%	-
Leg/Hip Abnormalities	33%	20%	18%	33%	-	-	8%
Wing Abnormalities	-	-	-	-	-	-	-
Misc.[c]	-	-	18%	-	20%	36%	46%

[a] NI = non-injected, VC = vehicle control, doses expressed as μg PCB congener/kg egg
[b] Statistically different from vehicle control at $p<0.05$
[c] Unusually tiny embryos and embryos with questionable looking heads/jaws and abnormal organs

Table 9. Abnormalities caused by congener combinations in White Leghorn chicken embryos.

	NI	VC	105/77	105/126	77/126	105/77/126
#Dead by Day 11	4/58	16/60	6/60	23/59	17/60	21/60
#Abnormal Embryos/#Eggs	2/58	6/60	3/60	4/59	9/60	7/60
Total Abnormalities	2	8	3	4	11	7
% Abnormalities by Type						
Head/Neck Edema	-	-	-	25%	36%	57%
Blister Edema	-	25%	-	-	-	-
Abdominal Edema	-	-	-	50%	-	-
Skull/Brain	50%	13%	-	-	-	-
Beak						
-Crossed	-	13%	-	-	-	-
-Short upper/lower	-	13%	-	-	9%	14%
Small/Missing Eye(s)	-	13%	-	-	-	-
Curved/Curled Toes	50%	-	33%	-	-	14%
Leg/Hip Abnormalities	-	13%	33%	25%	-	-
Wing Abnormalities	-	13%	-	-	-	-
Misc.[b]	-	-	33%	-	55%	14%

[a] NI = non-injected, VC = vehicle control, doses of congeners 105, 77, and 126 were 110, 0.98, and 0.80 μg PCB congener/kg egg, respectively

[b] Unusually tiny embryos, abnormal head, neck, or jaw, and abnormal organs

Table 10. Effect of congener 126 on the body weights of White Leghorn chickens through three weeks of age.

Dose	n[a]	SE[b]	Hatch[c]	1 Week	2 Weeks	3 Weeks
Vehicle	28	2.37	45.4	69.7	123.3	184.8
0.1 μg/kg	35	2.12	47.2	69.8	122.6	183.7
0.3 μg/kg	26	2.46	47.1	67.2	117.8	180.1
0.9 μg/kg	9	4.19	45.7	61.1	98.7[d]	146.7[d]
2.7 μg/kg	2	8.88	45.7	58.3	94.2[d]	142.8[d]

[a] Sample size
[b] Standard error (Not appropriate for comparisons across time)
[c] Data expressed as mean (g)
[d] Significantly different from vehicle control at $p < 0.05$

Table 11. Effect of congener 77 on the body weights of White Leghorn chickens through three weeks of age.

Dose	n[a]	SE[b]	Hatch[c]	1 Week	2 Weeks	3 Weeks
Vehicle	36	2.53	46.2	68.3	121.4	189.8
1 μg/kg	44	2.29	45.6	67.9	122.4	191.8
3 μg/kg	35	2.57	45.1	65.0	114.4	177.5[d]
9 μg/kg	16	3.80	44.9	64.1	108.6[d]	168.3[d]

[a] Sample size
[b] Standard error (Not appropriate for comparisons across time)
[c] Data expressed as mean (g)
[d] Significantly different from vehicle control at $p < 0.05$

Table 12. Effect of congener 105 on the body weights of White Leghorn chickens through three weeks of age.

Dose	n[a]	SE[b]	Hatch[c]	1 Week	2 Weeks	3 Weeks
Vehicle	39	2.32	45.2	74.8	132.0	196.6
100 μg/kg	30	2.65	45.4	73.1	129.3	191.7
300 μg/kg	31	2.61	44.7	70.5	123.4[d]	186.0[d]
900 μg/kg	19	3.33	43.7	70.3	124.0	183.1[d]
2700 μg/kg	20	3.24	44.8	69.8	123.4	187.1[d]
8100 μg/kg	7	5.49	42.7	60.9[d]	103.5[d]	153.3[d]

[a] Sample size
[b] Standard error (Not appropriate for comparisons across time)
[c] Data expressed as mean (g)
[d] Significantly different from vehicle control at $p < 0.05$

Table 13. Effect of congener combinations on the body weights of White Leghorn chickens through three weeks of age.

Treatments	n[a]	SE[b]	Hatch[c]	1 Week	2 Weeks	3 Weeks
Vehicle	24	3.38	45.3	69.8	124.5	190.2
105/77	33	2.88	44.6	70.1	124.8	192.9
105/126	9	5.52	44.1	60.7	104.8[d]	161.3[d]
77/126	9	5.52	45.5	63.7	108.6[d]	166.4[d]
105/77/126	4	8.28	42.2	56.4	96.6[d]	149.3[d]

[a] Sample size
[b] Standard error (Not appropriate for comparisons across time)
[c] Data expressed as mean (g)
[d] Significantly different from vehicle control at $p < 0.05$

Table 14. Effect of congener 126 on the actual and relative brain weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	28	1.64 ± 0.019	0.88 ± 0.017
0.1	34	1.66 ± 0.018	0.90 ± 0.016
0.3	26	1.59 ± 0.020	0.89 ± 0.018
0.9	9	1.51 ± 0.034[d]	1.03 ± 0.031[d]
2.7	2	1.43 ± 0.072[d]	1.00 ± 0.065

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 15. Effect of congener 126 on the actual and relative bursa weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	28	1.07 ± 0.052	0.572 ± 0.0221
0.1	34	1.06 ± 0.048	0.567 ± 0.0201
0.3	26	1.05 ± 0.054	0.567 ± 0.0229
0.9	9	0.62 ± 0.096[d]	0.414 ± 0.0390[d]
2.7	2	0.56 ± 0.196	0.389 ± 0.0827

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 16. Effect of congener 126 on the actual and relative heart weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	28	1.12 ± 0.035	0.602 ± 0.0155
0.1	34	1.12 ± 0.032	0.604 ± 0.0141
0.3	26	1.14 ± 0.037	0.624 ± 0.0161
0.9	9	1.24 ± 0.062	0.851 ± 0.0274[d]
2.7	2	0.77 ± 0.132[d]	0.545 ± 0.0581

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p<0.05$

Table 17. Effect of congener 126 on the actual and relative liver weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	28	4.95 ± 0.158	2.65 ± 0.058
0.1	34	5.00 ± 0.143	2.69 ± 0.053
0.3	26	5.10 ± 0.164	2.81 ± 0.060
0.9	9	3.64 ± 0.279[d]	2.47 ± 0.103
2.7	2	4.25 ± 0.591	2.99 ± 0.217

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p<0.05$

Table 18. Effect of congener 126 on the actual and relative spleen weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	28	0.24 ± 0.019	0.131 ± 0.0091
0.1	34	0.27 ± 0.017	0.147 ± 0.0083
0.3	26	0.28 ± 0.020	0.151 ± 0.0095
0.9	9	0.26 ± 0.033	0.174 ± 0.0161
2.7	2	0.18 ± 0.071	0.122 ± 0.0341

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 19. Effect of congener 126 on the actual and relative testes weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	14	0.046 ± 0.0030	0.023 ± 0.0015
0.1	20	0.045 ± 0.0026	0.023 ± 0.0013
0.3	11	0.037 ± 0.0034	0.020 ± 0.0017
0.9	7	0.034 ± 0.0043	0.023 ± 0.0022
2.7	0	--	--

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 20. Effect of congener 77 on the actual and relative brain weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	36	1.62 ± 0.019	0.86 ± 0.015
1	44	1.62 ± 0.018	0.86 ± 0.014
3	35	1.58 ± 0.020	0.90 ± 0.016
9	16	1.56 ± 0.029	0.93 ± 0.023[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 21. Effect of congener 77 on the actual and relative bursa weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	36	0.98 ± 0.050	0.510 ± 0.0202
1	44	1.00 ± 0.046	0.512 ± 0.0182
3	35	0.90 ± 0.051	0.495 ± 0.0205
9	15	0.81 ± 0.078	0.466 ± 0.0313

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 22. Effect of congener 77 on the actual and relative heart weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	36	1.24 ± 0.032	0.657 ± 0.0149
1	44	1.26 ± 0.029	0.661 ± 0.0135
3	34	1.27 ± 0.033	0.727 ± 0.0153[d]
9	16	1.46 ± 0.048[d]	0.870 ± 0.0223[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 23. Effect of congener 77 on the actual and relative liver weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	36	5.76 ± 0.138	3.04 ± 0.059
1	44	5.73 ± 0.125	3.01 ± 0.054
3	35	5.03 ± 0.140[d]	2.76 ± 0.060[d]
9	16	4.56 ± 0.207[d]	2.74 ± 0.089

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 24. Effect of congener 77 on the actual and relative spleen weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	36	0.29 ± 0.014	0.154 ± 0.0068
1	44	0.27 ± 0.013	0.141 ± 0.0061
3	35	0.29 ± 0.014	0.158 ± 0.0069
9	16	0.35 ± 0.021	0.211 ± 0.0102[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 25. Effect of congener 77 on the actual and relative testes weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	16	0.048 ± 0.0034	0.024 ± 0.0018
1	21	0.043 ± 0.0030	0.021 ± 0.0015
3	18	0.048 ± 0.0032	0.025 ± 0.0017
9	6	0.044 ± 0.0055	0.024 ± 0.0029

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 26. Effect of congener 105 on the actual and relative brain weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	39	1.67 ± 0.017	0.86 ± 0.017
100	30	1.62 ± 0.020	0.87 ± 0.020
300	31	1.65 ± 0.019	0.90 ± 0.019
900	19	1.65 ± 0.025	0.91 ± 0.025
2700	20	1.67 ± 0.024	0.90 ± 0.024
8100	7	1.53 ± 0.041[d]	1.01 ± 0.041[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 27. Effect of congener 105 on the actual and relative bursa weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight(g)[c]	% Body Weight[c]
Vehicle	39	1.23 ± 0.044	0.573 ± 0.0192
100	30	1.08 ± 0.051	0.553 ± 0.0219
300	31	1.07 ± 0.050	0.577 ± 0.0215
900	19	1.02 ± 0.064	0.556 ± 0.0275
2700	20	1.01 ± 0.062	0.535 ± 0.0268
8100	7	0.69 ± 0.105[d]	0.450 ± 0.0453

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 28. Effect of congener 105 on the actual and relative heart weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	39	1.14 ± 0.031	0.579 ± 0.0119
100	30	1.13 ± 0.036	0.586 ± 0.0136
300	31	1.12 ± 0.035	0.603 ± 0.0134
900	19	1.11 ± 0.045	0.603 ± 0.0171
2700	20	1.15 ± 0.043	0.617 ± 0.0167
8100	7	1.09 ± 0.074	0.714 ± 0.0282[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 29. Effect of congener 105 on the actual and relative liver weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	39	5.28 ± 0.129	2.68 ± 0.059
100	30	5.18 ± 0.147	2.75 ± 0.067
300	31	5.01 ± 0.144	2.70 ± 0.066
900	19	5.00 ± 0.185	2.73 ± 0.084
2700	20	5.23 ± 0.180	2.81 ± 0.082
8100	7	3.96 ± 0.304[d]	2.60 ± 0.138

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 30. Effect of congener 105 on the actual and relative spleen weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	39	0.26 ± 0.013	0.133 ± 0.0071
100	30	0.24 ± 0.015	0.122 ± 0.0081
300	31	0.26 ± 0.015	0.141 ± 0.0079
900	19	0.25 ± 0.019	0.136 ± 0.0101
2700	20	0.29 ± 0.018	0.158 ± 0.0099
8100	7	0.26 ± 0.031	0.167 ± 0.0167

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 31. Effect of congener 105 on the actual and relative testes weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	18	0.044 ± 0.0031	0.021 ± 0.0014
100	15	0.041 ± 0.0034	0.020 ± 0.0016
300	15	0.042 ± 0.0034	0.021 ± 0.0016
900	7	0.040 ± 0.0050	0.020 ± 0.0023
2700	9	0.043 ± 0.0044	0.021 ± 0.0020
8100	4	0.038 ± 0.0067	0.023 ± 0.0030

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 32. Effect of congener combinations on the actual and relative brain weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	1.64 ± 0.022	0.87 ± 0.021
105/77	33	1.62 ± 0.019	0.85 ± 0.018
105/126	9	1.54 ± 0.036	0.96 ± 0.034
77/126	9	1.49 ± 0.036[d]	0.92 ± 0.034
105/77/126	4	1.53 ± 0.054	1.03 ± 0.051[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 33. Effect of congener combinations on the actual and relative bursa weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	0.92 ± 0.060	0.481 ± 0.0227
105/77	33	1.01 ± 0.051	0.515 ± 0.0193
105/126	9	0.63 ± 0.099	0.384 ± 0.0370
77/126	9	0.67 ± 0.099	0.396 ± 0.0370
105/77/126	4	0.48 ± 0.148[d]	0.321 ± 0.0555[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 34. Effect of congener combinations on the actual and relative heart weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	1.31 ± 0.046	0.690 ± 0.0218
105/77	33	1.33 ± 0.039	0.693 ± 0.0186
105/126	9	1.21 ± 0.075	0.769 ± 0.0357
77/126	9	1.41 ± 0.075	0.857 ± 0.0357[d]
105/77/126	4	1.36 ± 0.112	0.916 ± 0.0535[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 35. Effect of congener combinations on the actual and relative liver weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	5.68 ± 0.204	2.98 ± 0.064
105/77	33	5.62 ± 0.174	2.92 ± 0.055
105/126	9	4.33 ± 0.333[d]	2.66 ± 0.105[d]
77/126	9	4.75 ± 0.333	2.85 ± 0.105
105/77/126	4	4.32 ± 0.500	2.91 ± 0.157

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 36. Effect of congener combinations on the actual and relative spleen weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	0.30 ± 0.029	0.157 ± 0.0139
105/77	33	0.27 ± 0.025	0.139 ± 0.0119
105/126	9	0.25 ± 0.048	0.155 ± 0.0227
77/126	9	0.41 ± 0.048	0.242 ± 0.0227
105/77/126	4	0.30 ± 0.072	0.205 ± 0.0341

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 37. Effect of congener combinations on the actual and relative testes weight of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	13	0.042 ± 0.0042	0.022 ± 0.0020
105/77	17	0.043 ± 0.0036	0.021 ± 0.0017
105/126	3	0.037 ± 0.0087	0.022 ± 0.0041
77/126	5	0.046 ± 0.0067	0.026 ± 0.0032
105/77/126	3	0.049 ± 0.0087	0.032 ± 0.0041

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

DISCUSSION

Populations of fish-eating waterbirds such as double-crested cormorants (Weseloh et al., 1983) have been adversely affected by contaminants in the Great Lakes (Peakall, 1988). These effects have included increased embryo mortality and increased incidence of abnormalities (Peakall, 1988; Gilbertson et al., 1991; Giesy et al., 1994). Based on concentrations of specific PCB congeners in cormorant eggs collected from Green Bay, WI and toxic equivalency factors (TEFs) derived by Safe (1991, 1992), congeners 126, 77, and 105 were shown to account for 83%, 1.2%, and 13%, respectively, of the toxic equivalents (TEQs) in these cormorant eggs or over 97% of the toxicity attributed to dioxin-like compounds specifically quantified by instrumental analysis (Yamashita et al., 1993).

Mortality: All three congeners produced significantly lower hatchability of eggs than the vehicle despite significant mortality in the vehicle control group. The lower rate of hatching (60%) in the vehicle control group was believed to be a result of injection on day 0 as opposed to day 4. Injections are generally made on day 4 of incubation in studies of this type (Brunström and Darnerud, 1983; Brunström and Reutergardh, 1986; Brunström, 1991) for two reasons: the first reason is that fertility of the eggs can easily be determined at this time, thus only fertile eggs are injected; the second reason is that embryo lethality due to the injection is less when injection is done on day 4 as compared to earlier injection (Brunström, personal communication; Hashizume et al., 1992). However, lipophilic environmental contaminants like PCBs are deposited in the yolk by

the female and are thus present from the time the egg is laid. This early exposure to contaminants may affect the critical developmental stages of the embryo during the first nine days (organogenesis) and cause early mortality (Carlson and Duby, 1973). As PCB concentrations in the yolk increase, embryonic development ceases at earlier stages (Tumasonis et al.,1973). For this reason, injection on day 0 was thought to best mimic the exposure situation in the wild. This factor was important enough to outweigh possible negative consequences associated with early injection.

In the present study, all three PCB congeners had an adverse effect on the hatching rate of chicken embryos. Injection of congener 126 into the yolk on day 0 produced almost 100% mortality at 2.7 μg/kg egg. These results agree with those reported by Brunström and Andersson (1988) in which 2.0 μg/kg egg of congener 126 injected into the yolk of chicken eggs on day 4 of incubation resulted in 90% mortality by day 18 of incubation. Injection of 3 μg/kg egg of congener 77 into the yolk on day 0 resulted in 60% hatchability. This is comparable to the 64% hatchability of chicken eggs injected on day 4 with 4 μg/kg egg of congener 77 by Brunström and Darnerud (1983). Congener 105 resulted in only 85% mortality (day 21) at the greatest dose used in this study, 8100 μg/kg egg. In contrast, 2500 μg/kg egg of congener 105 injected on day 4 resulted in 85% mortality by day 18 of incubation (Brunström, 1990). One explanation for this discrepancy is in the preparation of the stock solution of congener 105. Unlike the other two congeners which dissolved in the L/PO directly, this congener had to be placed in methylene chloride first, and it is possible that not all the solvent evaporated off. Thus, the stock solution may have been less concentrated than desired.

The present investigation confirms studies establishing the relative toxicity of these three congeners with congener 126 being the most toxic followed by congeners 77 and 105 (Tanabe et al., 1987; Brunström, 1989, 1990). Since these congeners have been shown to have detrimental effects on the hatchability of chickens, it is possible that environmental concentrations of these congeners could be having negative effects on the hatchability of wild avian species. In particular, congener 126 has been found at greater concentrations (3.6 μg/kg egg) in the eggs of double-crested cormorants fro m Green Bay, WI (Yamashita et al., 1993) than the dose (2.7 μg/kg egg) which caused nearly 100% mortality in chicken embryos in this study. While no significant differences were observed in mortality post-hatch, one study in which chicks of hens fed 50 mg total PCBs weekly for 17 weeks did report increased mortality between hatching and six weeks of age (Dahlgren and Linder, 1971). It is conceivable that increased mortality in those birds with lower body weights would have been observed had this study continued for another three weeks.

Teratogenicity: All three congeners caused greater incidences of abnormalites than the vehicle control. Congener 126 produced significantly ($p < 0.10$) more abnormal embryos than the vehicle at 0.9 μg/kg egg. While miscellaneous abnormalities were the most frequently observed type of abnormality in this dose group, when all PCB dose groups were combined, the most frequently observed abnormality was beak deformities. Other predominant abnormalities included edema and eye deformities. These types of abnormalities were also observed by Brunström and Andersson (1988). Congener 77 produced significantly ($p < 0.10$) more abnormal embryos than the vehicle at 27.0 μg/kg egg. The predominant abnormalities in this group as well as when all congener 77 dose

groups were considered together were beak deformities. Eye deformities and edema were also commonly observed. Brunström and Darnerud (1983) observed these abnormalities in their injection studies with congener 77. Congener 105 produced significantly ($p<0.05$) more abnormal embryos than the vehicle at 8100 μg/kg egg. Abnormally small embryos, edema, and beak deformities were the most frequently observed abnormalities in all dose groups combined. None of the congener combinations produced any significant differences in the number of abnormal embryos when compared to the vehicle control. Combining all the congener combination groups, edema of the head and neck was the most common type of abnormality followed by miscellaneous abnormalities. The lack of a consistent pattern in most frequently observed types of abnormalities is not surprising. Inhibition of cell-cell communication (Loch-Caruso and Trosko, 1985; Kavanagh et al., 1987) is one potential mechanism for teratogenesis. The location of this interrupted intercellular communication will determine the resulting types of abnormalities.

This study has demonstrated the teratogenic capabilities of each of these congeners when injected singly. Many of the abnormalities observed, such as edema and bill deformities which included crossed and/or shortened bills, have been commonly observed in double-crested cormorants (Yamashita et al., 1993). All abnormalities were observed in either dead embryos or chicks upon hatching. No new abnormalities were observed during the three weeks post-hatch, suggesting that all abnormalities resulting from these congeners occur early in the development of the embryo and do not arise in response to continual uptake of the yolk following hatch.

Body weight: Wasting syndrome, a condition in which animals experience decreases in body weight which ultimately lead to death, was not observed in this study. However, significantly lower body weight gains were observed at the greater doses of all three congeners and the combinations containing congener 126. In a previous injection study with congener 77, a delay in the onset of weight gain was observed in chicks hatching from eggs injected with 4 μg/kg egg. The lower body weights were significantly different from the control weights at three to 10 days post-hatch (Brunström and Darnerud, 1983). Growth retardation has also been seen in chicks hatching from eggs injected with Aroclor® 1242 on day 0 (Carlson and Duby, 1973). It was suggested that this growth retardation may be due to a combination of effects on the liver and hematopoietic tissue. Dahlgren and Linder (1971) found that 50 mg Aroclor® 1254 fed to adult hens weekly for 17 weeks resulted in chicks with lower body weights than control chicks at six weeks of age. Harris and associates (1976) fed White Leghorn chickens 5, 10, and 20 ppm Aroclor® 1242 or 1248 for eight weeks. Body weights of progeny from these PCB-fed hens were not different at hatch, but three-week-old chicks of hens fed 20 ppm of either Aroclor® were significantly lighter than control progeny. These results support the conclusion reached in the present study; PCBs have an adverse effect on body weight gain in chicks exposed *in ovo*.

Brain: In the present study, relative brain weights increased in a dose-dependent manner when compared to the vehicle control group. However absolute brain weights were either not affected (congener 105) or were lower (congener 126, combination of 126 and 77) when compared to vehicle control absolute brain weights. The changes observed

in the brain weight relative to body weight probably do not represent a real effect. As with congener 105, where the absolute brain weights did not change, the apparent increase was due to lower body weights rather than greater brain weights. Brain weight was also unaffected in nestling pelicans fed 100 mg Aroclor® 1254 daily for 10 weeks followed by two weeks of imposed food stress prior to necropsy (Greichus et al., 1975).

Bursa of Fabricius: Bursa weights, while significantly lower only in chicks hatching from eggs injected with congener 126 and the combination of all three congeners, were numerically lower in birds exposed to congeners 77 and 105 as well as the remaining combinations containing congener 126. The injection of congener 77 into the air cell of chicken eggs after 13 days of incubation and killed on day 19 of incubation resulted in lower bursa weights (73% of control weights at 30 μg/kg egg and 58% of control weights at 300 μg/kg egg) (Nikolaidis et al., 1988). Histological assessment of the bursae indicated a dose-dependent decrease in the number bursal follicles as well as in the number of lymphoid cells (Nikolaidis et al., 1988). Bursae of 10-day-old chicken embryos cultured in media containing TCDD for 24 hours and subsequently transplanted to the chorioallantoic membrane of 10-day-old chicken embryos had lower numbers of lymphoid cells (Nikolaidis et al., 1990). Thus, dioxin and dioxin-like compounds can inhibit lymphoid development and perhaps compromise the immune system. While histological examination of the bursa in the present study indicated no significant alteration when compared to vehicle control, the trend toward lower relative bursa weights could indicate a potentially compromised immune system as a result of PCB exposure. However, Harris et al. (1976) reported that feeding of Aroclor® 1248 to hens for 8 weeks resulted in lower

bursa weights in one-day-old chicks but not 11-week-old chicks. This decrease in bursa weight was not associated with a decrease in antibody production.

Heart: There was a general trend toward greater relative heart weights with increasing doses of all congeners and with all combinations containing congener 126. No gross malformations of the heart were observed, however, cardiovascular teratogenicity was not specifically assessed as has been done in embryos exposed to TCDD. Chicken embryos exposed to TCDD via injection into the albumin on day 0 of incubation had ventricular septal defects, aortic arch anomalies, and conotruncal malformations (Cheung et al., 1981). Previous studies involving exposure of various avian species to Aroclor® 1254 via the feed reported a PCB-induced increase (finches), decrease (chickens), and no change (pelicans) in heart weight (Prestt et al., 1970; Greichus et al., 1975; Cecil et al., 1978). Although there were no obvious cases of pericardial edema in the present study, the increase in relative heart weight observed in PCB-exposed chicks could be due to internal fluid accumulation. Chicks fed 0.04% chlorinated biphenyls for four weeks had hydropericardium, hydroperitoneum, and enlarged hearts (McCune et al., 1962). Flick et al. (1963) fed one-day-old cockerels a fatty by-product known to cause chick edema disease. Birds consuming this "toxic fat" had increased fluid accumulation in the pericardium and decreased hematocrits. The authors suggested that the chick edema factor may increase the permeability of the vascular bed of the heart. Another possible explanation could be an increase in the muscle mass of the heart (Walker, personal communication).

Liver: Changes in liver weight did not show a consistent pattern. Congener 77 caused a significant decrease in relative liver weight at 3 μg/kg egg. While not significant due to the small sample size, the 9 μg/kg egg group also had lower liver weights. The combination of congeners 105 and 126 also produced significantly lower relative liver weights. In another study in which PCBs were injected into chicken eggs (day 5), a decrease in actual liver weight was observed (Hatano and Hatano, 1994). In contrast, liver weights were greater in cockerels fed 50 or 500 ppm of either Aroclor® 1254 or 1268 for 30 days which was associated with induction of hepatic mixed function oxidases (Cecil et al., 1978). Liver weights were also greater when compared to the liver weights of controls in nestling pelicans fed 100 mg/day of Aroclor® 1254 (Greichus et al., 1975) as well as in mice (C57BL/Rij) administered weekly *ip* injections of 100 mg congener 77/kg body weight for four weeks (Brouwer and Van den berg, 1984).

Each congener, 126, 77, or 105, has been shown to cause induction of hepatic 7-ethoxyresorufin O-deethylase (EROD) in chick embryos. These embryos were exposed via injection into the air cell on day 7 and their livers were removed 72 hour later for EROD analysis (Brunström and Andersson, 1988; Brunström, 1990). Brunström (1986) reported that five-day-old chicken embryos had measurable activity of hepatic 7-ethoxycoumarin O-deethylase (ECOD) and aryl hydrocarbon hydroxylase (AHH). This enzyme activity increased from day 5 to day 10 of incubation, and then plateaued until day 19. Activity peaked at one day following hatch. Livers of five-day-old embryos taken 48 hour after injection of 5 μg congener 77/kg egg into the air cell had induced AHH (14 times control) and ECOD (two times control) activity. It is questionable whether any

changes in enzyme activity would have been observed in the chicks in the present study since these chicks were initially exposed on day 0 before any development of the liver or enzyme systems. Additionally, these chicks were not subject to necropsy until 21 days post-hatch (42 days from initial exposure). In the above mentioned studies, livers were removed two to three days following dosage of the embryos with PCB congeners.

Spleen: Spleen weights also lacked a definitive trend but results seemed to suggest an increase over the vehicle control with increasing dose. This was only significant with congener 77 at 9 μg/kg egg. In contrast, spleen weights were lower in chicks of hens fed various Aroclors® for eight weeks (Harris et al., 1976). Aroclor® 1254 at 500 ppm caused decreased spleen weights in chickens after 30 days of dietary exposure (Cecil et al., 1978). However, actual spleen weights were greater in day-18 chicken embryos injected with 60 nmol congener 77 on day 9 of incubation than in embryos injected with vehicle (dioxane) only (Rifkind and Muschick, 1983). Relative spleen weights were also significantly greater in pelicans exposed daily to 100 mg Aroclor® 1254 in the diet for two and half months (Greichus et al., 1975).

Testes: Testes weights were not significantly different in any of the congener groups or combinations used in the present study. Similarly, testes were unaffected in hatchlings of Aroclor®-fed hens (Harris et al., 1976). However, Cecil et al. (1978) reported that Aroclor® 1242 or 1254 at 500 ppm caused decreased testes weights in chickens after 30 days of dietary exposure. Similarly, cockerels fed 500 ppm showed lower testes weights at nine and 13 weeks of feeding (Platonow and Funnell, 1971). However, rats exposed to Aroclor® 1254 during lactation had testes with weights, absolute

and relative, significantly greater than controls. This increase in testes weight supported a hypothesis that PCB exposure neonatally and during lactation (dams treated on days 1-3, 5, 7, and 9 of lactation) results in a hypoandrogenic condition in adult male rats. These male offspring also experienced impaired mating and fertility (Sager, 1993).

SUMMARY AND CONCLUSIONS

In summary, all three PCB congeners caused adverse effects in chicken embryos exposed prior to incubation. Congener 126 caused significantly greater mortality, increased incidence of abnormalities, lower post-hatch body weights, lower relative bursa weights, and greater relative heart weights at the 0.9 μg/kg egg dose when compared to the vehicle. Congener 77 caused significantly greater mortality at the 9 μg/kg egg dose than the vehicle. Increased incidence in abnormalities occurred at the 27 μg/kg egg dose of congener 77. A dose of 3 μg/kg egg of congener 77 caused lower post-hatch body weights and greater relative heart and liver weights than the vehicle. Relative spleen weights were greater at the 9 μg/kg egg dose when compared to the vehicle. Congener 105 caused significantly greater mortality at the 8100 μg/kg egg dose than the vehicle. There were also increased incidences of abnormalities and greater relative heart weights at this dose group. Post-hatch body weights were significantly lower beginning with the 300 μg/kg egg dose. The LD_{50}s were 0.6, 4.9, and 5,592 μg/kg egg for congeners 126, 77, and 105, respectively. Post-hatch body weight was the most sensitive of the parameters studied. LOAELs and NOAELs were determined for each of the congeners

using doses that had an adverse effect on body weight . The LOAELs were 0.5, 5.2, and 173 μg/kg egg for congeners 126, 77, and 105, respectively. The NOAELs were 0.3, 1, and 100 μg/kg egg for congeners 126, 77, and 105, respectively.

REFERENCES

Brouwer, A. and K.J. Van den Berg. 1984. Early and differential decrease in natural retinoid levels in C57BL/Rij and DBA/2 mice by 3,4,3',4'-tetrachlorobiphenyl. Toxicol. Pharmacol., 73:204-209.

Brunström, B. 1986. Activities in chick embryos of 7-ethoxycoumarin O-deethylase and aryl hydrocarbon (benzo[a]pyrene) hydroxylase and their induction by 3,3',4,4'-tetrachlorobiphenyl in early embryos. Xenobiotica, 16:865-872.

Brunström, B. 1988. Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. .PoultrySci., 67:52-57.

Brunström, B. 1989. Toxicity of coplanar polychlorinated biphenyls in avian embryos. Chemosphere, 19:765-768.

Brunström, B. 1990. Mono-*ortho*-chlorobiphenyls: toxicity and induction of 7-ethoxyresorufin O-deethylase (EROD) activity in chick embryos. Arch. Toxicol., 63:188-192.

Brunström, B. 1991. Toxicity and EROD-inducing potency of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in avian embryos. Comp. Biochem. Physiol., 100:241-243.

Brunström, B. and L. Andersson. 1988. Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated biphenyls (PCBs) chick embryos. Arch. Toxicol., 62:263-266.

Brunström, B. and P.O. Darnerud. 1983. Toxicity and distribution in chick embryos of 3,3',4,4'-tetrachlorobiphenyl injected into eggs. Toxicology, 27:103-110.

Brunström, B. and J. Lund. 1988. Differences between chick and turkey embryos in sensitivity to 3,3',4,4'-tetrachlorobiphenyl and in concentration /affinity of the hepatic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Comp. Biochem. Physiol., 91:507-512.

Brunström, B. and J. Orberg. 1982. A method for studying embryotoxicity of lipophilic substances experimentally introduced into hens' eggs. Ambio, 11:209-211.

Brunström, B. and L. Reutergardh. 1986. Differences in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3',4,4'-tetrachlorobiphenyl, injected into the egg. Environ. Pollut., 42:37-45.

Carlson, R.W. and R.T. Duby. 1973. Embryotoxic effects of three PCB's in the chicken. Bull. Environ. Contam. Toxicol., 9:261-266.

Cecil, H.C., S.J. Harris, and J. Bitman. 1978. Liver mixed function oxidases in chicken: induction by polychlorinated biphenyls and lack of induction by DDT. Arch. Environ. Contam. Toxicol., 7:283-290.

Cheung, M.O., E.F. Gilbert, and R.E. Peterson. 1981. Cardiovascular teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the chick embryo. Toxicol. Appl. Pharmacol., 61:197-204.

Dahlgren, R.B., and R.L. Linder. 1971. Effects of polychlorinated biphenyls on pheasant reproduction, behavior, and survival. J. Wildl. Manage., 35:315-318.

Flick, D.F., C.D. Douglass, and L. Gallo. 1963. Studies of chick edema disease. Poultry Sci., 42:855-862.

Giesy, J.P., J.P. Ludwig, and D.E. Tillitt. 1994. Deformities in birds of the Great Lakes region. Environ. Sci. Technol., 28:128-135.

Gilbertson, M., T. Kubiak, J. Ludwig, and G.A. Fox. 1991. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J. Toxicol. Environ. Health, 33:455-520.

Gilman, A.P., G.A. Fox, D.B. Peakall, S.M. Teeple, T.R. Carroll, and G.T. Haymes. 1977. Reproductive parameters and egg contaminant levels of Great Lake herring gulls. J. Wildl. Manage., 41:458-468.

Gill, J.L. 1986. Repeated measurement: sensitive tests for experiments with few animals. J. Animal Science, 63:943-954.

Greichus, Y.A., D.J. Call, B.M. Ammann, A. Greichus, and H. Shave. 1975. Physiological effects of polychlorinated biphenyls or a combination of DDT, DDD, and DDE in penned white pelicans. Arch. Environ. Contam., 3:330-343.

Harris, S.J., H.C. Cecil, J. Bitman, and R.J. Lille. 1976. Antibody response and reduction in bursa of fabricius and spleen weights of progeny of chickens fed PCBs. Poultry Sci., 55:1933-1940.

Hashizume, R., A. Noda, M. Itoh, Y. Yamamoto, S. Masui, M. Oka, and T. Nakamura. 1992. Studies on teratological testing using chicken embryos-effects of solvents, injection sites, and the age of the embryo. Exp. Anim., 41:349-356.

Hatano, Y. and A. Hatano. 1994. Influence of polychlorinated biphenyls on the growth of chicken embryos. J. Toxicol. Environ. Health, 42:357-364.

Jones, P.D., J.P. Giesy, J.L. Newsted, D.A. Verbrugge, J.P. Ludwig, M.E. Ludwig, H.J. Auman, R. Crawford, D.E. Tillitt, T.J. Kubiak, and D.A. Best. 1994. Accumulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents by double-crested cormorant (*Phalacrocorax auritus*, Pelicaniformes) chicks in the North American Great Lakes. Ecotoxicol. Environ. Safety, 27:192-209.

Kavanagh, T.J., C.C. Chang, and J.E. Trosko. 1987. Effect of various polybrominated biphenyls on cell-cell communication in cultured human teratocarcinoma cells. Fund. Appl. Toxicol., 8:127-131.

Kubiak, T.J., H.J. Harris, L.M. Smith, T.R. Schwartz, D.L. Stalling, J.A. Trick, L. Sileo, D.E. Docherty, and T.C. Erdman. 1989. Microcontaminants and reproductive impairment of the Forster's Tern on Green Bay, Lake Michigan-1983. Arch. Environ. Contam. Toxicol., 18:706-727.

Lock-Caruso, R. and J.E. Trosko. 1985. Inhibited intercellular communication as a mechanistic link between teratogenesis and carcinogenesis. Crit. Rev. Toxicol., 16:157-174.

McCune, E.L., J.E. Savage, and B.L. O'Dell. 1962. Hydropericardium and ascites in chicks fed a chlorinated hydrocarbon. Poultry Sci., 41:295-299.

Mineau, P. and M. Pedrosa. 1986. A portable device for non-destructive determination of avian embryonic viability. J. Field Ornithol., 57:53-56.

Nikolaidis, E., B. Brunström, and L. Dencker. 1988. Effects of the TCDD Congeners 3,3',4,4'-tetrachloroazoxybenzene on lymphoid development in the bursa of fabricius of the chick embryo. Toxicol. Appl. Pharmacol., 92:315-323.

Nikolaidis, E., B. Brunström, L. Dencker, and T. Veromaa. 1990. TCDD inhibits the support of B-cell development by the bursa of fabricius. Pharmacology and Toxicology, 67:22-26.

Peakall, D.B. 1988. Known effects of pollutants on fish-eating birds in the Great Lakes of North America. in: Toxic Contamination in Large Lakes. Vol. I. Chronic Effects of Toxic Contaminants in Large Lakes. ed. N.W. Schmidtke. Proceedings of the World Conference on Large Lakes. May 18-21, 1986. Mackinac Island, MI, pp. 39-54.

Platonow, J.S. and H.S. Funnell. 1971. Anti-androgenic-like effect of polychlorinated biphenyls in cockerels. Vet. Rec., 88:109-110.

Prestt, I., D.J. Jefferies, and N.W. Moore. 1970. Polychlorinated biphenyls in wild birds in Britain and their avian toxicity. Environ. Pollut., 1:3-26.

Rifkind, A.B. and H. Muschick. 1983. Benoxaprofen suppression of polychlorinated biphenyl toxicity without alteration of mixed function oxidase function. Nature, 303:524-526.

Safe, S. 1991. Polychlorinated dibenzo-*p*-dioxins and related compounds: sources, environmental distribution and risk assessment. Environ. Carcinog. & Ecotox. Rev., C9(2):261-302.

Safe, S. 1992. Development, validation, and limitations of toxic equivalency factors. Chemosphere, 25:61-64.

Sager, D.B. 1983. Effect of postnatal exposure to polychlorinated biphenyls on adult male reproduction function. Environ. Res., 31:76-94.

Tanabe, S., N. Kannan, An. Subramanian, S. Watanabe, and R. Tatsukawa. 1987. Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. Environ. Pollut., 47:147-163.

Tillitt, D.E., G.T. Ankley, D.A. Verbrugge, J.P. Giesy, J.P. Ludwig, and T.J. Kubiak. 1991. H4IIE rat hepatoma cell bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in colonial fish-eating waterbird eggs from the Great Lakes. Arch. Environ. Contam. Toxicol., 21:91-101.

Tumasonis, C.F., B. Bush, and F.D. Baker. 1973. PCB levels in egg yolks associated with embryonic mortality and deformity of hatched chicks. Arch. Environ. Contam. Toxicol., 1:312-324.

Weseloh, D.V., S.M. Teeple, and M. Gilbertson. 1983. Double-crested cormorants of the Great Lakes: egg-laying parameters, reproductive failure, and contaminant residues in eggs, Lake Huron 1972-1973. Can. J. Zool., 61:427-436.

Yamashita, N., S. Tanabe, J.P. Ludwig, H. Kurita, M.E. Ludwig, and R. Tatsukawa. 1993. Embryonic abnormalities and organochlorine contamination in Double-Crested Cormorants (*Phalacrocorax auritus*) and Caspian Terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ. Pollut., 79:163-173.

CHAPTER 2

INJECTION OF 3,3',4,4',5-PENTACHLOROBIPHENYL INTO THE YOLKS OF DOUBLE-CRESTED CORMORANT (*Phalacrocorax auritus*) AND CHICKEN (*Gallus domesticus*) EGGS

ABSTRACT

Unincubated double-crested cormorant (*Phalacrocorax auritus*) eggs were collected from a relatively uncontaminated colony at Lake Winnipegosis, Manitoba, Canada in May, 1994. Eight doses of 3,3',4,4',5-pentachlorobiphenyl (IUPAC #126) were injected into the yolks of these eggs prior to their artificial incubation. Congener 126 was also injected into the yolks of White Leghorn chicken (*Gallus domesticus*) eggs on day 0 of incubation. Upon hatching, chicks of both species were subjected to necropsy. The brain, bursa, heart, liver, and spleen were removed and weighed. Assessment of the rate of hatching indicated LD_{50}s of 246 and 2.4 μg/kg egg for cormorants and chickens, respectively. No significant differences in the incidence of abnormalities were observed in the cormorant, while in the chicken significantly more abnormalities were observed at 3.2, 6.4, and 12.8 μg/kg egg than the vehicle control. There was no effect on body weight in hatchling cormorants. Relative heart, liver, and spleen weights were increased in the cormorant at 5 μg/kg egg. In the chicken, a dose of 3.2 μg/kg egg caused lower hatchling weights and greater relative brain, heart, and liver weights when compared to the vehicle control group. Relative spleen weight was unchanged, and while not significant, there was a trend toward lower relative bursa weights with increasing dose in both the cormorant and chicken.

INTRODUCTION

Polychlorinated biphenyls (PCBs) have become widespread contaminants in the Great Lakes. They are known to cause a variety of adverse effects in birds: increased embryo mortality, hydropericardium, bill deformities, subcutaneous edema, liver lesions, and induction of cytochrome P-450 enzymes (Brunström, 1989). Coplanar PCBs are structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and thus, can act through the same mechanism of toxicity as TCDD; binding to the Ah receptor. The most toxic of these PCB congeners is 3,3',4,4',5-pentachlorobiphenyl (IUPAC# 126) (Tanabe et al., 1987; Brunström, 1989). This congener represents approximately 83% of the toxic equivalents in cormorant eggs collected from Green Bay, Wisconsin, one of the most contaminated sites in the Great Lakes basin (Safe, 1991; Yamashita et al., 1993).

Double-crested cormorants (*Phalacrocorax auritus*) experienced reproductive problems from the late 1950s through the 1970s (Weseloh et al., 1983; Ludwig, 1984). While cormorant populations have been increasing in recent years (Weseloh and Ewins, 1994), they are still experiencing reproductive impairment including embryo lethality and anatomic deformities (Fox et al., 1991).

Research, thus far, has focused on correlations between contaminant concentrations at colony sites and biological end points such as mortality, incidence of abnormalities, and induction of liver enzymes (Yamashita et al., 1993; Sanderson et al., 1994). To my knowledge, there have been no controlled laboratory studies which assessed the effects of a particular PCB congener injected into relatively uncontaminated cormorant eggs prior

to incubation. Additionally, it would also be valuable to determine the difference in sensitivity to specific PCB congeners between the cormorant and the chicken. The chicken has been extensively studied and is easy to use in controlled laboratory experiments.

MATERIALS AND METHODS

Cormorant: Double-crested cormorant (*Phalacrocorax auritus*) eggs, provided by the FWS, were collected from a relatively uncontaminated colony at Lake Winnipegosis in Manitoba, Canada. Eggs were selected from nests containing only two eggs to ensure fresh (unincubated) eggs. Upon arrival at MSU, less than 24 hours after collection, the eggs were weighed and candled for air cell location. The eggs were returned to egg cartons and placed in coolers such that the eggs were laying horizontally. They were kept at room temperature for approximately 12 hours prior to injection, during which time they were rotated 90° about their long axis twice.

The eggs were divided into 11 groups, 60 eggs per group with the exception of the greatest dose group which contained only 15 eggs due to the amount of PCB congener required. There were 8 doses of congener 126 (5, 10, 25, 50, 100, 200, 400, and 800 μg/kg egg). There were also three control groups with 60 eggs each: non-injected, mock-injected (insertion of the syringe needle only), and vehicle control.

Congener 126 (AccuStandard, New Haven, CT) was diluted in triolein (Sigma, St. Louis, MO). Due to the instability of this oil in the presence of oxygen, all injection vials were flooded with argon following preparation of the doses. Congener 126 was dissolved

directly in the triolein to make stock solutions (5 μg/μl for the first seven doses and 10 μg/μl for the greatest dose). Each dose was cold filtered with a 0.22 micron syringe filter to sterilize the solution prior to injection.

All injections were made directly into the yolk prior to incubation. The injection volume was 0.1 μl/g egg. The surface of the egg was cleaned with 75% ethanol and a small hole was made into the air cell with a sterile pin. A sterile 25μl syringe (Hamilton Company, Reno, NV) (22S gauge) was inserted horizontally approximately 30 mm into the egg which was positioned on its side. Following injection of the solution and removal of the needle, the hole was sealed with melted paraffin.

Cormorant eggs were incubated on their sides in a Petersime (Gettysburg, OH) poultry incubator for up to 29 days. The temperature (99.0°F) and relative humidity (61-63%) were maintained using 99°F dry bulbs and a 87.5°F wet bulb. Eggs were automatically rotated every two hours. In addition, they were rotated 180° about their long axis by hand once a day. Approximately 80 additional eggs were incubated and weighed every two to three days to evaluate mass loss during incubation. These eggs were not rotated once a day by hand. All eggs were candled on days 7, 14, and 21 of incubation. Eggs were transferred to hatching baskets on day 25 or when they began to pip, whichever came first. All unhatched eggs were opened and assessed for abnormalities and approximate stage of development.

Upon hatching at approximately 26 days of incubation, cormorants were weighed and body length (crown of head to tip of tail) was measured. They were killed by decapitation, and the brain, bursa, heart, liver, and spleen were removed and weighed.

Five livers from each group, where possible, were wrapped in foil and put in liquid nitrogen for later analysis of 7-ethoxyresorufin O-deethylase (EROD) induction.

Chicken: White Leghorn chicken (*Gallus domesticus*) eggs were provided by the MSU Poultry Science Teaching and Research Center. They were weighed and candled for the location of the air cell the day before injection. In addition, the eggs were laid on their sides to allow the germ spot to raise to the top and minimize potential injury during injection. Eggs were kept in a cooler at 58°F prior to injection.

There were eight doses of congener 126 (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 μg/kg egg) as well as non-injected and vehicle controls. Previous injections of chicken eggs eliminated the need for a mock-injected control group. The doses were prepared from a stock solution (0.2 μg/μl) which was made with the same lot of congener 126 used for the cormorant. The same vehicle and injection techniques were used for both the chicken and the cormorant. Since availability of chicken eggs was not a limiting factor, the chicken egg injections were done in a series of three replicates with 20 eggs/group in each replicate to give the same total number of eggs used for the cormorant.

Chicken eggs were also incubated in a Petersime poultry incubator. They were positioned with their blunt end up and incubated according to standard conditions for this species, 99.5-99.75°F during the first 19 days, 97-99°F for days 21 and 22, and relative humidity approximately 60% (North, 1978). Eggs were candled on days 4 and 11. Viability was assessed at the time of transfer (day 18) with an EVD provided by the FWS. Any embryos dead on days 11 or 18 were opened and assessed for abnormalities and age at death was estimated.

Hatchling chicks were weighed and killed by decapitation. The brain, bursa, heart, liver, and spleen were removed and weighed. Five livers from each group were frozen for EROD comparisons with the cormorant.

Statistics: Mortality and abnormalities were evaluated using a 2x2 contingency table and Bonferroni Chi-square. The LD_{50}s were calculated by linear regressions on log-probit transformed data. Analysis of all other parameters was done using one-way ANOVA followed by Dunnett's test. All comparisons were made relative to the vehicle control group due to the low rate of hatching experienced by the cormorant vehicle control group. Statements of significance were based on a level of 0.05, unless otherwise stated.

RESULTS

Congener 126 caused embryo mortality in both the cormorant and chicken and increased incidence of abnormalities in the chicken. The LD_{50}s of congener 126 were 246 and 2.4 μg 126/kg egg (Tables 38-40) for the cormorant and chicken, respectively. There were no significant differences in the incidence of abnormalities between cormorant embryos in PCB treatment groups and those in the vehicle control group. There were unhatched cormorant chicks with edema in every group except the greatest dose group where the majority of embryos died before day 21. One embryo in the 10 μg/kg egg dose group had no eyes or bill. One embryo in the 400 μg/kg egg dose group had a hooked upper bill, and at 800 μg/kg egg, one embryo had no lower jaw. In the chicken, no abnormal embryos were observed in the vehicle control group while 13/60, 19/59, and

10/60 embryos had one or more types of abnormalities in the 3.2, 6.4, and 12.8 μg/kg egg dose groups, respectively. These incidences of abnormalities were significantly greater than in the vehicle control group ($p < 0.01$). The types of abnormalities observed included skull, eye, beak, and toe deformities as well as edema.

Congener 126 also caused sublethal effects such as changes in body weights and various organ weights. There were no differences in body weights (Tables 41-42) of cormorant hatchlings from the vehicle control group while the body weights of chicks in the greatest dose group which hatched (3.2 μg/kg egg) were significantly lower than those in the vehicle control group. Relative organ weights, organs expressed as a percent of body weight, were used for statements of significance. Relative heart, liver, and spleen weights in cormorant were all significantly greater at the lowest dose (5 μg/kg egg) relative to the vehicle control group. In the chicken, relative brain, heart, and liver weights were all significantly greater at 3.2 μg/kg egg than the values of the same parameters in the vehicle control group. There were no significant differences in spleen weights relative to the vehicle control group.

Table 38. Effect of injection of congener 126 into the yolks of double-crested cormorant eggs on day 0 of incubation on hatchability.

Dose μg/kg egg	Dose nmol/kg egg	# dead/ # eggs	% Mortality
Non-injected	0	18/60	30.0
0[a]	0	32/60	53.3
5	15	17/60	28.8
10	31	17/59	28.8
25	77	23/60	38.3
50	153	20/60	33.3
100	306	24/59	40.7
200	613	36/60	60.0
400	1225	52/60[b]	86.7
800	2451	15/15[b]	100

[a] Vehicle (triolein)
[b] Significantly different from the vehicle control at $p < 0.05$

Table 39. Effect of injection of congener 126 into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability.

Dose μg/kg egg	Dose nmol/kg egg	# dead/ # eggs	% Mortality
Non-injected	0	3/53	5.7
0[a]	0	5/59	8.5
0.1	0.3	11/60	18.3
0.2	0.6	18/59[b]	30.5
0.4	1.2	15/59	25.4
0.8	2.5	12/59	20.3
1.6	4.9	13/60	21.7
3.2	9.8	55/60[b]	91.7
6.4	19.6	59/59[b]	100
12.8	39.2	60/60[b]	100

[a] Vehicle (triolein)
[b] Significantly different from the vehicle control at $p < 0.05$

Table 40. LD_{50}s for congener 126 injected into the yolks of White Leghorn chicken or double-crested cormorant eggs on day 0 of incubation.

	Chicken	Cormorant
LD_{50} μg(nmol)/kg egg 95% Confidence limits	2.4 (7.4) 1.7 (5.2) - 2.7 (8.3)	246 (754) 179 (548) - 302 (925)
Intercept ± Standard Error 95% Confidence limits	1.3 ± 1.30 -1.29 - 3.82	- 4.7 ± 2.36 - 9.31 - - 0.04
Slope ± Standard Error 95% Confidence limits	9.9 ± 2.71 4.59 - 15.22	4.1 ± 0.96 2.17 - 5.92

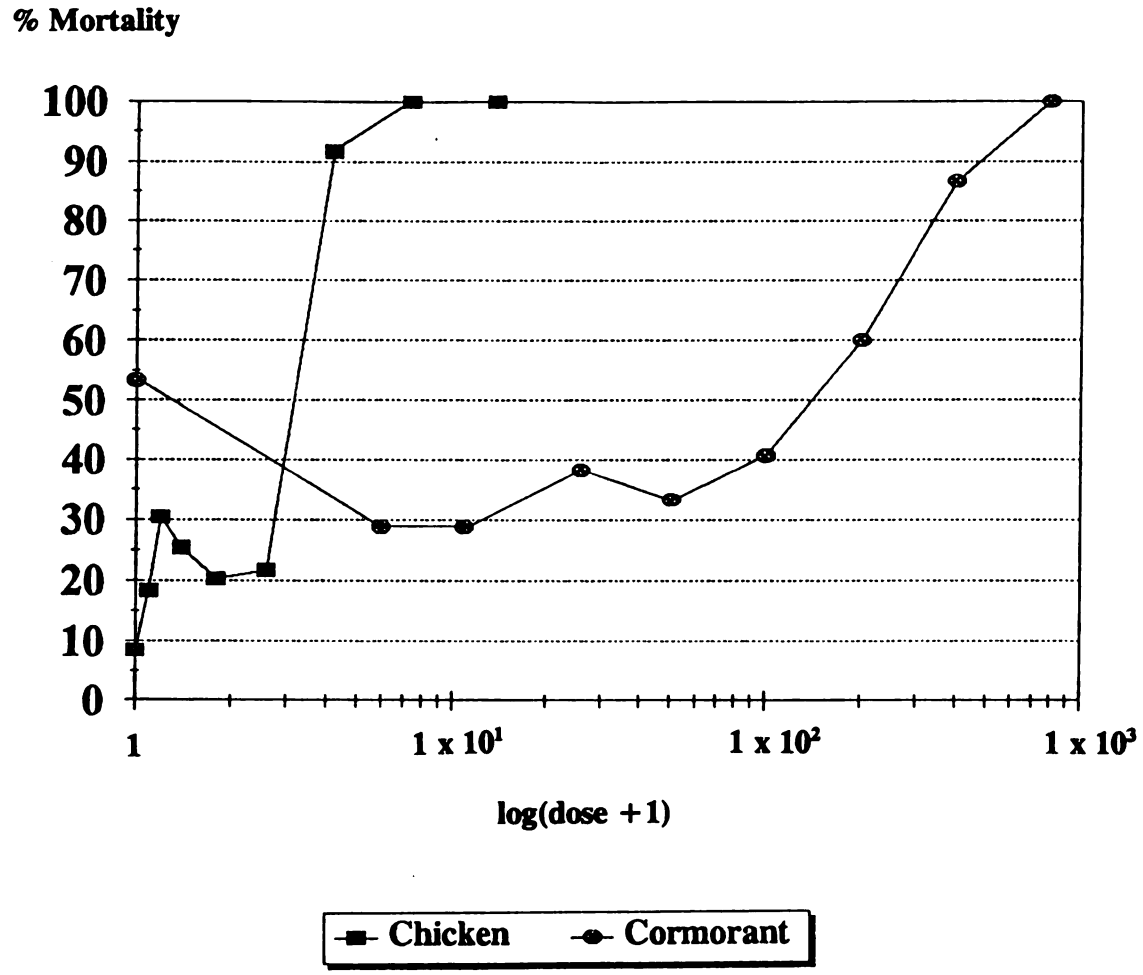

Figure 4. Effect of congener 126 on the mortality of White Leghorn chicken and double-crested cormorant embryos when exposed prior to incubation. One was added to each dose to allow inclusion of the vehicle control on a log scale. For example, vehicle control (0 μg/kg egg) = 1 and the highest dose of congener 126 in the cormorant (800 μg/kg egg) = 801.

Table 41. Effect of congener 126 on the hatch body length and body weight of double-crested cormorants.

Dose[a]	n[b]	Length (cm)[c]	n[b]	Body Weight (g)[c]
Non-injected	49	11.22 ± 0.063	53	34.99 ± 0.479
Vehicle	28	11.01 ± 0.084	29	34.63 ± 0.647
5	37	11.33 ± 0.073	38	34.14 ± 0.565
10	42	11.15 ± 0.069	44	33.09 ± 0.525
25	32	11.21 ± 0.078	34	34.70 ± 0.598
50	39	11.16 ± 0.071	39	34.50 ± 0.558
100	31	11.15 ± 0.080	31	34.94 ± 0.626
200	24	11.02 ± 0.091	24	33.84 ± 0.711
400	8	10.98 ± 0.157	8	36.40 ± 1.232

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 42. Effect of congener 126 on the hatch body weights of White Leghorn chickens.

Dose[a]	n[b]	Body Weight[c] (g)
Non-injected	50	43.0 ± 0.49
Vehicle	54	41.4 ± 0.47
0.1	49	42.4 ± 0.50
0.2	41	42.4 ± 0.54
0.4	42	41.9 ± 0.54
0.8	45	41.6 ± 0.52
1.6	47	41.8 ± 0.51
3.2	8	36.1 ± 1.23[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 43. Effect of congener 126 on the actual and relative brain weight of hatchling double-crested cormorants.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	48	0.788 ± 0.0105	2.27 ± 0.048
Vehicle	29	0.780 ± 0.0135	2.30 ± 0.061
5	36	0.785 ± 0.0121	2.34 ± 0.055
10	42	0.783 ± 0.0112	2.41 ± 0.051
25	32	0.769 ± 0.0128	2.23 ± 0.058
50	37	0.748 ± 0.0119	2.19 ± 0.054
100	31	0.775 ± 0.0130	2.23 ± 0.059
200	24	0.769 ± 0.0148	2.29 ± 0.067
400	8	0.762 ± 0.0257	2.11 ± 0.116

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 44. Effect of congener 126 on the actual and relative bursa weight of hatchling double-crested cormorants.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	48	0.030 ± 0.0011	0.087 ± 0.0032
Vehicle	28	0.030 ± 0.0014	0.088 ± 0.0042
5	36	0.031 ± 0.0013	0.092 ± 0.0037
10	41	0.026 ± 0.0012	0.080 ± 0.0035
25	32	0.027 ± 0.0013	0.078 ± 0.0040
50	36	0.029 ± 0.0013	0.084 ± 0.0037
100	30	0.028 ± 0.0014	0.080 ± 0.0041
200	24	0.025 ± 0.0016	0.074 ± 0.0046
400	8	0.028 ± 0.0027	0.075 ± 0.0079

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error

Table 45. Effect of congener 126 on the actual and relative heart weight of hatchling double-crested cormorants.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	48	0.244 ± 0.0050	0.702 ± 0.0150
Vehicle	29	0.223 ± 0.0065	0.653 ± 0.0192
5	36	0.251 ± 0.0058[d]	0.744 ± 0.0173[d]
10	42	0.239 ± 0.0054	0.725 ± 0.0160
25	32	0.242 ± 0.0062	0.700 ± 0.0183
50	37	0.235 ± 0.0057	0.686 ± 0.0170
100	31	0.226 ± 0.0063	0.646 ± 0.0186
200	24	0.228 ± 0.0071	0.679 ± 0.0211
400	8	0.211 ± 0.0123	0.581 ± 0.0366

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 46. Effect of congener 126 on the actual and relative liver weight of hatchling double-crested cormorants.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	48	0.709 ± 0.0147	2.04 ± 0.047
Vehicle	29	0.659 ± 0.0189	1.92 ± 0.060
5	36	0.763 ± 0.170[d]	2.26 ± 0.054[d]
10	42	0.676 ± 0.157	2.06 ± 0.050
25	32	0.691 ± 0.0180	2.00 ± 0.057
50	37	0.703 ± 0.0168	2.06 ± 0.053
100	31	0.666 ± 0.0183	1.92 ± 0.058
200	24	0.672 ± 0.0208	2.00 ± 0.066
400	8	0.684 ± 0.0361	1.88 ± 0.114

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 47. Effect of congener 126 on the actual and relative spleen weight of hatchling double-crested cormorants.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	48	0.070 ± 0.0040	0.202 ± 0.0119
Vehicle	29	0.066 ± 0.0051	0.192 ± 0.0153
5	36	0.092 ± 0.0046[d]	0.272 ± 0.0138[d]
10	41	0.081 ± 0.0043	0.248 ± 0.0129
25	32	0.070 ± 0.0049	0.203 ± 0.0146
50	37	0.081 ± 0.0045	0.237 ± 0.0136
100	30	0.072 ± 0.0050	0.207 ± 0.0151
200	24	0.056 ± 0.0056	0.163 ± 0.0168
400	8	0.068 ± 0.0097	0.186 ± 0.0292

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 48. Effect of congener 126 on the actual and relative brain weight of hatchling White Leghorn chickens

Dose[a]	n[b]	Weight (g)[c]	n[b]	% Body Weight[c]
Non-injected	50	0.871 ± 0.0090	50	2.04 ± 0.030
Vehicle	54	0.846 ± 0.0087	54	2.05 ± 0.029
0.1	49	0.851 ± 0.0091	49	2.02 ± 0.030
0.2	41	0.853 ± 0.0099	41	2.02 ± 0.033
0.4	43	0.853 ± 0.0097	42	2.04 ± 0.033
0.8	45	0.843 ± 0.0095	45	2.04 ± 0.032
1.6	47	0.853 ± 0.0093	47	2.05 ± 0.031
3.2	10	0.847 ± 0.0201	8	2.37 ± 0.075[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 49. Effect of congener 126 on the actual and relative bursa weight of hatchling White Leghorn chickens.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	50	0.061 ± 0.0025	0.14 ± 0.006
Vehicle	53	0.059 ± 0.0024	0.14 ± 0.006
0.1	49	0.055 ± 0.0025	0.13 ± 0.006
0.2	40	0.060 ± 0.0028	0.14 ± 0.007
0.4	42	0.059 ± 0.0027	0.14 ± 0.006
0.8	45	0.053 ± 0.0026	0.13 ± 0.006
1.6	47	0.055 ± 0.0026	0.13 ± 0.006
3.2	9	0.036 ± 0.0058[d]	0.11 ± 0.016

[a] Doses expressed as μg/kg egg
[b] Sample size (For % Body Weight n=41 for 0.4 μg/kg egg and n=7 for 3.2 μg/kg egg)
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 50. Effect of congener 126 on the actual and relative heart weight of hatchling White Leghorn chickens.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	50	0.26 ± 0.004	0.61 ± 0.011
Vehicle	54	0.25 ± 0.004	0.60 ± 0.011
0.1	49	0.23 ± 0.005	0.55 ± 0.011
0.2	41	0.24 ± 0.005	0.56 ± 0.012
0.4	43	0.24 ± 0.005	0.58 ± 0.012
0.8	45	0.25 ± 0.005	0.60 ± 0.012
1.6	47	0.25 ± 0.005	0.59 ± 0.012
3.2	10	0.25 ± 0.010	0.70 ± 0.028[d]

[a] Doses expressed as μg/kg egg
[b] Sample size (For % Body Weight n=42 for 0.4 μg/kg egg and n=8 for 3.2 μg/kg egg)
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 51. Effect of congener 126 on the actual and relative liver weight of hatchling White Leghorn chickens.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	50	0.87 ± 0.015	2.03 ± 0.039
Vehicle	54	0.86 ± 0.014	2.09 ± 0.037
0.1	49	0.88 ± 0.015	2.07 ± 0.039
0.2	41	0.88 ± 0.016	2.09 ± 0.043
0.4	43	0.88 ± 0.016	2.10 ± 0.042
0.8	45	0.88 ± 0.016	2.13 ± 0.041
1.6	47	0.89 ± 0.015	2.14 ± 0.040
3.2	10	0.90 ± 0.033	2.71 ± 0.096[d]

[a] Doses expressed as μg/kg egg
[b] Sample size (For % Body Weight n=42 for 0.4 μg/kg egg and n=8 for 3.2 μg/kg egg)
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p<0.05$

Table 52. Effect of congener 126 on the actual and relative spleen weight of hatchling White Leghorn chickens.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected	50	0.015 ± 0.0005	0.034 ± 0.0012
Vehicle	54	0.014 ± 0.0005	0.034 ± 0.0012
0.1	49	0.013 ± 0.0005	0.031 ± 0.0012
0.2	41	0.014 ± 0.0006	0.033 ± 0.0013
0.4	43	0.014 ± 0.0006	0.033 ± 0.0013
0.8	45	0.013 ± 0.0006	0.032 ± 0.0013
1.6	46	0.014 ± 0.0006	0.033 ± 0.0013
3.2	10	0.013 ± 0.0012	0.036 ± 0.0030

[a] Doses expressed as μg/kg egg
[b] Sample size (For % Body Weight n=42 for 0.4 μg/kg egg and n=8 for 3.2 μg/kg egg)
[c] Data expressed as mean ± standard error

DISCUSSION

Mortality: Seventy percent hatchability of cormorant eggs was considered successful since the eggs were artificially incubated from day 0. Previous attempts to incubate fresh cormorant eggs have met with limited success. No cormorants hatched with an incubation temperature of 37.5°C and relative humidity of 67% near the day of hatch (Larson, 1991). It has been noted that laboratory incubation of cormorant eggs is more successful if eggs are collected after approximately a week of natural incubation by the parents (Sotherland, unpublished data). Cormorant eggs naturally incubated for two weeks prior to their artificial incubation at 35.4°C with relative humidity of 46% had 77% hatchability (Sanderson et al., 1994).

Eggs collected in excess of those required for the PCB injection experiment were used to collect additional information on the effects of artificial incubation on the comorant. The average mass lost during incubation under the previously described conditions was about 8.5%. Cormorant eggs naturally incubated lose approximately 11% of their initial mass (Sotherland, unpublished data). Only 54% of the eggs which were not rotated by hand once a day hatched. Thus, this additional rotation improved the rate of hatching. None of the fifteen eggs incubated with their blunt end up throughout incubation hatched. Sixty percent of these embryos died after 23 days, which indicates that positioning or rotation is particularly important in the later stages of development.

Greater mortality in the vehicle control group may be due to random occurrence of infertile eggs. Hatchability of the mock-injected eggs (70%) was equal to that of the

non-injected group thus suggesting that the injection procedure itself was not harmful. The two smallest doses also had good hatchability (71%) which would indicate that the vehicle was not harmful. The vehicle had no toxic effects in the chicken since the rate of hatching in the vehicle control group was almost 92%. Despite great mortality in the cormorant vehicle control, significant increases in mortality were observed at 400 and 800 μg/kg egg. In another study there were no significant differences in mortality in cormorant eggs injected into the yolk on day 0 with 18.4 μg congener 126/kg egg which is comparable to results in the present study (Larson, 1991).

Mortality induced by exposure to congener 126 has been assessed in other chicken egg injection studies. An LD_{50} of 3.1 μg/kg egg for congener 126 in chickens was calculated when this congener was injected into the air cell on day 7 and the eggs were assessed for mortality 72 hours later (Brunström and Andersson, 1988). When 0.2 μg/kg egg was injected into the yolk on day 4, they observed only 10% mortality by day 18. Ninety percent mortality was observed when 2 μg/kg egg of congener 126 was injected the yolk on day 4 (Brunström, 1991), whereas the LD_{50} determined in the present study was 2.4 μg/kg egg. In Chapter 1, the LD_{50} for congener 126 was reported as 0.6 μg/kg egg. The only differences in methods described in Chapter 1 and the methods reported in this chapter are the vehicle and injection volume. In the initial trial, the vehicle used was that described by Brunström and colleagues (Brunström and Orberg, 1982; Brunström and Darnerud, 1983) at a volume of 1 μl/g egg as opposed to triolein at 0.1 μl/g egg which was used in the present trial. Thus, both vehicle and injection volume can significantly affect the LD_{50} of a given compound. Given the greater rate of hatching in the present

trial, 2.4μg/kg egg is a more appropriate LD_{50} for congener 126 in the chicken than the LD_{50} determined in chapeter 1.

Teratogenicity: The predominance of edema in cormorants from all groups is most likely due to humidity rather than PCB exposure. High humidity reduces evaporation from the eggs and chicks that do not evaporate water fast enough are larger than normal. This could explain the swollen appearance of most of the cormorants upon hatching. Sotherland (unpublished data) also observed that dead embryos with edema had significantly less fractional mass losses than dead embryos without edema or live hatchlings. Fox and associates (1991) observed 52.1 bill defects per 10,000 cormorant chicks from Green Bay, WI. Crossed or abnormal bills were also the most frequently reported abnormalities in other colonial waterbirds (Fox et al., 1991). The present study did not show a causal link between congener 126 and abnormalities such as bill deformities. However, given such a low rate of occurrence in the wild, it is difficult to show the same effects in the laboratory with the relatively small number of eggs injected. Injecting concentrations greater than those observed in the environment would seem to produce detectable increases in abnormalities, but, greater doses increased early embryo mortality thus reducing the number of embryos in which abnormalities may be observed.

Despite the lack of the abnormalities in the cormorant under laboratory conditions, significant increases in abnormalities were observed in the chicken at the three greatest doses. Edema, microphthalmia, and beak deformities were common types of abnormalities observed. The teratogenic capabilities of congener 126 have also been reported for the chicken by Brunström and Andersson (1988).

Body weight: Body weights and crown/rump lengths were unaffected in hatchling cormorants. Several parameters have been compared in cormorant hatchlings from two different colonies in the Netherlands, each with different levels of organochlorine contamination (Van den Berg et al., 1994). Hatchlings from the more contaminated site did not have body weights significantly different than chicks from the less contaminated site although there was a tendency for them to be slightly lower. These authors reported an inverse correlation between lower body weights and increased respiration and energy metabolism and suggested that these effects inhibit growth. The authors further suggested that wasting syndrome may be responsible for this increase in respiration.

Chickens hatching from the 3.2 μg/kg egg dose group had significantly lower body weights than those in the vehicle control group. This is in contrast to results in the first trial in which body weights of hatchling chicks injected with congener 126 on day 0 did not show any differences when compared to the vehicle control group (Chapter 1). However, the highest hatching dose in first trial was 2.7 μg/kg egg which was lower than the dose which caused a change in hatch body weights in the present trial.

Brain: Relative brain weights were affected in hatchling chicks but not in hatchling cormorants. Relative brain weights were greater in chicks hatchling from the 3.2 μg/kg egg dose group. However, this group of chicks also had lower hatchling body weights. Thus, the apparent effect observed in relative brain weight was influenced by the change in body weight. Actual brain weights were not different when compared to the vehicle control group. Brain weights were also unaffected in pelicans fed 100 mg of PCBs for 10 weeks (Greichus et al., 1975).

Bursa of Fabricius: Relative bursa weights were not significantly different in either hatchling cormorants or chickens. Actual bursa weights were significantly lower in hatchling chickens in the 3.2 μg/kg egg dose group. Bursa weights were also lower in hatchling chicks of hens fed PCBs for eight weeks (Harris et al., 1976). Both chicken and cormorant hatchlings did show a trend toward lower relative bursa weights with increasing dose indicating a potential effect on the immune system. Van den Berg and associates (1994) recorded bursa weights in hatchling cormorants from contaminated and uncontaminated colonies in the Netherlands, however they excluded them from the study since the small size of the bursa did not allow for reliable measurement.

Heart: Congener 126 caused an increase in relative heart weights in both cormorants (5 μg/kg egg) and chickens (3.2 μg/kg egg). The greater heart weights could be due to an internal accumulation of fluid in the heart. Other studies with chlorinated biphenyls have reported pericardial edema in exposed chicks (Mc Cune et al., 1962; Flick et al., 1963; Brunström, 1988; Brunström, 1990; Brunström et al., 1990). An alternate explanation is an increase in the heart muscle itself (Walker, personal communication), which seems to be the more plausible explanation since there were no obvious cases of pericardial edema in the present study.

Liver: Relative liver weights were greater in both the cormorant and chicken at a dose of 5 μg/kg egg and 3.2 μg/kg egg, respectively. A positive correlation between concentrations of PCDDs, PCDFs, or mono-ortho PCBs in the yolk sacs of one-day-old cormorants (*Phalacrocorax auritus*) and increased liver weights was observed in a field study in Europe (Van den Berg et al., 1994). Greater liver weights have also been

observed in cockerels fed 50 ppm Aroclor® 1242, 1254, and 1268 or 500 ppm Aroclor® 1268 for 30 days (Cecil et al., 1978) and in pelicans fed 100 mg Aroclor® 1254 for 10 weeks (Greichus et al., 1975). Increases in liver weight have been associated with induction of detoxifying enzymes (Cecil et al., 1978). Congener 126 is known to induce hepatic 7-ethoxyresorufin O-deethylase (EROD) in chicken embryos (Brunström and Andersson, 1988; Brunström, 1990).

Spleen: Cormorants hatching from the lowest dose group had greater relative spleen weights than the vehicle control group, while relative spleen weights were unaffected in chicken hatchlings. Spleen weights in White Leghorn chicks of hens fed Aroclor® mixtures for eight weeks were less than spleen weights in control chicks (Harris et al., 1976). Spleen weights were also lower in cockerels fed PCBs for 30 days (Cecil et al., 1978). However, like the cormorant, pelicans fed 100 mg PCBs for 10 weeks had significantly greater spleen weights than controls (Greichus et al., 1975).

SUMMARY AND CONCLUSIONS

Cormorants are less sensitive to the adverse effects of congener 126 than the domestic chicken. In terms of mortality, the cormorant embryo is approximately 100 times less sensitive to the effects of congener 126 than the domestic chicken embryo. Cormorants appear to be even less sensitive to the teratogenic effects of this congener. The LOAEL (NOAEL) for the cormorant based on mortality was 283 (200)μg/kg egg, while the LOAEL (NOAEL) for the chicken based on mortality, incidence of

abnormalities, body weight, and relative heart and liver weight was 2.3 (1.6) μg/kg egg. TCDD exposure in the last third of incubation can cause induction of hepatic microsomal EROD in both hatchling chickens and cormorants (Sanderson and Bellward, 1995). The magnitude of induction indicated that the cormorant is one to two orders of magnitude less sensitive than the chicken. Relative species sensitivities are helpful in assessing the potential effects of contaminants on wild populations. However, it would not be advisable to extrapolate from effects seen in a domestic precocial species to a wild altricial species. Wild species have much greater genetic diversity than the domestic species. In addition, the organ systems of the altricial and precocial chick develop at different stages (Dunn, 1975). These differences can lead to different toxicities making it difficult to predict the outcome of contaminant exposure for one species based on the effects seen in another that develops at a different rate.

REFERENCES

Brunström, B. 1988. Sensitivity of embryos from duck, goose, herring gull, and various breeds to 3,3',4,4'-tetrachlorobiphenyl. Poultry Sci., 67:52-57.

Brunström, B. 1989. Toxicity of coplanar polychlorinated biphenyls in avian embryos. Chemosphere, 19:765-768.

Brunström, B. 1990. Mono-ortho-chlorobiphenyls: toxicity and induction of 7-ethoxyresorufin O-deethylase (EROD) activity in chick embryos. Arch. Toxicol., 63:188-192.

Brunström, B. 1991. Toxicity and EROD-inducing potency of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in avian embryos. Comp. Biochem. Physiol., 100:241-243.

Brunström, B. and L. Andersson. 1988. Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated biphenyls (PCBs) chick embryos. Arch. Toxicol., 62:263-266.

Brunström, B. and P.O. Darnerud. 1983. Toxicity and distribution in chick embryos of 3,3',4,4'-tetrachlorobiphenyl injected into eggs. Toxicology, 27:103-110.

Brunström, B. and J. Orberg. 1982. A method for studying embryotoxicity of lipophilic substances experimentally introduced into hens' eggs. Ambio, 11:209-211.

Brunström, B., D. Broman, C. Näf. 1990. Embryotoxicity of polycyclic aromatic hydrocarbons (PAHs) in three domestic avian species, and of PAHs and coplanar polychlorinated biphenyls (PCBs) in common eider. Environ. Pollut., 67:133-143.

Cecil, H.C., S.J. Harris, and J. Bitman. 1978. Liver mixed function oxidases in chicken: induction by polychlorinated biphenyls and lack of induction by DDT. Arch. Environ. Contam. Toxicol., 7:283-290.

Dunn, E.H. 1975. Growth, body components and energy content of nestling double-crested cormorants. The Condor, 77:431-438.

Flick, D.F., C.D. Douglass, and L. Gallo. 1963. Studies of chick edema disease. Poultry Sci., 42:855-862.

Fox, G.A., B. Collins, E. Hayakawa, D.V. Weseloh, J.P. Ludwig, T.J. Kubiak, and T.C. Erdman. 1991. Reproductive outcomes in colonial fish-eating birds: a bioindicator for developmental toxicants in Great Lakes food chains. J. Great Lakes Res., 17:158-167.

Greichus, Y.A., D.J. Call, B.M. Ammann, A. Greichus, and H. Shave. 1975. Physiological effects of polychlorinated biphenyls or a combination of DDT, DDD, and DDE in penned white pelicans. Arch. Environ. Contam., 3:330-343.

Harris, S.J., H.C. Cecil, J. Bitman, and R.J. Lille. 1976. Antibody response and reduction in bursa of Fabricius and spleen weights of progeny of chickens fed PCBs. Poultry Sci., 55:1933-1940.

Larson, J.M. 1991. Reproduction and developmental anomalies in double-crested cormorants (*Phalacrocorax auritus*) on Lake Michigan: an environmental contaminants study. Master's Thesis, University of Wisconsin, Madison, WI, 120 pp.

Ludwig, J.P. 1984. Decline, resurgence, and population dynamics of Michigan and Great Lakes double-crested cormorants. Jack-Pine Warbler, 62:90-102.

North, Mack O. Commercial Chicken Production Manual. Westport, Connecticut: AVI Publishing Company, Inc., 1978. pp. 692.

McCune, E.L., J.E. Savage, and B.L. O'Dell. 1962. Hydropericardium and ascites in chicks fed a chlorinated hydorcarbon. Poultry Sci., 41:295-299.

Safe, S. 1991. Polychlorinated dibenzo-*p*-dioxins and related compounds: sources, environmental distribution and risk assessment. Environ. Carcinog. & Ecotox. Rev., C9(2):261-302.

Sanderson, J.T., R.J. Norstrom, J.E. Elliott, L.E. Hart, K.M. Cheng, and G.D. Bellward. 1994. Biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in double-crested cormorant chicks (*Phalacrocorax auritus*). J. Toxicol. Environ. Health, 41:247-265.

Sanderson, J.T., G.D. Bellward. 1995. Hepatic microsomal ethoxyresorufin O-deethylase-inducing potency in ovo and cytosolic Ah receptor binding affinity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: comparison of four avian species. Toxicol. Appl. Pharmacol., (in press).

Tanabe, S., N. Kannan, An. Subramanian, S. Watanabe, and R. Tatsukawa. 1987. Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. Environ. Pollut., 47:147-163.

Van den Berg, M., B.L.H.J. Craane, T. Sinnige, S. van Mourik, S. Dirksen, T. Boudewijn, M. van der Gaag, I.J. Lutke-Schipholt, B. Spenkelink, and A. Brouwer. 1994. Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant after in ovo exposure. Environ. Toxicol. Chem., 13:803-816.

Weseloh, D.V. and P.J. Ewins. 1994. Characteristics of a rapidly increasing colony of double-crested cormorants (*Phalacrocorax auritus*) in Lake Ontario: Population size, reproductive and band recoveries. J. Great Lakes Res., 20:443-456.

Weseloh, D.V., S.M. Teeple, and M. Gilbertson. 1983. Double-crested cormorants of the Great Lakes: egg-laying parameters, reproductive failure, and contaminant residues in eggs, Lake Huron 1972-1973. Can. J. Zool., 61:427-436.

Yamashita, N., S. Tanabe, J.P. Ludwig, H. Kurita, M.E. Ludwig, and R. Tatsukawa. 1993. Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ. Pollut., 79:163-173.

CHAPTER 3

THE EFFECTS OF AN EXTRACT DERIVED FROM CONTAMINATED DOUBLE-CRESTED CORMORANT (*Phalacrocorax auritus*) EGGS ON THE CHICKEN (*Gallus domesticus*)

ABSTRACT

White Leghorn chicken (*Gallus domesticus*) eggs were injected prior to incubation with one of four concentrations (0.001, 0.01, 0.1, and 1.0 egg equivalent) of an extract derived from double-crested cormorant (*Phalacrocorax auritus*) eggs collected from Green Bay, WI. Injection of 1.0 egg equivalent (the concentration of contaminants corresponded to what was present in an average cormorant egg) resulted in 77% mortality at hatch. The incidence of abnormalities was not affected by injection of the extract. Body weight gain was retarded in the 1.0 egg equivalent dose group in the second and third week post-hatch. At three weeks of age, chicks were subject to necropsy. Relative brain weights were greater and bursa weights were lower in the 1.0 egg equivalent dose group when compared to the vehicle control. There were no significant differences in the relative weights of the heart, liver, spleen, or comb.

INTRODUCTION

Double-crested cormorants (*Phalacrocorax auritus*) are one of the species of piscivorous colonial waterbirds residing in the Great Lakes that have experienced reproductive problems. Cormorant populations began to decrease in the 1950s and 1960s due to destruction by commercial fisherman and egg breakage. They stopped breeding in Lake Michigan in 1963 and by the 1970s they were no longer breeding in Lake Superior or the Canadian waters of Lake Ontario. They were the only species of Great Lakes waterbirds to cease breeding which suggests that they may be more sensitive to some contaminants than other waterbird species. (Ludwig, 1984; Fox et al., 1991c).

DDT/DDE are environmental contaminants known to have had an adverse effect on cormorant populations (Weseloh et al., 1983). Cormorants nesting in Lake Huron were reported to have egg shells 24% thinner than normal (1972) leading to egg breakage (Weseloh et al., 1983; Fox et al., 1991c). Since then, concentrations of DDT/DDE have been decreasing, and as a result, egg shell thickness has increased. Thus, beginning in the early 1980s cormorant populations have been increasing (Ludwig, 1984; Fox et al., 1991a; Weseloh and Ewins, 1994).

Despite recovering populations, embryo lethality (Tillitt et al., 1991) and congenital malformations have been observed in cormorant populations of the Great Lakes since the late 1970s (Fox et al., 1991c). These malformations have included deformed feet and legs, missing or abnormal eyes, and crossed or abnormal bills (Fox et al., 1991b). Since such malformations are uncommon in most wild avian populations (Fox et al.,

1991b), these observations prompted concern as to etiology. It has been suggested that polyhalogenated diaromatic hydrocarbons (PHDHs) capable of inducing the hepatic microsomal enzyme, aryl hydrocarbon hydroxylase (AHH), are responsible for these effects (Fox et al., 1991a; Giesy et al., 1994a and 1994b). Observations of malformations in cormorants and Forster's terns in Green Bay, WI were correlated with concentrations of polychlorinated biphenyls (PCBs) in the environment (Gilbertson et al., 1991). PCB concentrations have also been correlated with decreased hatchability of Forster's tern eggs (Kubiak et al., 1989).

Toxic equivalency factors (TEFs) are frequently used in evaluating the toxicity of PHDHs. PCB, polychlorinated dibenzo-*p*-dioxin (PCDD), and polychlorinated dibenzofuran (PCDF) isomers are assigned a TEF value based on their relative toxicity compared to the most toxic of these PHDHs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Environmental extracts can be assessed for potential toxicity by multiplying the concentrations of the dioxin-like contaminants by their respective TEFs to obtain toxic equivalents (TEQs). Great concentrations of TEQs have been reported in the eggs of waterbirds from polluted industrialized areas of the Great Lakes where reproductive impairment has been the most severe (Tillitt et al., 1991; Yamashita et al., 1993). These areas include Green Bay, Lake Michigan; Saginaw Bay, Lake Huron; Hamilton Harbor, Lake Huron; and the Detroit River (Fox, 1993).

In this study, the domestic chicken (*Gallus domesticus*) embryo was used to examine the potential effects of an extract derived from cormorant eggs collected from Green Bay, Lake Michigan, an area of relatively great contamination by PCDHs with a

history of compromised reproduction in resident colonies of double-crested cormorants (Fox et al., 1993; Yamashita et al., 1993; Giesy et al., 1994a and 1994b).

MATERIALS AND METHODS

Egg Collection: Double-crested cormorant eggs were collected in 1988 from Spider Island in Green Bay, Lake Michigan (45°13'N, 86°59'W). All three, four, and five egg clutches were collected on May 5, 13, and 19. Eggs were refrigerated until opening. Egg contents were pooled and placed into chemically clean jars (I-Chem, Hayward, CA). Samples were then frozen and transported on dry ice to the U.S. National Biological Survey (Columbia, MO) on Feb. 26, 1991 where they were processed. Samples were composites of 10 to 20 eggs. These composites were given individual data-base identification numbers and kept frozen at -20°C until sample preparation.

Extract Preparation: Egg contents were thawed and dehydrated with anhydrous sodium sulfate. The mixture was homogenized, packed in an extraction column, and extracted with methylene chloride. This extract was then concentrated by rotoevaporation and consolidated. Egg lipids were dialyzed in 50 ml aliquants with 80:20 (v/v) hexane/methylene chloride in 80 cm by 5 cm sections of layflat PE tubing (Brentwood Plastics, Inc., St. Louis, MO) (Meadows et al., 1993). Dialysates for all extracts were concentrated and combined. Cholesterol was removed by crystallization and filtration from cold pentane. Gravity-flow gel permeation chromatography (GPC) was used to remove any residual lipids in the egg extract. The final extract was in a volume of 10 mls.

Analysis of Extract: Capillary gas chromatography/electron capture detection (CGC/ECD) was used to analyze aliquants of purified sample extracts for residues of organochlorine pesticides (Schmitt et al., 1990) and mono-ortho-PCBs (Feltz et al., 1995). CGC and mass spectrometry were used to analyze for PCDDs, PCDFs, and non-ortho-PCBs (Feltz et al., 1995).

Egg Extract Injection Doses: The 1001 cormorant eggs used to prepare the extract weighed 42,127 grams. Thus, an average cormorant egg weighed 42 g. Doses were expressed in egg equivalents. One egg equivalent contained the amount of extract equal to that derived from a cormorant egg weighing 42.085 g. The doses chosen were 1, 0.1, 0.01, and 0.001 egg equivalent. Doses were prepared with cold-filtered triolein as the vehicle. The injection volume was 10 μl per egg. Approximately 1.2 μl of the 10 μl injected (vehicle or extract) was methylene chloride.

Injections: The extract doses and vehicle arrived at MSU in July, 1994. They were kept frozen between injection days. There were three replications with 20 eggs per group. Egg injections were performed as described in previous studies (Chapters 1 and 2).

Incubation, Post-Hatch, and Necropsy: Eggs were incubated as described in Chapter 1. Mortality was assessed at hatching and abnormalities were looked for in all nonviable embryos and hatchling chicks. Chicks were raised for three weeks as described in Chapter 1. Body weights were recorded weekly. Three-week-old chicks were killed by cervical dislocation and the brain, bursa, heart, liver, spleen, testes, and comb were removed and weighed.

Data Analysis: All comparisons were made with respect to the vehicle control, due to the relatively low hatchability of the vehicle control group. Mortality data were evaluated using a 2x2 contingency table and Bonferroni Chi-Square ("infertiles" were considered early deads since it was often difficult to distinguish between the two). The same analysis was used to test the occurrence of abnormalities. Analysis of body weight was conducted using univariate analysis of variance for split-plot repeated measures (Gill, 1986) followed by Dunnett's test for comparisons with control. Organ weights were analyzed using a one-way ANOVA and Dunnett's test. Analysis of body and organ weights was performed with the statistical software SAS (SAS Institute Inc., 1992). The level of significance was 0.05, unless otherwise stated.

RESULTS

Concentrations of predominant contaminants and their relative contribution to the toxicity of the cormorant egg extract based on TEFs derived for the chicken (Bosveld et al., 1995) are given in Table 53. The major contributors to toxic equivalents were PCB congener 126 [3,3',4,4',5-PeCB, (79.95%)]; 1,2,3,7,8-PeCDD (5.11%), PCB congener 77 [3,3',4,4'-TCB, (3.48%)]; 2,3,7,8-TCDD (3.37%); PCB congener 105 [2,3,3',4,4'-PeCB, (3.03%)]; and 2,3,4,7,8-PeCDD (2.65%).

One egg equivalent (1.0) was the only dose to produce significantly greater mortality than the vehicle control (Table 54). Body weights of both males and females were significantly lower at the second and third week post-hatch in the 1.0 egg equivalent

dose group (Table 55). Relative brain weights (Table 56) were greater in this group and actual and relative bursa weights (Table 57) were less when compared to the vehicle control values. The heart, liver, and spleen were not significantly affected by injection of the cormorant egg extract (Tables 58-60). Comb weight (Table 61-62) was also unaffected, although there was a tendency toward lower relative comb weights in the extract-exposed males. Relative testes weights (Tables 63-64) showed no statistical differences.

Table 53. Concentrations of selected polyhalogenated aromatic hydrocarbons and TEQ in cormorant egg extract.

Contaminant	(pg/g)[a]	TEF[b]	TEQ[c] (pg/g)	% TEQs
2,3,7,8-TCDD	14.0	1.0	14.0	3.37
1,2,3,7,8-PeCDD	17.7	1.2	21.24	5.11
1,2,3,4,7,8-HxCDD	3.4	0.05	0.17	0.04
1,2,3,6,7,8-HxCDD	22.3	0.01	0.223	0.05
1,2,3,7,8,9-HxCDD	5.2	0.1	0.52	0.13
1,2,3,4,6,7,8-HpCDD	16.7	0.001	0.017	0
2,3,7,8-TCDF	0.70	0.9	0.63	0.15
1,2,3,7,8-PeCDF	0.27	0.3	0.081	0.02
2,3,4,7,8-PeCDF	10.00	1.1	11.0	2.65
1,2,3,4,7,8-HxCDF	2.00	0.01	0.02	0
2,3,4,6,7,8-HxCDF	1.30	0.1	0.13	0.03
1,2,3,6,7,8-HxCDF	1.60	0.4	0.64	0.15
3,3,4',4',5-PeCB (126)	3,320	0.1	332	79.95
3,3,4',4'-TCB (77)	722	0.02	14.44	3.48
3,3',4,4',5,5'-HxCB (169)	376	0.001	0.376	0.09
2,3,3',4,4'-PeCB (105)	247,000	5.1×10^{-5}	12.60	3.03
2,3',4,4',5-PeCB (118)	711,000	4.1×10^{-6}	2.92	0.70
2,3,3',4,4',5-HxCB (156)	50,000	6.1×10^{-5}	3.05	0.73
2,3,3',4,4',5'-HxCB (157)	15,000	7.1×10^{-5}	1.07	0.26
2,3',4,4',5,5'-HxCB (167)	50,000	3.1×10^{-6}	0.155	0.04
			415.269	100%

[a] Values are expressed on a wet weight basis
[b] Toxic Equivalency Factor (Bosveld et al., 1995) based on cytochrome P450 induction studies in chicken embryos
[c] Toxic Equivalents, relative to 2,3,7,8-TCDD

Table 54. Effect of injection of cormorant egg extract into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability.

Dose[a]	TEQ[b]	TEQs/egg[c]	# 126/egg[d]	# dead/ # eggs	% Mortality
Non-injected	0	0	0	3/60	5.0
Vehicle	0	0	0	12/60	20.0
$1*10^{-3}$	0.415	$1.7*10^{1}$	$1.4*10^{-4}$	16/62	25.8
$1*10^{-2}$	4.15	$1.7*10^{2}$	$1.4*10^{-3}$	6/61	9.8
$1*10^{-1}$	41.5	$1.7*10^{3}$	$1.4*10^{-2}$	13/64	20.3
1.0	415	$1.7*10^{4}$	$1.4*10^{-1}$	46/60*	76.7

[a] Doses expressed in units of an egg equivalent (vehicle = triolein)
[b] Doses expressed in Toxic Equivalents (pg/g)
[c] Doses expressed in pg/egg (avg. cormorant egg = 42g)
[d] Doses expressed in μg PCB congener 126/egg (avg. cormorant egg = 42g)
* Significantly different from vehicle control at $p < 0.05$

Table 55. Effect of cormorant egg extract on the body weights of White Leghorn chickens through three weeks of age.

Dose[a]	n[b]	SE[c]	Hatch[d]	1 Week[d]	2 Weeks[d]	3 Weeks[d]
Vehicle	47	2.15	43.6	72.2	125.6	187.0
$1*10^{-3}$	44	2.22	43.9	72.1	124.3	186.4
$1*10^{-2}$	54	2.00	43.6	71.4	124.2	185.2
$1*10^{-1}$	51	2.06	44.1	68.9	119.4	181.1
1.0	13	4.08	44.5	62.8	106.5*	160.0*

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Standard error (Not appropriate for comparison across time)
[d] Data expressed as mean (g)
* Significantly different from vehicle control at $p < 0.05$

Table 56. Effect of cormorant egg extract on the actual and relative brain weights of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	47	1.69 ± 0.014	0.914 ± 0.0149
$1*10^{-3}$	43	1.67 ± 0.015	0.909 ± 0.0156
$1*10^{-2}$	54	1.66 ± 0.013	0.908 ± 0.0139
$1*10^{-1}$	51	1.64 ± 0.013[d]	0.913 ± 0.0143
1.0	13	1.55 ± 0.026[d]	0.994 ± 0.0283[d]

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p<0.05$

Table 57. Effect of cormorant egg extract on the actual and relative bursa weights of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	47	1.13 ± 0.047	0.601 ± 0.0202
$1*10^{-3}$	43	1.09 ± 0.050	0.582 ± 0.0211
$1*10^{-2}$	54	1.13 ± 0.044	0.600 ± 0.0188
$1*10^{-1}$	51	1.07 ± 0.046	0.584 ± 0.0194
1.0	12	0.62 ± 0.094[d]	0.385 ± 0.0399[d]

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p<0.05$

Table 58. Effect of cormorant egg extract on the actual and relative heart weights of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	47	1.21 ± 0.026	0.645 ± 0.0094
$1*10^{-3}$	43	1.24 ± 0.027	0.668 ± 0.0098
$1*10^{-2}$	54	1.23 ± 0.024	0.667 ± 0.0088
$1*10^{-1}$	51	1.21 ± 0.025	0.667 ± 0.0090
1.0	13	1.01 ± 0.049[d]	0.642 ± 0.0179

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p<0.05$

Table 59. Effect of cormorant egg extract on the actual and relative liver weights of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	47	5.21 ± 0.132	2.79 ± 0.044
$1*10^{-3}$	43	5.11 ± 0.138	2.73 ± 0.046
$1*10^{-2}$	54	5.29 ± 0.123	2.86 ± 0.041
$1*10^{-1}$	51	5.05 ± 0.126	2.79 ± 0.042
1.0	13	4.54 ± 0.250	2.85 ± 0.084

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error

Table 60. Effect of cormorant egg extract on the actual and relative spleen weights of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	47	0.247 ± 0.0118	0.131 ± 0.0054
$1*10^{-3}$	43	0.258 ± 0.0123	0.137 ± 0.0057
$1*10^{-2}$	54	0.253 ± 0.0110	0.136 ± 0.0051
$1*10^{-1}$	50	0.245 ± 0.0114	0.134 ± 0.0053
1.0	13	0.243 ± 0.0224	0.146 ± 0.0103

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error

Table 61. Effect of cormorant egg extract on the actual and relative comb weights of female three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	22	0.052 ± 0.0027	0.030 ± 0.0015
$1*10^{-3}$	24	0.050 ± 0.0026	0.028 ± 0.0014
$1*10^{-2}$	28	0.051 ± 0.0024	0.029 ± 0.0013
$1*10^{-1}$	28	0.049 ± 0.0024	0.029 ± 0.0013
1.0	6	0.050 ± 0.0052	0.032 ± 0.0029

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error

Table 62. Effect of cormorant egg extract on the actual and relative comb weights of male three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	0.376 ± 0.0404	0.193 ± 0.0213
$1*10^{-3}$	19	0.242 ± 0.0454	0.122 ± 0.0240
$1*10^{-2}$	26	0.235 ± 0.0388[d]	0.120 ± 0.0205
$1*10^{-1}$	21	0.254 ± 0.0432	0.135 ± 0.0228
1.0	7	0.192 ± 0.0749	0.115 ± 0.0395

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 63. Effect of cormorant egg extract on the actual and relative left testes weights of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	0.028 ± 0.0016	0.015 ± 0.0008
$1*10^{-3}$	19	0.026 ± 0.0018	0.013 ± 0.0009
$1*10^{-2}$	26	0.028 ± 0.0015	0.014 ± 0.0008
$1*10^{-1}$	23	0.025 ± 0.0016	0.013 ± 0.0009
1.0	7	0.021 ± 0.0029	0.013 ± 0.0016

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error

Table 64. Effect of cormorant egg extract on the actual and relative right testes weights of three-week-old White Leghorn chicks.

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Vehicle	24	0.025 ± 0.0015	0.013 ± 0.0008
$1*10^{-3}$	19	0.024 ± 0.0017	0.012 ± 0.0008
$1*10^{-2}$	26	0.025 ± 0.0015	0.013 ± 0.0007
$1*10^{-1}$	23	0.022 ± 0.0016	0.011 ± 0.0008
1.0	7	0.018 ± 0.0028	0.011 ± 0.0014

[a] Doses are expressed in units of an egg equivalent
[b] Sample size
[c] Data expressed as mean ± standard error

DISCUSSION

Total TEQs present in the cormorant egg extract (Table 53) were approximately 415 pg/g. These toxic equivalents were calculated using TEFs for chickens derived by Bosveld et al. (1995). Total TEQs reported for cormorant eggs collected from Green Bay, WI in 1988 by Yamashita et al. (1993) were around 1300 pg/g. The majority of this difference in total TEQs is due to the difference in TEFs used to calculate the TEQ for congener 126. Yamashita and associates used 0.3 while a TEF of 0.1 was used in the present study. Total TCDD-EQs in cormorant eggs collected from Green Bay in the summer of 1989 were approximately 382 pg/g (Jones et al., 1994).

There is an association between abnormalities in live cormorant chicks and areas with eggs contaminated with 2,3,7,8-TCDF and coplanar PCBs (Yamashita et al., 1993). No correlation was found for other organochlorine contaminants. Of the TEQs reported by Yamashita et al. (1993), PCB congener 126 accounted for 83% of the TEQs and PCB congener 105 accounted for 13%. Congener 126 also accounted for the majority of the TEQs in the present cormorant extract (80%). This congener was present at 3,320 pg/g egg which is consistent with the concentration (3,600 pg/g egg) determined in cormorant eggs collected by Yamashita and associates (1993). PCB congeners 77 and 105 each accounted for approximately 3% of the TEQs in the present cormorant egg extract and 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD accounted for over 8% of the TEQs.

Mortality: The injection of the cormorant egg extract in the present study produced increased mortality at the same concentration as found in cormorant eggs in Green Bay,

WI in 1988. Since congener 126 was present in the extract at 3,320 pg/g egg and an average cormorant egg weighed 42 g, each chicken egg received approximately 139,440 pg of congener 126. This corresponds to an injection dose of approximately 2.32 μg congener 126/kg egg which is very close to the LD_{50} for congener 126 in chickens, 2.42 μg/kg egg (Chapter 2). While greater than 50% mortality occurred at 2.32 μg/kg egg (77%), which may be attributed to the remaining components in the cormorant extract, the primary effect still seems to be due to the concentration of congener 126. It is unlikely that cormorants would experience increased mortality with this concentration of congener 126 since the LD_{50} for this congener in cormorants is approximately 246 μg/kg egg (Chapter 2).

Teratogenicity: There were no significant differences in the incidences of abnormalities among any of the dose groups. The highest dose group, 1.0 egg equivalent, provided a lower concentration of congener 126 (2.32 μg/kg egg) than did the doses of congener 126 which caused an increased incidence of abnormalities in a previous injection study, (3.2 - 12.8 μg/kg egg) (Chapter 2).

Body weights: The cormorant extract had a significant effect on body weights at the second and third weeks post-hatch. Chicks from eggs injected with congener 126 (0.9, 2.7 μg/kg egg) also experienced lower body weights at the second and third week of rearing (Chapter 1).

Organ and comb weights: Relative brain weights were greater in the 1.0 egg equivalent dose group, however, this was probably a result of the significantly lower body weights also observed in chicks of this dose group. The weights of the bursa of Fabricius

were significantly lower in chicks exposed to 1.0 egg equivalent prior to incubation. Chicks exposed to congener 126 prior to incubation also had lower relative bursa weights at three weeks post-hatch (Chapter 1). Relative heart, liver, and spleen weights were not significantly different when compared to the vehicle control chicks. While not significantly different in either extract-exposed males or females, the comb weights of three-week-old males were numerically lower with increasing concentration of extract. This suggests that the extract may have had an anti-androgenic effect. Fry and associates (1987) have reported that organochlorine compounds can cause feminization of male germ cells in the testes. New evidence is suggesting that feminizing effects are the result of androgen blockage. One organochlorine contaminant, pp'-DDE, has been shown to bind to androgen receptors, thus blocking the binding of testosterone (Culotta, 1995). When one-month-old cockerels were fed 500 ppm Aroclor® 1254 over a 12 week period, they showed no change in comb weights, while controls showed a significant increase in weight. These PCB-fed birds also had retarded gain in testes weight (Platonow and Funnell, 1971). Mature cockerels fed 500 ppm of the same Aroclor® for 30 days also had lower comb and testes weights than the control birds (Cecil et al., 1978).

It is questionable whether any adverse effects would be observed if this extract were to be injected into cormorant eggs at the levels naturally present in a single cormorant egg. Gilman and associates (1978) injected organochlorine contaminants (DDE, PCBs, mirex, photomirex, and hexachlorobenzene) extracted from Lake Ontario herring gulls into relatively uncontaminated, unincubated eggs collected from New Brunswick, Canada. All eggs were injected in the field and naturally incubated. Contaminant-injected eggs had

no increase in embryo mortality suggesting that organochlorine contaminants in the egg are not directly responsible for the increased incidence of embryo mortality observed in Lake Ontario herring gulls. However, it should be noted than the injection procedure alone reduced the rate of hatching from 91 to 52%. The solvent (10% acetone in DMSO) further reduced the rate of hatching, and 14 to 29% of all eggs used in the study were destroyed by predators. In addition, most of the photomirex and about half the mirex were lost in the preparation of the extracts. Gilman and associates (1978) suggested that the toxic effects of contaminants may be expressed before the egg is laid. They offered chromosomal alterations, sex cell viability, yolk quality, and changes in the structural characteristics of the egg as possible explanations for the reduction in the rate of hatching observed in herring gull eggs.

SUMMARY AND CONCLUSIONS

Injection of the contents of an average cormorant egg (1.0 egg equivalent) from Green Bay, WI into White Leghorn chicken eggs prior to incubation had adverse effects on the developing embryo. Mortality during incubation was greater, post-hatch body weights were lower, and there was a reduction in the relative weight of the bursa of Fabricius. Total TEQs in this extract were approximately 415 pg/g. Toxic equivalents determined by the H4IIE bioassay (TCDD-EQs) have ranged from 49 to 415 pg/g in eggs from double-crested cormorants and caspian terns residing in the Great Lakes (Tillitt et al., 1991). Tillitt et al. (1992), using the H4IIE bioassay, demonstrated a dose-response

relationship between PCBs and mortality of cormorant eggs from the Great Lakes. PCBs accounted for nearly 90% of the TEQs determined in the extract used for this egg injection study and congener 126 accounted for nearly 80% of this toxicity.

REFERENCES

Bosveld, A.T.C., J. Gradener, A.J. Murk, A. Brouwer, M. van Kampen, E.H.G. Evers, and M. Van den Berg. 1995. Effects of PCDDs, PCDFs, and PCBs on common terns (*Sterna hirundo*), breeding in estuarine and coastal colonies in the Netherlands and Belgium. Environ. Toxicol. Chem., 14:99-115.

Cecil, H.C., S.J. Harris, and J. Bitman. 1978. Liver mixed function oxidases in chicken: induction by polychlorinated biphenyls and lack of induction by DDT. Arch. Environ. Contam. Toxicol., 7:283-290.

Culotta, E. 1995. St. Louis Meeting Showcases "Creature Features" Science, 267:330-331.

Feltz, K.P., D.E. Tillitt, R.W. Gale, and P.H. Peterman. 1995. Automated HPLC fractionation of PCDDs and PCDFs and planar and nonplanar PCBs on C_{18}-dispersed PX-21 carbon. Environ. Sci. Technol., 29:709-718.

Fox, G.A., B. Collins, E. Hayakawa, D.V. Weseloh, J.P. Ludwig, T.J. Kubiak, and T.C. Erdman. 1991a. Reproductive outcomes in colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains. J. Great Lakes Res., 17:153-167.

Fox, G.A., M. Gilbertson, A.P. Gilman, and T.J. Kubiak. 1991b. A rationale for the use of colonial fish-eating birds to monitor the presence of developmental toxicants in Great Lakes fish. J. Great Lakes Res., 17:151-152.

Fox, G.A., D.V. Weseloh, T.J. Kubiak, and T.C. Erdman. 1991c. Reproductive outcomes in colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains. J. Great Lakes Res., 17:153-157.

Fox, G.A. 1993. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. J. Great Lakes Res., 19:722-736.

Fry, D.M., C.K. Toone, S.M. Speich, and R.J. Peard. 1987. Sex ratio skew and breeding patterns of gulls: demographic and toxicological considerations. Studies in Avian Biology, 10:26-43.

Giesy, J.P., J.P. Ludwig, D.E. Tillitt. 1994a. Dioxins, dibenzofurans, PCBs and Colonial, Fish-Eating Water Birds. In: Schecter, A. (ed) Dioxins and Health. Plenum Press, New York. pp. 249-307.

Giesy, J.P., J.P. Ludwig, D.E. Tillitt. 1994b. Deformities in birds of the Great Lakes region: assigning causality. Environ. Sci. Technol., 28:128A-136A.

Gilbertson, M., T. Kubiak, J. Ludwig, and G.A. Fox. 1991. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J. Toxicol. Environ. Health, 33:455-520.

Gill, J.L. 1986. Repeated measurement: sensitive tests for experiments with few animals. J. Animal Science, 63:943-954.

Gilman, A.P., D.J. Hallett, G.A. Fox, L.J. Allan, W.J. Learning, and D.B. Peakall. 1978. Effects of injected organochlorines on naturally incubated herring gull eggs. J. Wildl. Manage., 42:484-493.

Jones, P.D., J.P. Giesy, J.L. Newsted, D.A. Verbrugge, J.P. Ludwig, M.E. Ludwig, H.J. Auman, R. Crawford, D.E. Tillitt, T.J. Kubiak, and D.A. Best. 1994. Accumulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents by double-crested cormorant (*Phalacrocorax auritus*, Pelicaniformes) chicks in the North American Great Lakes. Ecotox. Environ. Safety, 27:192-209.

Kubiak, T.J., H.J. Harris, L.M. Smith, T.R. Schwartz, D.L. Stalling, J.A. Trick, L. Sileo, D.E. Docherty, and T.C. Erdman. 1989. Microcontaminants and reproductive impairment of the Forster's Tern on Green Bay, Lake Michigan-1983. Arch. Environ. Contam. Toxicol., 18:706-727.

Ludwig, J.P. 1984. Decline, resurgence, and population dynamics of Michigan and Great Lakes double-crested cormorants. Jack-Pine Warbler, 62:90-102.

Meadows, J., D. Tillitt, J. Huckins, and D. Schroeder. 1993. Large-scale dialysis of sample lipids using a semipermeable membrane device. Chemosphere, 26:1993-2006.

Platonow, J.S. and H.S. Funnell. 1971. Anti-androgenic-like effect of polychlorinated biphenyls in cockerels. Vet. Rec., 88:109-110.

Schmitt, C.J., J.L. Zajicek, P.H. Peterman. 1990. National contaminant biomonitoring program: residues of organochlorine chemicals in U.S. freshwater fish, 1976-1984. Arch. Environ. Contam. Toxicol., 19:748-781.

Tillitt, D.E., G.T. Ankley, D.A. Verbrugge, J.P. Giesy, J.P. Ludwig, and T.J. Kubiak. 1991. H4IIE rat hepatoma cell bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in colonial fish-eating waterbird eggs from the Great Lakes. Arch. Environ. Contam. Toxicol., 21:91-101.

Tillitt, D.E., G.T. Ankley, J.P. Giesy, J.P. Ludwig, H. Kurita-Matsuba, D.V. Weseloh, P.S. Ross, C.A. Bishop, L. Sileo, K.L. Stromborg, J.Larson, T.J. Kubiak. 1992. Environ. Toxicol. Chem., 11:1281-1288.

Weseloh, D.V. and P.J. Ewins. 1994. Characteristics of a rapidly increasing colony of double-crested cormorants (*Phalacrocorax auritus*) in Lake Ontario: Population size, reproductive and band recoveries. J. Great Lakes Res., 20:443-456.

Weseloh, D.V., S.M. Teeple, and M. Gilbertson. 1983. Double-crested cormorants of the Great Lakes: egg-laying parameters, reproductive failure, and contaminant residues in eggs, Lake Huron 1972-1973. Can. J. Zool., 61:427-436.

Yamashita, N., S. Tanabe, J.P. Ludwig, H. Kurita, M.E. Ludwig, and R. Tatsukawa. 1993. Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ. Pollut., 79:163-173.

APPENDIX

Table 1. Concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) in cormorant egg extract.

Contaminant	(pg/g)	TEF[a]	TEQ (pg/g)[b]
2,3,7,8-TetraCDD	14.0	1.0	14.0
1,2,3,7,8-PentaCDD	17.7	0.5	8.85
1,2,4,7,8-PentaCDD	ND[c]	-	-
1,2,3,4,7,8-HexaCDD	3.4	0.1	0.34
1,2,3,6,7,8-HexaCDD	22.3	0.1	2.23
1,2,3,7,8,9-HexaCDD	5.2	0.1	0.02
1,2,3,4,6,7,8-HeptaCDD	16.7	0.01	0.133
OctaCDDs	202.3	0.001	0.2023

[a] Toxic Equivalency Factor (TEF) (Safe, 1992)
[b] Toxic-Equivalent
[c] Not Detected at specified detection limit (0.1 pg/g)

Table 2. Concentrations of polychlorinated dibenzofurans (PCDFs) in cormorant egg extract.

Contaminant	(pg/g)	TEF[a]	TEQ (pg/g)[b]
2,3,7,8-TetraCDF	0.70	0.1	0.070
1,2,3,7,8-PentaCDF	0.27	0.1	0.027
2,3,4,7,8-PentaCDF	10.00	0.5	5.00
1,2,3,4,7,8-HexaCDF	2.00	0.1	0.20
1,2,3,6,7,8-HexaCDF	1.60	0.1	0.16
1,2,3,7,8,9-HexaCDF	NQ[c]	0.1	-
2,3,4,6,7,8-HexaCDF	1.33	0.1	0.133
1,2,3,4,6,7,8-HeptaCDF	0.80	0.1	0.08
1,2,3,4,7,8,9-HeptaCDF	0.67	0.1	0.067
OctaCDFs	1.73	0.001	0.00173

[a] Toxic Equivalency Factor (TEF) (Safe, 1992)
[b] Toxic-Equivalent
[c] Not Quantitated at specified average concentration (0.2 pg/g)

Table 3. Concentrations of non-ortho-polychlorinated biphenyl congeners in cormorant egg extract.

PCB Congener	(pg/g)	TEF[a]	TEQ (pg/g)[b]
126	3,320	0.1	322
77	722	0.01	7.22
169	376	0.05	18.8
81	852	-	-

[a] Toxic Equivalency Factor (TEF) (Safe, 1992)
[b] Toxic-Equivalent

Table 4. Concentrations of mono-ortho-polychlorinated biphenyl congeners in cormorant egg extract.

PCB Congener	(ng/g)	TEF[a]	TEQ (ng/g)[b]
123	6	0.001	0.006
118	711	0.001	0.711
114	16	0.001	0.016
105	247	0.001	0.247
167	50	0.001	0.050
156	50	0.001	0.050
157	15	0.001	0.015
189	6	0.001	0.006

[a] Toxic Equivalency Factor (TEF) (Safe, 1992)
[b] Toxic-Equivalent

Table 5. Concentrations of organochlorine pesticides in cormorant egg extract.

Contaminant	(pg/g)	Contaminant	(pg/g)
Hexachlorobenzene	11.7	*cis* Chlordane	23.6
alpha-BHC	3.8	*o,p'*-DDE	<MQL
Lindane	<MQL[a]	Dieldrin	255.1
beta-BHC	4.9	*p,p'*-DDE	1147.2
Heptachlor	<MDL[b]	*o,p'*-DDD	4.9
delta-BHC	<MQL	Endrin	12.8
Dacthal	<MDL	*cis* Nonachlor	43.1
Oxychlordane	80.3	*o,p'*-DDT	<MDL
Heptachlor epoxide	88.3	*p,p'*-DDD	23.8
trans Chlordane	<MQL	*p,p'*-DDT	29.8
trans Nonachlor	13.8	Mirex	23.8
		Total PCB	8872

[a] Method Quantitation Limit (2.9 ng/g)
[b] Method Detection Limit (1.0 ng/g)

Table 6. Concentrations of polychlorinated biphenyl congeners in cormorant egg extract.

Congener	(ng/g)	Congener	(ng/g)
004, 010	<1.1	064	10.6
007, 109	<1.1	040	<2.9
006	<1.1	067	4.8
005, 008	<1.1	063	9.0
019	<1.1	074	206.1
018	<1.1	070, 076	24.4
017, 015	<1.1	066, 095, 088	408.6
024, 027	<1.1	091	14.9
016, 032	<1.1	056, 060	34.0
029	<1.1	092	29.9
026	8.0	084	11.6
025	5.9	101, 090	65.4
031	<2.0	099	450.8
028	81.8	119	18.0
020, 033, 053	<1.1	083	<2.9
051	<1.1	097	5.5
022	<1.1	087	31.1
045	<1.1	110	18.0
046	<1.1	082, 151	48.3
052	45.3	135, 144, 124	6.9
049, 043	51.1	107	25.1
047, 048	221.1	123, 149	36.6
044	24.1	118	251.7
042	<2.0	134	<2.9
041	9.7	146	166.7

Table 6 (cont'd)

Congener	(ng/g)	Congener	(ng/g)
153	466.4	185	5.3
132	9.2	174	5.5
105	34.1	177	69.6
141	9.9	202, 171, 156	74.5
179	<1.1	157, 201	13.9
137	37.5	172	24.2
176	<1.1	180	307.2
130	48.1	193	25.7
138	568.6	191	9.3
158	47.6	200	<1.1
129, 178	46.2	170, 190	127.5
182, 187	249.8	199	69.3
183	122.4	196, 203	65.3
128	172.4	208, 195	22.4
167	3.9	194	47.7
		Total PCBs	5352

REFERENCES

Safe, S. 1992. Development, validation and limitations of toxic equivalency factors. Chemosphere, 25:61-64.