PCB EFFECTS ON BRAIN TYPE II 5'DEIODINASE ACTIVITY IN DEVELOPING BIRDS

By

Leslie Ann Fowler

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In

BIOLOGY

APPROVED:

_____

Dr. F.M. Anne McNabb, Chair

_____ _____

Dr. Jeff R. Bloomquist Dr. Marion F. Ehrich

September 5, 2000

Blacksburg, Virginia

Key words: thyroxine, brain type II 5'Deiodinase, PCBs, developing bird

PCB Effects on Brain Type II 5'Deiodinase Activity in Developing Birds

By

Leslie Ann Fowler

Dr. FMA McNabb, Chairman

Department of Biology

(ABSTRACT)

PCBs are known to cause thyroid disruption in laboratory rats and are thought to be the causal agent in thyroid gland alterations in herring gulls in the Great Lakes. This study examined the regulation of thyroid hormone supply during development in (1) domestic chicken embryos (*Gallus domesticus*) exposed to a specific dioxin-like PCB congener (PCB-126) and (2) herring gull (*Larus argentatus*) embryos and pre-fledglings from Great Lakes sites with different chemical pollutant exposures. Specifically, PCB effects on thyroid status were evaluated by measuring plasma thyroid hormone concentrations and brain type II 5'D activity (to determine if PCB exposure was associated with alteration in brain 5'D type II activity that could maintain local T3 supply to the brain). If PCB-126 and PCB mixtures altered thyroid function, we expected to see decreased plasma thyroid hormone concentrations and subsequent increases in 5'D-II activity. Chicken eggs were injected (into the air cell) before incubation with five dose levels (0.0512, 0.128, 0.32, 0.64, 0.8 ng/g) of PCB-126 (3,3, 4,4',5-pentachlorobiphenyl), or vehicle (sunflower oil); sampling was on day 20 of the 21-day incubation period. Studies on PCB-treated embryos included a preliminary study and a larger study encompassing a serious of smaller studies. Herring gull embryos (at pipping, on day 25 of the 26 day incubation), and 28-day pre-fledgling chicks were sampled (for two field seasons) at several Great Lakes sites with different contaminant exposures (with Kent Island being the reference site). In PCB-treated chicken embryos, there were no statistically significant decreases in plasma T4 or T3 concentrations and no significant increases in brain 5'D-II activity in either the preliminary or the larger study. We found no clear pattern of altered thyroid function in herring gulls from polluted Great Lakes' sites. Plasma TH concentrations were not significantly decreased and 5'D-II activity did not significantly increase in birds from more contaminated sites in comparison to birds from Kent Island or sites with less contamination. Although pipped embryos from Strachnan Island had a significant increase in 5'D-II activity when compared to Kent Island, there were no differences in plasma TH concentrations, and brain 5'D-II activity was not significantly increased in birds from sites with greater PCB loads than Strachnan Island. Plasma T4 and T3 concentrations were significantly decreased in prefledglings from West Sister Island and Detroit Edison in comparison to Kent Island, but there was no subsequent increase in brain 5'D-II activity. The present study is the first to evaluate the potential effects of PCBs, alone and in a mixed environmental exposure, on circulating THs and brain 5'D-II activity in developing birds. Although thyroid function was not altered by the specific PCB congener used in my study or by

exposure to environmental pollutants, more complete evaluations are needed before determining whether PCBs alter thyroid function in birds.

ACKNOWLEDGMENTS

I would like to give special thanks to Dr. Anne McNabb, my committee chair, mentor, and friend, for her guidance, patience, wisdom, and faith. I would also like to thank the other members of my committee, Dr. Jeff Bloomquist, Dr. Marion Ehrich, and Dr. Alan Heath, for their advice, suggestions, and support.

I would also like to thank my parents and siblings for their constant support and for listening when I needed to voice both joys and frustrations.

Lastly, to friends and colleagues who took time to assist me with different aspects of my project: Ryan McCleary, Cathy Parsons, Melissa Goode, Travis Schmidt, Eric Belling, Jenny Honaker, Keesha Steed, Jesper Lorentzen and John Baumgartner.

This research was supported by funding from the EPA, Sigma Xi Grant -In-Aid, and the Biology Department of Virginia Tech.

Table of Contents

LITERATURE REVIEW

I. Endocrine Disruption

Alterations in endocrine development at early stages (or during ontogeny) can have permanent and detrimental effects throughout the life of the organism. In brief, studies of endocrine disruption by pollutant chemicals have focused mainly on alterations in reproductive development and function. For example, Beluga whales from the St. Lawrence seaway, where the waters are highly contaminated with organochlorines [e.g. polychlorinated biphenyls (PCBs)], show hermaphroditic qualities. Some males not only have male organs (epididymis, vas deferens, and testes) but also female organs (uterus and ovaries) (Colborn et al., 1996). Likewise, Everglades National Park and Big Cypress Swamp in Southern Florida lie downstream from large agriculture areas where pesticide use is heavy. Panthers that reside in this area show signs of sterility, decreased sperm count, and undescended testes (Crain et al., 1997). In Lake Apopka in Florida, male alligators have abnormally small penises and defective testes, and females show follicle and ovary abnormalities. The hormone ratios in these male alligators are like those of a normal female. Females also have estrogen levels above normal (Guillette and Crain, 1996). Such observations led to the hypothesis that chemical pollutants bind to hormone receptors for reproductive steroids and either trigger actions similar to those of the native hormone (hormone agonists) or prevent the native hormone from binding but don't trigger cellular action (hormone antagonists) (Gray, 1992).

Carnivorous birds, residing at the top of the food change, mainly are exposed to pollutants through bioaccumulation in the food chain. Before the banning of DDT and dieldrin use, bald eagles had problems with egg-shell thinning and embryo death, both thought to be a result of pollutant exposure. Reproduction rates were also impaired (Bowerman et al., 1995). In polluted areas, such as the Great Lakes, and in laboratory tests, herring gulls show estrogenic effects similar to those in other birds and mammals from similarly polluted areas. Males are feminized and have some female organs, such as ovaries. Females sometimes have extra ovaries and often nest with other females, presumably due to lack of fertile males (Fry and Toone, 1981).

Disruption of endocrine systems that control developmental processes could alter many aspects of animal function for the life of that organism. Thyroid hormones (TH) are essential for the development and continued function of many organs and tissues, including the central nervous system, as well as being necessary for proper growth and metabolism in vertebrates. Studies suggest that thyroid function may be altered in some vertebrates exposed to organochlorines. This literature review will (1) briefly describe a major organochlorine group, PCBs, which are thought to cause thyroid alterations upon exposure in vertebrates (2) outline important aspects of thyroid function (3) review the literature that suggests alterations in thyroid function in laboratory rats and laboratory and field-caught birds exposed to PCBs.

II. Polychlorinated Biphenyls (PCBs)

In the Great Lakes, PCBs are abundant and widespread contaminants and are found in the tissues of fish-eating birds (Fox et al., 1993). Until their banning in 1979, PCBs were largely used as fire retardant fluids in capacitors, coolant fluids in transformers, and as wetting agents

and surfactants. Due to their long half-life and lipophilic properties, PCBs are incorporated into organisms low in the food chain and are biomagnified, they increase in concentration higher in the food chain. Given their high position on the food web, fish-eating (piscivorous) birds, such as herring gulls, potentially are exposed to biomagnification of PCBs and have been used as sentinel species to monitor the concentrations and biological effects of environmental contaminants in the Great Lakes (Ness et al., 1993).

The coplanar PCB congeners are dioxin-like and their toxicity appears to be mediated mainly through the arylhydrocarbon receptor (AhR). Dioxin-like toxicity is estimated by using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) potency as the standard, either as toxic equivalency factor (TEF) for individual compounds or as the TCDD toxic equivalent (TEQ) for mixtures (Safe, 1990). The nonplanar congeners do not bind to the AhR but are found in high concentrations in the tissues of wild animals (Nessel et al., 1992). To date, most of the reports of thyroid disruption in birds have been on wild birds exposed to mixtures of pollutants (Fox, 1993; Grasman et al., 1996; Murk et al., 1994; Van den Berg et al., 1994). Thyroid alterations in laboratory rodents have been associated with treatments of several specific congeners, both coplanar (e.g. PCB-169, 126, 156, 77) (Grasman et al., 1996, Murk et al., 1994) and non-coplanar (e.g. PCB-13, 52, 153) (Van der Kolk et al., 1992; Morse et al., 1993; Van Birgelen et al., 1992) and with PCB mixtures (e.g. Arochlor 1254; Bastomsky, 1974; Morse et al., 1996).

III. Thyroid Function

The vertebrate thyroid gland secretes two hormones, thyroxine (T4), and triiodothyronine (T3) that are important for development and metabolism. T4 is the most abundant hormone, whereas T3 is the most active with respect to its function in biological systems (i.e. main hormone that binds to nuclear receptor). The thyroid gland is under the control of the hypothalamic-pituitary-thyroid (HPT) axis. Thyrotropin-releasing hormone (TRH), from the hypothalamus, stimulates the release of thyroid stimulating hormone (TSH), from the anterior pituitary, which in turn causes the thyroid gland to produce and release hormone. TH feedback to the anterior pituitary regulates the control of TSH production, as well as TRH release, which is also modulated by feedback from TSH (McNabb, 1992). TSH stimulation of the thyroid gland increases when circulating concentrations of thyroid hormones (THs) are low.

Although small amounts of T3 are produced by the thyroid gland, most of the T3 in the body is produced by deiodination of T4. Deiodination of T4 occurs when iodine atoms are removed from either the phenolic (outer) or tyrosyl (inner) ring. In 5'deiodination (5'D), an iodine is removed from the outer ring of T4, yielding T3 (the most active hormone), and in 5deiodination (5D), an iodine atom is removed from the inner ring, yielding rT3 (which is inactive), (figure). These products can then be deiodinated further to yield diiodothyronines (T2) (other inactive forms). There are three types of deiodination pathways: type I, type II, and type III. Type I deiodination provides a circulating source of T3 to peripheral tissues. This type of deiodination is found mainly in the thyroid, liver, and kidney. Hyperthyroidism causes Type I deiodination to increase and hypothyroidism causes it to decrease, i.e. the enzyme activity is substrate driven. Type II deiodination occurs in the brain, pituitary, and brown adipose tissue (BAT). In the brain and pituitary, T3 produced by type II remains largely within the tissue where it is produced, maintaining the intracellular T3 supply at euthyroid levels despite hypothyroid conditions in the rest of the body. In mammals, when T3 is limited, BAT type II 5'D releases T3

to peripheral tissues, providing a circulating source of T3. . In a hyperthyroid state, type II decreases its activity. Type III deiodination involves removal of an iodine atom from the inner ring of iodothyronines. It is a major route for T3 degradation and inactivation of T4, producing rT3 (Leonard and Koehrle, 1996).

Binding proteins transport THs in the blood and ensure sustained TH supply to tissues despite fluctuations in thyroid gland function. They are present in different concentrations, with different numbers of hormone binding sites depending on the species. Transthyretin (TTR), the major binding protein in birds, binds to T4 with greater affinity than T3, and the amount of hormone bound greatly exceeds the amount of free hormone in circulation. Binding proteins play important roles in the availability and distribution of THs in the extracellular and intracellular compartments, and thus in regulating TH metabolism and kinetics (McNabb, 1992).

IV. Thyroid Development

Birds and mammals, can be divided into two categories with respect to their patterns of development: precocial and altricial. Precocial animals (quail, chickens, sheep, humans) are born/hatched in a relatively advanced state whereas altricial species (doves, mice, rabbits) are in a more immature state at birth/hatching.

Precocial young have more mature body development and the muscular and skeletal systems are more advanced than in altricial young. At hatching/birth, precocial young are capable of coordinated locomotion, their nervous systems are well developed, and their respiratory and gastrointestinal organs are functionally mature (McNabb, 1999). The thyroid gland appears early and develops considerable function during the latter half of embryonic life. Thyroids come under HPT control by mid incubation/mid gestation and circulating TH concentrations peak during the perihatch/perinatal period. The perihatch/perinatal peak in T4 concentrations is due to TSH stimulation, whereas the peak in T3 concentrations is due to increased 5'D activity at this time (birds, Thommes 1987; Freeman and McNabb, 1991; mammals, Fisher et al., 1977). Hormone concentrations decrease after hatching/birth.

Altricial young have relatively immature body development and of muscular, skeletal, and nervous systems. Hatchlings/young are incapable of coordinated locomotion and the respiratory and gastrointestinal organs have not yet reached functional maturity at hatch/birth (McNabb, 1999). The thyroid gland develops little during embryonic life and does not come under the control of the HPT axis until after hatching/birth (birds, McNichols and McNabb 1988; mammals, Fisher et al., 1977). TH concentrations are low at hatching/birth and do not increase until several weeks after hatching/birth (McNabb and King, 1993).

Herring gulls, however, are interesting in that their development cannot be described as strictly precocial or altricial, but is described as either semiprecocial (Nice, 1962; Starck, 1993) or semialtricial (Skutch, 1976). Herring gull hatchling characteristics include: downy hatchling plumage, more developed motor and locomotor activity (precocial characteristics), and dependence on parents for food (altricial characteristic) (Starck and Ricklefs, 1998). Embryonic thyroid development has not yet been studied in herring gulls, so we know nothing about the development of thyroid gland function and hormone production.

V. Disruption Of Thyroid Function In Lab Rodents

Studies on PCB effects on thyroid function in rats suggest that many coplanar (dioxin-like) PCBs alter thyroid function indirectly by altering thyroid hormone dynamics, rather than by interfering with thyroid hormone/receptor binding (as if often the case with reproductive endocrine disruption) or hormone production. Two main mechanisms have been suggested: enhanced glucuronidation of T4, resulting in increased hormone excretion from the body, and decreased binding of THs to thyroid hormone binding protein, TTR. Competitive displacement of hormones from binding proteins could increase hormone excretion by increasing the amount of free hormone.

A. Enhanced Glucuronidation of Thyroid Hormones

UDP-glucuronosyltransferase (UDP-GT), a Phase II liver microsomal enzyme, glucuronidates T4, which renders it more hydrophilic and thereby facilitates its excretion in bile, feces, and/or urine (Leonard and Koehrle, 1996). In adult rats, PCB mixtures act as direct UDP-GT inducers, increasing the glucuronidation of T4 and decreasing serum T4 concentrations (Barter and Klassen, 1994). Coplanar PCBs [#169 (3,3',4,4',5,5'-hexachlorobiphenyl), #77 (3,3',4,4'-tetrachlorobiphenyl), Morse et al., 1993; #126 (,3,3',4,4',5-pentachlorobiphenyl), and #156 (2,3,3',4,4',5-hexachlorobiphenyl), Van Birgelen et al., 1995], cause a decrease in total T4 and free T4 concentrations, and an increase in T4 UDP-GT activity in maternal, fetal, and neonatal rats.

These studies suggest that PCBs induce T4-UDP-GT activity, increase T4 excretion, and thus decrease circulating T4 concentrations. A decrease in plasma T4 concentrations facilitates (through negative feedback) TSH stimulation of the thyroid gland to produce and secrete more hormone. Increased TSH concentrations have been measured in rats when glucuronidation was induced by pollutants (Barter and Klassen, 1994).

B. Competitive Binding of PCBs to Transthyretin

PCBs (especially the dioxin-like PCBs and their hydroxylated metabolites) have some structural similarities to thyroid hormones (McKinney et al., 1985), and this observation led to the suggestion that PCBs might be competing with T4 for their thyroid binding protein, transthyretin (TTR). Thus binding of these pollutants to TTR should cause free T4 to increase, at least transiently, due to its displacement from the protein. This could enhance T4 removal from the circulation (possibly by rapid conjugation of the hormone by the liver), and excretion from the body, resulting in a decrease in circulating T4.

Pregnant mice injected with 14C-labeled CB-77 (a coplanar PCB congener), have a dose-dependent uptake of 14C-radioactivity in both maternal and fetal plasma and liver with fetal plasma levels of total radioactivity 4 to 9 times that of the mother. Electrophoresis confirms that the 14C-radioactivity in fetal plasma is bound to TTR, and 125-I bound to TTR is reduced by 50% compared to controls. Fetal plasma total and free T4 concentrations are also significantly decreased (Darnerud et al., 1996).

PCBs undergo hydroxylation (a type of metabolism) within biological systems, and it is PCB metabolites that bind with highest affinity to TTR. Competitive binding studies show that hydroxylated PCBs and their metabolites have a 3-10X higher binding affinity for TTR than for

thyroid hormones (Rickenbacher, 1986; Brouwer and Van den Berg, 1986; Lans et al., 1993). For example, 4-OH-pentaCB (2,3,3',4,5-pentachloro-4-biphenylol, a major metabolite of PCB-105), binds to TTR in vitro with a 10-fold higher affinity than T4 (Morse et al., 1995), and 4-OH-2',3,3',4',5-pentachlorobiphenyl has a 6X stronger affinity for TTR than T4 (Darnerud et al., 1996). In competitive inhibition experiments with PCBs, hydroxylated PCBs (OH-PCBs), DDT and metabolites, only OH-PCBs competed for binding to TTR (Cheek et al., 1999).

C. Adaptive Responses to Alterations in Thyroid Function in Lab Rodents

Whether PCBs alter thyroid function by displacing T4 from its binding proteins and/or by enhancing T4 glucuronidation, if circulating T4 decreases, the HPT axis will initiate feedback effects to maintain euthyroid concentrations of circulating thyroid hormones. Increased TSH concentrations (by negative feedback; HPT axis) have been observed when glucuronidation was induced by PCBs in rats (Barter and Klassen, 1994). However, Morse and Brouwer (1994) found no increase in TSH concentrations in PCB-dosed rats with low TH concentrations, suggesting that PCBs may inhibit HPT axis function.

Brain 5'D type II, which converts T4 to T3, is stimulated in PCB-exposed rodents (Morse et al., 1993, 1996), and this T3 remains in the CNS where it is produced thereby protecting CNS function. The CNS is dependent on THs for normal development and function and 5'D-II activity is known to be increased in hypothyroid conditions to maintain the intratissue T3 concentrations (Leonard and Visser, 1986). Eventually, if PCB exposure continues, 5'D-II conversion of T4 to T3 may no longer compensate for the decrease in TH concentrations (Morse et al., 1996).

In mammals, if HPT responses are insufficient to maintain normal concentrations of circulating thyroid hormones, increased 5'D type II activity in BAT may supply adequate amounts of T3 to the circulation. In rats exposed to dioxin, type II 5'D activity in BAT increases significantly; despite a decrease in serum T4 concentrations, T3 concentrations are maintained at euthyroid levels (Raasmaja et al., 1996).

VI. Alterations Of Thyroid Gland Function In Birds

To date, there is less known about PCBs effects on thyroid function in birds than in laboratory rodents. However, many developmental and physiological abnormalities in birds exposed to pollutants are suggestive of thyroid alterations. Most goiters are a compensatory response of the thyroid gland to stimulation by TSH. Thyroid gland enlargement (goiter) and epithelial hyperplasia has been observed in herring gulls from polluted Great Lakes sites (Fox et al., 1993; Moccia et al., 1986) and in PCB-dosed lesser black-backed gulls and guillemots (Jefferies and Parslows, 1972). Homing pigeons fed DDT, DDE, dieldrin, and PCBs also show signs of goiter and alterations in thyroid histology (Jefferies and French, 1972). Lower incubation attentiveness, resulting from nervous system dysfunction, could be due to deficient thyroid hormone supply to the CNS. Wild birds exposed to pollutants [Forster's terns from Green bay and herring gulls from Lake Ontario (Fox et al., 1978)] have lower incubation attentiveness. Likewise, in laboratory studies on ring doves fed PCBs (Peakall and Peakall, 1973), and in ring doves dosed with a PCB-organochlorine mixture (McArthur et al., 1983) incubation attentiveness was altered. Thyroid hormones are also necessary for maintenance of normal metabolic function. Alterations in metabolic rate and 02 consumption have been

observed in PCB-fed morning doves (Mayer and Tori, 1981) and pigeons fed dieldrin and DDE (Jefferies and French, 1971).

More recent studies of laboratory and wild-caught birds have measured thyroid-related variables to access possible thyroid alterations from PCB exposure. In 28-day old Eider ducklings injected with PCB-77 (3,3',4,4'-tetrachlorobiphenyl, a coplanar dioxin-like congener) plasma TT4 and TT3 concentrations decreased (Murk et al., 1994). Van den Berg (1994) found significant reductions in plasma thyroid hormones in cormorants at 1 of 2 polluted sites studied in the Netherlands. However, though plasma TT4, FT4 and TT3 levels were reduced, only FT4 concentrations were significantly correlated with site PCB concentrations.

Surprisingly, in breeding doves fed PCB mixtures, there was a significant increase in circulating thyroxine concentrations (McAuthur et al., 1983). Increased thyroxine levels were correlated with total wing-flipping and in-bowl activities of courtship pairs, suggesting thyroxine-induced hyperactivity. There were also decreases in incubation attentiveness and brooding activity, and the incubation period was extended by 3 days. Hyperactivity also was observed by Peakall and Peakall (1973) in PCB-dosed doves.

Other studies on PCB exposed birds show no alterations in circulating thyroid hormones. In Japanese Quail dosed with two mixed PCB preparations, there were no differences in plasma thyroid hormone concentrations despite signs of goiter development (Grassle and Biessmann, 1982). Likewise, no changes were found in plasma thyroid hormone concentrations in 3-week old herring gulls injected with two doses of PCB-77 (Brouwer, 1991). The herring gulls also showed no signs of thyroid hyperplasia. Some wild-caught birds from polluted sites also show no alterations in circulating thyroid hormones. In herring gull and Caspian tern chicks, plasma T4 levels did not differ in Great Lakes sites with different PCB concentrations (Grasman et al., 1996). Murk et al (1994) also found no consistent pattern of a decrease in TH concentrations with an increase in PCB exposure in chicks of the common tern in coastal waters of the Netherlands and Belgium.

From these studies, it is difficult to evaluate how PCBs affect thyroid function in birds. There is no clear pattern showing that PCB exposure alters thyroid hormone dynamics. Also, the lack of obvious alterations in plasma T4 concentrations in some birds exposed to PCBs suggests that these birds may be compensating for PCB effects by increasing TSH secretion. Whereas in mammals, BAT 5'D type II activity supplies T3 to the circulation even if circulating thyroid hormones concentrations are low, birds lack BAT, so this source of T3 production is not available in avian systems. To date, no studies have addressed whether brain 5'D type II activity increases upon pollutant exposure in birds.

With respect to possible mechanisms causing alterations in thyroid function in birds, one study illustrates induction of UDP-GT in Japanese Quail exposed to PCBs (Riviere et al., 1978). To date, neither PCB binding to avian TTR nor the consequences of this binding have been addressed experimentally.

Future studies need to address the extent to which adaptive thyroid responses may compensate for the effects of PCBs mixtures and specific congeners, and the exposure time and concentration of congeners that elicit a thyroid specific biological response. Many more studies need to be conducted both on field-caught birds and in laboratory species. Many important wildlife species are oviparous, and it is important to understand how PCBs may be affecting developing embryos in the egg (verses mammals developing *in utero*), hatchlings (after absorption of possible contaminated yolk; feeding on contaminated fish) and adult birds (longer

period of exposure through contaminated food). Fundamental differences in thyroid function between birds and mammals include: differences in the proportions of T4 and T3 in the circulation, differences in TH binding proteins, and lack of BAT in birds. More studies need to be done to evaluate PCB effects on thyroid function in birds and to determine if PCB effects on thyroid function in birds are comparable to that seen in mammals.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are abundant and widespread contaminants in the Great Lakes and are found in the tissues of fish-eating birds (Fox et al., 1993). Exposure to PCBs is thought to cause endocrine problems resulting in alterations in growth and development of vertebrate species (Fox et al., 1993; Brucker-Davis, 1999). To date, the majority of the focus of endocrine disruption research has been on effects on reproductive function and development, with lesser attention to disruption of other endocrine systems. Thyroid hormones (TH) are essential for the development and continued function of many organs and tissues, including the central nervous system, as well as being necessary for growth and metabolism in vertebrates.

Studies of laboratory rodents indicate that PCB mixtures and a number of specific PCB congeners alter thyroid function indirectly by effects on thyroid hormone dynamics, rather than by interfering with hormone production or hormone action (i.e. thyroid hormone to receptor binding). Two main mechanisms of disruption have been suggested: enhanced glucuronidation of T4 and decreased binding of THs to the thyroid binding protein, transthyretin (TTR). Dioxin-like PCBs, administered to rats, induce the microsomal enzyme UDP-glucuronosyltransferase (UDP-GT), which facilitates TH excretion (by rendering them more hydrophilic), and decreases circulating TH concentrations (Barter and Klassen, 1994; Van Birgelen et al., 1994). PCBs also may affect thyroid function in rats by binding to TTR, displacing T4 and facilitating its excretion by liver enzymes, resulting in decreased plasma T4 concentrations (Bastomsky, 1974; Darnerud et al., 1996). In PCB exposed rats, FT4 and total T4 (TT4) concentrations are decreased (Darnerud et al., 1996, Morse et al., 1996).

Decreased circulating T4 concentrations may trigger fundamental adaptive responses by the HPT axis to maintain TH homeostasis in rats. TSH stimulation of the thyroid gland increases when circulating concentrations of THs are low and increased TSH concentrations have been observed (Barter and Klassen, 1994) when increased glucuronidation was induced by PCB exposure. However, Morse and Brouwer (1994) found no increase in TSH concentrations in PCB-dosed rats with low circulatingTH concentrations, suggesting that PCBs may inhibit HPT axis function. It is unclear why discrepancies occur among these different studies. When PCB exposure is high or prolonged, and circulating THs are low, the HPT axis stimulation of thyroid gland activity may not be able to maintain circulating TH concentrations, and there may be an adaptive enhancement of type II 5'D activity. Type II 5'D circulating enzyme increases the conversion of T4 to T3 in the brain and pituitary and thus "protects" these tissues from alterations in circulating TH supply (Morse et al., 1993, 1996).

To date, little is known about PCB affects on thyroid function in birds. Field studies examining birds from polluted areas, such as the Great Lakes, have measured few thyroid-related variables, and the limited laboratory studies done on birds give no clear indication of alterations in thyroid function. Wild birds from the most contaminated areas of the Great Lakes have developmental, behavioral, and reproductive abnormalities that could be indicative of thyroid deficiencies. Herring gulls from Lake Ontario and Forster's terns from Green Bay were shown to have lower incubation attentiveness (Fox et al., 1978). Incubation attentiveness also was altered in ring doves fed diets containing PCBs (Peakall and Peakall, 1973) and a PCB-organochlorine mixture (McArthur et al., 1983). The metabolic rate of PCB-fed mourning doves was decreased, suggesting an inability to thermoregulate (Mayer and Tori, 1981). Thyroid gland enlargement

(goiter) and epithelial hyperplasia have been observed in thyroids of Great Lakes' herring gulls (Moccia et al., 1986) and PCB-dosed lesser black-backed gulls and guillemots (Jefferies and Parslow, 1972). Feeding birds PCBs resulted in goiter, alterations in thyroid histology, decreased metabolic rate and O2 consumption (Jefferies and French, 1972).

In several more recent studies that measured thyroid-related variables, there were no clear patterns showing that PCB exposure altered thyroid hormone dynamics in birds. Plasma T4 concentrations did not differ in herring gull and Caspian tern chicks from Great Lakes sites with different amounts of PCB contamination (Grasman et al., 1996). Murk et al (1994) also found no consistent pattern of decreased TH concentrations in PCB exposed common tern chicks in coastal waters of the Netherlands and Belgium. Likewise, in laboratory studies on Japanese quail dosed with two PCB preparations, there were no differences in plasma THs at any dose levels despite some thyroid gland hypertrophy (Grassle and Biessmann, 1982). Van den Berg et al (1994) found decreases in FT4 concentrations in cormorants exposed to PCBs in the Netherlands but no overall relationship with site PCBs. Some studies evaluating PCB effects on thyroid function in birds have shown decreased plasma T4 concentrations. In 28-day-old eider ducklings injected with PCB-77 (3,3',4,4'-tetrachlorobiphenyls; a coplanar dioxin-like congener) there were decreases in plasma TT4 and TT3 (Murk et al., 1994).

From these studies, it is difficult to evaluate how PCBs affect thyroid function in birds. The lack of obvious alterations in plasma T4 concentrations, despite evidence of TG changes, in some birds exposed to PCBs suggests that these birds may be able to compensate for PCB effects by increased TSH stimulation of thyroid gland activity to maintain TH supply. Whereas in mammals, BAT 5'D type II activity increases T3 supply to the circulation if circulating THs are low, birds lack BAT, so this source of T3 production is not available. To date, no studies have addressed whether brain 5'D type II activity increases upon pollutant exposure in birds.

The general objective of this study was to examine the regulation of thyroid hormone supply during development in (1) herring gull (*Larus argentatus*) embryos and pre-fledglings from Great Lakes sites with different chemical pollutant exposures and in (2) domestic chicken embryos (*Gallus domesticus*) exposed to a specific dioxin-like PCB congener (PCB-126). To address this objective, I evaluated the effects of environmental pollutant exposure and PCB-126 on thyroid status by measuring (1) plasma thyroid hormone concentrations and (2) brain T4 to T3 conversion (type II 5'D activity) to determine if mixed pollutant exposure of herring gulls or PCB-126 exposure of chicken embryos was associated with alteration in brain 5'D type II activity that could maintain local T3 supply to the brain.

MATERIALS AND METHODS

Animals

Chicken eggs were injected (into the air cell) with 5 dose levels (0.0512, 0.128, 0.32, 0.64, 0.8 ng/g) of PCB-126 (3,3',4,4',5-Pentachlorobiphenyl, Ultra Scientific, North Kingston, RI), or vehicle (sunflower oil), before incubation; sampling was on day 20 of the 21-day incubation period. Dr. Keith Grasman's laboratory, at Wright State University, Dayton, OH, did the injection and sampling. Herring gull embryos (at pipping; 26 days of incubation), and 28-day pre-fledgling chicks were sampled by Dr. Keith Grasman and Mr. Glen Fox (Canadian Fish and Wildlife Service, Quebec, Canada) at several Great Lakes sites with different contaminant exposures (Table 1). Ten individuals were sampled at each site. Both the chicken and herring gull tissues and plasma were stored at –80C until shipping to our laboratory, on dry ice, for thyroid studies.

Plasma hormone analysis

Plasma T4 and T3 concentrations were measured with a double antibody RIA by the method of McNabb and Hughes (1983) using standards prepared in hormone-stripped chicken plasma. Primary antibodies were purchased from Endocrine Sciences (Calabasas,CA), ^{125}I-T4 and ^{125}I-T3 were from New England Nuclear (Boston, MA; high specific activity: T4, 1250 µCi/µg; T3, 1200 µCi/µg), and carrier immunoglobulin was from Antibodies Incorporated (Davis, CA). Secondary Antibody was kindly provided by Dr. John McMurty (USDA; Beltsville, MD). Assay volumes were 12.5 µl for T4 and 25 µl for T3. The plasma assay was validated for use in chickens and Herring gulls by demonstrating parallelism of serially diluted and spiked samples with the standard curve.

Brain Homogenate

Brain tissue (approximately 0.7 gram) was thawed on ice and homogenized with 3 volumes of phosphate buffer using a hand-held homogenizer (Kontes Potter-Elvehjem tissue grinder with glass pestle). Phosphate buffer was composed of 0.2 M monobasic sodium phosphate, monohydrate ($NaH_2PO_4 \cdot H_20$) and 0.2 M dibasic sodium phosphate, heptahydrate ($NaHPO_4 \cdot 7H_20$), pH 7.4). For Herring gull assays, this crude homogenate was used directly for analysis of deiodinase activity. For chicken embryo assays, a postmitochondrial fraction (PMF) was prepared in which the crude homogenate was centrifuged at 13,000*g* at 4C for 2 min. and the supernatant held on ice. The precipitate was washed with a volume of phosphate buffer equal to the volume of supernatant and recentrifuged as before. The supernatant and wash were combined to yield a 12.5% PMF. Both the crude homogenate and PMF were stored at –80C until analysis.

Brain type II thyroxine 5'-deiodinase

Validation of the 5'D assay was done by demonstrating proportionality of activity to enzyme concentration and linearity of activity over assay time. Initial assay conditions were determined using brains from Japanese Quail available in our laboratory before final validations were tested on pooled tissue from PCB-dosed chicken embryos and herring gulls. These validations determined the concentrations of homogenate and the reaction time used for the standard assays. Measurements of deiodinase activity were adapted from the methods of Freeman and McNabb (1991), Morse et al. (1993, 1996), and Rudas et al. (1993). Freeman and McNabb's work was on liver type I deiodinase activity in birds, Morse et al. studied brain type II deiodinase in rats, and Rudas et al. studied brain type II deiodinase in birds. PMF was used in assays on PCB-treated chicken embryos because assays with homogenate had high variability in replicate tubes from individual samples. Homogenate (instead of PMF) was used in assays on herring gulls because PMF gave little to no 5'D-II activity.

The brain 5'D assay, with an incubation volume of 180 µl, consisted of the following components: 100 µl homogenate or PMF, 15 µl phosphate buffer (pH 7.4), 20 µl dithiothreitol (DTT cofactor, Sigma, St. Louis, MO), 10 µl T3 (to inhibit inner-ring deiodination of T4 by type III deiodinase), 10 µl propylthiouracil (PTU, Sigma, St. Louis, MO, to inhibit type I deiodinase), 15 µl T4 and 10 µl ^{125}I-T4 (10^5 cpm, SA, 750-1250 µCi/µg; New England Nuclear Corp., Boston, MA). Final concentrations in the reaction mixture were: 100 mM phosphate buffer, 25 mM DTT, 100 nM T3, 1 mM PTU, 4.3 nM T4 (appoximately 2x the K*m* of the enzyme, Rudas et al., 1993). Incubations were carried out at 37ºC for 30 min. The reaction was stopped by addition of 75 µl 4% bovine serum albumin (BSA) and precipitated by 500 µl 20% trichloroacetic acid (TCA). Blanks consisted of tissue-free incubations, samples in which the reaction was terminated immediately after the addition of substrate, or samples with boiled tissue, each giving the same results. After TCA addition, the tubes were held on ice for 15 minutes then centrifuged at 4,000*g* at –10C for 30 minutes. Supernatant (500 µl) was applied to Bio-Rad "Dowex" AG 50W-X8 (H+) ion-exchange columns with a 2 ml bed volume (poly-prep chromatography columns; Bio-Rad Laboratories, Hercules, CA) that had been prewashed with 10 ml 1.74 M acetic acid. Labelled iodide released was eluted with three 5 ml aliquots of 1.74 M acetic acid and counted. Iodide released was calculated by subtracting the sum of the 3 washes from the total cpm in the original sample and expressed as a percent of the total cpm. This value was then corrected for the percent iodide in the blank and multiplied by two because of random labeling at the 3' or 5' positions of the phenolic ring of T4 (the labelled iodide released accounts for only half of the 5'D activity). Homogenate protein was measured by Pierce Coomassie Protein Assay (Pierce, Rockford, IL). Results were expressed as femtomoles T4 deiodinated per hour per milligram protein (fmol I rel/hr mg protein).

Statistical Analysis

For validation of 5'D assay, an unweighted least-squares procedure was used to determine regression analyses. All other data were analyzed using students t-test and one-way analysis of variance (ANOVA). Values of $p < 0.05$ were considered indicative of statistically significance differences.

RESULTS

Validation Studies for 5'Deiodinase Assay

Chicken embryos

Proportionality of enzyme activity with enzyme concentration was demonstrated using PMF up to 5.54 mg PMF protein/ml in the reaction mixture (Fig 1). Specific activity (fmol I released/min.mg tissue) was constant over the proportional range. Enzyme activity also was linear with time up to at least 70 minutes of incubation using 2.77 mg PMF protein/ml (Fig 2). Standard assay conditions of 2.77 mg PMF protein/ml and a 30-minute incubation time were based on these validation studies.

Herring gulls

Proportionality of activity with enzyme concentration was demonstrated using homogenate, up to 14.68 mg homogenate protein/ml in the reaction mixture (Fig 3). Linearity of activity (fmol I released) with incubation time, up to at least 45 minutes, was demonstrated using 14.68 mg homogenate protein/ml (Fig 4). Standard assay conditions of 14.68 mg homogenate protein/ml and a 30-minute incubation time were based on these validation studies.

Analysis of Data

Studies on PCB-treated embryos included a preliminary study, April 1998, and a larger study encompassing August 1998, October 1999, and January 2000.
In 1998, herring gull egg homogenates were sampled and analyzed for PCB concentrations. All 1998 data were graphed with Kent Island being the reference site (see table 1). Egg PCB concentrations for 1999 have not been analyzed yet, so 1999 data are graphed in relation to 1998 PCB concentrations.

Plasma Hormones

PCB-treated Chicken Embryos

There were no statistically significant differences in plasma T4 concentrations in treated embryos compared to controls (non-injected or vehicle injected) in both the preliminary and the larger study (Fig 5a, 5b). Likewise, plasma T3 concentrations did not differ with PCB treatment in the preliminary study (Fig 6a). In the larger study, plasma T3 concentrations were significantly increased at a dose of .05 ng/g egg (increased by 224% compared to N.I, increased by 233% compared to 0) and .48 ng/g egg [(increased by 234% compared to N.I, increased by 244% compared to 0), Fig 6b].

1998 Herring Gulls Pipped Embryos

There were no significant differences in plasma T4 or T3 at any of the sampling sites when compared to Kent Island (Figs 7,8).

1999 Herring Gulls

Although pipped embryos were not sampled at Kent Island in 1999, Chantry Island, having low PCB concentrations, may also be considered a reference site. Of the 3 sites that pipped embryos were sampled (Chantry Island, Detroit Edison, and West Sister Island), there were no significant differences in plasma T4 concentrations (Fig 9a).

In pre-fledglings, plasma T4 concentrations were significantly less from Detroit Edison (147%) and West Sister Island (146%) when compared to pre-fledglings from Kent Island (Fig 9b).

In pipped embryos, plasma T3 concentrations were significantly less from West Sister Island when compared to both Chantry Island and Detroit Edison [(29% and 36% decrease respectively), Fig 10a].

In pre-fledglings, plasma T3 concentrations were significantly less from Detroit Edison (68%) and West Sister Island (67%) when compared to pre-fledglings from Kent Island (Fig 10b).

Brain Type II Thyroxine 5'Deiodinase

PCB-treated Chicken Embryos

The preliminary study used 1.3 nM T4, (instead of 4.3 nM T4 that was used in the larger study), which resulted in low deiodinase activity (see Fig 11). The preliminary assay was then rerun using 4.3 nM T4, after tissue had been stored as PMF at –80C for 1 year. The activity was very low, indicating a loss of enzyme activity (though the actual pattern of activity across doses was similar to that seen in prior assays).

In the preliminary study, there were no significant differences in 5'D-II activity in control versus PCB-treated birds, though 5'D-II activity tended to increase with an increase in PCB dose (Fig 11a). In the larger study, there was a significant decrease in 5'D-II activity in embryos treated with .05 ng/g PCB-126 (82% compared to N.I, 67% compared to 0), but no significant differences in activity in embryos treated with higher doses (Fig 11b).

1998 Herring Gull Pipped Embryos

There was a significant increase in 5'D-II activity in pipped embryos from Strachnan Island (70%) when compared to pipped embryos from Kent Island (Fig 12).

In pre-fledglings, there was a significant decrease in 5'D-II activity from Detroit Edison (43%) when compared to Kent Island (Fig 13b).

1999 Herring Gulls

In the three sites sampled, 5’D-II activity was significantly lower in pipped embryos from West Sister Island in comparison to both Chantry Island and Detroit Edison [(decreased by 36% and 37% respectively), Fig 13a].

DISCUSSION

The present study of brain 5'deiodinase activity was conducted as part of a comprehensive evaluation of the effects on thyroid function of PCBs and environmental pollutant mixtures rich in PCBs, in both chicken embryos exposed to different doses of PCB-126 and field-caught herring gulls exposed to mixed pollutants in the Great Lakes. PCBs are known to disrupt thyroid function in laboratory rats; exposure to specific PCB congeners results in decreased circulating TH concentrations (Barter and Klassen, 1994, Morse et al., 1993, 1996), resulting in increased TSH stimulation of the thyroid gland (Barter and Klassen, 1994), and increased brain 5'D type II activity that is thought to maintain T3 supply to the brain (Morse et al., 1993, 1996). In the present study, we hypothesized that in chicken embryos, exposure to PCB-126 would result in dose-dependent decreases in circulating thyroid hormones and subsequent increases in brain 5'D type II activity. The maintenance of circulating thyroid hormone concentrations in PCB exposed embryos, compared to those in control embryos, suggest that these levels of PCB exposure do not alter function in a manner that would have developmental consequences. However, circulating hormone measurements alone do not address whether adaptive responses could be occurring to maintain normal thyroid hormone concentrations in the circulation and in critical tissues. Adaptive responses that could occur include (1) increased brain 5'D-II activity to maintain local T3 supply and (2) increased TSH stimulation of the thyroid gland to produce and secrete more hormone. In this study, there were no increases in brain 5'D-II activity at any of the PCB doses. Also, increased TSH stimulation was not indicated by analysis of thyroid gland weight and thyroid gland hormone content (personal communication with Dr. Anne McNabb and Kathy Parsons). Overall, there was no indication that PCB-126 altered thyroid function in chicken embryos.

PCB-126 has been known to decrease T4 concentrations in rats (Van Birgelen et al., 1995), as well as cause developmental abnormalities in chickens (Powell et al., 1996). A laboratory study on Eider ducklings dosed with another coplanar PCB (#77) showed decreases in TH concentrations (Murk et al., 1994), while another study on Japanese quail fed Arochlor 1254 (a PCB mixture) showed no differences in plasma TH concentrations despite thyroid gland hypertrophy (Grassle and Biessmann, 1982).

Field studies on birds, environmentally exposed to pollutant mixtures, show no clear pattern of altered thyroid function among sites with different pollutant concentrations (Grasman et al., 1996, Murk et al., 1994, Van den Berg et al., 1994). In the present study, we hypothesized that herring gull embryos and pre-fledglings from Great Lakes' sites with higher contaminant loads would have greater alterations in thyroid function than herring gulls from sites with lower contaminant loads. We found no clear pattern of altered thyroid function in herring gulls from polluted Great Lakes' sites. As mentioned before, when circulating TH concentrations are low, brain 5'D-II activity increases to protect intratissue T3 concentrations (Leonard and Visser, 1986). In this study, 5'D-II activity did not significantly increase in birds from more contaminated sites in comparison to birds from Kent Island or sites with less contamination. Although 1998 pipped embryos from Strachnan Island showed significant increases in 5'D-II activity when compared to Kent Island, there were no differences in brain 5'D-II activity at sites with greater PCB loads than Strachnan Island. Also, pipped embryos from Strachnan Island

showed no differences in plasma TH concentrations. A previous study on field-caught herring gulls from the Great Lakes showed no alterations in TH concentrations (Grasman et al., 1996). However, Eider ducks and cormorants in polluted sites from coastal regions of the Netherlands and Belgium have decreased plasma T4 concentrations compared to those from more pristine locations (Murk et al., 1994; Van den Berg et al., 1994).

The present study is the first to evaluate the potential effects of PCBs, alone and in a mixed environmental exposure, on circulating THs and brain 5'D-II activity in developing birds. Although thyroid function was not altered by the specific PCB congener used in my study, more complete evaluations are needed before determining whether PCBs alter thyroid function in birds. Future studies on PCB dosed chicken embryos should include non-coplanar PCBs (e.g. PCB-153) and PCB mixtures. Non-coplanar PCBs are found in high concentrations in the environment and show interactions (additive effects) with coplanar PCBs in laboratory studies of embryonic mortality, deformities and immune function (Van der Kolk et al., 1992). Wild birds are exposed to a mixture of PCBs, rather than one specific PCB congener, and some of the strongest evidence for TH alterations has been with PCB mixtures. Studies should assess PCB effects on several developmental stages during different times of growth and maturation of specific tissues such as the brain. Herring gulls are exposed to pollutants mainly through their diet (herring gulls are top predators; fish-eating birds), therefore older birds would be expected to have greater PCB loads and show greater thyroid alterations. Future studies on field-caught herring gulls should include more age groups to determine if thyroid function is being altered.

LITERATURE CITED

Barter RA & Klassen CD (1994). Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-Glucuronoyltransferase inducers in rats. *Toxicol. and Appl. Pharmacol.* **128,** 9-17.

Bastomsky CH (1974) Effects of a polychlorinated biphenyl mixture (Arochlor 1254) and DDT on biliary thyroxine excretion in rats. *Endocrinology* **95**, 1150-1155.

Bowerman WW, Giesy JP, Best DA & Kramer VJ (1995). A Review of Factors Affecting Productivity of Bald Eagles in the Great Lakes Region: Implications for Recovery. *Env. Health Perspec*. **103**, 51-59.

Brouwer A (1991). Role of biotransformation in PCB-induced alterations in vitaminA and thyroid hormone metabolism in laboratory and wildlife species. *Biochem. Soc. Trans.* **13**, 731-737.

Brouwer A & Van den Berg KJ (1986) Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum Vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. *Toxicol. And Appl. Pharmacol.* **85**, 301-312.

Brucker-Davis F (1998) Effects of environmental synthetic chemicals on thyroid function. *Thyroid* **8**, 827-856.

Cheek AO, Kow K, Chen J and McLachlan JA (1999) Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Env. Health Perspec*. **107**, 273-278.

Colborn T, Dumanoski D, & Myers JP (1996) Our Stolen Future. New York: Penguin Books.

Darnerud PO, Morse D, Klasson-Wehler E, & Brouwer A (1996). Binding of a 3,3',4,4'-tetrachlorobiphenyl (CB-77) metabolite to fetal transthyretin and effects on fetal thyroid hormone levels in mice. *Toxicology* **106**, 105-114.

Fisher DA, Dussault JS & Chopra IJ (1997). Ontogenesis of hypothalamic-pituitary-thyroid function and metabolism in man, sheep, and rat. *Rec. Progr. Horm. Res.* **33**, 59-116.

Fox GA (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. *Journal of Nutrition* **125**, 722-736.

Fox GA, Gilman AP, Peakall DB & Anderka FW (1978). Behavioral abnormalities of nesting Lake Ontario herring gulls. *J. Wildl. Manage.* **42**, 477.

Freeman TB & McNabb FMA (1991). Hepatic 5'-Deiodinase Activity of Japanese Quail Using Reverse-T3 as Substrate: Assay Validation, Characterization, and Developmental Studies. *Journ. of Exper. Zool*. **258**, 212-220.

Fry DM & Toone CK (1981). DDT-induced fertilization of gull embryos. *Science* **213**, 922-924.

Grasman KA, Fox GA, Scanlon PF, Ludwig JP (1996) Organochlorine-associated immunosuppression in prefledgling Caspian terns and herring gulls from the Great Lakes: An ecoepidemiological study. *Env. Health Perspec*. **104**, 829-842.

Grassle B & Biessmann A (1982) Effects of DDT, polychlorinated biphenyls and thiouracil on circulating thyroid hormones, thyroid histology and eggshell quality in Japanese quail (*Coturnix coturnix japonica)*. *Chem-Biol Interactions* **42**, 371-377.

Guillette Jr LJ, Crain DA, Endocrine-disrupting contaminants and reproductive abnormalities in reptiles. *Toxicology* **5**, 381-399.

Jefferies DJ & French MC (1973). Changes induced in the pigeon thyroid by p,p'DDE and dieldrin. *Journal of Wildlife Management* **36** (1), 24-30.

Jefferies DJ & French MC (1971). Hyper- and hypothyroidism in pigeons fed DDT: an explanations for the thin eggshell phenomenon. *Environ. Pollut*. **1**, 235-242.

Jefferies DJ & Parslow JFL (1972). Effect of one polychlorinated biphenyl on size and activity of the gull thyroid. *Bull Environ. Contamin. Toxicol.* **8**, 306-310.

Lans MC, Klasson-Wehler E, Willemsen M, Meussen E., Safe S & Brouwer A. 1993. Structure–dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-*p*-dioxins and dibenzofurans with human transthyretin. *Chem-Biol Interactions* **88**:7-21.

Leonard JL & Koehrle J (1996). Werner and Ingbar's The Thyroid a Fundamental and Clinical Text 7th ed. Philadelphia: Harper & Row Inc., 125-161.

Leonard JL & Visser TJ (1986). Biochemistry of deiodination In: Hennemann G. ed. Thyroid Hormone Metabolism. W. B. Saunders, Philadelphia, 189-229.

Mayer LP & Tori GM (1981). Effects of polychlorinated biphenyls on the metabolic rates of morning doves exposed to low ambient temperatures. *Bull Environm. Contam. Toxicol.* **27**, 678-682.

McArthur MLB, Fox GA, Peakall DB & Philogene BJR (1983). Ecological Significance of behavioral and hormonal abnormalities in breeding ring doves fed an organochlorine chemical mixture. *Environ. Contam. And Tox.* **12**, 343-353.

McKinney JD, Fawkes J, Jordan S, Chae K, Oatley S, Coleman RE, & Briner W. (1985). 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) as a Potent and Persistent Thyroxine Agonist: A Mechanistic Model for Toxicity Based on Molecular Reactivity. *Environ. Health Perspectives* **61**, 41-53.

McNabb FMA (1992) Thyroid Hormones New Jersey: Prentice Hall

McNabb, FMA & Hughes TE (1983). The role of serum binding proteins n determining free thyroid hormone concentrations during development in quail. *Endocrinology* **113**, 957-963.

McNabb FMA & King DB (1993). Thyroid Hormone Effects on Growth, Development, and Metabolism. pp. 393-417. Academic Press, New York.

McNabb FMA (1999). Altricial and precocial development in birds. *Encyclopedia of Reproduction* **vol 1**, 113-118.

McNichols MJ & McNabb FMA (1988). Development of thyroid function and its pituitary control in embryonic and hatchling precocial Japanese quail and altricial Ring doves. *Gen. Comp. Endocrinol.* **69**, 109-118.

Moccia RD, Fox GA & Britton A. (1986). A Quantitative assessmemt of thyroid histopathology of Herring Gulls (Larus Argentatus) from the great lakes and a hypothesis on the casual role of environmental contaminants. J. *Wildlife Disease* **22**(1), 60-70.

Morse D & Brouwer A (1994). Perinatal alterations of thyroid hormone homeostasis and long term neurochemical alterations in rats following maternal Aroclor 1254 exposure. *Organohalogen Compounds* **21**, 439.

Morse DC, Groen D, Veerman M, Van Amerongen CJ, Koeter DBWM, Smits Van Prooije, AE, Visser TJ, Koeman JH & Brouwer A. (1993). Interference of Polychlorinated Biphenyls in Hepatic and Brain Thyrid Hormone Metabolism in Fetal and Neonatal Rats. *Toxicol. Appl. Pharmacol*. **122**, 27-33.

Morse DC, Wehler EK, Wesseling W, Koeman JH & Brouwer A. (1996). Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicology and Applied Pharmacology* **136,** 269-279.

Murk AJ, Van den Berg JHJ, Fellinger M, Rozemeijer MJC, Swennen C, Duiven P, Boon JP, Brouwer A & Koeman JH (1994b) Toxic and biochemical effects of 3,3',4,4'-

tetrachlorobiphenyl (CB-77) and Clophen A50 on eider ducklings (*Somateria mollissima*) in a semi-field experiment. *Environ. Pollut*. **86**, 21-30.

Ness DK, Schantz SL, Moshtaghian J & Hansen LG (1993). Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. *Toxicology Lett*ers **68,** 311-323.

Nessel CS & Gallo MA (1992) Dioxins and Related Compounds. In:Environmental Toxicants. Human Exposures and their Health Effects. ed. M. Lippmann., New York: Van-Nostrand Reinhold. Ch. 6, 163-183.

Nice, MM (1962). Development of behavior in precocial birds. *Trans. Linn. Soc.* (NY) **8**, 1-211.

Peakall DB & Peakall ML (1973). Effect of a polychlorinated biphenyl on the reproduction of artificially and naturally incubated dove eggs. *J. Appl. Ecol.* **10**, 103.

Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, Stromborg KL & Bursian SJ (1996) *Environ. Contam. And Tox.* **31**, 404-409.

Raasmaja A, Viluksela M & Rozman KK (1996). Decreased liver type I 5'-deiodinase and increased brown adipose tissue type II 5'-deiodinase activity in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated Long-Evans rats. *Toxicology* **114,** 199-205.

Rickenbacher U, McKinney JD, Oatley SJ & Blake CCF (1986) Structurally specific binding of halogenated biphenyls to thyroxine transport protein. *Journal of Med. Chem*. **29,** 641-648.

Riviere JL, De Lavaur E & Grolleau G (1978). Effect of polychlorinated biphenyls on drug metabolism in Japanese quail and its progeny. *Toxicology* **11**, 329-334.

Robbins J (1996). Werner and Ingbar's The Thyroid a Fundamental and Clinical Text 7th ed. Philadelphia: Harper & Row Inc., 96-110.

Rudas P, Bartha T & Frenyo LV (1993). Thyroid hormone deiodination in the brain of young chickens acutely adapts to changes in thyroid status. *Acta Veterinaria Hungarica* **41**, 381-393.

Starck JM (1993). Evolution of avian ontogenies. *Curr. Orn.* **10**, 275-366.

Starck JM & Ricklefs RE (1998). Avian Growth and Development: Evolution with the Altricial-Precocial Spectrum New York: Oxford University Press, 3-29.

Skutch, AF (1976). Parent birds and their young. University of Texas Press, Austin.

Safe, SH (1990) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *CRC Crit. Rev. Toxicol.* **21**, 51.

Thommes RC (1987). Ontogenesis of thyroid function and regulation in the developing chick embryo. *J. Exp. Zool. Suppl.* **1**, 273-279.

Van Birgelen APJM, Van der Kolk J, Poiger H, Van den Berg M & Brouwer A (1992) Interactive effects of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on thyroid hormone, vitamin A, and viamin K metabolism in the rat. *Chemosphere* **25**, 1239-1244.

Van Birgelen APJM, Smit EA, Kampen IM, Groeneveld CN, Fase KM, Van der Kolk J, Poiger H, Van den Berg M, Koeman JH & Brouwer A (1995). Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. *European Journal of Pharmacology* **293,** 77-85.

Van den Berg M, Craane BLHJ, Sinnige T, Van Mourik S, Dirksen S, Boudewijn T, Van der Gaag M, Lutke-Schipholt IJ, Spenkelink B & Brouwer A (1994) Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after *ovo* exposure. *Env. Tox. And Chem.* **13**, 803-816.

Van der Kolk, J, Van Birgelen APJM, Poiger H & Schlatter C (1992). Interactions of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. *Chemosphere* **25**, 2023.

Table 1: 1998 Herring Gull Sampling

A: PCB Concentrations of Pooled Egg Homogenates

Sampling Site	Sum of PCBs (In PPM)	PCB Ranking
Kent Island (K)	1.508	1
Chantry Island	2.898	2
Scotch Bonnet (S)	7.856	3
Strachnan Island (STR)	11.207	4
Detroit Edison (DE)*	13.00	5
West Sister Island (WS)	16.092	6
Saginaw Bay (SB)	21.224	7

*Egg homogenates were sampled but not analyzed, so the PCB concentration at Detroit Edison is based on analysis in 1997.

B: Arochlor Concentrations of Pooled Egg Homogenates

Sampling Site	Arochlor Concentration (PPM)
Kent Island (K)	1.961
Scotch Bonnet (S)	Not analyzed
Strachnan Island (STR)	17.128
Detroit Edison (DE)*	15.3472
West Sister Island (WS)	21.838
Saginaw Bay (SB)	35.002

*Egg homogenates were sampled but not analyzed, so the Arochlor concentration at Detroit Edison is based on analysis in 1997.

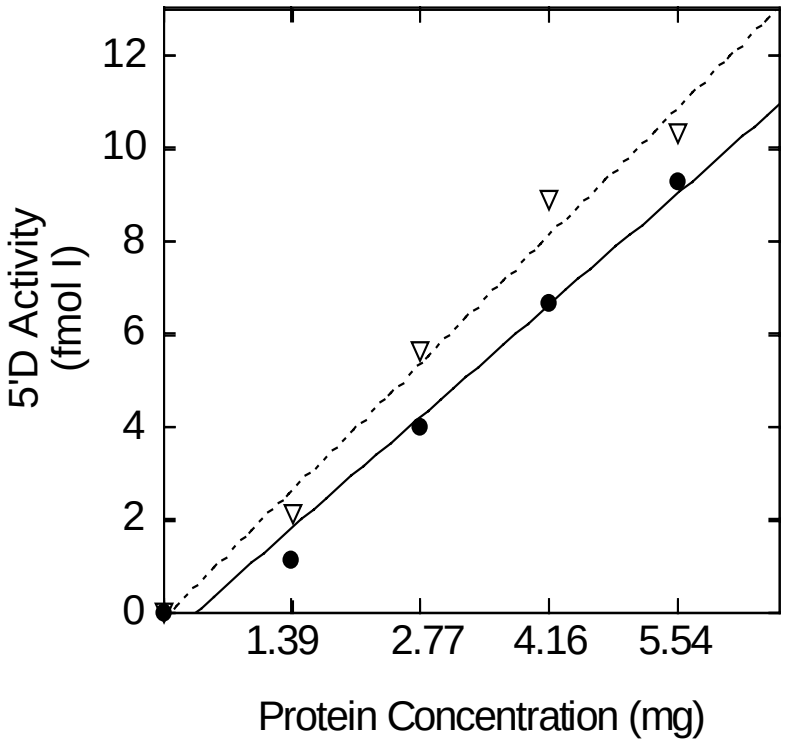

Fig 1. Proportionality of T4 5'D activity with enzyme concentration (postmitochondrial fraction) of pooled brain samples fromchicken. embryos. ● Control embryos ▽ PCB-treated embryos Assay conditions: 4.3 nM T4, 30 minute incubation. Data reported are the means of 4 replicates at each protein concentration. Activity is expressed as fmol iodide generated at a 30 minute incubation.

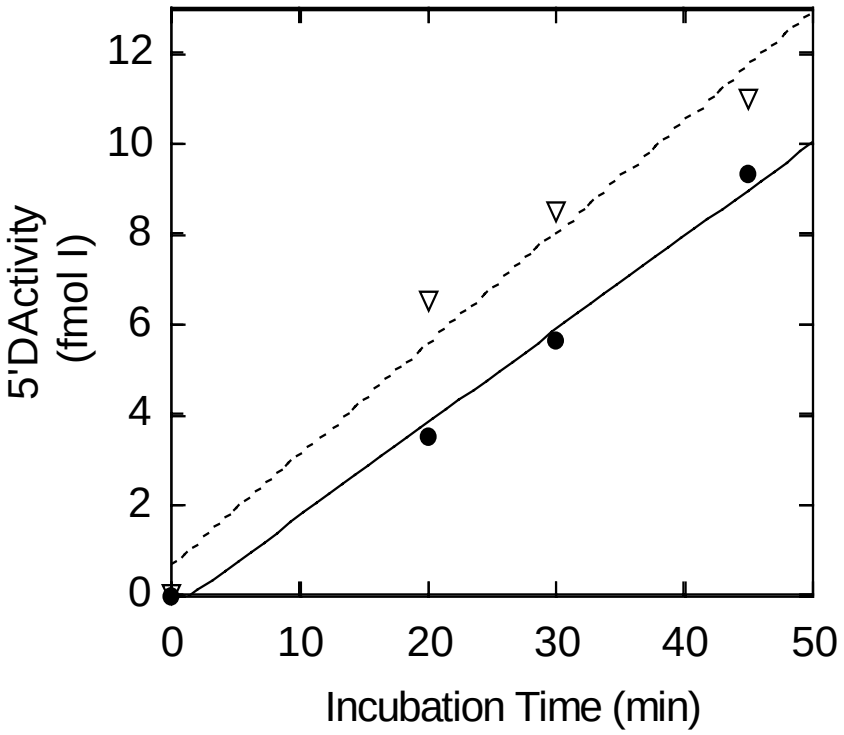

Fig 2. Linearity of T4 5'D activity with incubation time of postmitochondrial fraction frompooled brain samples from chicken embryos. ● Controls embryos ▽ PCB-treated embryos. Assay conditions: 2.77 mg protein concentration, 4.3 nM T4. Data reported are the means of 4 replicates at each protein concentration. Activity is expressed as fmol iodide generated.

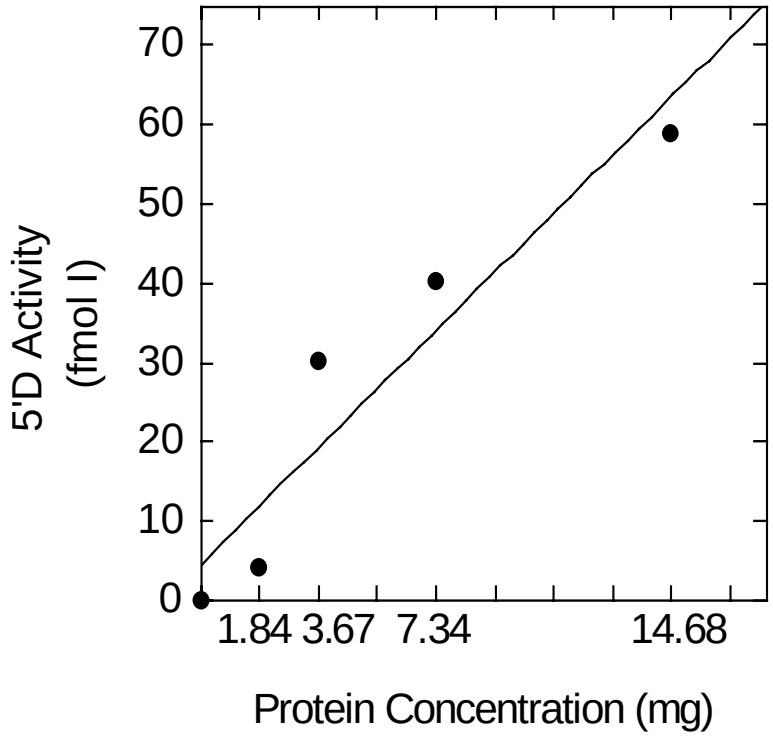

Fig 3. Proportionality of T4 5'D activity with enzyme concentration (crude homogenate) of pooled brain samples from herring gulls. Assay conditions: 4.3 nM T4, 30 minute incubation. Data reported are the means of 4 replicates at each protein concentration. Activity is expressed as fmol iodide generated in a 30 minute incubation.

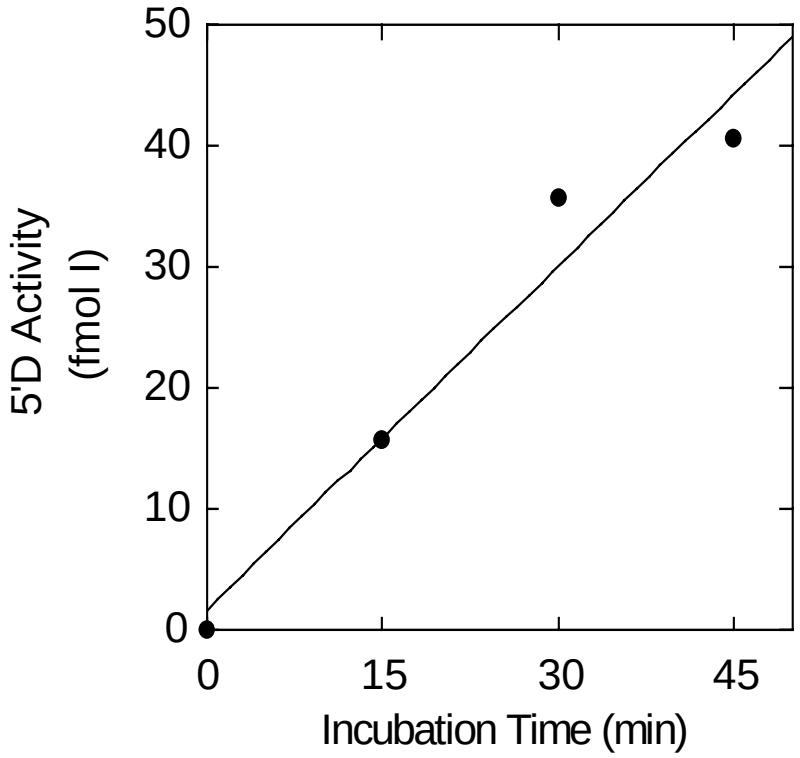

Fig 4. Linearity of T4 5'D activity with incubation time of crude homogenate from pooled brain samples from herring gulls. Assay conditions: 14.68 mg protein concentration, 4.3 nM T4. Data reported are the means of 4 replicates at each protein concentration. Activity is expressed as fmol iodide generated.

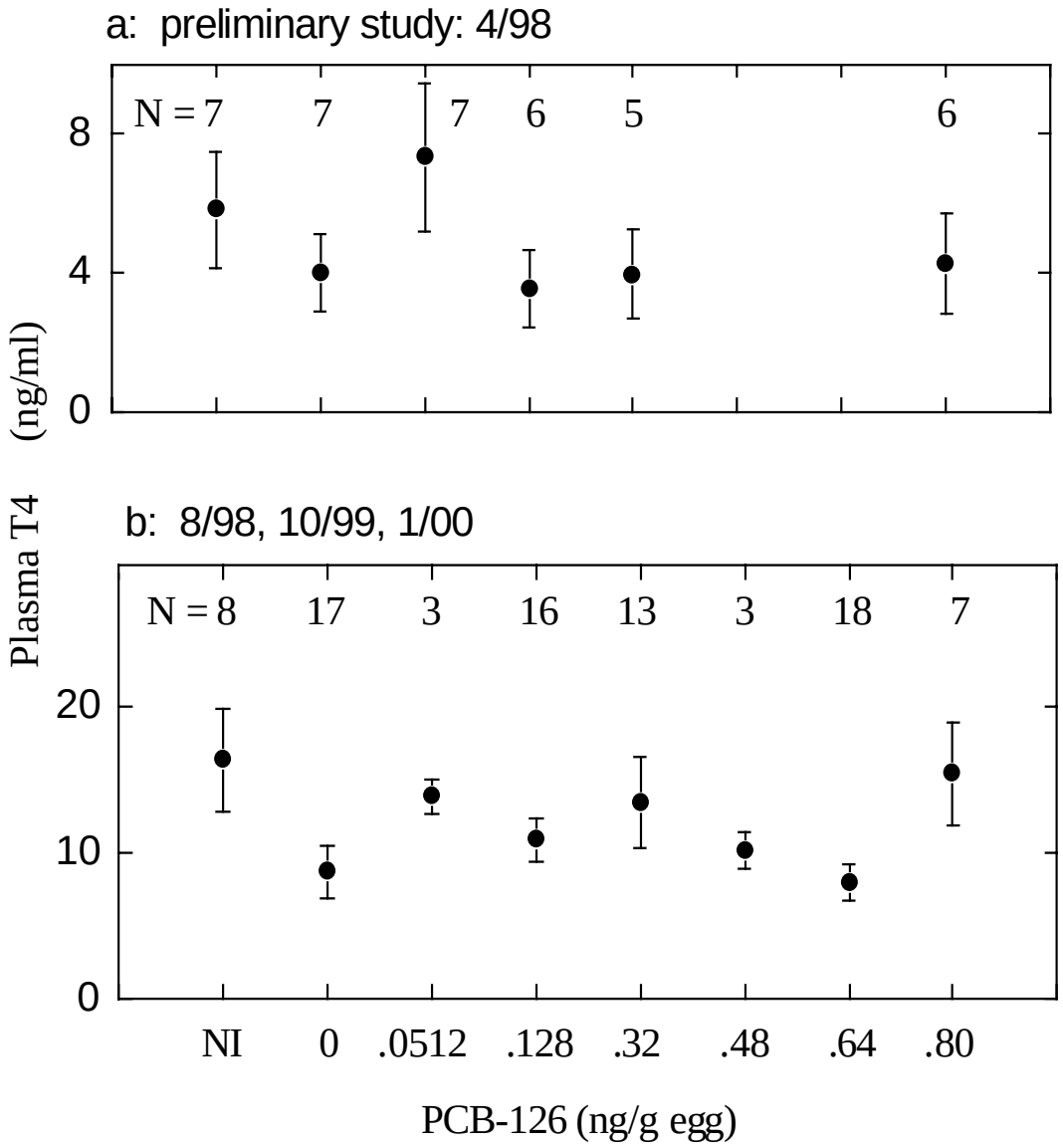

Fig 5. Plasma T4 concentrations in chicken embryos treated with PCB-126. a: preliminary study (4/98). b: larger study (samples combined from 8/98, 10/99, 1/00). N: number of embryos/dose. N.I: non-injected control. 0: injected control. Symbols represent mean +/- 1 SE; There are no significant differences between controls and embryos treated with any of the PCB doses.

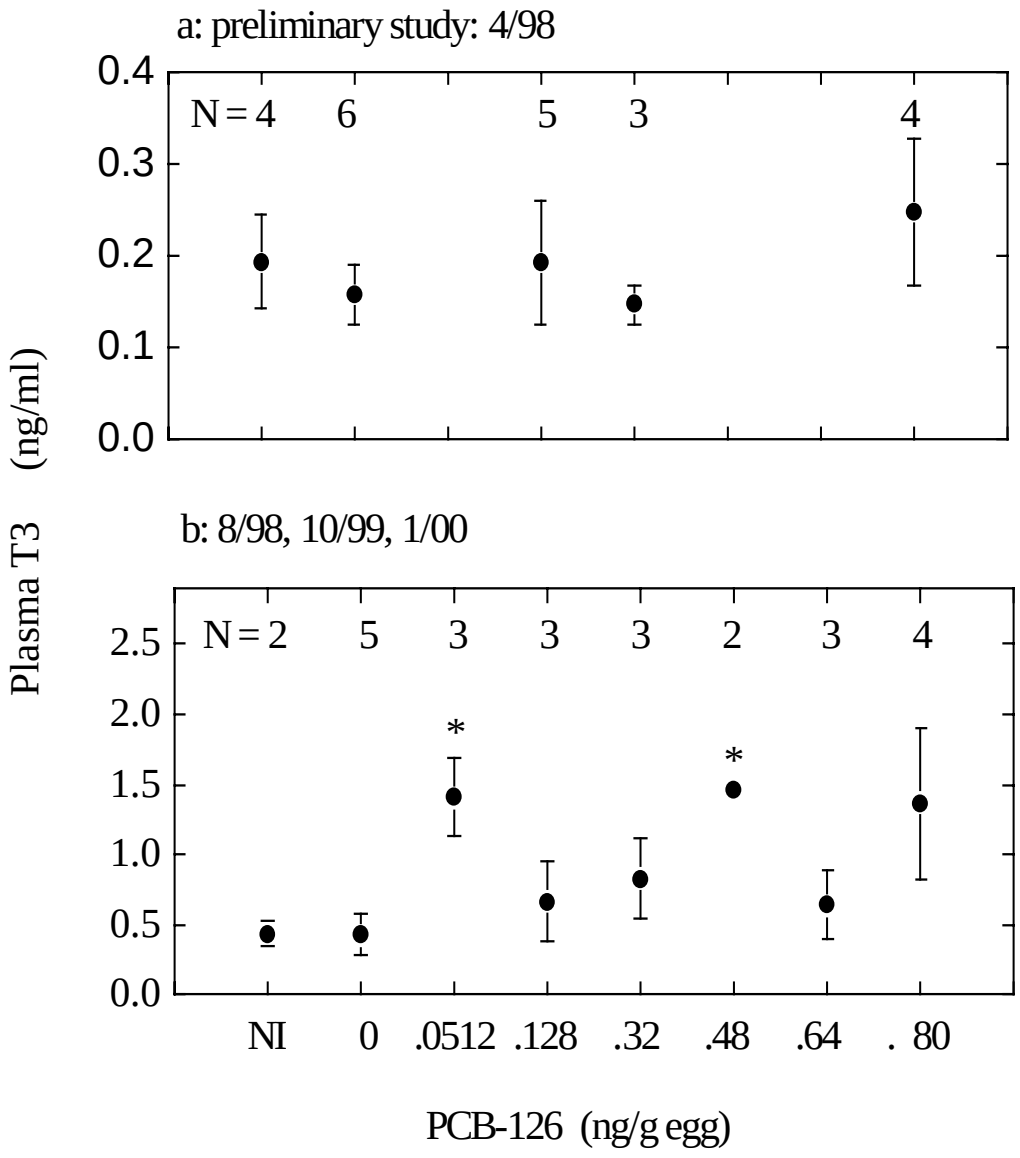

Fig 6. Plasma T3 concentrations in chicken embryos treated with PCB-126. a: preliminary study (4/98). b: larger study (samples combined from 8/98, 10/99, 1/00). N: number of embryos/dose. N.I: non-injected. 0: injected control. Symbols represent mean +/- 1 SE; *Indicates significantly different from control values at $p < .05$.

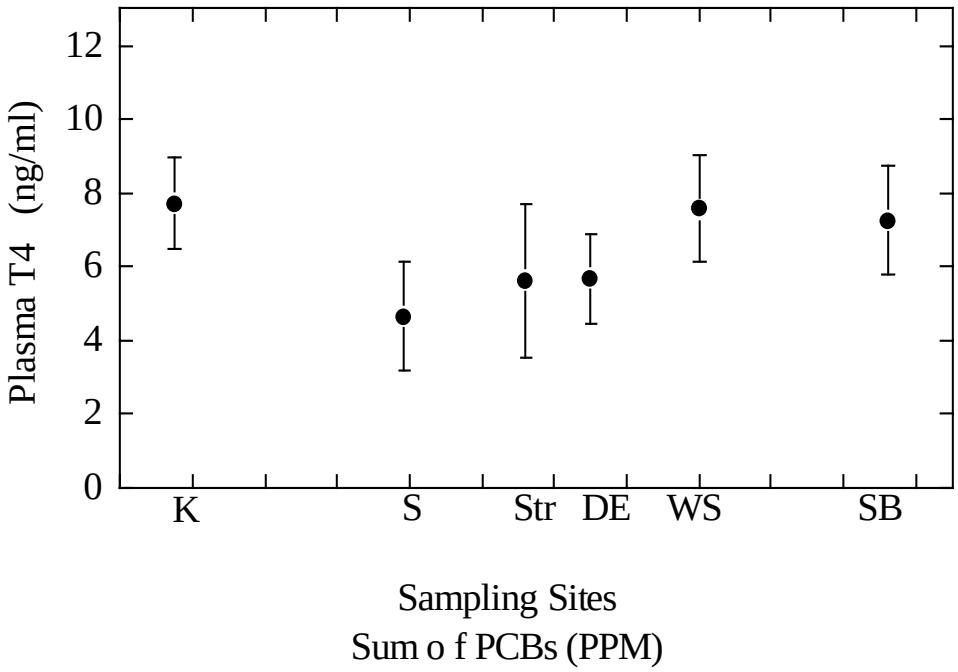

Fig 7. Plasma T4 concentrations in herring gulls from K (Kent Island, reference site) and Great Lakes sampling sites: S (Scotch Bonnet), Str (Strachnan Island), DE (Detroit Edison), WS (West Sister Island), SB (Saginaw Bay). PCB rankings based on pooled egg homogenates. Symbols represent mean +/- 1 SE; There are no significant differences in plasma concentrations in herring gull pipped embryos from Kent Island vs. Great Lakes sampling sites.

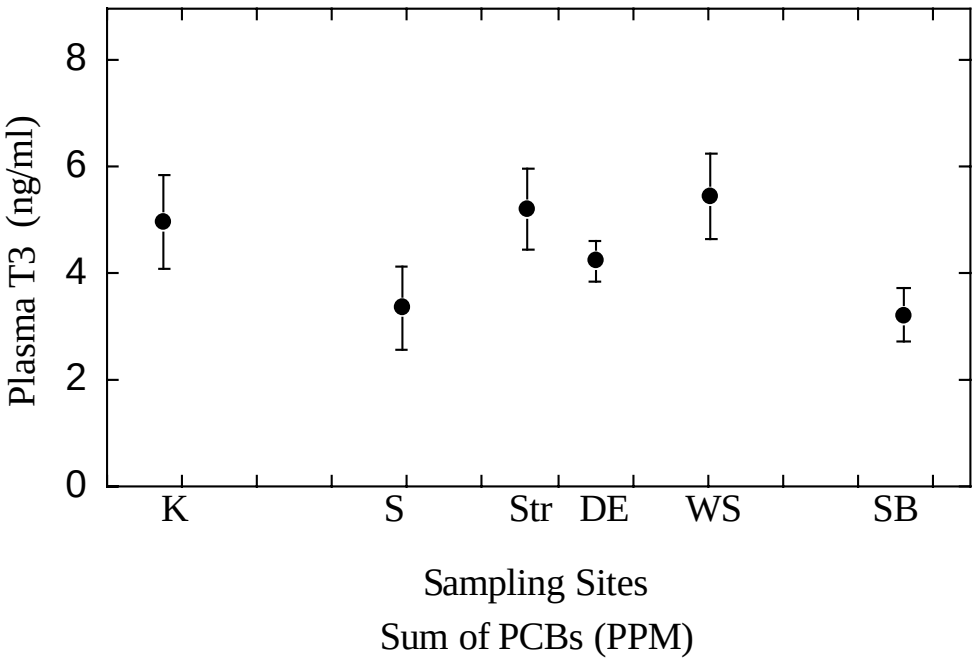

Fig 8. Plasma T3 concentrations in herring gulls from K (Kent Island , reference site) and Great Lakes sampling sites: S (Scotch Bonnet), Str (Strachnan Island), DE (Detroit Edison), WS (West Sister Island), SB (Saginaw Bay). PCB rankings based on pooled egg homogenates. Symbols represent mean +/- 1 SE;
There are no significant differences in plasma T3 concentrations in herring gull pipped embryos from Kent Island vs. Great Lakes sampling sites.

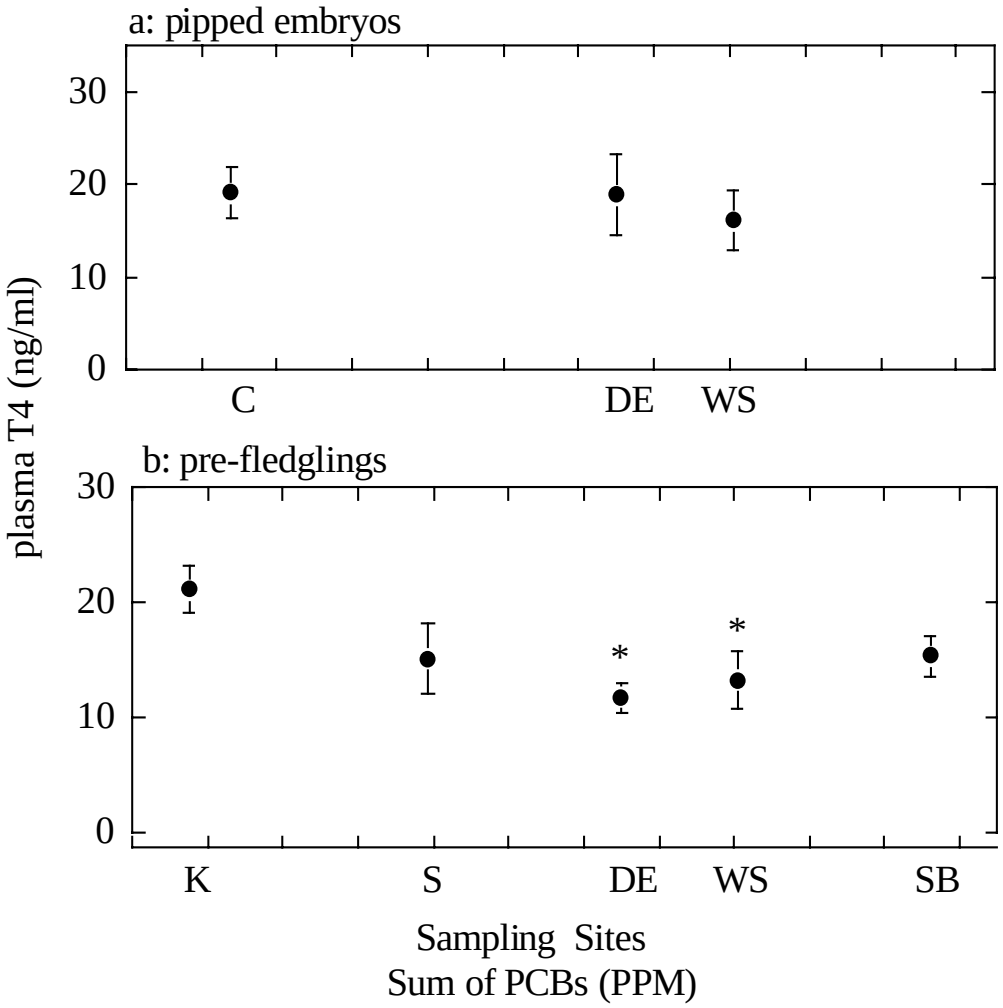

Fig 9. Plasma T4 concentrations in 1999 herring gull: a. pipped embryos b. pre-fledglings from K (Kent Island, reference site), S (Scotch Bonnet), DE (Detroit Edison), WS (West Sister Island), and C (Chantry Island). PCB rankings based on 1998 PCB concentrations. Symbols represent mean +/- 1 SE. a: Kent Island pipped embryos were not sampled, therefore, the three sites sampled are compared to each other with no significant differences. b: *indicates significantly different from Kent Island at $p < .05$.

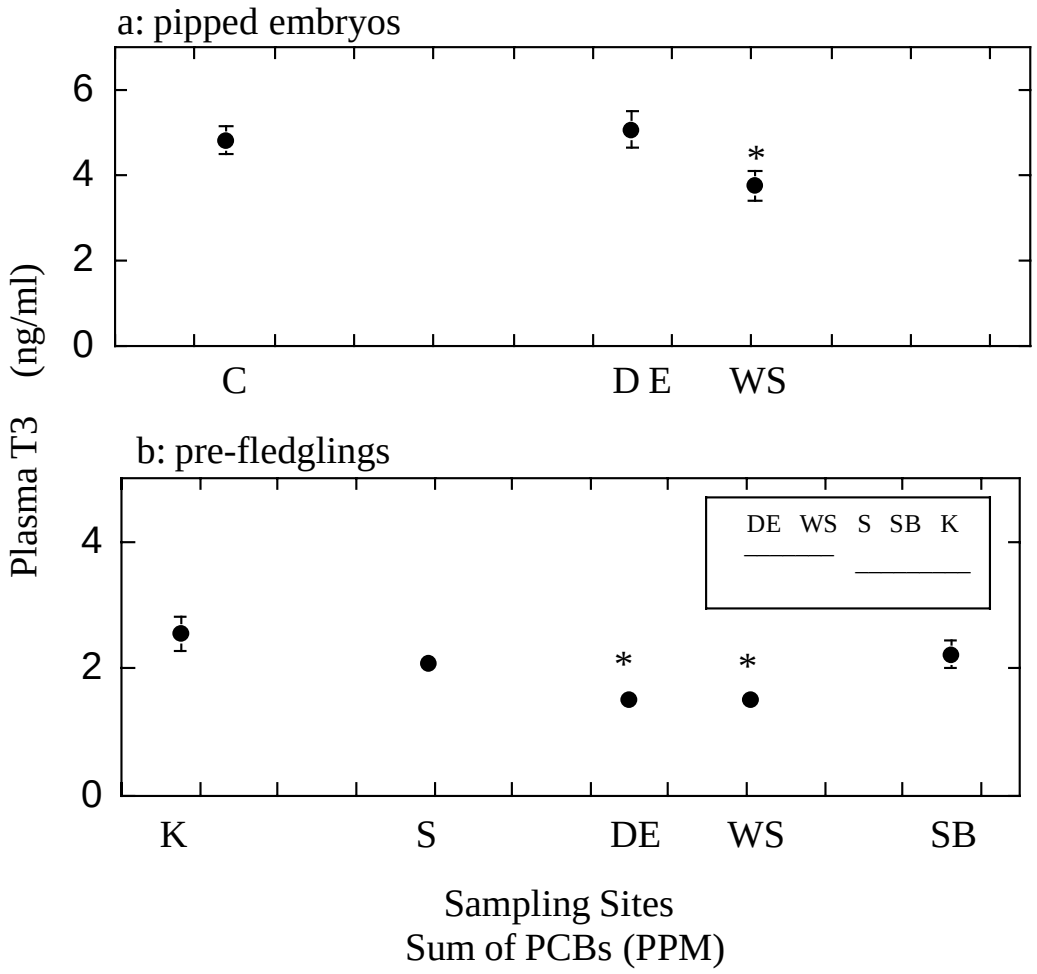

Fig 10. Plasma T3 concentrations in 1999 herring gull: a. pipped embryos b. pre-fledglings from K (Kent Island, reference site), S (Scotch Bonnet), DE (Detroit Edison), WS (West Sister Island), and C (Chantry Island). PCB rankings based on 1998 PCB concentrations. Symbols represent mean +/- 1 SE. a: Kent Island pipped embryos were not sampled, therefore, the three sites sampled are compared to each other. a: *Indicates WS significantly different from both C and DE at $p < .05$. b: *Indicates significantly different from Kent Island at $p < .05$.

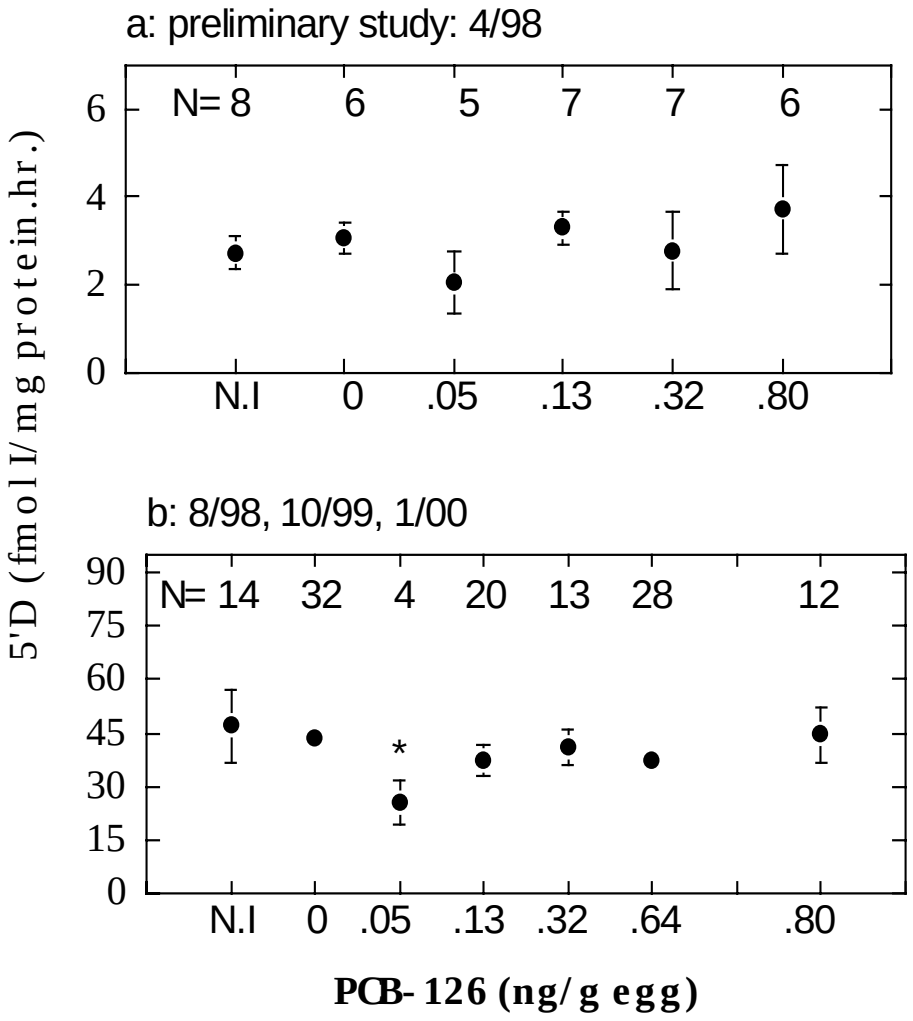

Fig 11. Brain 5'D-II activity in chicken embryos treated with PCB-126.
a: preliminary study (4/98), assay conditions: 1.3 nM T4, 30 minute incubation.
b: larger study (samples combined from 8/98, 10/99, 1/00), assay conditions: 4.3 nM T4, 30 minute incubation. N; number of embryos/dose. N.I: non-injected control. 0: injected control. Symbols represent mean +/- 1 SE. a: There are no significant differences between controls and embryos treated with any of the PCB doses. b: *Indicates significantly different from control values at $p < .05$.

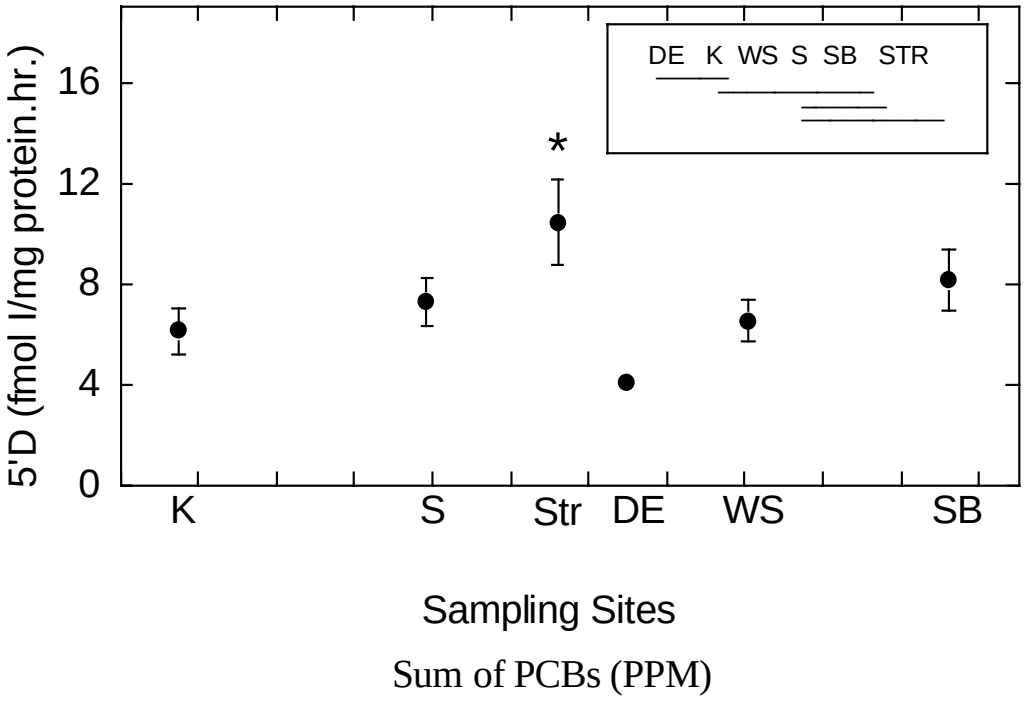

Fig 12. Brain 5'D-II activity in herring gull pipped embryos from K (Kent Island, reference site) and Great Lakes sampling sites: S (Scotch Bonnet), Str (Strachnan Island), DE (Detroit Edison), WS (West Sister Island), SB (Saginaw Bay). PCB rankings based on pooled egg homogenates. Symbols represent mean +/- 1 SE. *Indicates significantly different from Kent Island at $p < .05$.

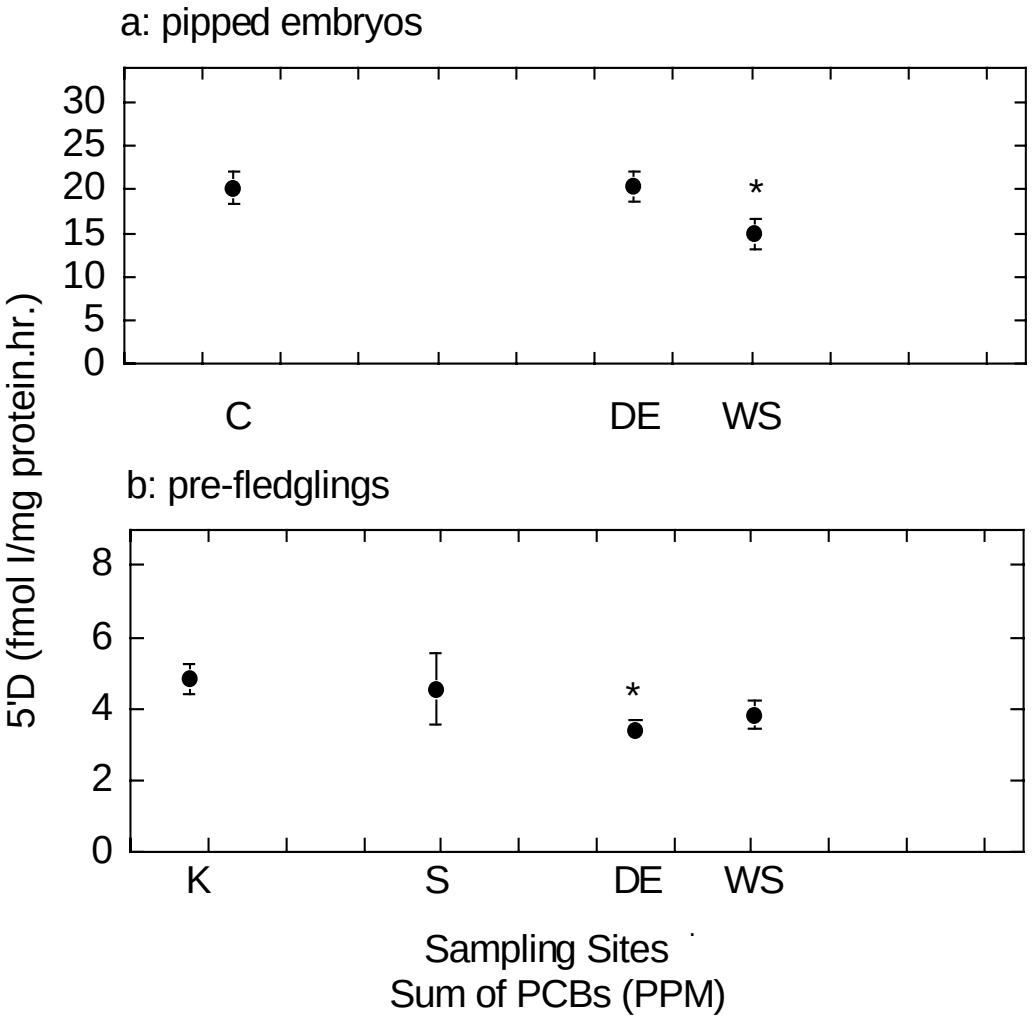

Fig 13. Brain 5'D-II activity in 1999 herring gull: a. pipped embryos b. pre-fledglings from K (Kent Island, reference site), S (Scotch Bonnet), DE (Detroit Edison), WS (West Sister Island), C (Chantry Island). PCB rankings based on 1998 PCB concentrations. Symbols represent mean +/- 1 SE. a: Kent Island pipped embryos were not sampled, therefore the three sites are compared to each other. *Indicates WS significantly different from both C and DE at $p < .05$. b: *Indicates significantly different from Kent Island at $p < .05$.

CURRICULUM VITAE

Leslie Fowler

Education

May 1996	B.S., Biology, Virginia Tech, Blacksburg, VA. Overall QCA: 3.3, In major QCA: 3.4
May 1997	B.A., English, Virginia Tech, Blacksburg, VA. Overall QCA: 3.3, In major QCA: 3.6 Undergraduate Research: Pollutant Effects On Thyroid Hormones.
September 2000	M.S., Biology, Virginia Tech, Blacksburg, VA. Overall QCA: 3.7 "PCB Effects On Brain Type II 5'Deiodinase Activity in Developing Birds"

Employment

August 1993- May 1994	Assistant Animal Lab Technician, Derring Hall, Virginia Tech, Blacksburg, VA.
August 1997- December 1997	Supplemental Instructor, Department of Academic Enrichment, Virginia Tech, Blacksburg, VA.
August 1997- August 1999	Graduate Teaching Assistant, Principles of Biology Laboratory, Virginia Tech, Blacksburg, VA. Evaluation: 3.7/4.0
August 1999- December 1999	Graduate Teaching Assistant, Majors of Biology Laboratory, Virginia Tech, Blacksburg, VA.

Awards

May 1992	Rebekah Lodge Scholarship for freshman year of college (awarded each year to 1 in a class of three hundred students).
June 1992	Precious Blood Church Scholarship for freshman year of college (awarded each year to 1 in a class of fifty students).
May 1993	Rebekah Lodge Scholarship for sophomore year of college (first time ever awarded to the same person twice).

Organizations

January 1993- May 1996	Block and Bridle, Virginia Tech, Blacksburg, VA.
January1993- May 1996	Phi Sigma Honor Society, Virginia Tech, Blacksburg, VA.
August 1997- July 2000	Biology Graduate Student Association (BGSA), Virginia Tech, Blacksburg, VA.
January 1999- January 2000	Treasurer, BGSA, Virginia Tech, Blacksburg, VA.
January 1999- Present	The Society for Integrative and Comparative Biology (SICB).

Grants

November 1998	Sigma Xi: Chemical Pollutant Effects on Thyroid Hormones in Brain Development of Birds, $600.00. Biology Department matching funds, $500.00.
January 1999	Graduate Research Development Project (GRDP) Travel Fund: Presentation at SICB meeting, Denver, CO, January 1999, $176.00.

Presentation/Abstract

January 1998	Thyroid Function In Chicken Embryos Exposed to PCB-126. L.A. Fowler*, F.M.A. McNabb and K. Grasman**. Virginia Tech, Blacksburg and **Wright State, Dayton. Abstract, *American Zoologist*, December,1998. Presented at SICB annual meeting (Division of Comparative Endocrinology), Denver, CO.