URIDINEDIPHOSPHATE-GLUCURONOSYLTRANSFERASE (UDP-GT) ONTOGENY AND PCB EFFECTS IN GALLIFORM BIRDS

Ryan J. R. McCleary

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

MASTER OF SCIENCE
in
Biology

Dr. F. M. Anne McNabb, Chair
Dr. David Bevan
Dr. Alan Heath

June 26, 2001
Blacksburg, Virginia

Keywords: PCBs, uridinediphosphate-glucuronosyltransferase (UDP-GT), thyroid hormones, avian development, Japanese quail

Abstract

Hepatic UDP-GTs are partly responsible for metabolism of the thyroid hormone, thyroxine (T_4), in mammals, but little is known of UDP-GT activity in birds. To determine the ontogenic pattern of UDP-GT activity in precocial birds, we measured activity in Japanese quail (*Coturnix japonica*) liver at days 12 and 14 of the 16.5-day incubation, 3 perihatch stages and <1, 1, 4, 6, 7, 20 and 42 days posthatch. We used an enzymatic reaction with *para*-nitrophenol (*p*NP) as substrate that was validated for quail tissue. The pattern of UDP-GT development included low embryonic activity, increased activity beginning in the perihatch period, a peak in activity at day 4 posthatch and a return to lower activity levels from day 6 to adults. The profile of UDP-GT activity, in relation to the ontogeny of circulating T_4 and triiodothyronine (T_3) in quail, is consistent with UDP-GT playing a role in regulating circulating T_4 and with the perihatch peak in T_3 stimulating the posthatch peak in UDP-GT activity. To examine the effects of polychlorinated biphenyls (PCBs) on UDP-GT in developing precocial birds, we dosed chicken (*Gallus domesticus*) eggs with concentrations of PCB 126 from 0 to 0.80 ng/g egg (in sunflower oil) prior to incubation. Tissues were sampled at day 20 of the 21-day incubation and assayed for plasma hormones and UDP-GT activity. Eggs also were dosed with 0 or 0.25 ng PCB 126/g egg or with 0 or 0.64 ng/g egg of the coplanar PCB 77, allowed to hatch, and sampled at 42 days posthatch. There was no consistent pattern of altered thyroid hormones or UDP-GT activity in developing chickens exposed to either of these coplanar PCBs although previous studies indicated developmental alterations from exposure to the higher doses.

This research was supported by funding from the U. S. Environmental Protection Agency, Sigma Xi, the Biology Department at Virginia Tech and the Graduate Student Association of Virginia Tech.

Acknowledgements

The work before you would not have been possible without the kindness, patience and understanding of Dr. F. M. Anne McNabb. Without her help and extraordinary efforts, this thesis would still be a simple imagining. I would like to especially thank her for taking a chance with someone not initially knowledgeable in the field of endocrinology and giving me a wonderful opportunity to learn science under her tutelage. I would also like to thank the other members of my committee, Dr. David Bevan and Dr. Alan Heath, for their knowledge, expertise, suggestions, comments and guidance. Further, although he was not a part of my final committee, I would like to thank Dr. Joseph Falkinham for his advice and understanding.

I would particularly like to thank my mother, Karen Rogers, my father, Dr. Ken McCleary, my stepmother, Dr. Pamela Weaver, and my best friend in the world, Jennifer Swartz for all their words of encouragement and unending moral support. They have heard the good and the bad, *ad nauseum*, and have borne it all with nothing but kind words.

I also owe a debt of gratitude for the help of previous and current members of the McNabb lab, including Leslie Fowler, Cathy Parsons, John Baumgartner, Erica McFarland, Julie Gibson, Jesper Lorentzen, Jenn Claflin, Stewart Milton, Sarah Hood, Jenny Honaker, Eric Belling and Keesha Steed. Further support, both technical and moral, was provided by members of the Esen lab (Muzzo Cicek, Sukanda Vichitphan, and David Blanchard), Walker lab (Dr. Richard Walker, Bettina Deavours and Arzu Karabay), and other fellow graduate students including Daniel Warner, Matt Lovern, Bill Zabaronick, Memuna Kahn and Jody Riley. I would further like to extend thanks to Dr. Keith Grasman at Wright State University and Dr. Glen Fox of Environment Canada for all their help.

Table of Contents

Chapter 1: Literature Review

Pollutant Chemicals in Natural Environments

Due to previous widespread use of halogenated chemicals, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and pesticides such as DDT, by industry, manufacturing and agriculture, many of these chemicals are now ubiquitous pollutants in natural environments.

Polyhalogenated aromatic hydrocarbons (PHAHs) have had some drastic effects on wildlife (Carson 1962) and continue to exert other, non-lethal effects (Colborn *et al*. 1996) because of their persistence. Although lethal chemical effects have historically been the focus of toxicological studies, non-lethal effects, which are more difficult to quantify in terms of their damage to individuals, populations and species, are receiving much current attention.

One particularly disturbing category of non-lethal effects of some pollutant chemicals is their ability to disrupt endocrine control. This disruption may occur because the chemical mimics or blocks hormone action on target tissues (*i.e*. it acts as an agonist or antagonist, respectively, and binds to receptors) or because the chemical alters the production or metabolism of hormones. Colborn *et al*. (1996) chronicled some of the known problems related to endocrine disruption in natural animal populations, and McLachlan and Arnold (1996) reviewed evidence showing that some pollutants can act as estrogenic hormones. An important example of reproductive endocrine disruption can be seen in American alligators (*Alligator mississippiensis*), from polluted lakes in Florida, that have altered concentrations of circulating steroid hormones (Crain *et al*. 1998). Indeed, pollutant chemicals have been shown to produce sex reversals in alligators and turtles from these polluted lakes, thereby affecting population sex ratios (Guillette and Crain 1996).

Alterations in thyroid function, especially during ontogeny, can interfere with the normal development of many other, non-thyroidal systems in vertebrates. Low thyroid hormone (TH) concentrations during development can lead to slowed skeletal growth and maturation, and problems with muscle and central nervous system development (McNabb and King 1993). In amphibians, THs play crucial roles in the control of metamorphosis, so alterations in TH during development can result in abnormalities in the nature and timing of morphological and physiological development.

Descriptions of PCBs

PCBs, the chemicals of concern in this review, are widespread pollutants generated from many industrial processes. They have historically been used for many purposes, including as dielectric fluids in capacitors and transformers, as heat transfer fluids, as plasticizers in paints, in adhesives, as organic diluents, in laminating agents and in waxes (Bastomsky 1974; Safe 1990). Because of their widespread use and their

persistence, PCBs are ubiquitous. Because of their lipophilic nature, PCBs are stored in adipose tissue, and their bioaccumulation is magnified up the food chain. The presence of PCBs in animal tissues has been correlated with developmental and reproductive abnormalities in animals (see section on General Toxicology and Endocrine Disruption).

The basic structure of a PCB is two phenyl rings connected by a covalent bond. Each phenyl ring has five positions (numbered 2-6 and 2'-6'—Figure 1) where chlorines can potentially substitute in place of hydrogen atoms for bonding to carbons in the ring structure. On each ring, there are two *ortho* positions (those closest to the shared covalent bond between the rings), two *meta* positions (those adjacent to the *ortho* positions, but distal to the shared bond) and one *para* position (located most distal from the shared bond—see Figure 2). The naming of PCBs is based on the presence and location of the chlorine atoms on the phenyl rings (see Table 1). When chlorine atoms are absent from the *ortho* positions, the phenyl rings can rotate about the shared bond, due to the lower steric hindrance associated with hydrogen atoms than with chlorine atoms. In this conformation, PCBs have a higher probability of being in a planar position, one where both phenyl rings are in the same geometric plane. The steric hindrance of *ortho* chlorine atoms, however, reduces the probability of planarity (De Voogt *et al*. 1993).

Those PCBs with no *ortho*-substituted chlorines, but with both *para* and at least one *meta* position with chlorines, have the highest probability of being in a planar conformation. These coplanar PCBs (PCBs 77, 81, 126 and 169—Figure 3) have the highest toxicity when compared with other PCBs (Safe 1990). This is perhaps due to their chemical resemblance to the planar 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD—Figure 4), the most toxic of the PHAHs. TCDD binds to the aryl hydrocarbon receptor (AhR), which mediates its toxic response (Whitlock *et al*. 1996), and coplanar PCBs are thought to act at least partially through this mechanism. Safe (1990) proposed the use of toxic equivalency factors (TEFs), which express the relative toxicity of other PHAHs in terms of TCDD and found the coplanar PCBs (specifically PCB 126) to have the highest toxicity among PCB congeners.

General Toxicology and Endocrine Disruption

The exposure and some effects of pollutant chemicals on birds have been studied for years in Great Lakes herring gulls (*Larus argentatus*). In the 1970s, many abnormalities were noticed in gulls from colonies at heavily polluted sites. These included lower nest attentiveness, less nest defense, lower fecundity and lower embryo survival. These Great Lakes sites had some of the highest organochlorine pollutant loads of any areas tested (Peakall *et al*. 1978). Rattner *et al*. (1984) reviewed some of the known effects of pollutant chemicals on avian endocrine systems, including gonadal and thyroidal systems.

The herring gull has been considered a particularly good sentinel species for monitoring the overall health of an ecosystem because it is a top predator that consumes primarily fish and thus bioaccumulates persistent, lipophilic, organic pollutants. Gulls

nest colonially (so are relatively easy to monitor), have a wide geographic distribution (making it relatively easy to compare different sites with different pollutant loads), and adults generally remain on one territory year-round (Mineau *et al*. 1984).

The effects of organochlorine pollutants on thyroid gland size and histology were assessed in Great Lakes herring gulls from 1977 (shortly after PCB manufacture was outlawed) to 1982. A majority of the animals sampled were found to have goiter, and thyroid histopathology was highly correlated with the presence of environmental PHAHs (Moccia *et al*. 1986). Although the hatching success of these gulls has increased and reproductive and developmental toxicities have decreased over the past 25-year period of monitoring, the continued presence of high concentrations of organochlorines is sufficient to cause or exacerbate other physiological problems (Fox 1993; Peakall and Fox 1987). For example, a more recent study (Grasman *et al.* 1996) showed an association between environmental pollutant loads and immunological dysfunction in herring gulls and Caspian terns (*Sterna caspia*).

The possibility of organochlorine pollutant effects on birds in western Europe also has been studied and several lines of evidence are suggestive of thyroid alterations at the most polluted sites in the Netherlands and Belgium (Brouwer *et al*. 1998 and see section below on Effects of PCBs, Birds).

General Background

Vertebrate Thyroid Function:

Thyroid hormones are critical in the regulation of normal growth and development throughout the vertebrate classes and are involved in the regulation of metabolism in birds and mammals (McNabb and King 1993). Thus, alterations in circulating TH concentrations can have drastic permanent consequences for developing organisms.

Thyroid hormones [thyroxine (T_4) and triiodothyronine (T_3)] are produced by the iodination of tyrosine residues within thyroglobulin by thyroid follicle cells and are stored in colloid in the center of each thyroid follicle. For hormone mobilization, colloid enters follicular cells by endocytosis, the thyroglobulin is cleaved by lysosomal proteases and THs are released into adjacent capillaries by diffusion. The major TH produced and released is T_4 (see Figure 5 for structure). T_4 (3,3',5,5'-tetraiodothyronine) is considered a prohormone because it can be converted to T_3 (3,3',5-triiodothyronine) by 5' deiodination. T_3, the thyroid hormone for which thyroid receptors have highest binding affinity, has higher biological potency than T_4 and is produced primarily from the deiodination of T_4 to T_3 in extrathyroidal tissues. Both hormones, which are relatively lipophilic (hydrophobic), are transported in the blood primarily bound to carrier proteins (McNabb 1992).

Depending on the species, there are three major carrier proteins: thyroxine-binding globulin (TBG, found only in some mammals), transthyretin (TTR) and albumin.

In birds, TTR is the highest affinity T_4 carrier protein. In general, bound THs cannot enter cells and exert biological activity, but are important reservoirs of hormone for release to the small free hormone pool. Free THs can enter cells and produce physiological responses by binding to nuclear T_3 receptors. However, there is some evidence that TTR is involved in T_4 transport in at least one case, namely across the blood-brain barrier into the central nervous system (Robbins 1996). TH availability within extrathyroidal tissues also is influenced by deiodinases, which can alter the concentrations of T_3 by the deiodination of T_4 (Type I and II; outer-ring or 5'-deiodination) or by the degradation of T_4 to reverse T_3 (3,5,5'-triiodothyronine), which has no known biological activity (Type III; inner-ring or 5-deiodination; Leonard and Visser 1986; Leonard and Koehrle 1996).

In adult vertebrates, concentrations of T_4 are maintained homeostatically by a balance involving production in the thyroid gland and extrathyroidal deiodinations and degradation/excretion. A major mechanism of T_4 metabolism is conjugation, in which another molecule is added to T_4 to facilitate its excretion. For example, hepatic uridinediphosphate-glucuronosyltransferases (UDP-GTs—an important pathway altered by PCBs) make T_4 more water-soluble by glucuronidating it, thus facilitating its excretion in bile (Visser 1990; also see Biometabolic Enzymes section below).

Overall regulation of euthyroid TH concentrations is by the hypothalamic-pituitary-thyroid (HPT—Figure 6) axis. When TH concentrations in the blood are low, negative feedback is exerted on the anterior pituitary, which increases the output of thyroid stimulating hormone (TSH). Elevated TSH levels cause a subsequent increase in the production and release of THs from the thyroid, thereby increasing circulating TH concentrations (McNabb 1992).

Avian Thyroid Development:

Avian developmental patterns range from chicks that are fairly independent at the time of hatching (precocial) to those that are completely dependent on parental care after hatching (altricial). Precocial birds, like domestic chickens and Japanese quail, have their eyes open, are covered with down feathers, are capable of locomotion and self-feeding at hatching and show metabolic responses to cooling starting from time of hatching. In contrast, altricial birds like pigeons, sparrows and starlings, have closed eyes, lack down, are incapable of locomotion and are completely dependent on parental care for some period after hatch (Starck and Ricklefs 1998).

The patterns of thyroid development differ in these two modes of ontogeny (McNabb *et al*. 1998). In precocial birds, which show considerable maturation of their anatomical and physiological systems by the time of hatching, and which initiate thermoregulatory responses at hatch, most thyroid gland maturation occurs during the latter part of embryonic life and circulating concentrations of both THs have large peaks during the perihatch period. After hatching, T_4 concentrations decline, and then increase slightly to reach adult concentrations during early juvenile life. T_3 concentrations also decline posthatch then change little during juvenile life (McNabb and Hughes 1983).

Altricial birds, which are hatched at a less developed stage and do not show metabolic responses to cooling until some time (usually 1-3 weeks) after hatch, have relatively immature thyroid function at hatch. Their thyroid function increases posthatch and stabilizes at about the time when they develop thermoregulation (McNabb and Cheng 1985). These different patterns of avian thyroid development have implications for the time during which chemical pollutants are likely to alter thyroid function and cause other downstream effects on morphology and physiological development.

Biometabolic Enzymes:

Some endogenous and many exogenous chemicals are partly metabolized prior to excretion in vertebrates by Phase I and II hepatic biotransformation enzymes. These enzymes are important in the context of this project in two ways: (1) because chemicals like PCBs typically induce these enzymes, and (2) because some of these enzymes are involved in thyroid hormone regulation and turnover. Phase I and II biotransformation enzymes are categorized by their functions. Phase I cytochrome P450 enzymes generally catalyze degradative reactions (oxidation, reduction or hydrolysis) resulting in products with a polar functional group. Substances with exposed functional groups are more water-soluble, may be excreted in urine and are subject to conjugation by Phase II biometabolic enzymes. Phase II enzymes catalyze reactions that are synthetic, *i.e*. they attach a chemical moiety to the substrate. This results in a water-soluble product that is excreted more readily than the original substrate (Tephly and Burchell 1990; O'Flaherty 2000).

Two widely used assays of Phase I activity examine the activity of the enzymes ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin-*O*-deethylase (PROD). Although these enzymatic processes usually detoxify toxic compounds, in a few cases P450s render compounds more fat-soluble and/or more toxic (Klasson-Wehler *et al*. 1989).

Phase II biometabolic enzymes, which are mostly found in the liver [but also occur in the skin, lungs, small intestine (rats—Li and Hansen 1997) spleen and head kidney (carp—Tayesse *et al*. 1998)], catalyze synthetic reactions (by adding a molecule that is covalently bound, *i.e*. conjugated to the substrate), such as attaching a glucuronic acid (for UDP-GTs) or glutathione [for glutathione S-transferase (GST)] to many potentially harmful exogenous chemicals. These reactions also are part of the pathway for the excretion of steroid and thyroid hormones that are relatively lipophilic. This enzymatic action makes relatively lipophilic compounds more water-soluble (hydrophilic), which increases their excretion in bile and urine. Although all UDP-GTs utilize endogenous uridine 5'-diphosphoglucuronic acid (UDPGA) as a reaction cofactor, each UDP-GT isozyme reacts with different substrates to different degrees. Basically, each isozyme has a range of compatible substrates and many isozymes may overlap activity for a specific substance. UDP-GTs have traditionally been grouped into categories based on either the substances they glucuronidate or the types of chemicals that induce their activity (Burchell and Coughtrie 1989; Mulder 1992).

UDP-GTs are separated into two large isozyme groups, depending on their substrates. UDP-GT1s are considered drug-glucuronidating (although their activity is not strictly limited to clinical compound substrates) and UDP-GT2s are considered steroid-glucuronidating (Emi *et al*. 1996). UDP-GT1A and 2A isozymes are considered active toward aromatic compounds and UDP-GT1B and 2B isozymes are considered active toward bilirubin. Thus, in this scheme, UDPGT1A would be considered a drug-glucuronidating enzyme with activity toward aromatic compounds.

The gene containing information for transcription of UDP-GTs has a variable exon 1 region followed by 4 conserved exons. Each exon 1 codes for the N-terminus of the subsequently translated protein and gives the UDP-GT its specificity (Masmoudi *et al*. 1997). Humans have at least 13 different UDP-GT1s (Masmoudi *et al*. 1997), and rats have at least 12 (Metz and Ritter 1998); the total number of UDP-GTs per species is continually being revised as more are discovered. While the functions of many individual isozymes have been determined in humans, some have not shown activity toward any tested substrate (Beaulieu *et al*. 1998). Currently, there is no published information on activities of individual UDP-GT isozymes in birds.

Effects of PCBs

Mammals:

The effects of PCBs on endocrine systems in mammals have been extensively investigated for one animal model, laboratory rats. Studies utilizing laboratory rats have shown significant effects on thyroid function in rats exposed to PCBs. In general, in this model, PCBs produce hypothyroid conditions by increasing TH excretion as a result of increased glucuronidation and the displacement of T_4 from TTR binding.

Bastomsky (1974) analyzed the effects of the PCB mixture Aroclor 1254 on biliary excretion of T_4 in male hooded rats. Using radioiodine (^{131}I) and labeled T_4 (^{125}I-T_4) administered to rats, he found that Aroclor 1254 treatment increased the thyroidal uptake of ^{131}I, the biliary excretion of ^{125}I-T_4, and the proportion of ^{125}I-T_4 that was present in bile in the glucuronidated form. These results indicate that UDP-GTs were induced by PCBs, leading to increased glucuronidation and excretion of T_4. The increase in ^{131}I uptake suggests that the thyroid gland was stimulated by HPT axis activation through negative feedback by decreased circulating THs to compensate for the excreted T_4. Further, this study showed decreased binding of THs to binding proteins, which also may have increased T_4 excretion. The ability of some PCBs to displace T_4 from TTR has been demonstrated in a number of *in vitro* studies (*e.g*. McKinney and Waller 1994; Cheek *et al*. 1999), but the importance of this displacement in the regulation of thyroid function has not been adequately investigated.

Barter and Klaassen (1992b) characterized the types of hepatic UDP-GT in laboratory rats. They showed that hepatic glucuronidation of T_4 was increased in rats dosed with Aroclor 1254, and that these rats experienced a 70-75% reduction in total and

free serum T_4 (Barter and Klaassen 1992a). By using thyroidectomized rats infused with TH solutions, they were able to demonstrate that these Aroclor 1254-induced reductions in circulating T_4 were due to an extrathyroidal mechanism *i.e*. the enhancement of T_4 excretion presumably due to the induction of hepatic UDP-GT. Similar results were obtained in a study in which thyroidectomized and T_4-supplemented rats were dosed with TCDD and showed increased induction of T_4-specific UDP-GT accompanied by decreases in plasma total and free T_4 concentrations (Schuur *et al*. 1997). Likewise, these results are consistent with the idea that it is the coplanar, TCDD-like congeners that are primarily responsible for the alterations in thyroid function associated with PCB exposure. Barter and Klaassen (1994) noted increased UDP-GT activity toward T_4 as well as reductions in total and free T_4 of 80-90% when rats were dosed with Aroclor 1254. Dosing with Aroclor 1254 also led to a 40% increase in TSH concentration, a 30% increase in thyroid gland weight and a 100% increase in ^{131}I uptake, all changes indicative of feedback effects on the HPT axis and TSH effects on the thyroid gland.

Pregnant Sprague-Dawley rats exposed to coplanar PCB 77 showed high perinatal mortality of embryos (Harris and Bradshaw 1984). In the surviving offspring, many alterations in liver morphology were noted as well as an increase in the induction of hepatic UDP-GTs. Treated animals showed enzyme activity three times as high as in untreated controls. Weanlings of female Sprague-Dawley rats exposed to PCB 77 or TCDD *in utero* showed significantly decreased concentrations of plasma total T_4 (Seo *et al*. 1995). These rats, as well as those dosed with PCB 126, showed increases in UDP-GT activity toward the phenolic test substrate, *para*-nitrophenol (*p*NP) as well as accompanying increases in EROD activity. An examination of normal UDP-GT activity in adult Wistar rats found that females have significantly lower activities than males (Damanhouri and Tayeb 1994). Henry and Gasiewicz (1987) studied the effects of TCDD on TH concentrations and UDP-GT activity in adult male Sprague-Dawley rats and found significant decreases in T_4, significant increases in UDP-GT activity toward T_4 and, surprisingly, no change in TSH. This last part is significant because the decrease in T_4 does not bring a concomitant increase in TSH, as would normally be expected (see also Hood *et al*. 1999). Despite the rather consistent picture of PCB induction of hepatic UDP-GT in the studies above, one study showed the converse, namely decreases in UDP-GT activity in juvenile male Wistar rats exposed to either PCB 77 or 126, with 126 showing the greatest effect (Koga *et al*. 1994).

Although most of the effects of PCBs are attributed to coplanar (non-*ortho*) congeners, the mono-*ortho* congener 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) increases the activity of many liver enzymes, including Phase I enzymes P4501A1 and 1A2, as well as liver size and pathology in female Sprague-Dawley rats (Haag-Grönlund *et al*. 1997). Unfortunately this study did not specifically analyze the effects of these changes on thyroid function.

PCBs and PCB mixtures have been shown to cause alterations in circulating TH concentrations in both adult rats and the pups of PCB-exposed mothers. When pregnant female Long-Evans rats were treated with Aroclor 1254 (0, 1, 4 and 10 mg/kg) from day 6 of gestation through day 21 *post partum*, offspring of animals treated with the highest

doses had reduced circulating total T_4 and T_3 (TT_4 and TT_3) and free T_4 and T_3 (fT_4 and fT_3) concentrations by 21 days after birth, and some hormone concentrations were reduced earlier (Goldey *et al*. 1995). Weanling Sprague-Dawley rats exposed to coplanar PCB 77 (10, 100, 1000 or 10,000 ppb) or mono-*ortho* PCB 28 (2,4,4'-trichlorobiphenyl; at 50, 500, 5000 or 50,000 ppb) in their diets for 90 days had altered circulating T_4 concentrations (Desaulniers *et al*. 1997). Consistent with most other studies of coplanar PCBs, circulating T_4 was decreased in high-dose females exposed to PCB 77. With mono-*ortho* PCB 28, circulating T_4 also was decreased in females receiving the highest dose. An unexpected result was that T_4 was significantly decreased in both low-dose females and high-dose males receiving PCB 77. In the same study, adult male Sprague-Dawley rats dosed twice with coplanar 126 (6.25, 25, 100 or 400 µg/kg) had significantly reduced T_4 in the three groups with the highest doses and reduced T_3 in the groups with the two highest doses.

Birds:

In laboratory studies, chickens have been the main models used to elucidate the effects of pollutant chemicals in birds. In terms of embryonic mortality and Phase I biometabolic enzyme induction (measured as EROD activity), there is a hierarchy of declining effects of PCB congeners in the following order: 126 > 77 > 169 > 105 (Brunström 1990). PCBs 126, 77 and 169 are coplanar, or dioxin-like, congeners and PCB 105 is a mono-*ortho* substituted congener that has some properties similar to the coplanar congeners (Kannan *et al*. 1988). PCB 126 caused 90% mortality in day 10 embryos dosed at day 7 of incubation with a relatively low concentration of 2 ppb. Brunström and Andersson (1988) showed a similar hierarchy in terms of LD_{50}, with PCB 126 being most toxic, followed by PCBs 77 and 169 (LD_{50}s of 3.1, 8.6 and 170 ppb, respectively). Powell *et al*. (1996) injected PCB 126 in triolein into yolks and saw a dose-dependent increase in developmental abnormalities and mortality. They also found that doubling the dose from 1.6 ppb to 3.2 ppb increased mortality from 21.7% to 91.7%. Lorenzen *et al*. (1997) showed *in vitro* induction of EROD in chicken embryo hepatocytes by PCB 126 in a dose-dependent manner. The site of chemical injection into eggs can influence effects on embryos as illustrated by the work of Henshel *et al*. (1997) that indicated TCDD injected into the yolk of chicken eggs had a greater toxic effect (in terms of mortality) than when injected into the air cell.

One study used embryos of chicken eggs dosed with different PCB congeners (PCBs 54, 77 or 80) or mixtures (Aroclor 1242 or 1254) prior to incubation and killed on the final day of the 21-day incubation period (Gould *et al*. 1999). Embryos with the highest doses of either mixture (100 ng/egg or ~6.7 ppb in yolk) showed significant decreases in plasma T_4 concentration. Embryos dosed with the highest concentration of Aroclor 1242 also exhibited a significant decrease in plasma T_3.

Galliform species other than chickens also have been manipulated in the laboratory to determine the effects of pollutant chemicals. Riviere *et al*. (1978) used Japanese quail (*Coturnix japonica*) to study the effects of Phenochlor, a PCB mixture with about 50% chlorine, on enzyme induction in treated adults and their offspring.

Dosed adult animals showed significant increases in liver weight, cytochrome P450 concentrations, and several enzyme activities, including UDP-GT activity toward *p*NP.

Most studies on wild birds have been done on animals that have been exposed to PCBs in their natural environments, although in some cases wild-caught animals have been treated with PCBs in the laboratory. Murk *et al*. (1994b) injected one intraperitoneal dose of either PCB 77 or Clophen A50, a PCB mixture, into 28-day old wild-caught eider ducklings (*Somateria mollissima*) and found significant increases in EROD activity 10 days after injection. There were significant correlations between high body lipid PCB concentrations and decreases in the rates of body weight gain and beak length growth in the Clophen A50-dosed groups, increased relative liver weight and EROD activity in both PCB 77 and Clophen-dosed groups, and reduced plasma T_4 and T_3 and hepatic retinoid concentrations in PCB 77-dosed groups.

Female mallard ducks (*Anas platyrhynchos)* have shown rapid bioaccumulation of organochlorine pollutants when maintained at a sewage plant (Custer *et al*. 1996). These birds showed a linear increase in PCB concentration with time, and P450 enzyme activity increased proportionately with PCB loads. Adult male mallard ducks, gavaged twice weekly for five weeks with different concentrations of Aroclor 1254 (0, 4, 20, 100, 250 or 500 mg/kg), showed significant EROD and PROD induction at 20 mg/kg and higher, with the greatest induction in those dosed at 100-500 mg/kg. Relative liver and thyroid weights in these birds were increased at the higher doses, while plasma total T_3 showed a dose-dependent decrease (Fowles *et al*. 1997). It is unfortunate that this study did not analyze Aroclor 1254 effects on T_4 because the enhanced excretion expected to result from PCB treatment is specific to T_4, and the effects on T_3 are much more complex.

The effects of some pollutant chemicals on cormorant embryos also have been studied. Powell *et al*. (1998) injected eggs of double-crested cormorants (*Phalacrocorax auritus*) with PCB 126 (0, 70, 175, 349 or 698 µg/kg egg) or TCDD (0, 1.3, 5.4, 10.7 or 21.4 µg/kg egg) prior to incubation and sampled the chicks just after hatching. They found increased hepatic EROD activity in all dosed groups. The LD_{50} of TCDD was about 44.25 times lower than the LD_{50} for PCB 126.

A further study used artificially incubated eggs of the common tern (*Sterna hirundo*) collected from a natural habitat in the Netherlands (Bosveld *et al*. 2000). Hatchlings were fed fish with different concentrations of coplanar PCB 126 alone or in conjunction with non-coplanar PCB 153 for 21 days posthatch. There was a statistically significant negative relationship between concentrations of coplanar PCBs (including PCB 126) in liver and plasma T_4 concentrations.

Organochlorine Pollutant Effects on Avian Liver Biotransformation Enzymes:

Birds from habitats highly contaminated with organochlorine pollutants typically have significant increases in activity of hepatic Phase I biotransformation enzymes such as EROD. However, there is little basic knowledge about Phase II biotransformation enzymes that are assumed to be important in thyroid homeostasis in birds.

There are posthatching developmental increases in liver UDP-GT activity in chickens. Coulet *et al*. (1996) examined changes in UDP-GT activity at different ages in male Sasso broiler chickens ranging in age from 3 to 12 weeks. UDP-GT steadily increased over the time of the study, with activities at 9 and 12 weeks being significantly higher than at 3 weeks. However, embryonic development of UDP-GT has not been investigated, although most thyroid maturation occurs during embryonic life in these precocial birds. A comparative study of hepatic UDP-GT activity in 10-month old ostriches (*Struthio camelus*), adult white Leghorn chickens of both sexes and 12-week old male Sprague-Dawley rats showed that ostriches had much lower activity per gram of liver than chickens, and the activity in both avian species was significantly lower than in the rats (Amsallem-Holtzman and Ben-Zvi 1997). No studies have addressed the role of UDP-GT in thyroid hormone homeostatsis or the importance of UDP-GT in altering thyroid state in birds. However, one study (Murk *et al*. 1994a) did attempt to evaluate the importance of UDP-GT alterations in PCB-exposed birds (see below).

Phase I enzymes (immunodetected P4501A *i.e*. CYP1A) appear to be induced by environmental PCBs in pipping embryos of black-crowned night herons (*Nycticorax nycticorax*) in the United States. Samples taken from three polluted sites in Wisconsin and California exhibited significant increases in Phase I enzymes compared to the Virginia reference site, and Phase I enzyme activity was correlated with total concentrations of PCBs and 11 specific congeners thought to exert toxicity through the aryl hydrocarbon receptors (AhR; Rattner *et al*. 1994).

A study of common tern embryos in western Europe found no differences in Phase II UDP-GT activity toward T_4 and mean site PHAHs in yolk sacs (Murk *et al*. 1994a). Although this study did find strong correlations between UDP-GT activity and Phase I (EROD) enzyme activity, it is difficult to interpret because circulating thyroid hormones were not altered in relation to site PCBs. Unfortunately, this study did not include validation of the enzyme assay techniques for use on avian tissues.

In general, the literature on PCB effects on thyroid status in birds is limited, and the results of different studies often are inconsistent with each other. Specifically, the relationships between environmental PCB load, induction of Phase II liver biotransformation enzymes and alterations of thyroid hormones are unclear. To understand PCB effects on thyroid function in birds, future studies must examine the normal ontogeny of UDP-GT activity and the effects of pollutant chemicals on UDP-GT activity and thyroid status in the wild and in a laboratory setting.

Chapter 2: Introduction

The presence of high concentrations of pollutant chemicals, including polychlorinated biphenyls (PCBs), in natural environments has traditionally been of great concern because of their toxicity. Since the production of PCBs was outlawed in 1976, the concentrations found in wildlife and natural environments have declined (Peakall and Fox 1987; Fox 1993). However, the persistence of such chemicals is of concern not only in terms of acute toxicity, but also in terms of their potential for endocrine disruption.

It has been suggested that PCBs may be causing disruption of thyroid function in wild birds (Fox 1993). Herring gulls (*Larus argentatus*) exposed to pollutant loads in the Great Lakes have exhibited increased ontogenic and immunological problems compared to reference populations (Peakall *et al*. 1978; Moccia *et al*. 1986; Grasman *et al*. 1996). Thyroid gland histopathology in gulls with high environmental PCB exposure (Moccia *et al*. 1986) and developmental effects in chicken embryos exposed to PCBs *in ovo* (Powell *et al*. 1996) are suggestive of thyroid abnormalities. Herring gulls are considered important indicators of habitat quality because they are piscivorous top predators that are exposed to concentrated pollutant loads through bioaccumulation in the food chain. Recent work in our laboratory has demonstrated that Herring gulls from highly polluted PCB sites in the Great Lakes have significantly depleted thyroid hormone stores in their thyroid glands compared to gulls from reference sites (McNabb *et al*. 2001).

Effects of PCBs on thyroid physiology have been studied mainly using laboratory rats as models, and the thyroid alterations observed in these studies serve as a frame of reference for studies in birds. PCBs are known to disrupt thyroid function in rats by indirect mechanisms such as induction of hepatic Phase II biotransformation enzymes that increases T_4 conjugation and excretion in bile and displacement of T_4 from binding proteins that presumably also increases T_4 excretion (Brouwer *et al*. 1998). If enhanced T_4 excretion exceeds the capacity of the thyroid gland to produce T_4, despite HPT axis stimulation, the resulting hypothyroidism would be expected to have serious consequences for organismal development (McNabb 1992).

Despite the relatively well-developed picture of PCB effects on thyroid function in laboratory studies of rats, it is not clear whether birds are affected in the same ways. Studies of PCB effects on birds have yielded conflicting results about whether circulating thyroid hormones (both total and free) are altered and whether there is thyroid gland hypertrophy indicative of HPT axis activation (reviewed in Dawson 2000). Common tern (*Sterna hirundo*) chicks from PCB-polluted sites along the Rhine and Meuse rivers in the Netherlands and Belgium did not show an inverse relationship between colony PCBs and plasma THs (Murk *et al*. 1994a). Likewise, van den Berg *et al*. (1994) found no significant concentration-effect relationship between yolk sac PCBs and plasma THs in hatchling cormorants. These results were surprising because they found significantly decreased plasma TT_4, TT_3 and fT_4 in the colony of cormorants from the PCB-polluted site compared to the reference colony.

The few studies of PCB induction of liver enzymes, that are presumed to facilitate T_4 excretion in birds, also have yielded inconsistent results. Female Japanese quail (*Coturnix japonica*) dosed with a PCB mixture showed an increase in UDP-GT activity (Riviere *et al*. 1978), but chicks from laboratory-incubated common tern (*Sterna hirundo*) eggs collected at environmentally polluted sites showed no significant differences in hepatic UDP-GT activity compared to a reference site (Murk *et al*. 1994a). However, hepatic Phase I biometabolic enzyme activity was correlated with PCB concentrations in individual terns from these sites (Bosveld *et al*. 1995).

Because we are concerned with the disruptive effects of PCBs on avian thyroid systems, we analyzed induction of activity of the Phase II biotransformation enzyme complex known as uridinediphosphate-glucuronosyltransferases (UDP-GTs). UDP-GTs are partially responsible for the removal of T_4 from the circulation in vertebrates, and are induced in laboratory rats exposed to the PCB mixture, Aroclor 1254 (Barter and Klaassen 1992a, 1994). Such induction, which leads to conjugation of xenobiotics, was found to not only affect the pollutant chemical, but also to increase the biliary clearance of glucuronidated T_4 (Bastomsky 1974), thereby decreasing circulating T_4 concentrations (Barter and Klaassen 1992a, 1994).

Although wild bird populations are normally exposed to mixtures of chemical pollutants, it is important to determine which components of mixtures are responsible for observed effects. This paper reports laboratory studies, conducted in galliform birds, that were designed to determine how PCBs might affect the key liver enzymes known to conjugate T_4 and facilitate its excretion in mammals. Our first objective was to investigate the developmental pattern of hepatic UDP-GT in embryonic and early posthatch life in Japanese quail (which have an abbreviated but otherwise identical pattern of thyroid development to that in chickens; McNabb 1988). This study is important for understanding of the role of this enzyme in the pattern of avian thyroid development and aids in the interpretation of the second objective, which included studying the effects of PCBs at a single developmental stage.

The second objective of this study was to determine the effects of the PCB congener 3,3',4,4',5-pentachlorobiphenyl (PCB 126) on hepatic UDP-GT in chicken embryos. PCB 126 is a co-planar, or dioxin-like, congener (Haag-Grönlund *et al*. 1997) that acts at least partially through the aryl hydrocarbon receptor (AhR) and is considered the most toxic of the PCBs (Brunström 1990; Safe 1990; Whitlock *et al*. 1996). PCB 126 is one of the most common PCBs (in terms of presence and concentration in natural environments; Safe 1990). We also report preliminary data on the effects of 3,3',4,4'-tetrachlorobiphenyl (PCB 77, also a co-planar congener) on UDP-GT activity in chicken embryos.

Chapter 3: Materials and Methods

Animals

Fertile eggs were obtained from a random-bred Japanese quail (*Coturnix japonica*) colony maintained in our laboratory at Virginia Tech. Quail eggs were incubated in a Humidaire Hatchette (incubator/hatcher) at a temperature of 38˚C and 90% relative humidity. Chicks were maintained in a brooder with a temperature gradient for the first three weeks posthatch and fed Sporting Bird Starter Diet (Southern States, Christiansburg, VA).

Fertile White Leghorn chicken (*Gallus domesticus*) eggs from a random bred/out bred colony were purchased from Truslow Farms for PCB experiments conducted in K. Grasman's laboratory at Wright State University (WSU; Dayton, OH). They were incubated at 37.5˚C and 55% relative humidity until day 20 of the 21-day incubation period for the embryonic experiments. For the experiments on chicks, the eggs were placed in hatching trays in a horizontal position 3 days prior to the predicted hatching date. Chicks were allowed to hatch and dry before being moved to the animal care facility for maintenance and were maintained on chicken starter feed and water *ad libitum* until time of sampling.

Experimental Design

Quail UDP-GT Ontogeny:

For our study of the development of UDP-GT in a precocial bird (Japanese quail) we used embryos sampled on days 12, 14, and the perihatch period of the 16- to 17-day incubation period (N = 5-17 per developmental age). Perihatch embryos sampled on days 16 and 17 were separated into three stages: beak not into air cell (NI), internal pipping (*i.e*. beak into the air cell—I) and external pipping (*i.e*. beak pipped through the eggshell—S). Chicks were sacrificed by decapitation at <1, 1, 4, 6, 7 and 20 days posthatch. Adult quail also were sampled. Samples were frozen at –80ºC until analysis. Livers were homogenized individually if they were 0.25 mg or larger, or pooled to yield a 0.33 mg or larger sample in the case of smaller embryos or chicks.

Effects of PCBs on Hepatic UDP-GT Activity in Developing Chickens:

To examine different aspects of UDP-GT activity on precocial birds, we performed three experiments. In Experiment 1, day 20 embryos from eggs dosed with PCB 126 were sampled to assess hepatic UDP-GT activity. Due to the constraints of sampling large numbers of eggs at one time, several sets of 10-17 eggs (runs) with different doses of PCB 126 were used. Because there were no statistically significant differences between runs, all runs were combined for the final analysis.

Chicken eggs were dosed with 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and embryos and chicks were sampled in the laboratory of K. Grasman at WSU. PCB 126

was administered in sunflower oil onto the air cell of eggs prior to incubation. Doses included were 0 (sunflower oil vehicle), 0.05, 0.13, 0.32, 0.48, 0.64 or 0.80 ng PCB 126/g egg. Non-injected controls also were included. Eggs were incubated, and the embryos were sacrificed and sampled on day 20 of the 21-day incubation period. Livers were removed, divided into right and left lobes and stored at –80˚C until analysis. Blood samples were collected in heparinized tubes and centrifuged, and separated plasma was stored frozen until analysis.

In Experiments 2 (embryonic exposure to PCB 126) and 3 (embryonic exposure to PCB 126 or 77), chicks from the PCB-dosed eggs were sampled at day 42 posthatch. For Exp. 2, eggs were dosed with PCB 126 at concentrations of 0, 0.25 and 0.50 ng/g egg weight. In Exp. 3, eggs were dosed with PCB 126 (0.25 ng/g egg weight), PCB 77 (3,3',4,4'-tetrachlorobiphenyl—0.64 ng/g egg weight) or sunflower oil only. The doses of PCBs we used were designed to investigate PCB effects at levels with relatively low toxicity [note that the highest dose (0.8 ng/kg) of PCB 126 resulted in 40% mortality].

Measurements of Hepatic UDP-GT Activity

Tissues were examined for activity toward *para*-nitrophenol (*p*NP), a substrate utilized by all UDP-GT isozymes induced by phenolic compounds, and preliminary studies were done using labeled T_4 as substrate.

Tissue Preparations:

Crude liver homogenate (HOM) was prepared by 10 strokes of a ground glass pestle in a 3 ml Kontes 21 glass homogenizer containing liver and buffer. Depending on the desired tissue dilution for different parts of the study, HOM was one part liver by weight plus two (3X) or four (5X) parts Tris-HCl buffer (50 mM Tris with 150 mM KCl) by volume. HOM samples were frozen, thawed and filtered through one layer of nylon organdy to remove congealed lipids and fragments of connective tissue, then was stored at -80ºC.

For comparison with HOM preparations, microsomes (MIC) were prepared from HOM samples by differential centrifugation. First, HOM was centrifuged at 10,000*g* for 20 min at 0ºC to obtain a supernatant "post-mitochondrial fraction" (PMF) which in turn was centrifuged at 125,000*g* for 60 min at 4ºC in a Beckman TL-100 ultracentrifuge. After the supernatant was discarded, the microsomal pellet was resuspended in 0.25 M sucrose and stored at -80ºC (Barter and Klaassen 1992a). There was considerable loss of UDP-GT activity in MIC preparations compared to HOM, relative to original tissue used so all data reported in this study were generated using HOM.

Methods Validation:

To measure liver UDP-GT in birds, we first needed to validate the use of a UDP-GT assay, previously used for mammalian and fish tissue, for use with avian liver preparations. Our validations determined the assay conditions under which enzyme

activity was proportional to both enzyme concentration and incubation time for each experiment. We used adult quail liver for the initial validation studies, and then checked for valid assay conditions with liver from the embryonic chicken and quail used in the different experiments.

para-*Nitrophenol Assay for UDP-GT Activity:*

Hepatic UDP-GT activity toward phenolic substrates was measured as decolorization of *para*-nitrophenol (*p*NP), by modification of the methods of G. A. Fox (Environment Canada; personal communication) and Castren and Oikari (1983). The decolorization of *p*NP from yellow to clear with conjugation was measured as absorbance at 400 nm. In this method, 50 µl of HOM or MIC was mixed with 100 µl of 10.0 mM uridine 5'-diphosphoglucuronic acid (UDPGA) solution in buffer-diluent (0.2 M Tris-HCl w/0.02% Triton X-100, pH 7.4). This mixture was vortexed and pre-incubated for 5 min at 37°C to conjugate any potentially confounding endogenous phenolic compounds and to bring the mixture to reaction temperature. Then, 50 µl of 5.0 mM *p*NP solution in buffer-diluent was added. The mixture was vortexed and incubated at 37°C for 30-180 min, depending on the tissue preparation (HOM or MIC) and on other factors, such as animal age and species, which might affect the enzyme content of the tissues. At the end of incubation, the reaction was stopped by adding 900 µl of ice-cold 3% trichloroacetic acid (TCA), and the tubes were vortexed and left on ice for 10 min. The tubes were then centrifuged on a Jouan MR 18 12 centrifuge for 20 min at 10,000*g* and 0°C. Then 900 µl of the supernatant was added to tubes containing 100 µl 5 N NaOH. This alkaline mixture was vortexed and a 500 µl aliquot of each sample was placed in a disposable 4.5 ml polystyrene cuvette, diluted with 2.0 ml deionized H_2O and mixed by inversion. The absorbance of each sample was read on a Beckmann DU-640 spectrophotometer at 400 nm. Blanks were treated as above except that TCA was added immediately after the addition of *p*NP solution (zero time blanks). For each sample, we did triplicate tubes for enzyme activity and for zero time blanks. We tested several types of blanks (0 time blanks, incubated blanks that contained no tissue and incubated blanks that contained no UDPGA) and found no detectable difference in absorbance between the three types.

Standard conditions for *p*NP assays were as described above, but with the specifics that follow. The chicken embryo experiments were done with a 5X homogenate and a 180 min incubation time. The quail UDP-GT developmental study was done using 5X HOM for a 40 min incubation period and the 42-day posthatch chicken study was done using 12.5X HOM.

Labeled T_4 Assays for UDP-GT Activity:

To compare the amount of enzyme activity indicated by the *p*NP assay to the amount of T_4 glucuronidated, we investigated UDP-GT activity using labeled T_4 as substrate by a modification/combination of techniques (Beetstra *et al*. 1991; Barter and Klaassen 1992a). Each incubation tube contained 50 µl tissue HOM or other preparation, 80 µl buffer (100 mM Tris-HCl w/13.33 mM $MgCl_2$, 0.033% Brij-58, 1.87 mM saccharic acid-1,4-lactone, pH 7.4), 30 µl 33.33 mM UDPGA in buffer, 20 µl 1.0 mM unlabeled T_4

in H_2O and 20 µl ^{125}I-T_4 in buffer (~100,000 cpm). The concentration of the labeled T_4 was negligible (0.304 nM) and did not significantly increase the overall concentration of T_4 in the incubation mixture. The tubes were incubated in a 37°C water bath for 60 min, and the reaction was halted by the addition of 200 µl of ice-cold methanol (MeOH). This mixture was vortexed, then centrifuged for 15 min at 10,000*g* and 0°C. Supernatant (350 µl) was then added to 700 µl 0.1 N HCl, vortexed, and loaded onto Sephadex LH-20 columns (1.0 ml bed volume) that were prewashed with 0.1 N HCl. The sample components were eluted in a step-wise manner with 6 X 1.0 ml aliquots of 0.1 N HCl followed by 7 X 1.0 ml aliquots of deionized H_2O and 4 X 1.0 ml aliquots of 0.1 N NaOH/ethanol (1:1, v/v). These elution fractions were collected and counted. As Figure 7 shows, there are three distinct peaks of radioactivity that correlate with the three elution solvents. Unbound $^{125}I^-$ was eluted in the acid fraction, glucuronidated ^{125}I-T_4 (T_4G) was eluted in the water fraction, and the unbound ^{125}I-T_4 was eluted in the alkaline ethanol fraction (Beetstra *et al*. 1991; Finnson and Eales 1997).

For the *p*NP assay, Tris-HCl, Triton X-100, *p*NP, UDPGA and TCA were supplied by Sigma (St. Louis, MO); KCl and 4.5 ml disposable polystyrene cuvettes were supplied by Fisher Scientific (Pittsburgh, PA). For the labeled T_4 assay, high specific activity radiolabeled T_4 (1200 µCi/µg ^{125}I-T_4) was supplied by New England Nuclear (Boston, MA); $MgCl_2$, HCl, MeOH and NaOH were supplied by Fisher Scientific (Pittsburgh, PA); Brij-58, saccharic acid-1,4-lactone and Sephadex LH-20 were supplied by Sigma (St. Louis, MO); and Poly-Prep chromatography columns were supplied by Bio-Rad Laboratories (Hercules, CA).

Verification of Measurements of UDP-GT Activity Using Mammalian Tissue:

To demonstrate that these techniques could determine differences in UDP-GT activity between control animals and those dosed with pollutant chemicals, C57Bl/6++ mice were used. These mice, which are sensitive to TCDD and exhibit relatively high background levels of UDP-GT activity, were dosed with either 50 µg/kg body weight TCDD in corn oil or only corn oil (as control) and their livers were prepared as described previously.

Protein Determinations

Protein determinations were done spectrophotometrically at 595 nm using the Pierce Coomassie Protein Assay (Pierce, Rockford, IL). For the protein assay, homogenate samples were diluted 300X and a 25 µl aliquot was added to 1.0 ml of Coomassie blue reagent, mixed and read against a standard curve (0-1000 ng/ml) from dilutions of the bovine serum albumin solution provided by Pierce. All protein measurements were done in triplicate.

Statistics

Quail UDP-GT ontogeny data were analyzed using a one-way ANOVA (SPSS; Chicago, IL) with Tukey's HSD post hoc test. Differences in means were considered

significant at $p < 0.05$. Embryonic and 28-day chicken data were analyzed using 95% confidence intervals generated by SigmaPlot (SPSS). Mouse samples were analyzed by t-test for equality of means, equal variances not assumed (SPSS).

Chapter 4: Results

Validation Studies for p*NP Assay of UDP-GT Activity*

To calculate *p*NP glucuronidation activity, we determined the molar absorption coefficient for *p*NP solutions for our conditions, with different concentrations of *p*NP in buffer as the standard curve. From this standard curve (Figure 8), and using Beer's Law ($\Delta A = \ell \Delta c \varepsilon$, where ΔA is change in absorbance, ℓ is length of light path, Δc is change in solution concentration and ε is the molar absorption coefficient), the molar absorption coefficient for *p*NP was determined to be 14,587 $M^{-1}cm^{-1}$.

For quail liver, UDP-GT activity was shown to be proportional to enzyme concentration using HOM preparations up to 2.6 µg HOM protein/ml in the 200 µl reaction mixture (Figure 9). Also, using 2.8 µg HOM protein/ml, enzymatic activity was linear for times up to 90 minutes (Figure 10). These findings led to standard assay conditions, using homogenate concentrations containing approximately 2.8 µg HOM protein/ml and an incubation time of 40 minutes for adult quail liver samples. For the developmental study of quail liver, an incubation time of 40 minutes was also used, but, due to differences in protein content at different ages, homogenate concentrations ranged from 2.9 to 8.1 µg HOM protein/ml in the 200 µl incubation.

The standard assay conditions using chicken liver HOM were altered based on developmental age of the tissue. In general, we found embryonic chicken tissue to be lower in protein content than adult tissue. Enzyme activity in juvenile chicken HOM preparations was linear with time up to 120 minutes using 4.7 µg HOM protein/ml reaction mixture (Figure 11). Assays on juvenile chicken liver were done using approximately 6.8 µg HOM protein/ml and 20 minute incubations; embryonic assays were done using approximately 11.0 µg HOM protein/ml at 180 minutes.

To demonstrate that the *p*NP assay could detect induction of UDP-GT, we used liver HOM of TCDD-dosed C57Bl/6++ mice. These mice were dosed with up to 50 µg/kg TCDD in a sunflower oil vehicle. The assay clearly detected induction of *p*NP UDP-GT in TCDD-dosed compared to control mice that received corn oil (Figure 12).

Quail UDP-GT Ontogeny

The profile of UDP-GT activity toward *p*NP during development in Japanese quail is shown in Figure 13. Embryonic liver UDP-GT activity levels increased slowly until the end of the perihatch period (24 hours posthatch). Then, UDP-GT activity increased markedly between days one (>24 hours) and day 4 posthatch then declined to levels slightly higher than those in embryos by day 6 posthatch. Enzyme activity did not change significantly between day 6 and adult ages (sexually mature birds of >6 weeks of age). When compared to day 12 or 14 embryos, significantly higher activity was found at days 1, 4 and 7 posthatch. When compared to adults, liver UDP-GT activity was significantly higher at day 4 posthatch (one-way ANOVA, Tukey's HSD test, $p < 0.0001$).

Effects of PCBs on Hepatic UDP-GT Activity in Developing Chickens

Experiment 1. Embryonic Exposure to PCB 126/Embryos:

UDP-GT activity toward *p*NP was not significantly different from either vehicle-injected or non-injected controls in any of the groups dosed prior to incubation and sacrificed on day 20 of incubation. Whether all data were analyzed together (Figure 14) or as individual runs (8/98, 10/98 and 1/99, Figures 15, 16 and 17, respectively) there were no trends toward increasing activity (indicative of enzyme induction) with increasing PCB doses. Likewise, plasma T_4 did not differ between either control (vehicle-injected or non-injected) and any of the dosed groups. Plasma T_3 did not show any consistent pattern of significant differences in relation to PCB doses although T_3 was significantly increased, compared to both types of controls, at doses of 0.0512 and 0.48 ng/g egg weight (Figure 18).

Experiment 2. Embryonic Exposure to PCB 126/Chicks:

In chicks dosed with PCB 126 prior to incubation and sacrificed at day 42 posthatch there were no significant differences in UDP-GT activity between control and PCB 126-injected groups (Figure 19).

Experiment 3. Embryonic Exposure to PCB 126 or 77/Chicks:

Neither PCB 126-dosed (0.25 ng/g egg) nor PCB 77-dosed (0.64 ng/g egg) groups showed significantly altered UDP-GT activities toward *p*NP compared to vehicle-injected controls. These tissues were from chicks dosed prior to incubation and sacrificed at day 42 posthatch (Figure 20).

T_4 Assay

In preliminary experiments using the T_4 assay, we were able to show separation between control and TCDD-dosed (50 μg/kg) mouse liver HOM (Figure 21). This indicates the potential for utilizing radiolabeled T_4 as substrate to specifically examine UDP-GT activity in the context of thyroid function. In preliminary experiments with adult quail HOM, we have been able to show linearity and proportionality up to 90 minutes with 2.7 μg HOM protein/ml in the 200 μl reaction mixture (Figure 22).

Chapter 5: Discussion

The present study of UDP-GT activity in galliform birds was part of a larger project investigating PCB effects on avian thyroid development and function. Overall, this larger project addresses the effects of pollutant chemicals (including highly prevalent PCBs) on thyroid status in wild-caught herring gulls from the Great Lakes and the effects of specific PCB congeners on thyroid function in laboratory-reared chickens. Specifically, the present study investigated one hepatic enzyme system that is induced in some vertebrates by PCBs and is involved in the metabolism and excretion of T_4.

There were two main aspects to this study: (1) the description of the pattern of UDP-GT ontogeny in a precocial galliform bird throughout embryonic and early posthatch life, and (2) the investigation of UDP-GT responses to coplanar PCB exposure during embryonic development.

UDP-GT Ontogeny in Quail

Because UDP-GT is responsible, in part, for normal metabolism of T_4 (glucuronidation) resulting in increased hydrophilicity and consequent excretion in urine and bile, we were interested in comparing UDP-GT activity with T_4 concentrations during development. To understand the regulation of circulating thyroid hormones at different times during development, it is necessary to understand the pattern of UDP-GT ontogeny as well as the development of thyroid hormone production and secretion (McNabb *et al*. 1998). To date, only one study (Coulet *et al*. 1996) has examined hepatic UDP-GT activity in a developing bird. That study, however, examined only four ages in juvenile chickens (3, 6, 9 and 12 weeks posthatch). The present study is, to our knowledge, the first investigation of UDP-GT during the period of embryonic and early posthatch life when most thyroid development occurs.

Circulating thyroid hormone concentrations reflect a balance between hormone production/release from the thyroid gland and thyroid hormone metabolism/excretion. During the second half of incubation in precocial birds, embryonic plasma T_4 concentrations increase several-fold (McNabb *et al*. 1981). During the perihatch period, plasma T_4 increases even more dramatically to peak at concentrations several-fold those in late incubation. This peak of circulating T_4 is largely due to TSH signals from the pituitary as part of HPT axis maturation (Thommes 1987). The low UDP-GT activity we found during late incubation and the perihatch period are suggestive of low T_4 metabolism and excretion until after hatch. This low UDP-GT activity, in conjunction with increased thyroid gland function, must contribute to the perihatch peak in T_4. Just after hatch, plasma T_4 concentrations decrease precipitously, probably partly due to the posthatch increase in UDP-GT found in the present study (Figure 23).

Studies of UDP-GT regulation in juvenile thyroidectomized rats (Masmoudi *et al*. 1996) may help explain the role of UDP-GT in the developmental pattern of circulating T_4 in precocial birds. Masmoudi *et al*. (1996) found evidence that high T_3 can induce hepatic UDP-GT activity in young rats. As shown in Figure 23, T_3, like T_4, peaks during

the perihatch period (in this case due to changes in deiodination activity; Freeman and McNabb 1991; Darras *et al*. 1992). If UDP-GT regulation in avian development is similar to that in rats, the perihatch peak in plasma T_3 could be important in the induction of increased UDP-GT activity between days 1 and 4 posthatch in quail in the present study.

Embryonic and Posthatch Exposure to PCB 126 in Chickens

In previous studies, laboratory rats exposed to PCB mixtures, to coplanar PCB congeners and to TCDD typically had decreased circulating T_4 concentrations and increased hepatic UDP-GT activity (Brouwer *et al*. 1998). Because UDP-GTs are thought to be involved in T_4 metabolism/excretion in birds and because some PCBs have decreased circulating T_4 in some avian studies (*e.g*. Eider ducklings, Murk *et al*. 1994b; chicken embryos, Gould *et al*. 1999) we hypothesized that avian embryos exposed to PCBs in the egg would have decreased plasma T_4 and increased hepatic UDP-GT activity. PCB 126 was chosen as the first congener to investigate because it is similar structurally to TCDD (coplanar), it has high toxicity relative to other PCBs, it is present at high concentrations in many polluted environments and it is highly persistent. Also, PCB 126 exposure in embryonic chickens has been shown to cause developmental abnormalities, while in the same study TCDD exposure did not (Powell *et al*. 1996). Unfortunately, this study did not include information on TH concentrations so it is not clear whether altered thyroid function was the direct cause of these developmental abnormalities. In another study of embryonic chickens, TCDD induced the Phase I biotransformation enzyme ethoxyresorufin *O*-deethylase (EROD) but did not cause either developmental abnormalities or changes in TH levels (Janz and Bellward 1996). It should be noted that these authors did not measure UDP-GT. Murk *et al*. (1994a) did find a correlation between EROD and UDP-GT in the common tern (*Sterna hirundo*), but relatively little is known about the relationships between induction of these enzymes.

Surprisingly, in our experiments there was no consistent evidence of decreased thyroid function in chicken embryos or chicks that were exposed to PCB 126 throughout embryonic development. All variables measured—UDP-GT activity and plasma thyroid hormones in the present study, as well as thyroid gland mass, gland hormone content and brain for 5'-deiodination activity (McNabb *et al*. 2001)—supported the conclusion that thyroid development and function were not altered in either embryos or chicks by this exposure to PCB 126. Likewise, PCB 77 exposure during incubation did not affect UDP-GT activity or plasma TH concentrations in chicks at 6 weeks of age.

We know that the PCBs entered the embryos because of other physiological effects seen in dosed birds in our studies. Concurrent studies on other tissues from these same embryos indicated that a dose of 0.13 ng PCB 126/g egg (our second lowest dose) caused immunological alterations and that our highest dose caused 40% mortality (K. A. Grasman, personal communication).

The UDP-GT assay we used was shown to be capable of detecting increases in UDP-GT induced by TCDD in laboratory mice. The *p*NP substrate is a phenolic

compound that indicates the activity of UDP-GTs toward other phenolic compounds (including both PCBs and T_4). This substrate is conjugated by many UDP-GT isozymes and so is considered to indicate overall induction of UDP-GT-related conjugation. The common use of the assay (which detects multiple UDP-GT isozymes) has led to its use as a frame of reference for Phase II biometabolic activity. However, because this assay is not specific to T_4 glucuronidation, it may not detect subtle effects on the specific isozymes of UDP-GT that are important in thyroid function. The relationship between this measure of activity and the effects on T_4 metabolism remain to be investigated.

Although our studies indicate that exposure to PCB 126 throughout embryonic development does not induce UDP-GT activity in either chicken embryos or chicks, PCB 126 is only one of many different PCB congeners found in polluted environments. Most of the very limited number of studies that have shown alterations in thyroid function in birds have utilized commercial PCB mixtures such as Aroclor 1254, although some studies (Murk *et al*. 1994b; Gould *et al*. 1999) have shown decreases in avian thyroid function with exposure to PCB 77. It is important to note that in one of these studies (Gould *et al*. 1999), PCB 77 and Aroclor 1242 were correlated with negative effects on growth in embryonic chickens, but that the Aroclor 1242 and 1254 treatments were the only ones that resulted in decreased plasma T_4 concentrations. The apparent effectiveness of PCB mixtures in altering avian thyroid function suggests there may be synergistic reactions between the constituents of these mixtures.

Future Studies

Because effects of individual PCB constituents and PCB mixtures are not equivalent, it is important that mixtures be used in future studies to examine possible thyroid hormone alterations in birds. It also is important to examine the effects of other PCB congeners, such as the non-coplanar PCB 153, that compose a large percentage of industrial mixtures. Further, to truly determine whether ontogenic alterations occur, it may be necessary to examine more stages throughout embryonic development. Finally, these results are applicable only to the species described—to assess effects, whether in terms of toxicity or endocrine disruption, on birds in general, it will be necessary to examine the same parameters in other avian species.

Literature Cited

Amsallem-Holtzman, E and Z Ben-Zvi. 1997. Drug metabolizing enzymes in the ostrich (*Struthio camelus*): comparison with the chicken and the rat. Comparative Biochemistry and Physiology 116C(1):47-50.

Barter, RA and CD Klaassen. 1992a. UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. Toxicology and Applied Pharmacology 113:36-42.

Barter, RA and CD Klaassen. 1992b. Rat liver microsomal UDP-glucuronosyltransferase activity toward thyroxine: characterization, induction, and form specificity. Toxicology and Applied Pharmacology 115:261-267.

Barter, RA and CD Klaassen. 1994. Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. Toxicology and Applied Pharmacology 128:9-17.

Bastomsky, CH. 1974. Effects of a polychlorinated biphenyl mixture (Aroclor 1254) and DDT on biliary thyroxine excretion in rats. Endocrinology 95(4):1150-1155.

Beaulieu, M, É Lévesque, DW Hum and A Bélanger. 1998. Isolation and characterization of a human orphan UDP-glucuronosyltransferase, UGT2B11. Biochemical and Biophysical Research Communications 248:44-50.

Beetstra, JB, JG van Engelen, P Karels, HJ van der Hoek, M de Jong, R Docter, EP Krenning, G Hennemann, A Brouwer and TJ Visser. 1991. Thyroxine and 3,3',5-triiodothyronine are glucuronidated in rat liver by different uridine diphosphate-glucuronyltransferases. Endocrinology 128(2):741-746.

Bosveld, ATC, J Gradener, AJ Murk, A Brouwer, M van Kampen, EHG Evers and M Van den Berg. 1995. Effects of PCDDs, PCDFs, and PCBs in common tern (*Sterna hirundo*) breeding in estuarine and coastal colonies in the Netherlands and Belgium. Environmental Toxicology and Chemistry 14(1):99-115.

Bosveld, ATC, R Nieboer, A de Bont, J Mennen, AJ Murk, LA Feyk, JP Giesy and M van den Berg. 2000. Biochemical and developmental effects of dietary exposure to polychlorinated biphenyls 126 and 153 in common tern chicks (*Sterna hirundo*). Environmental Toxicology and Chemistry 19(3):719-730.

Brouwer, A, DC Morse, MC Lans, AG Schuur, AJ Murk, E Klasson-Wehler, Å Bergman and TJ Visser. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicology and Industrial Health 14(1/2):59-84.

Brunström, B. 1990. Mono-*ortho*-chlorinated chlorobiphenyls: toxicity and induction of 7-ethoxyresorufin *O*-deethylase (EROD) activity in chick embryos. Archives of Toxicology 64:188-192.

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

Burchell, B and MWH Coughtrie. 1989. UDP-glucuronosyltransferases. Pharmaceutical Therapeutics 43:261-289.

Carson, R. 1962. Silent Spring. Boston: Houghton Mifflin.

Castren, M and A Oikari. 1983. Optimal assay conditions for liver UDP-glucuronosyltransferase from the rainbow trout, *Salmo gairdneri*. Comparative Biochemistry and Physiology 76C(2):365-369.

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

Colborn, T, D Dumanoski and JP Myers. 1996. Our Stolen Future. New York: Dutton.

Coulet, M, C Eeckhoutte and P Galtier. 1996. Ontogenic development of drug-metabolizing enzymes in male chicken liver. Canadian Journal of Physiology and Pharmacology 74:32-37.

Crain, DA, LJ Guillette, Jr, DB Pickford, HF Percival and AR Woodward. 1998. Sex-steroid and thyroid hormone concentrations in juvenile alligators (*Alligator mississippiensis*) from contaminated and reference lakes in Florida, USA. Environmental Toxicology and Chemistry 17(3):446-452.

Custer, TW, DW Sparks, SA Sobiech, RK Hines and MJ Melancon. 1996. Organochlorine accumulation by sentinel mallards at the Winston-Thomas sewage treatment plant, Bloomington, Indiana. Archives of Environmental Contamination and Toxicology 30:163-169.

Damanhouri, ZA and OS Tayeb. 1994. Comparison of cytochrome P-450 content and conjugative enzymes in livers of camels (*Camelus dromedarius*), guinea pigs (*Cavia porcellus*) and rats (*Rattus norvegicus*). Comparative Biochemistry and Physiology 108B(3):349-355.

Darras, VM, TJ Visser, LR Berghman and ER Kuehn. 1992. Ontogeny of type I and type III deiodinase activities in embryonic and posthatch chicks: relationship with

changes in plasma triiodothyronine and growth hormone levels. Comparative Biochemistry and Physiology A 103(1):131-136.

Desaulniers, D, R Poon, W Phan, K Leingartner, WG Foster and I Chu. 1997. Reproductive and thyroid hormone levels in rats following 90-day dietary exposure to PCB 28 (2,4,4'-trichlorobiphenyl) or PCB 77 (3,3',4,4'-tetrachlorobiphenyl). Toxicology and Industrial Health 13(5):627-638

Dawson, A. 2000. Mechanisms of endocrine disruption with particular reference to occurrence in avian wildlife: a review. Ecotoxicology 9:59-69.

De Voogt, P, DE Wells, L Rutergardh and UAT Brinkman. 1993. Biological activity, determination and occurrence of planar, mono- and di-*ortho* PCBs. *In* Environmental Analytical Chemistry of PCBs (J Albaiges, ed.). Yverdon, Switzerland: Gordon and Breach, vol. 16, pp 151-196.

Emi, Y, S Ikushiro and T Iyanagi. 1996. Xenobiotic responsive element-mediated transcriptional activation in the UDP-glucuronosyltransferase family 1 gene complex. The Journal of Biological Chemistry 271(7):3952-3958.

Finnson, KW and JG Eales. 1997. Glucuronidation of thyroxine and 3,5,3'-triiodothyronine by hepatic microsomes in rainbow trout, *Oncorhynchus mykiss*. Comparative Biochemistry and Physiology 117C(2):193-199.

Fowles, JR, A Fairbrother, KA Trust and NI Kerkvliet. 1997. Effects of Aroclor 1254 on the thyroid gland, immune function, and hepatic cytochrome P450 activity in mallards. Environmental Research 75:119-129.

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 Great Lakes Research 19(4):722-736.

Freeman, TB and FMA McNabb. 1991. Hepatic 5' deiodinase activity of Japanese quail using reverse-T_3 as substrate: assay validation, characterization and developmental studies. Journal of Experimental Zoology 258(2):212-220.

Goldey, ES, LS Kehn, C Lau, GL Rehnberg and KM Crofton. 1995. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicology and Applied Pharmacology 135:77-88.

Gould, JC, KR Cooper and CG Scanes. 1999. Effects of polychlorinated biphenyls on thyroid hormones and liver type I monodeiodinase in the chick embryo. Ecotoxicology and Environmental Safety 43:195-203.

Grasman, KA, GA Fox, PF Scanlon and JP Ludwig. 1996. Organochlorine-associated immunosuppression in prefledgling Caspian terns and herring gulls from the Great Lakes: an ecoepidemiological study. Environmental Health Perspectives 104(Supplement 4):829-842.

Guillette, Jr., LJ and DA Crain. 1996. Endocrine-disrupting contaminants and reproductive abnormalities in reptiles. Comments on Toxicology 5(4-5):381-399.

Haag-Grönlund, M, Y Kato, R Fransson-Steen, G Scheu and L Wärngård. 1997. Promotion of enzyme altered foci in female rat livers by 2,3,3',4,4',5-hexachlorobiphenyl. Toxicology and Applied Pharmacology 147:46-55.

Harris, C and WS Bradshaw. 1984. Alterations in liver ultrastructure and induction of UDP-glucuronyltransferase in the rat following prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl. Archives of Environmental Contamination and Toxicology 13:715-721.

Henry, EC and TA Gasiewicz. 1987. Changes in thyroid hormones and thyroxine glucuronidation in hamsters compared with rats following treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicology and Applied Pharmacology 89:165-174.

Henshel, DS, B Hehn, R Wagey, M Vo and JD Steeves. 1997. The relative sensitivity of chicken embryos to yolk- or air-cell injected 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environmental Toxicology and Chemistry 16(4):725-732.

Hood, A, YP Liu, VH Gattone II and CD Klaassen. 1999. Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. Toxicological Sciences 49:263-271.

Janz, DM and GD Bellward. 1996. *In ovo* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure in three avian species. Toxicology and Applied Pharmacology 139:281-291.

Kannan, N, S Tanabe and R Tatsukawa. 1988. Toxic potential of non-*ortho* and mono-*ortho* coplanar PCBs in commercial PCB preparations: "2,3,7,8-T_4CDD toxicity equivalence factors approach." Bulletin of Environmental Contamination and Toxicology 41:267-276.

Klasson-Wehler, E, Å Bergman, I Brandt, PO Darnerud and CA Wachtmeister. 1989. 3,3',4,4'-tetrachlorobiphenyl: excretion and tissue retention of hydroxylated metabolites in the mouse. Drug Metabolism and Disposition 17(4):441-448.

Koga, Y, M Tsuda, N Ariyoshi, Y Ishii, H Yamada, K Oguri, Y Funae and H Yoshimura. 1994. Induction of bilirubin UDP-glucuronyltransferase and CYP4A1 P450 by co-planar PCBs: different responsiveness of guinea pigs and rats. Chemosphere 28(3):639-645.

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

Leonard, LJ and J Koehrle. 1996. Intracellular pathways of iodothyronine metabolism. *In* Werner and Ingbar's The Thyroid, a Fundamental and Clinical Text, 7th edition (LE Braverman and RE Utiger, eds.). Philadelphia: Lippincott-Raven Publishers.

Li, M-H and LG Hansen. 1997. Consideration of enzyme and endocrine interactions in the risk assessment of PCBs. Reviews in Toxicology 1:71-156.

Lorenzen, A, SW Kennedy, LJ Bastien and ME Hahn. 1997. Halogenated aromatic hydrocarbon-mediated porphyrin accumulation and induction of cytochrome P4501A in chicken embryo hepatocytes. Chemical Pharmacology 53:373-384.

Masmoudi, T, AK Hihi, M Vázquez, Y Artur, B Desvergne, W Wahli and H Goudonnet. 1997. Transcriptional regulation by triiodothyronine of the UDP-glucuronosyltransferase family 1 gene complex in rat liver: comparison with induction by 3-methylcholanthrene. The Journal of Biological Chemistry 272(27):17171-17175.

Masmoudi, T, R Planells, J Mounié, Y Artur, J Magdalou and H Goudonnet. 1996. Opposite regulation of bilirubin and 4-nitrophenol UDP-glucuronosyltransferase mRNA levels by 3,3',5 triiodo-L-thyronine in rat liver. FEBS Letters 379:181-185.

McKinney JD and CL Waller. 1994. Polychlorinated biphenyls as hormonally active structural analogues. Environmental Health Perspectives 102:290-297.

McLachlan, JA and SF Arnold. 1996. Environmental estrogens. American Scientist 84:452-461.

McNabb, FMA. 1988. Peripheral thyroid hormone dynamics in precocial and altricial avian development. American Zoologist 28:427-440.

McNabb, FMA. 1992. Thyroid hormones. Englewood Cliffs, New Jersey: Prentice Hall

McNabb, FMA and M-F Cheng. 1985. Thyroid development in altricial ring doves, *Streptopelia risoria*. General and Comparative Endocrinology 58:243-251.

McNabb, FMA and TE Hughes. 1983. The role of serum binding proteins in determining free thyroid hormone concentrations during development in quail. Endocrinology 113(3):957-963.

McNabb, FMA and DB King. 1993. Thyroid hormone effects on growth, development and metabolism. *In* The Endocrinology of Growth, Development, and

Metabolism in Vertebrates (PKT Pang, CG Scanes and MP Schreibman, eds.). New York: Academic Press, pp. 393-417.

McNabb, FMA, RJR McCleary, LA Fowler, CM Parsons, KA Grasman, and GA Fox. 2001. Thyroid function in PCB-exposed avian embryos and chicks. *In* Perspectives in Comparative Endocrinology: Unity and Diversity (JJTh Goos, RK Rastogi, H Vaudry and R Pierantoni, eds.). Bologna, Italy: Monduzzi Editore, pp. 275-280.

McNabb, FMA, CG Scanes and M Zeman. 1998. The endocrine system. *In* Avian Growth and Development, Evolution within the Altricial-Precocial Spectrum (JM Starck and RE Ricklefs, eds.). New York: Oxford University Press, pp. 174-202.

McNabb, FMA, RT Weirich and RA McNabb. 1981. Thyroid development in embryonic and perinatal Japanese quail. General and Comparative Endocrinology 43:218-226.

Metz, RP and JK Ritter. 1998. Transcriptional activation of the UDP-glucuronosyltransferase 1A7 gene in rat liver by aryl hydrocarbon receptor ligands and oltipraz. The Journal of Biological Chemistry 273(10):5607-5614.

Mineau, P, GA Fox, RJ Norstrom, DV Weseloh, DJ Hallett and JA Ellenton. 1984. Using the herring gull to monitor levels and effects of organochlorine contamination in the Canadian Great Lakes. *In* Toxic Contaminants in the Great Lakes (JD Nriagu, MS Simmons, eds.). New York: John Wiley & Sons, pp. 425-452.

Moccia, RD, GA Fox and A Britton. 1986. A quantitative assessment of thyroid histopathology of herring gulls (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environmental contaminants. Journal of Wildlife Diseases 22(1):60-70.

Mulder, GJ. 1992. Glucuronidation and its role in regulation of biological activity of drugs. Annual Review of Pharmacological Toxicology 32:25-49.

Murk, AJ, ATC Bosveld, M van den Berg and A Brouwer. 1994a. Effects of polyhalogenated aromatic hydrocarbons (PHAHs) on biochemical parameters in chicks of the common tern (*Sterna hirundo*). Aquatic Toxicology 30:91-115.

Murk, AJ, JHJ Van den Berg, M Fellinger, MJC Rozemeijer, C Swennen, P Duiven, JP Boon, A Brouwer and JH Koeman. 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. Environmental Pollution 86:21-30.

O'Flaherty, EJ. 2000. Absorption, distribution, and elimination of toxic agents. *In* Principles of Toxicology: Environmental and Industrial Applications, 2nd edition (PL Williams, RC James and SM Roberts, eds.). New York: John Wiley & Sons, Inc.

Peakall, DB and GA Fox. 1987. Toxicological investigations of pollutant-related effects in Great Lakes gulls. Environmental Health Perspectives 71:187-193.

Peakall, DB, GA Fox, AP Gilman, DJ Hallett and RJ Norstrom. 1978. Reproductive success of herring gulls as an indicator of Great Lakes water quality. *In* Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, Environmental Science Research, vol. 16 (BK Afghan and D Mackay, eds.). New York: Plenum, pp. 337-344.

Powell, DC, RJ Aulerich, JC Meadows, DE Tillitt, JP Giesy, KL Stromborg and SJ Bursian. 1996. Effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) injected into the yolks of chicken (*Gallus domesticus*) eggs prior to incubation. Archives of Environmental Contamination and Toxicology 31:404-409.

Powell, DC, RJ Aulerich, JC Meadows, DE Tillitt, ME Kelly, KL Stromborg, MJ Melancon, SD Fitzgerald and SJ Bursian. 1998. Effects of 3,3',4,4',5-pentachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin injected into the yolks of double-crested cormorant (*Phalacrocorax auritus*) eggs prior to incubation. Environmental Toxicology and Chemistry 17:2035-2040.

Rattner, BA, VP Eroschenko, GA Fox, DM Fry and J Gorsline. 1984. Avian endocrine responses to environmental pollutants. The Journal of Experimental Zoology 232:683-689.

Rattner, BA, JS Hatfield, MJ Melancon, TW Custer and DE Tillitt. 1994. Relation among cytochrome P450, Ah-active PCB congeners and dioxin equivalents in pipping black-crowned night-heron embryos. Environmental Toxicology and Chemistry 13(11):1805-1812.

Riviere, JL, E DeLavaur and G Grolleau. 1978. Effect of polychlorinated biphenyls on drug metabolism in Japanese quail and its progeny. Toxicology 11:329-334.

Robbins, J. 1996. Thyroid hormone transport proteins and the physiology of hormone binding. *In* Werner and Ingbar's The Thyroid, a Fundamental and Clinical Text, 7th edition (LE Braverman and RE Utiger, eds.). Philadelphia: Lippincott-Raven Publishers.

Safe, S. 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). Critical Reviews in Toxicology 21:51-88.

Schuur, AG, FM Boekhorst, A Brouwer and TJ Visser. 1997. Extrathyroidal effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on thyroid hormone turnover in male Sprague-Dawley rats. Endocrinology 138(9):3727-3734.

Seo, B-W, M-H Li, LG Hansen, RW Moore, RE Peterson and SL Schantz. 1995. Effects of gestational and lactational exposure to coplanar polychlorinated biphenyl (PCB) congeners or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on thyroid hormone concentrations in weanling rats. Toxicology Letters 78:253-262.

Starck, JM and RE Ricklefs. 1998. Patterns of development: the altricial-precocial spectrum. *In* Avian Growth and Development, Evolution within the Altricial-Precocial Spectrum (JM Starck and RE Ricklefs, eds.). New York: Oxford University Press, pp. 3-30.

Tayesse, L, C Chambras, D Marionnet, C Bosgiraud and P Deschaux. 1998. Basal level and induction of cytochrome P450, EROD, UDPGT and GST activities in carp (*Cyprinus carpio*) immune organs (spleen and head kidney). Bulletin of Environmental Contamination and Toxicology 60:300-305.

Tephly, TR and B Burchell. 1990. UDP-glucuronosyltransferases: a family of detoxifying enzymes. Trends in Pharmacological Science 11:276-279.

Thommes, RC. 1987. Ontogenesis of thyroid function and regulation in the developing chick embryo. Journal of Experimental Zoology, Supplement 1:273-279.

van den Berg, M, BLHJ Craane, T Sinnige, S van Mourik, S Dirksen, T Boudewun, M van der Gaag, IJ 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 (*Phalcrocorax carbo*) after *in ovo* exposure. Environmental Toxicology and Chemistry 13(5):803-816.

Visser, T. 1990. Importance of deiodination and conjugation in the hepatic metabolism of thyroid hormone. *In* The Thyroid Gland, Comprehensive Endocrinology Revised Series (MA Greer, ed.). New York: Raven Press, pp. 255-283.

Whitlock, Jr., JP, ST Okino, L Dong, HP Ko, R Clarke-Katzenberg, Q Ma and H Li. 1996. Induction of cytochrome P4501A1: a model for analyzing mammalian gene transcription. FASEB Journal 10:809-818.

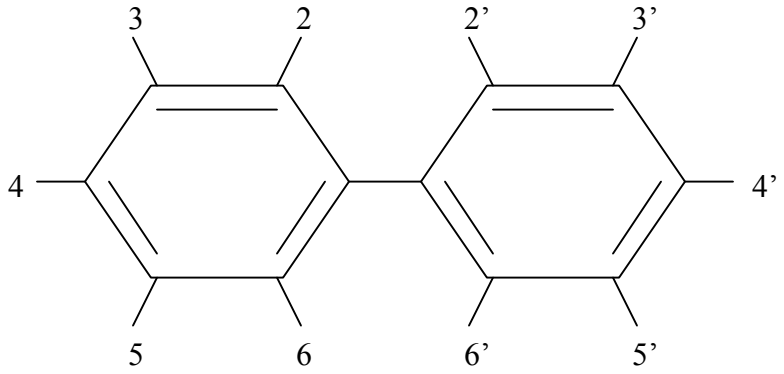


Figure 1. Numbering of Possible Chlorine Substitution Positions in PCBs. The diagram shows their relationship to PCB naming protocol.

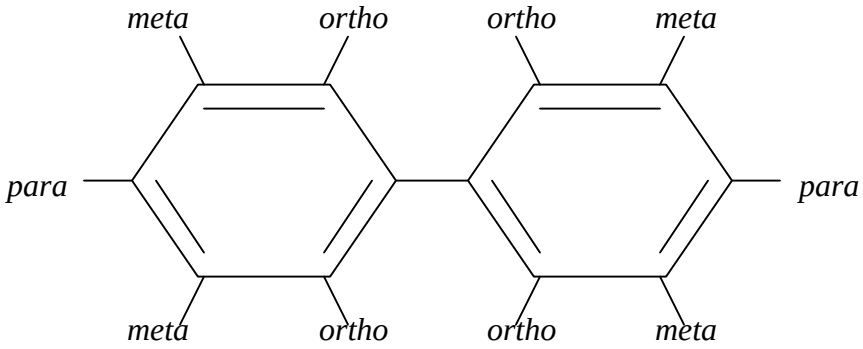


Figure 2. Chlorine Substitution Positions in PCBs.

Table 1. Naming System for Polychlorinated Biphenyl (PCB) Congeners.

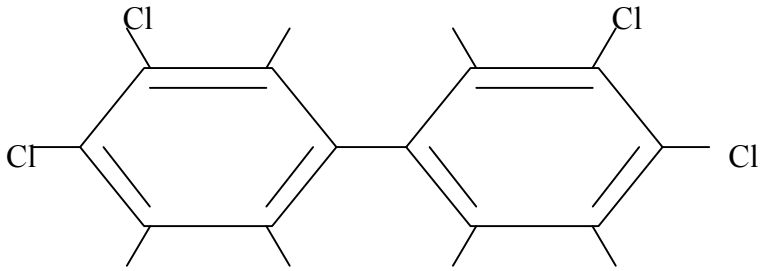
No.	Structure
	Monochlorobiphenyls
1	2
2	3
3	4
	Dichlorobiphenyls
4	2,2’
5	2,3
6	2,3’
7	2,4
8	2,4’
9	2,5
10	2,6
11	3,3’
12	3,4
13	3,4’
14	3,5
15	4,4’
	Trichlorobiphenyls
16	2,2’,3
17	2,2’,4
18	2,2’,5
19	2,2’,6
20	2,3,3’
21	2,3,4
22	2,3,4’
23	2,3,5
24	2,3,6
25	2,3’,4
26	2,3’,5
27	2,3’,6
28	2,4,4’
29	2,4,5
30	2,4,6
31	2,4’,5
32	2,4’,6
33	2’,3,4
34	2’,3,5
35	3,3’,4
36	3,3’,5
37	3,4,4’
38	3,4,5’
39	3,4’,5
	Tetrachlorobiphenyls
40	2,2’,3,3’
41	2,2’,3,4
42	2,2’,3,4’
43	2,2’,3,5
44	2,2’,3,5’
45	2,2’,3,6
46	2,2’,3,6’
47	2,2’,4,4’
48	2,2’,4,5
49	2,2’,4,5’
50	2,2’,4,6
51	2,2’,4,6’

No.	Structure
	Tetrachlorobiphenyls
52	2,2’,5,5’
53	2,2’,5,6’
54	2,2’,6,6’
55	2,3,3’,4
56	2,3,3’,4
57	2,3,3’,5
58	2,3,3’,5’
59	2,3,3’,6
60	2,3,4,4’
61	2,3,4,5
62	2,3,4,6
63	2,3,4’,5
64	2,3,4’,6
65	2,3,5,6
66	2,3’,4,4’
67	2,3’,4,5
68	2,3’,4,5’
69	2,3’,4,6
70	2,3’,4’,5
71	2,3’,4’,6
72	2,3’,5,5’
73	2,3’,5’,6
74	2,4,4’,5
75	2,4,4’,6
76	2’,3,4,5
77	3,3’,4,4’
78	3,3’,4,5
79	3,3’,4,5’
80	3,3’,5,5’
81	3,4,4’,5
	Pentachlorobiphenyls
82	2,2’,3,3’,4
83	2,2’,3,3’,5
84	2,2’,3,3’,6
85	2,2’,3,4,4’
86	2,2’,3,4,5
87	2,2’,3,4,5’
88	2,2’,3,4,6
89	2,2’,3,4,6’
90	2,2’,3,4’,5
91	2,2’,3,4’,6
92	2,2’,3,5,5’
93	2,2’,3,5,6
94	2,2’,3,5,6’
95	2,2’,3,5’,6
96	2,2’,3,6,6’
97	2,2’,3’,4,5
98	2,2’,3’,4,6
99	2,2’,4,4’,5
100	2,2’,4,4’,6
101	2,2’,4,5,5’
102	2,2’,4,5,6’
103	2,2’,4,5’,6
104	2,2’,4,6,6’

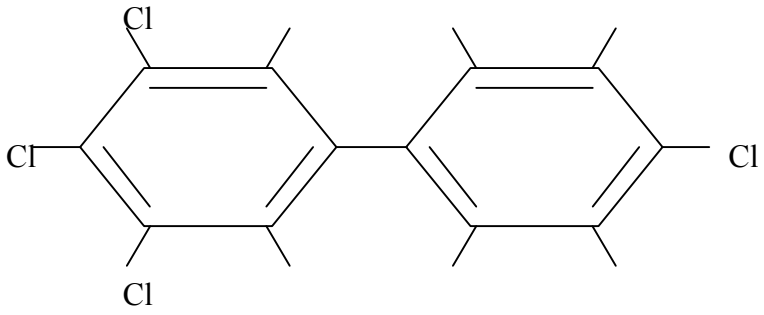
No.	Structure
	Pentachlorobiphenyls
105	2,3,3’,4,4’
106	2,3,3’,4,5
107	2,3,3’,4’,5
108	2,3,3’,4,5’
109	2,3,3’,4,6
110	2,3,3’,4’,6
111	2,3,3’,5,5’
112	2,3,3’,5,6
113	2,3,3’,5’,6
114	2,3,4,4’,5
115	2,3,4,4’,6
116	2,3,4,5,6
117	2,3,4’,5,6
118	2,3’,4,4’,5
119	2,3’,4,4’,6
120	2,3’,4,5,5’
121	2,3’,4,5’,6
122	2’,3,3’,4,5
123	2’,3,4,4’,5
124	2’,3,4,5,5’
125	2’,3’4,5,6’
126	3,3’,4,4’,5
127	3,3’,4,5,5’
	Hexachlorobiphenyls
128	2,2’,3,3’,4,4’
129	2,2’,3,3’,4,5
130	2,2’,3,3’,4,5’
131	2,2’,3,3’,4,6
132	2,2’,3,3’,4,6’
133	2,2’,3,3’,5,5’
134	2,2’,3,3’,5,6
135	2,2’,3,3’,5,6’
136	2,2’,3,3’,6,6’
137	2,2’,3,4,4’,5
138	2,2’,3,4,4’,5’
139	2,2’,3,4,4’,6
140	2,2’,3,4,4’,6’
141	2,2’,3,4,5,5’
142	2,2’,3,4,5,6
143	2,2’,3,4,5,6’
144	2,2’,3,4,5’,6
145	2,2’,3,4,6,6’
146	2,2’,3,4’,5,5’
147	2,2’,3,4’,5,6
148	2,2’,3,4’,5,6’
149	2,2’,3,4’,5’,6
150	2,2’,3,4’,6,6’
151	2,2’,3,5,5’,6
152	2,2’,3,5,6,6’
153	2,2’,4,4’,5,5’
154	2,2’,4,4’,5,6’
155	2,2’,4,4’,6,6’
156	2,3,3’,4,4’,5
157	2,3,3’,4,4’,5’
158	2,3,3’,4,4’,6
159	2,3,3’,4,5,5’
160	2,3,3’,4,5,6

No.	Structure
	Hexachlorobiphenyls
161	2,3,3’,4,5’,6
162	2,3,3’,4’,5,5’
163	2,3,3’,4’,5,5’
164	2,3,3’,4’,5’,6
165	2,3,3’,5,5’,6
166	2,3,4,4’,5,6
167	2,3’,4,4’,5,5’
168	2,3’,4,4’,5’,6
169	3,3’,4,4’,5,5’
	Heptachlorobiphenyls
170	2,2’,3,3’,4,4’,5
171	2,2’,3,3’,4,4’,5
172	2,2’,3,3’,4,5,5’
173	2,2’,3,3’,4,5,6
174	2,2’,3,3’,4,5,6
175	2,2’,3,3’,4,5’,6
176	2,2’,3,3’,4,6,6’
177	2,2’,3,3’,4’,5,6
178	2,2’,3,3’,5,5’,6
179	2,2’,3,3’,5,6,6’
180	2,2’,3,4,4’,5,5’
181	2,2’,3,4,4’,5,6
182	2,2’,3,4,4’,5,6’
183	2,2’,3,4,4’,5’,6
184	2,2’,3,4,4’,6,6’
185	2,2’,3,4,5,5’,6
186	2,2’,3,4,5,6,6’
187	2,2’,3,4’,5,5’,6
188	2,2’,3,4’,5,6,6’
189	2,3,3’,4,4’,5,5’
190	2,3,3’,4,4’,5,6
191	2,3,3’,4,4’,5’,6
192	2,3,3’,4,5,5’,6
193	2,3,3’,4’,5,5’,6
	Octachlorobiphenyls
194	2,2’,3,3’,4,4’,5,5’
195	2,2’,3,3’,4,4’,5,6
196	2,2’,3,3’,4,4’,5,6’
197	2,2’,3,3’,4,4’,6,6’
198	2,2’,3,3’,4,5,5’,6
199	2,2’,3,3’,4,5,6,6’
200	2,2’,3,3’,4,5’,6,6’
201	2,2’,3,3’,4,5,5’,6’
202	2,2’,3,3’,5,5’,6,6’
203	2,2’,3,4,4’,5,5’,6
204	2,2’,3,4,4’,5,6,6’
205	2,3,3’,4,4’,5,5’,6
	Nonachlorobiphenyls
206	2,2’,3,3’,4,4’,5,5’,6
207	2,2’,3,3’,4,4’,5,6,6’
208	2,2’,3,3’,4,5,5’,6,6’
	Decachlorobiphenyl
209	2,2’,3,3’,4,4’,5,5’,6,6’

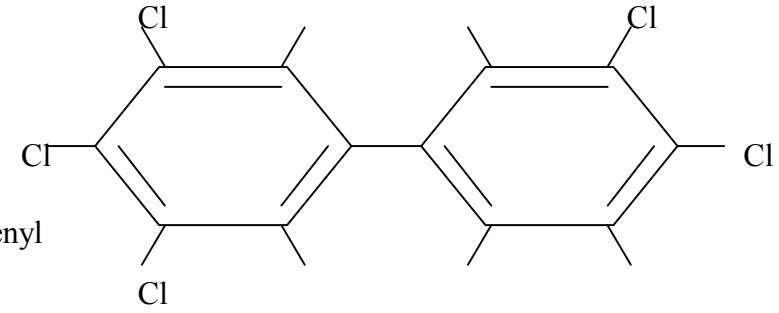
PCB 77
3,3’,4,4’-tetrachlorobiphenyl



PCB 81
3,4,4’,5-tetrachlorobiphenyl



PCB 126
3,3’,4,4’,5-pentachlorobiphenyl



PCB 169
3,3’,4,4’,5,5’-hexachlorobiphenyl

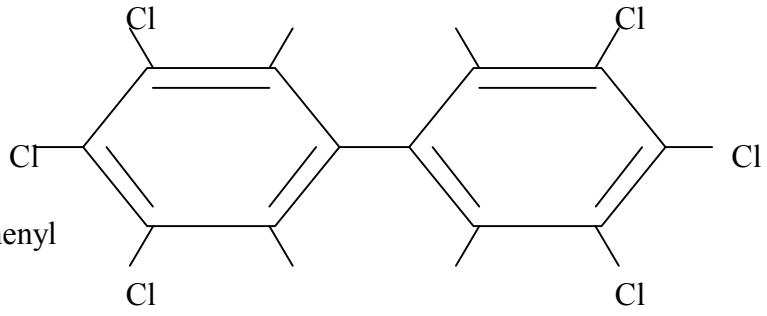


Figure 3. Diagrams of the Four Coplanar PCB Congeners: PCBs 77, 81, 126 and 169.

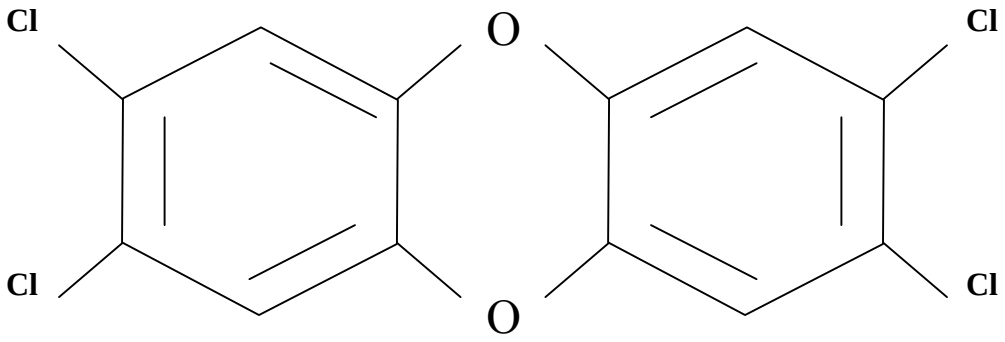


Figure 4. The Structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

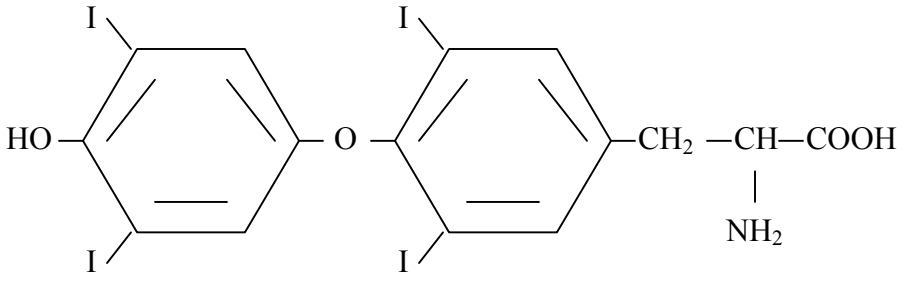


Figure 5. The Structure of Thyroxine (T_4).

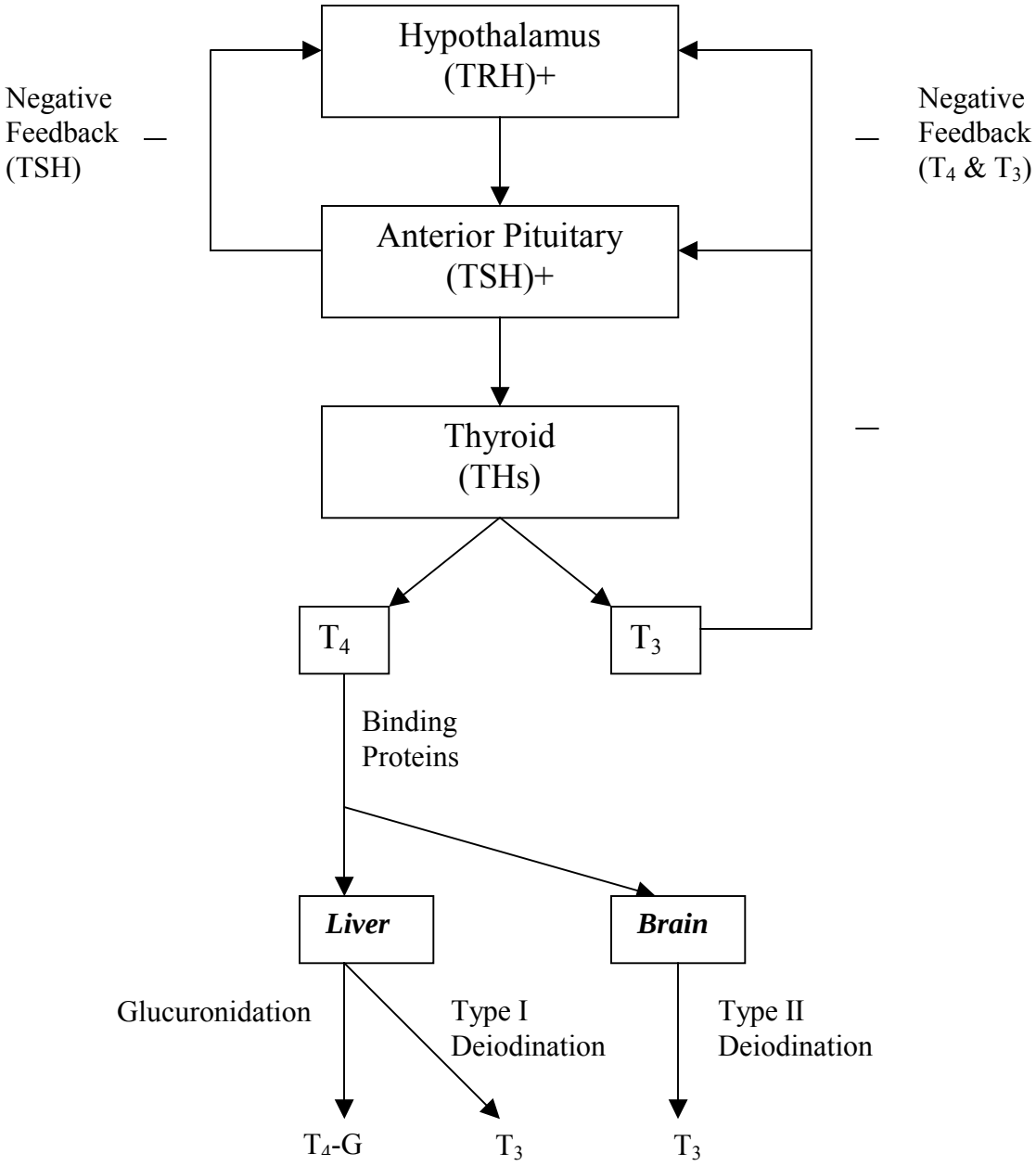


Figure 6. The Hypothalamic-Pituitary-Thyroid (HPT) Axis.

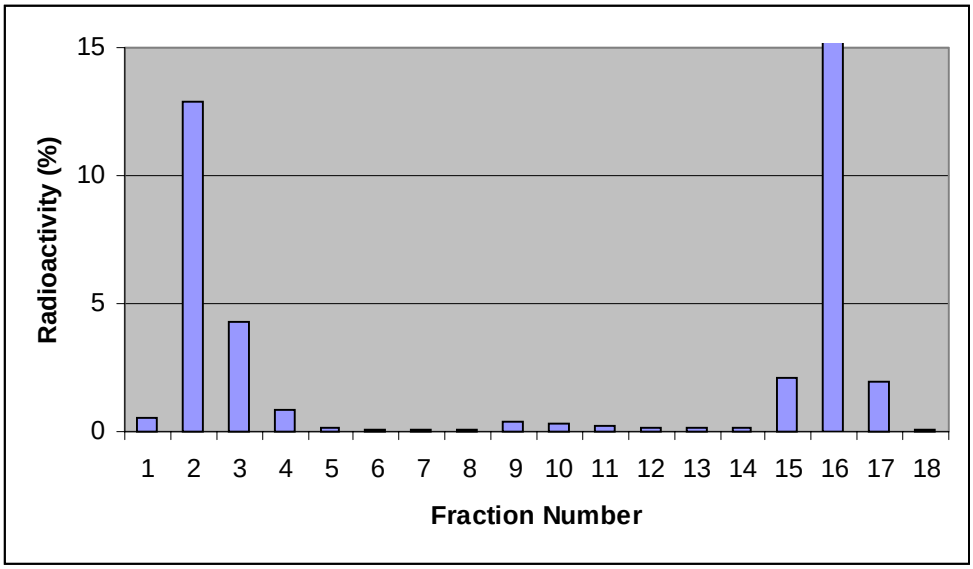


Figure 7. Elution Pattern of Radiolabeled T_4 Metabolites During Sephadex LH-20 Separation Chromatography. The fraction numbers correspond to the following solvents: 1—load (sample in 0.1 N HCl), 2-7—0.1 N HCl, 8-14—H_2O, 15-18—0.1 N NaOH/EtOH (1:1, v/v). Total radioactivity is 18.87% for acid fractions (unbound ^{125}I), 1.44% for water fractions (^{125}I-T_4-G) and 79.70% for alkaline ethanol fractions (^{125}I-T_4). Fraction #16 is truncated and accounts for approximately 75.6% of the overall radioactivity recovered.

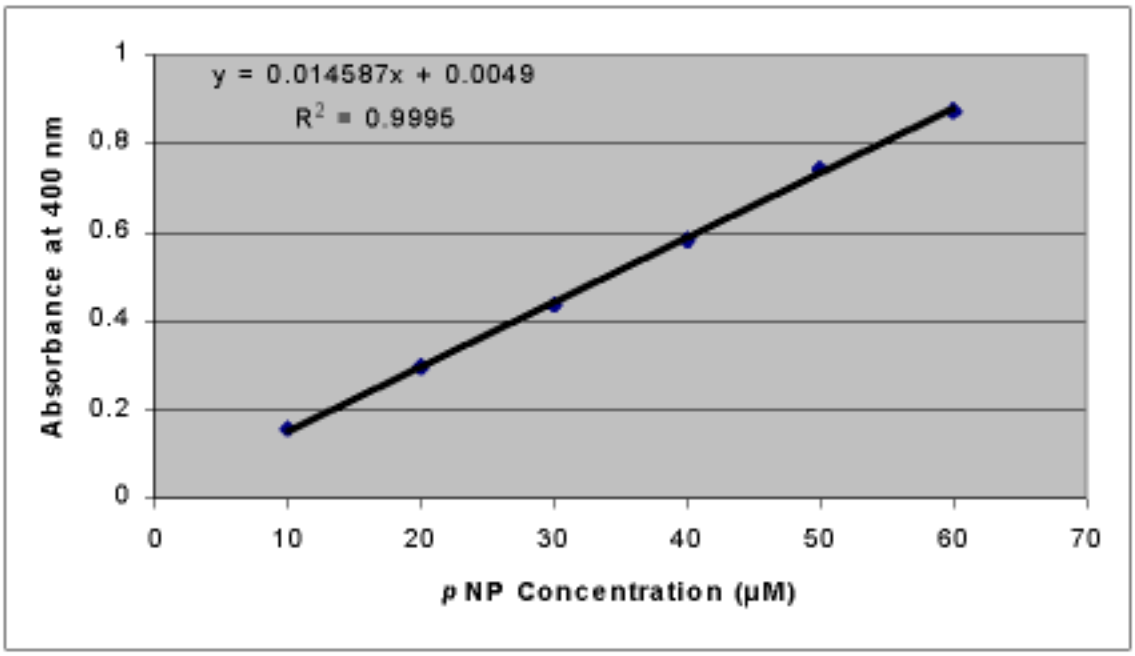


Figure 8. Standard Curve for Determination of Molar Absorption Coefficient for *p*NP. The molar absorption coefficient is determined by the slope of the line (14,587/M*cm).

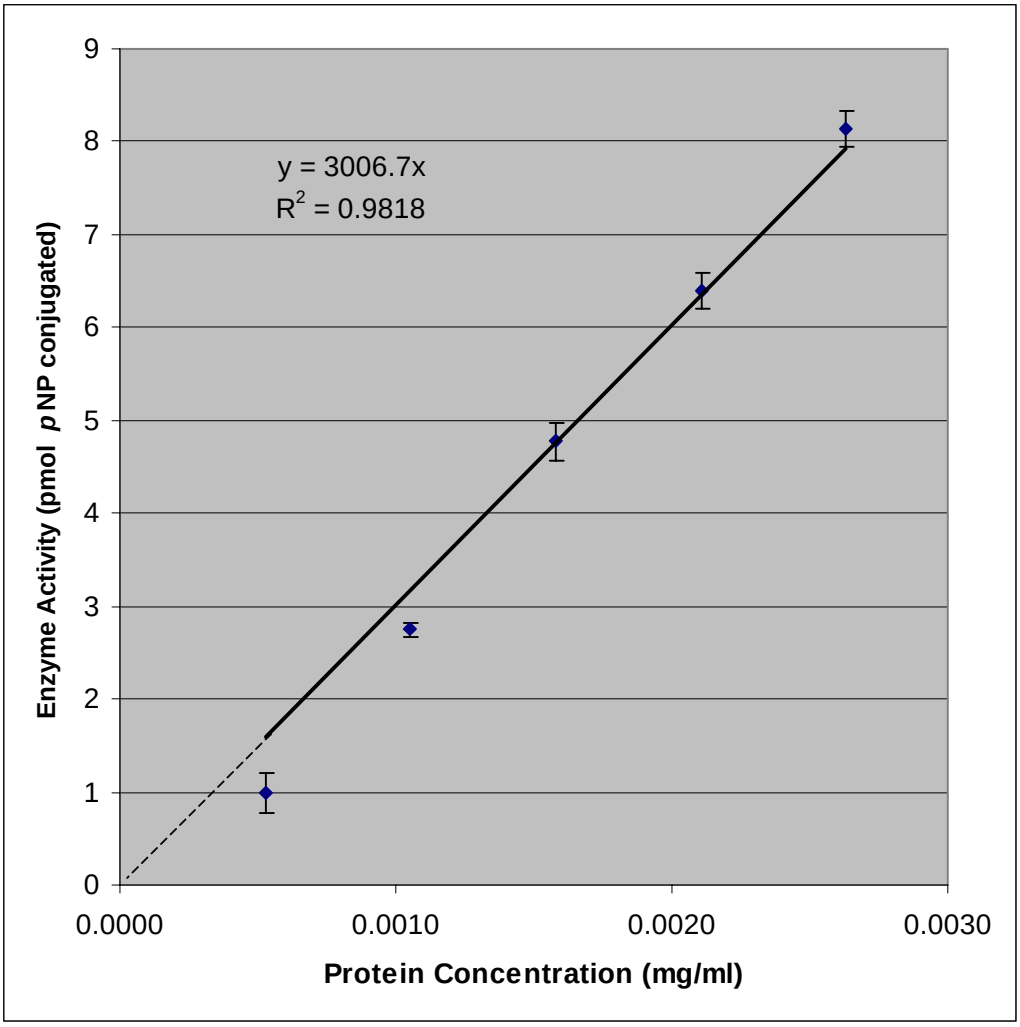


Figure 9. UDP-GT Assay Validation: Proportionality of Enzyme Activity with Enzyme Concentration (Protein Concentration in Homogenate Dilutions) in Adult Quail Liver Homogenate. Data points are the average of triplicates. Error bars show ± 1 standard error. Incubation time was 60 min.

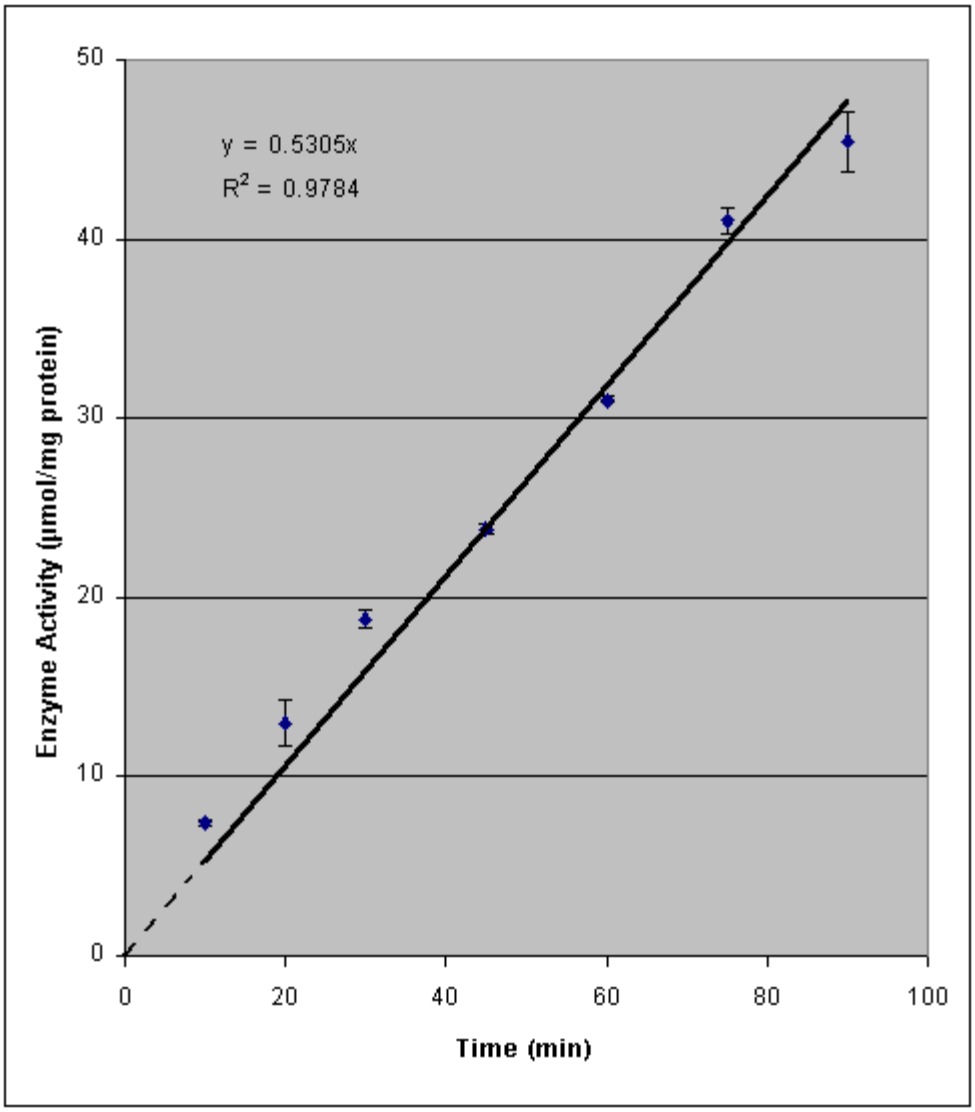


Figure 10. UDP-GT Assay Validation: Time Course for Adult Quail Liver Homogenate *p*NP Assay Validation. Data points are the average of triplicates. Enzyme activity is in µmol *p*NP conjugated/mg protein. Error bars show ± 1 standard error.

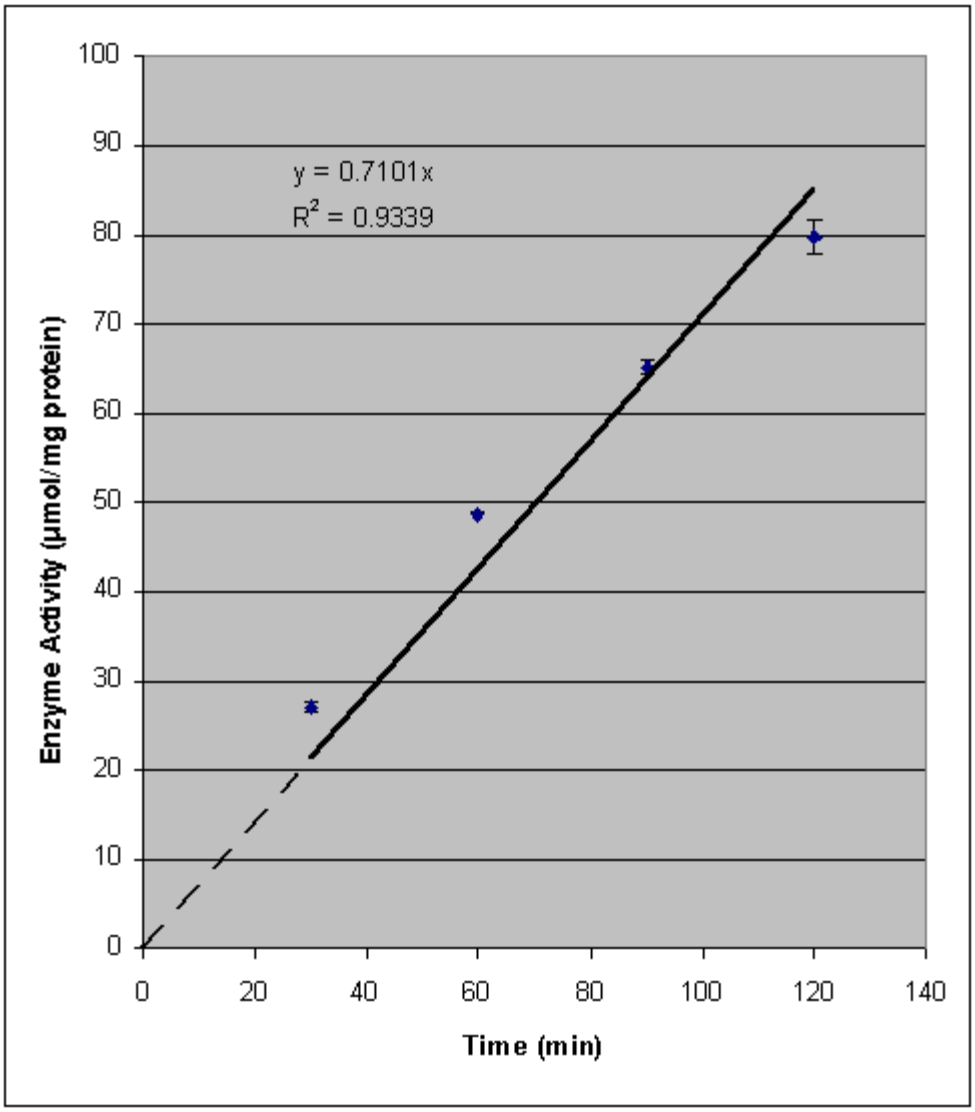


Figure 11. UDP-GT Assay Validation: Time Course for Juvenile Chicken Liver Homogenate *p*NP Assay Validation. Data points are the average of triplicates. Enzyme activity is in µmol *p*NP conjugated/mg protein. Error bars indicate ± 1 standard error.

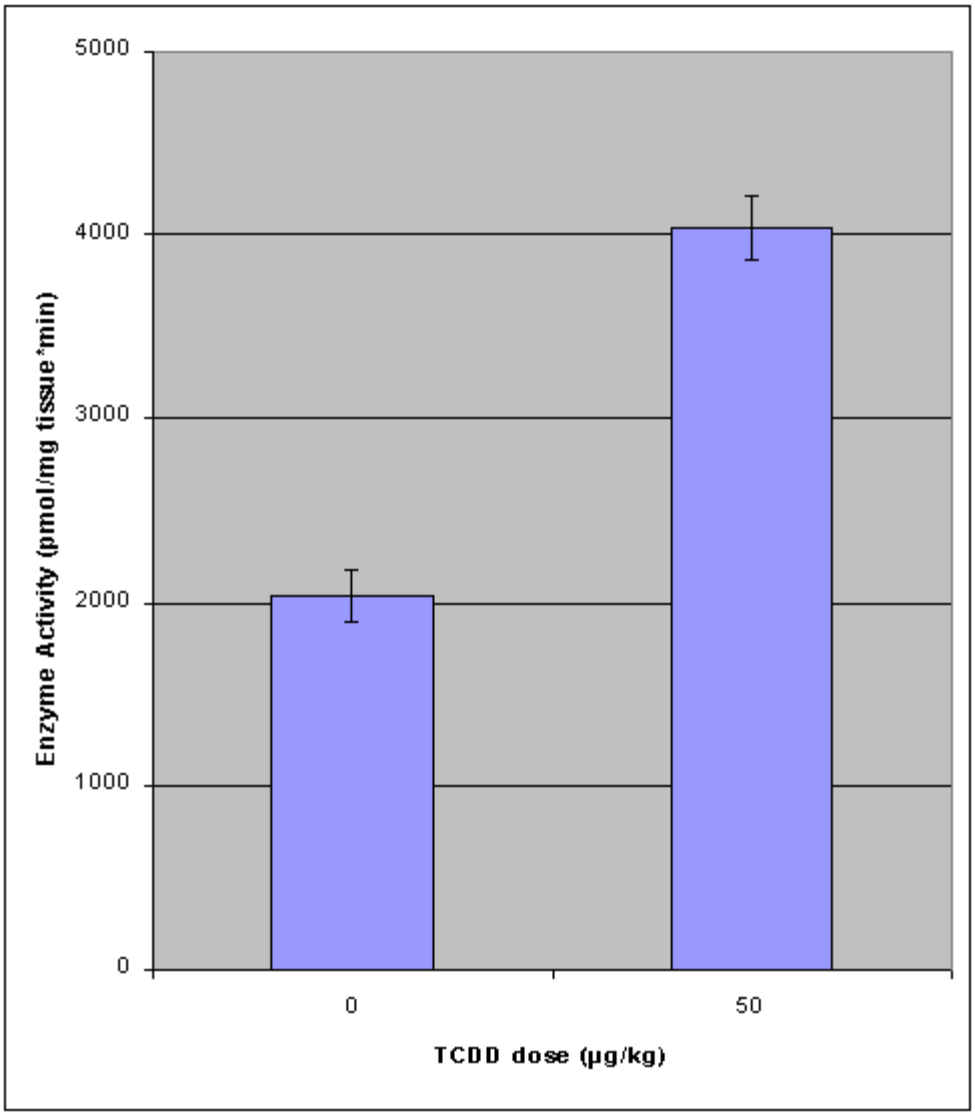


Figure 12. Induction of UDP-GT Activity toward *p*NP in TCDD-Treated Mice. Each data point is the average of multiple individuals (N = 6 for control and N = 8 for TCDD-treated). Mice were dosed with either corn oil vehicle or 50 µg/kg body weight TCDD in corn oil intraperitoneally and were sacrificed after 5 days. Enzyme activity is in pmol *p*NP conjugated/mg tissue*min. Error bars indicate ± 1 standard error. The groups are significantly different from one another (two-tailed t-test for equality of means, equal variances not assumed, $p < 0.001$).

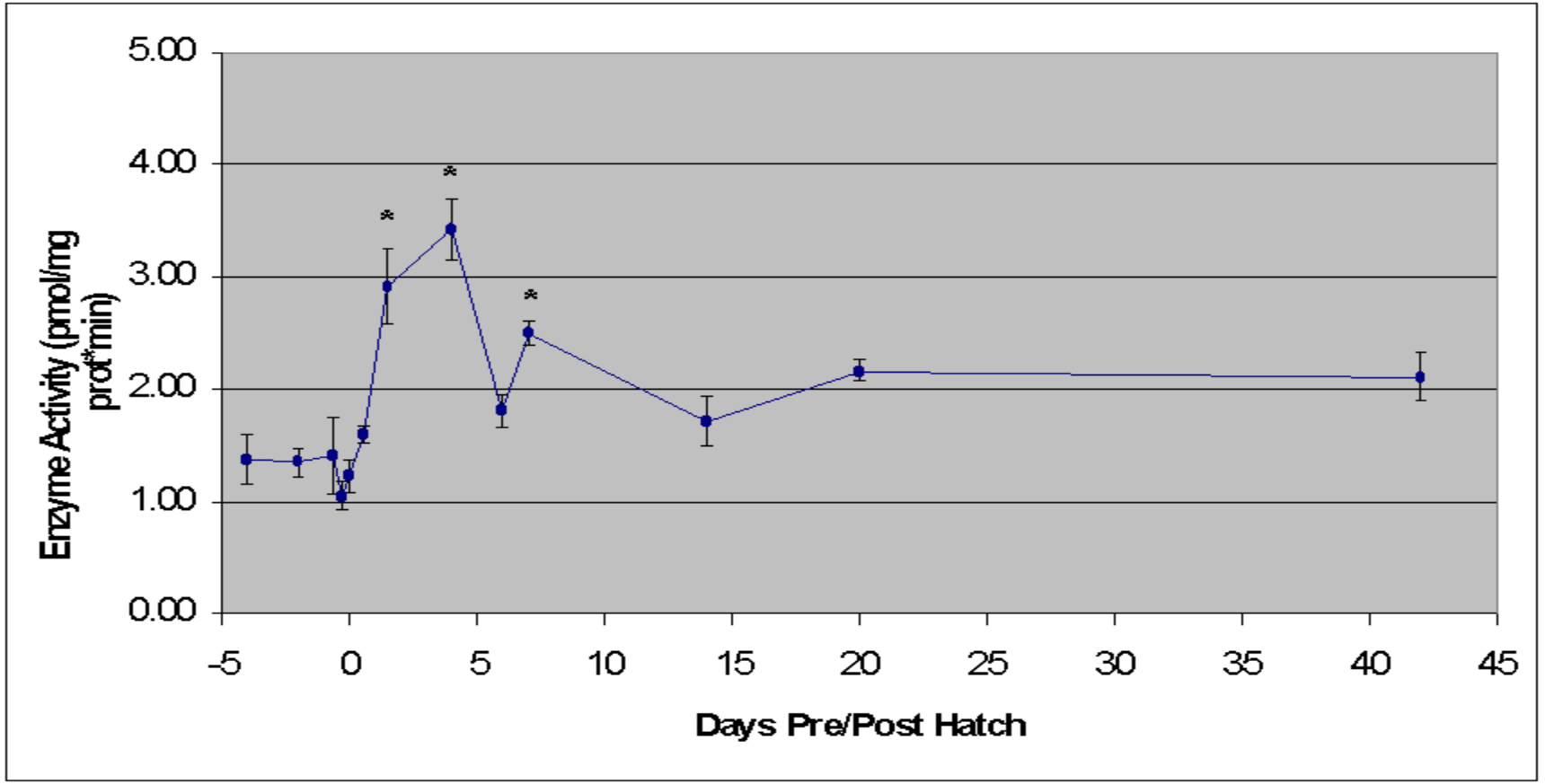


Figure 13. Profile of Ontogenic UDP-GT Activity in Japanese Quail. The graph shows ages of embryos from day 12 of incubation (-4 on graph) to approximately 42 days posthatch and includes activities during the perihatch period. For the perihatch period, NI samples are indicated at -0.67, I at -0.33 and S at 0 (NI = beak not into air cell, I = beak into air cell, not through shell, S = beak pipped through shell). All data points indicate the mean of individuals (N = 5-17/stage), and error bars indicate ± 1 standard error. Enzyme activity is in pmol *p*NP conjugated/mg protein*min. * Indicates significant differences from prehatch groups (ANOVA, Tukey's HSD test, $p < 0.001$).

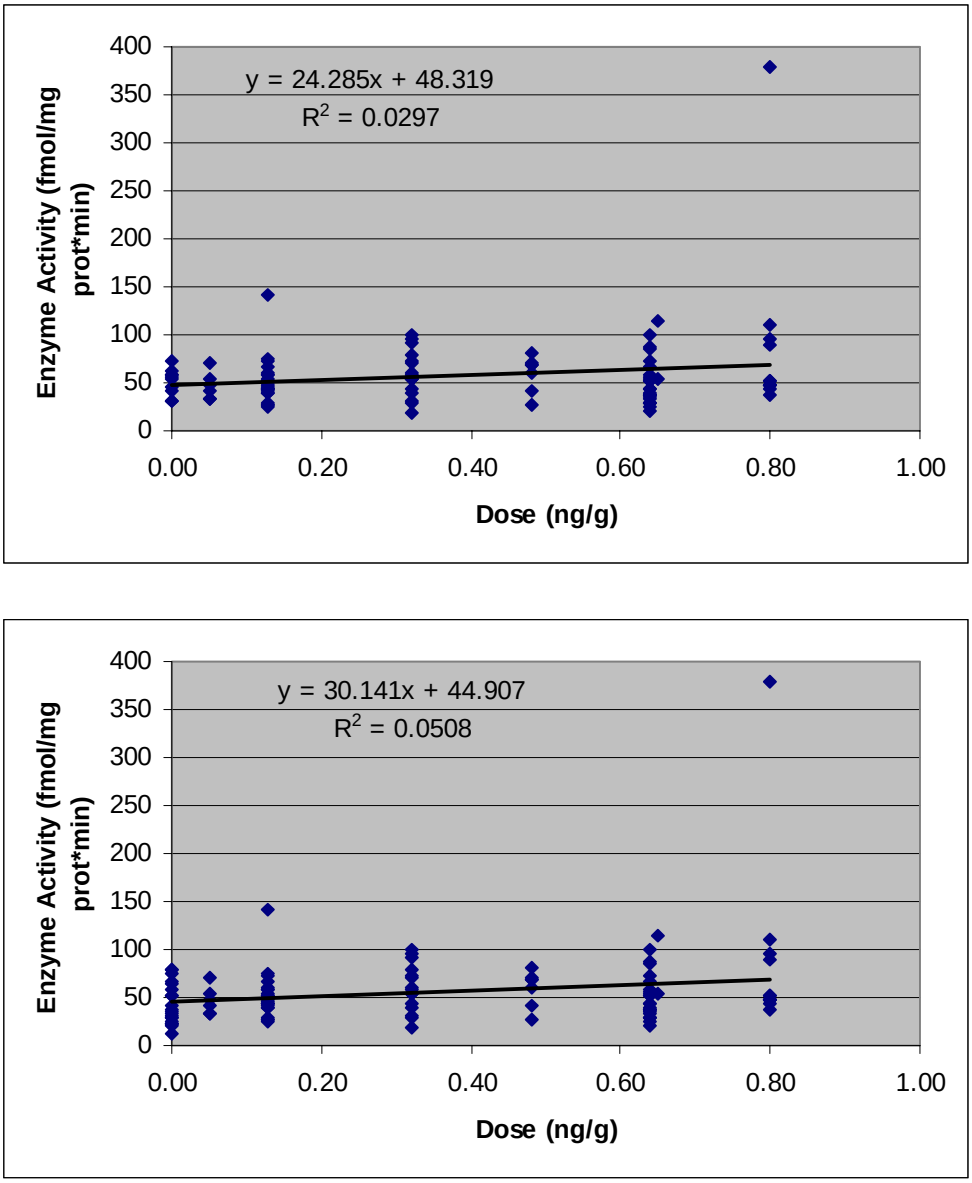
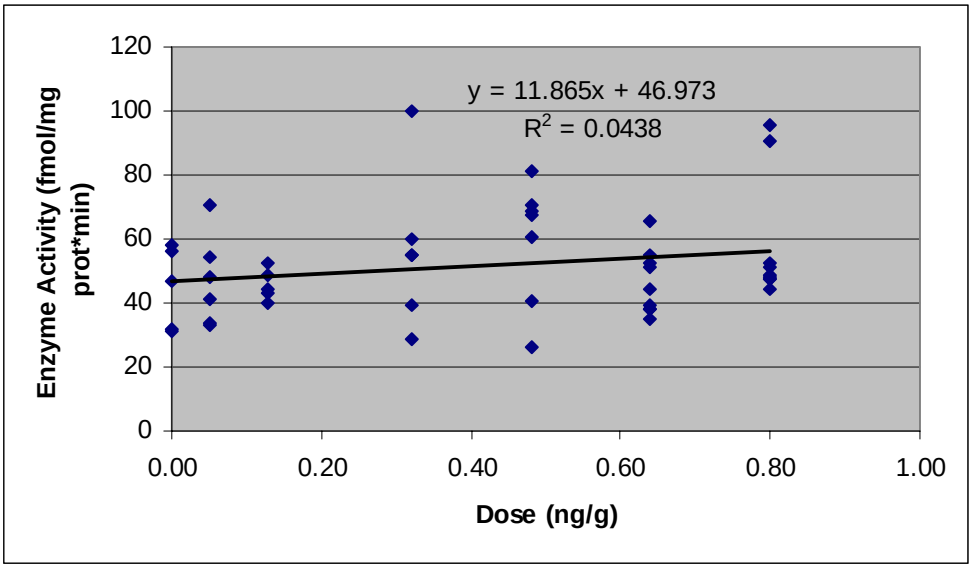


Figure 14. Hepatic UDP-GT Activity in Juvenile Chickens Exposed to PCB 126 *in ovo* (combined data). All data points are the average of triplicate samples. The upper graph shows non-injected controls as 0 dose; the lower graph shows injected controls as 0 dose. Enzyme activity is in fmol *p*NP conjugated/mg protein*min.



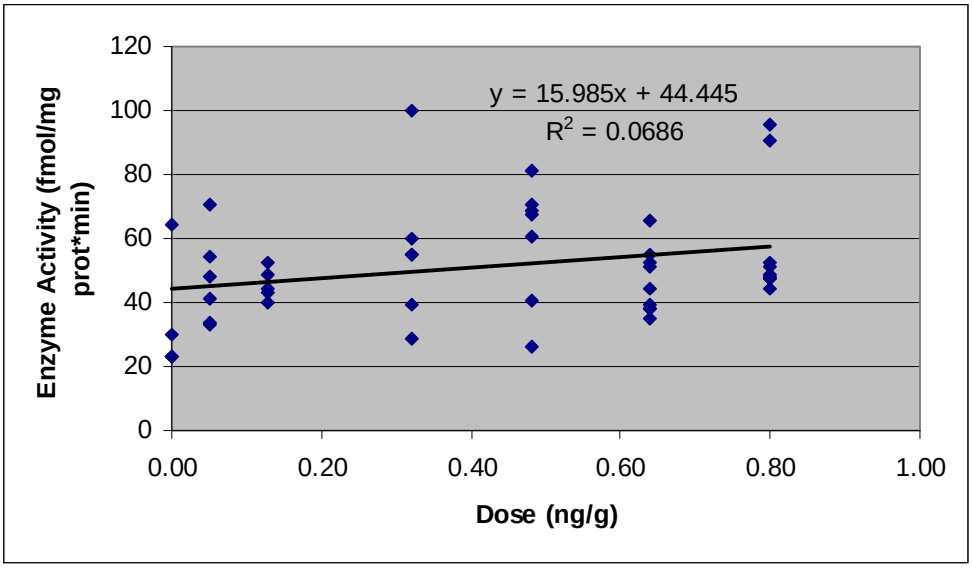
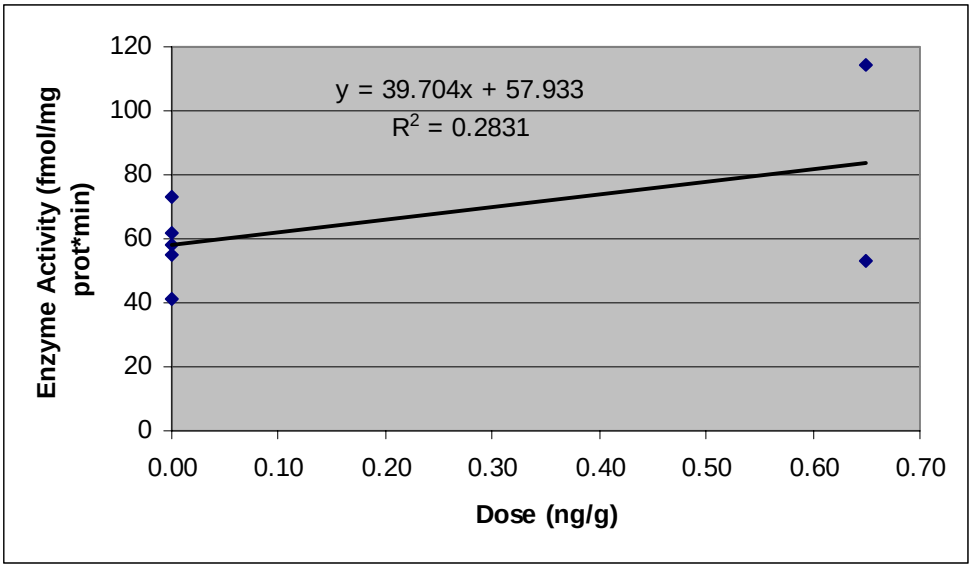


Figure 15. Hepatic UDP-GT Activity in Juvenile Chickens Exposed to PCB 126 *in ovo* (8/98). All data points are the average of triplicate samples. The upper graph shows non-injected controls as 0 dose; the lower graph shows injected controls as 0 dose. Enzyme activity is in fmol *p*NP conjugated/mg protein*min.



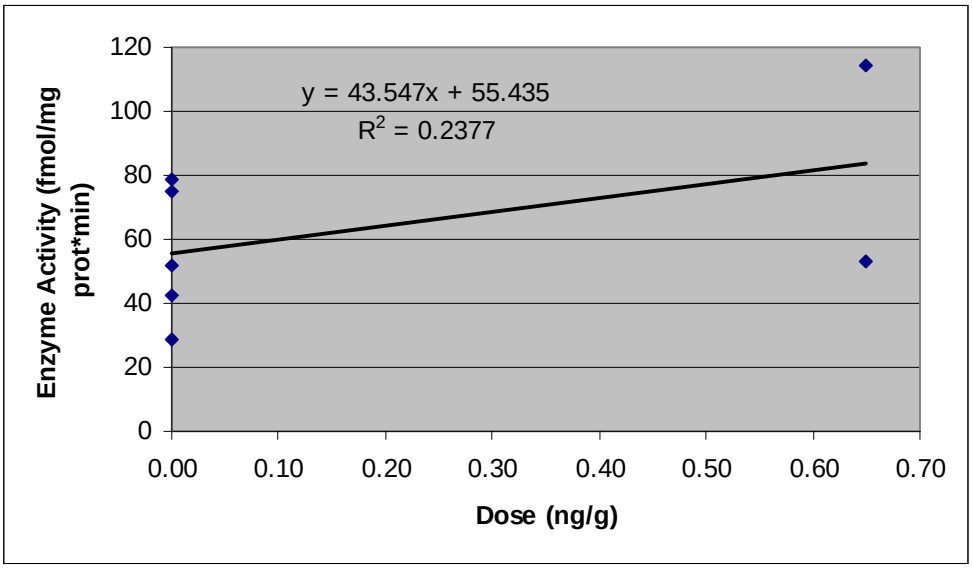


Figure 16. Hepatic UDP-GT Activity in Juvenile Chickens Exposed to PCB 126 *in ovo* (10/98). All data points are the average of triplicate samples. The upper graph shows non-injected controls as 0 dose; the lower graph shows injected controls as 0 dose. Enzyme activity is in fmol *p*NP conjugated/mg protein*min.

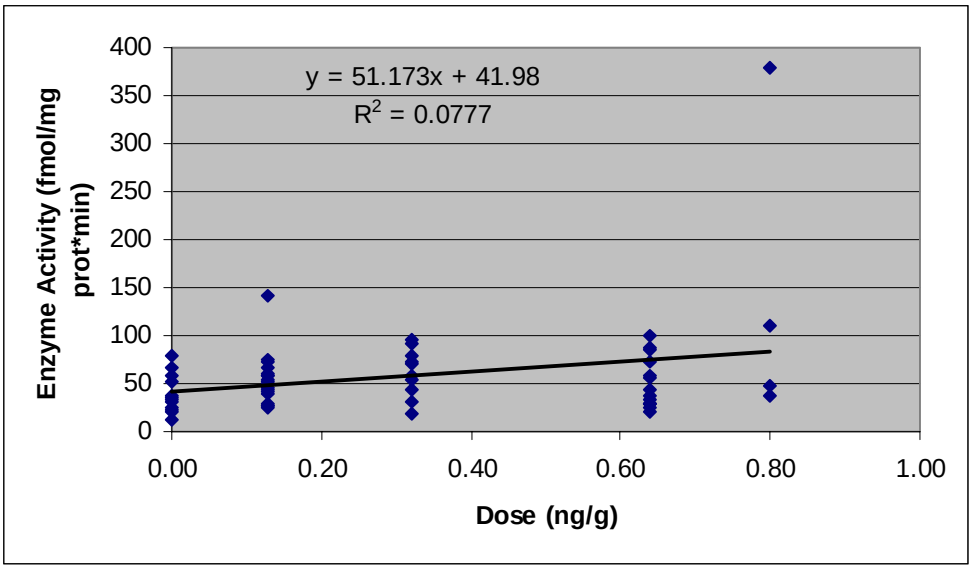


Figure 17. Hepatic UDP-GT Activity in Juvenile Chickens Exposed to PCB 126 *in ovo* (1/99). All data points are the average of triplicate samples. This graph shows injected controls as 0 dose; non-injected controls were not used in this experiment. Enzyme activity is in fmol *p*NP conjugated/mg protein*min.

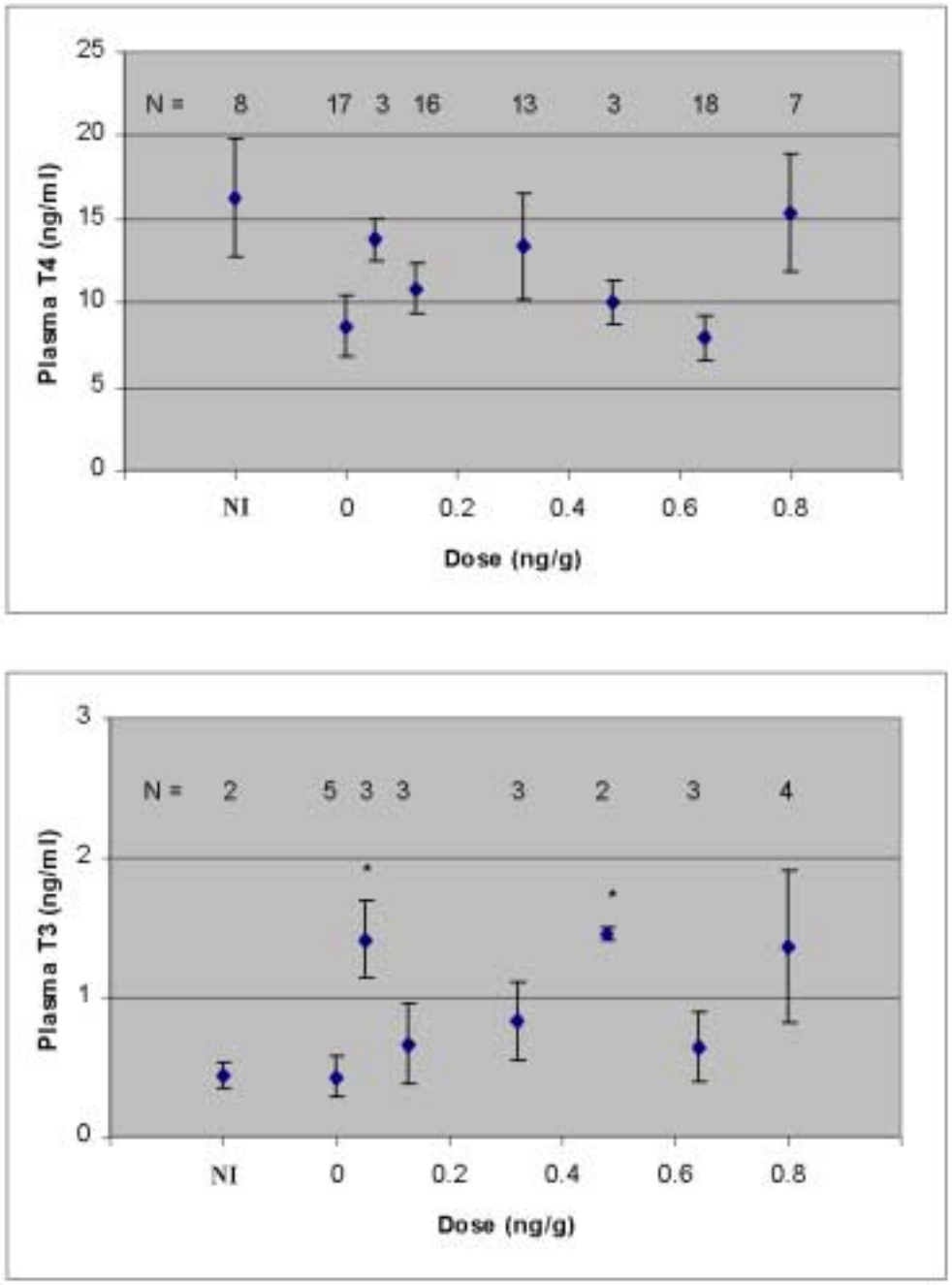


Figure 18. Plasma Thyroid Hormone Concentrations in Embryonic Chickens Exposed to PCB 126. The graphs show combined data for samples from 8/98, 10/98 and 1/99 experiments. Error bars indicate ± 1 standard error. *Indicates significant differences from both control values at $p < 0.05$. Injected controls are graphed at dose 0, and non-injected controls are graphed as NI.

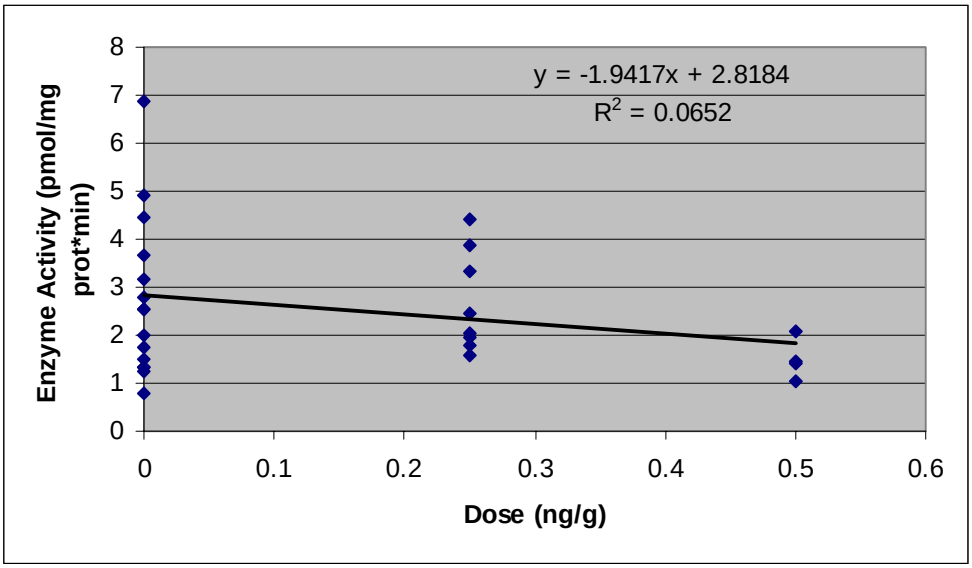


Figure 19. Hepatic UDP-GT Activity at Different Doses in PCB 126-Dosed Chickens. Eggs were dosed prior to incubation, and juvenile chickens were sacrificed 42 days posthatch. All data points are the average of triplicate samples. Enzyme activity is in pmol *p*NP conjugated/mg protein*min.

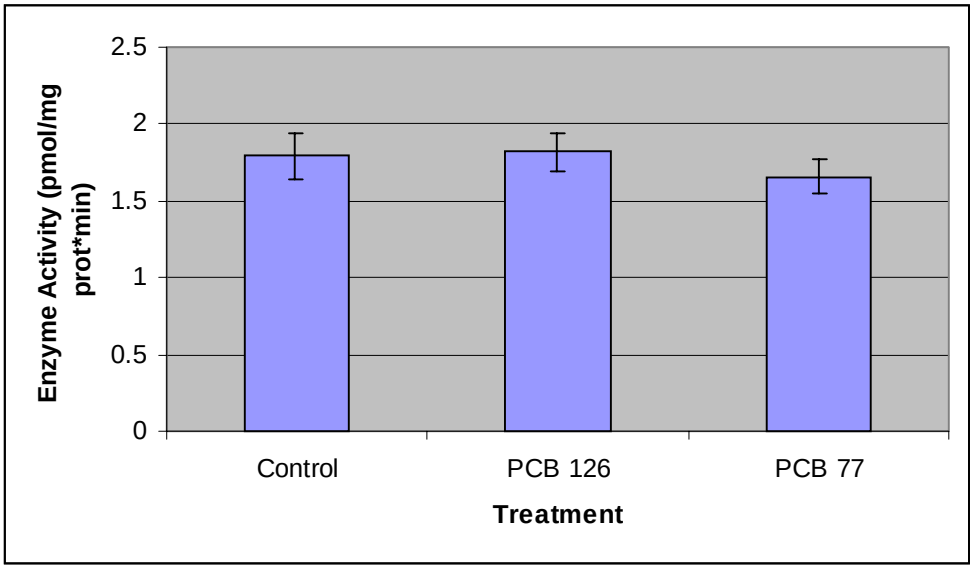


Figure 20. Hepatic UDP-GT Activity at Different Doses in PCB 126- or PCB 77-Dosed Chickens. Eggs were dosed prior to incubation, and juvenile chickens were sacrificed 42 days posthatch. PCB 126-dosed animals were dosed with 0.25 ng PCB/g egg, and PCB 77-dosed animals were dosed with 0.64 ng PCB/g egg. Data bars indicate ±1 standard error. Enzyme activity is in pmol *p*NP conjugated/mg protein*min. There were no statistically significant differences between controls and either of the treated groups.

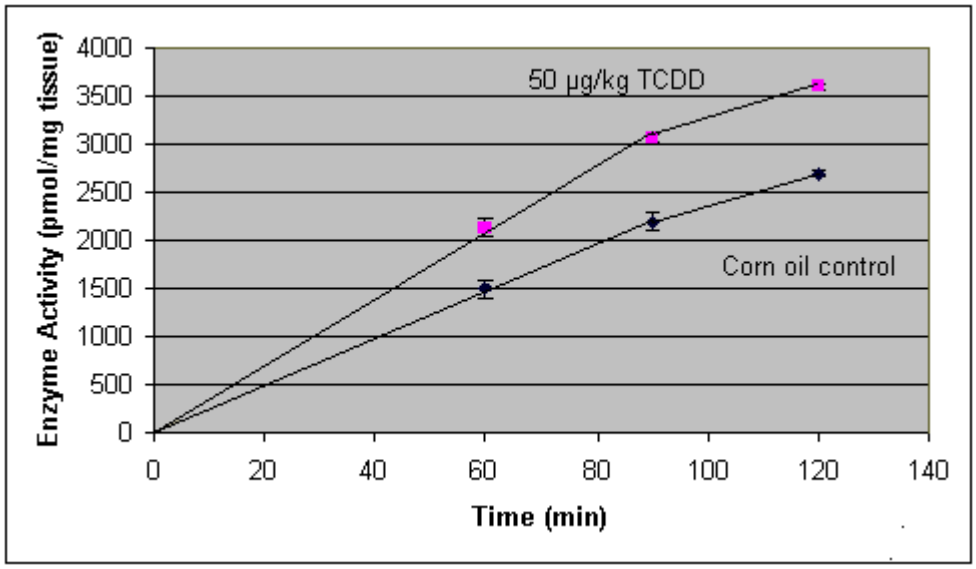


Figure 21. UDP-GT Glucuronidation of Labeled T_4 in Control and TCDD-Dosed C57Bl6++ Mice. Mice were either dosed with corn oil as a control or with 50 µg/kg TCDD. Each data point indicates the average of triplicates. Enzyme activity is in pmol T_4 conjugated/mg tissue. Error bars indicate ± 1 standard error.

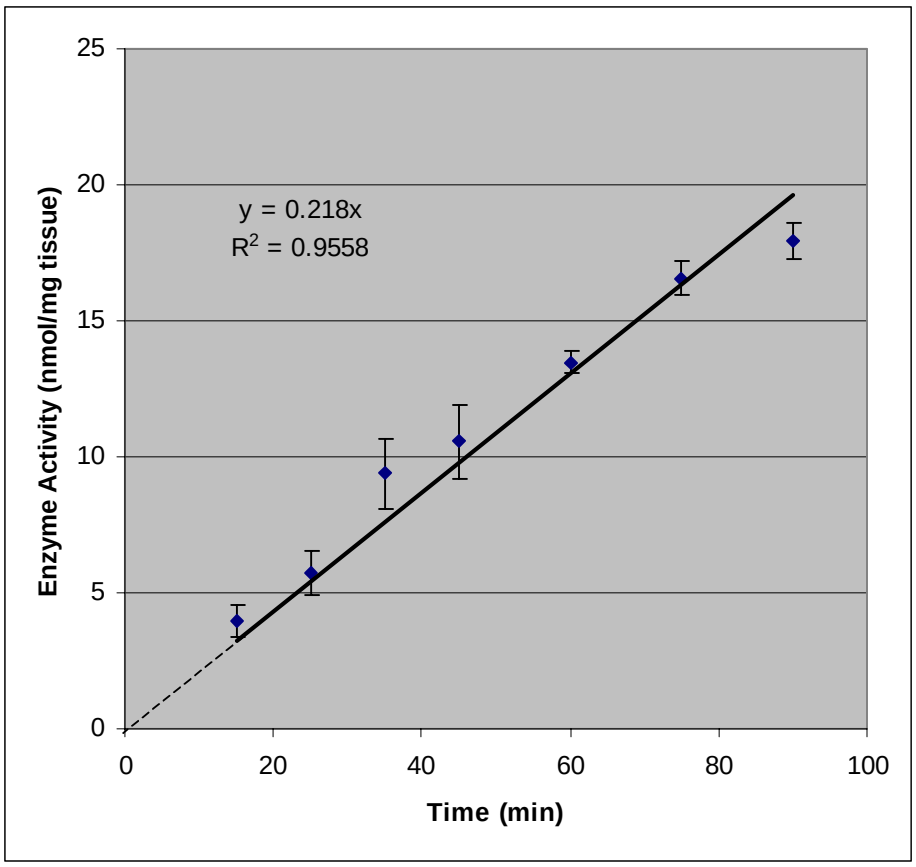
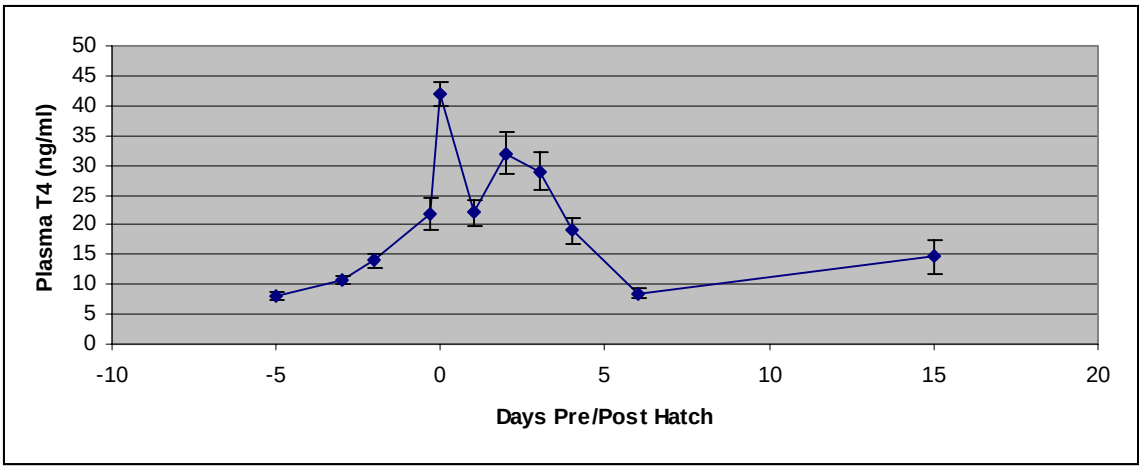
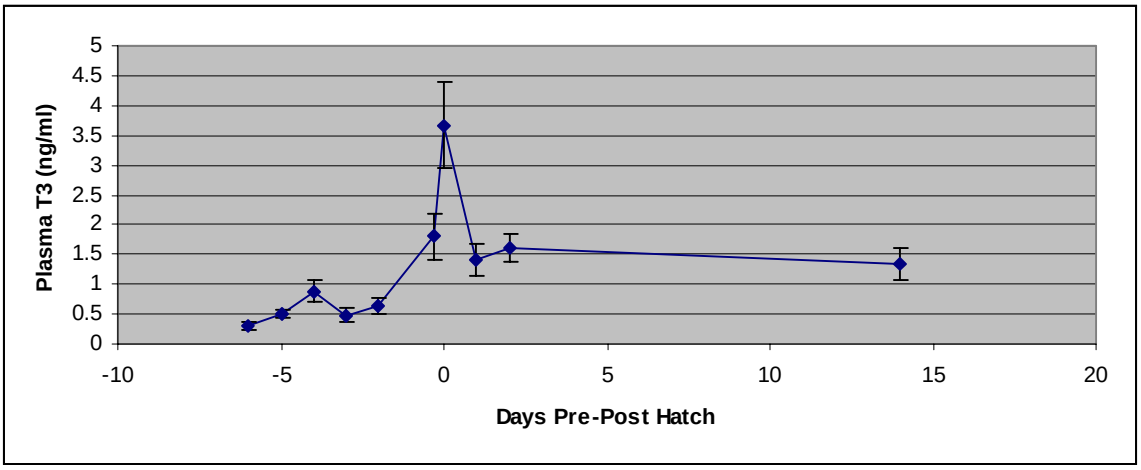


Figure 22. Validation of Labeled T_4 Assay for Measuring UDP-GT: Time Course Using Adult Quail Liver Homogenate. Each data point indicates the average of triplicates (except for 45 minute sample—duplicates). Enzyme activity is in nmol T_4 conjugated/mg tissue. Error bars indicate ± 1 standard error.





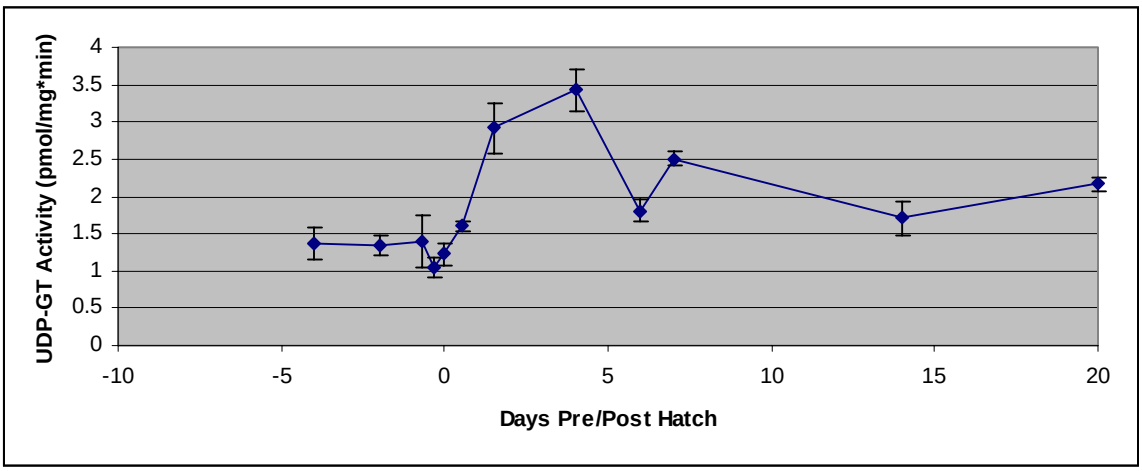


Figure 23. Comparison of Plasma T_4 and T_3 Concentrations with UDP-GT Activity throughout Embryonic Development in Japanese Quail. Perihatch period times are plotted as follows: NI = -0.67, I = -0.33 and S = 0. Enzyme activity is in pmol *p*NP conjugated/mg protein*min. Error bars indicate ± 1 standard error.

Curriculum Vitae

Ryan J. R. McCleary

Education

April 1993	B.S., Biology, Western Michigan University, Kalamazoo, MI Minor in Chemistry Overall GPA: 3.63 Major GPA: 3.73 Undergraduate Honors Thesis: The Use of Aquatic Insect Succession in Determining Post Mortem Interval for Forensic Purposes
December 2001	M.S., Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA Overall Graduate QCA: 3.71
January 2001--Present	Ph.D. Program, Zoology, University of Florida, Gainesville, FL Current GPA: 4.00

Employment

May 1991-August 1991	Summer Intern, Department of Vertebrate Zoology, National Museum of Natural History (Smithsonian), Washington, DC
September 1992-January 1993	Research Assistant, Ecology Laboratory, Western Michigan University, Kalamazoo, MI
May 1993-August 1993	Ed Almandarz Fellow in Herpetology, Lincoln Park Zoo, Chicago, IL
August 1993-July 1994	Graduate Teaching Assistant, General Biology Laboratories, Virginia Tech, Blacksburg, VA Teaching Evaluations: 3.3, 3.2, 3.6, 3.5, 3.4, 3.7, 3.7/4.0
August 1994-November 1994	Senior Animal Laboratory Technician, International Research and Development Corporation, Mattawan, MI
November 1994-September 1995	Reptile Specialist, Discount Pet Center, Portage and Kalamazoo, MI
June 1995-August 1995	Part-time Instructional Faculty, Kalamazoo Valley Community College, Texas Township, MI

February 1996- February 1997	Funding Specialist, American Auto Funding Corporation, Park Ridge, IL
February 1997- October 1998	Credit Manager and Funding Specialist, General Electric Capital Customized Auto Credit Services, Barrington, IL
January 1999- May 1999	Graduate Teaching Assistant, General and Principles of Biology Laboratories, Virginia Tech, Blacksburg, VA Teaching Evaluations: 3.3, 3.6, 3.5/4.0
August 1999- December 1999	Graduate Teaching Assistant, Majors Biology Laboratories, Virginia Tech, Blacksburg, VA Teaching Evaluations: 4.0, 4.0/4.0
January 2000- December 2000	Graduate Research Assistant, Endocrinology Laboratory, Virginia Tech, Blacksburg, VA
January 2001- Present	Graduate Teaching Assistant, Biology Laboratories, University of Florida, Gainesville, FL Teaching Evaluations: 4.60, 4.86, 4.92, 4.36/5.0

Awards

September 1989- April 1993	Medallion Scholarship, Western Michigan University, Kalamazoo, MI
December 1992	Undergraduate Creative Activities Award, Western Michigan University, Kalamazoo, MI

Memberships in Scientific Societies

January 2001- Present	Zoology Graduate Student Association (ZGSA), University of Florida, Gainesville, FL
January 1999- December 2000	Biology Graduate Student Association (BGSA), Virginia Tech, Blacksburg, VA
November 1999- Present	The Society for Integrative and Comparative Biology (SICB)
April 2000- Present	Sigma Xi, the Scientific Research Society
April 2001- Present	Society for the Study of Amphibians and Reptiles

May 2001-Present	Society for Environmental Toxicology and Conservation (SETAC)
May 2001-Present	American Society of Ichthyologists and Herpetologists

Grants

June 2000	Sigma Xi Grant-in-Aid of Research: Effects of UDP-GT Induction by Exogenous Pollutants on Thyroid Hormone Status in Lab-reared Chickens and Wild Herring Gulls, $800.00. Biology Department matching funds, $500.00
October 2000	Graduate Student Association Research Travel Fund for poster presentation at Gordon Research Conference on Environmental Endocrine Disruptors, Plymouth, NH, June 2000, $200.00. Biology Department matching funds, $200.00.

Publications

McCleary, RJR and RW McDiarmid. 1993. *Phyllorhynchus decurtatus*. Catalogue of American Amphibians and Reptiles 580.1-580.7.

McDiarmid, RW and RJR. McCleary. 1993. *Phyllorhynchus*. Catalogue of American Amphibians and Reptiles 579.1-579.5.

McNabb, FMA, RJR McCleary, LA Fowler, CM Parsons, KA Grasman and GA Fox. 2001. Thyroid function in PCB-exposed avian embryos and chicks. In Perspectives in Comparative Endocrinology: Unity and Diversity (JJTh Goos, RK Rastogi, H Vaudry and R Pierantoni, eds.). Bologna, Italy: Monduzzi Editore, pp. 275-280.

Published Abstracts

McCleary, RJR and FMA McNabb. 1999. Assay development for measuring pollutant effects on T_4 excretion in birds. American Zoologist 39(5): 123-124A.

McCleary, RJR, FMA McNabb and KA Grasman. 2000. Effects of pollutant chemicals on thyroid hormone excretion in birds. Virginia Journal of Science 51(2):78.

McNabb, FMA, RJR McCleary, LA Fowler, and KA Grasman. 2000. Thyroid function during development in chicken embryos exposed to PCB 126. 21st SETAC Annual Meeting Abstract Book A290, p.65.

McCleary, RJR, EC McFarland, KA Grasman and FMA McNabb. 2001. Ontogeny of UDP-GT activity in Japanese quail and effects of PCB 126 on UDP-GT activity in

chicken embryos. Society for Integrative and Comparative Biology Final Program and Abstracts 2001:293.

McCleary RJR, McFarland EC, Grasman KA, McNabb FMA. 2001. Hepatic UDP-GT activity in developing quail and PCB 126-dosed chickens. 22nd SETAC Annual Meeting Abstract Book PM130, p. 165.

Presentations; No Published Abstract

McCleary, RJR, FMA McNabb and KA Grasman. 2000. UDP-Glucuronosyltransferase (UDP-GT) activity in PCB-treated chicken embryos. Gordon Research Conference on Environmental Endocrine Disruptors, Plymouth, NH. *Poster*.