Thyroid Hormone Disruption and Biological Consequences for Aquatic Vertebrates

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The objectives of this presentation are (1) to review the control of thyroid function and thyroid hormone action in vertebrates, (2) to examine the evolution of thyroid function in vertebrates, (3) to review known mechanisms of thyroid disruption, and (4) to examine the potential consequences of thyroid disruption for development and metamorphosis of amphibians.

Thyroid hormones are essential for normal development and maturation in all vertebrates. Appropriate levels of thyroid hormones are necessary for nerve cell differentiation, for gonadal maturation in seasonally breeding animals, for metamorphosis in fish and amphibians, for molting in salamanders, lizards, snakes and birds, and for the onset of migratory restlessness in fish and birds (Norris, 1997; Gorbman et al. 1983). This presentation focuses on the roles of thyroid hormone in amphibians and fishes.

During amphibian metamorphosis, massive tissue remodeling occurs, including development of limbs and loss of the tail, transition from gill respiration to lung respiration, transition from a herbivore intestine to a carnivore intestine, remodeling of the nervous system and switching from a tadpole to adult form of hemoglobin. Thyroid hormones (TH) are both necessary and sufficient for initiation and completion of metamorphosis, but other hormones of the thyroid and interrenal (adrenal) axes are involved (Kaltenbach, 1996).

Review of thyroid function in vertebrates

Figure 1. Current concept of thyroid axis function in anuran (frog) tadpoles (reviews: Denver, 1996, 1998; Hayes, 1997; and Kaltenbach, 1996).
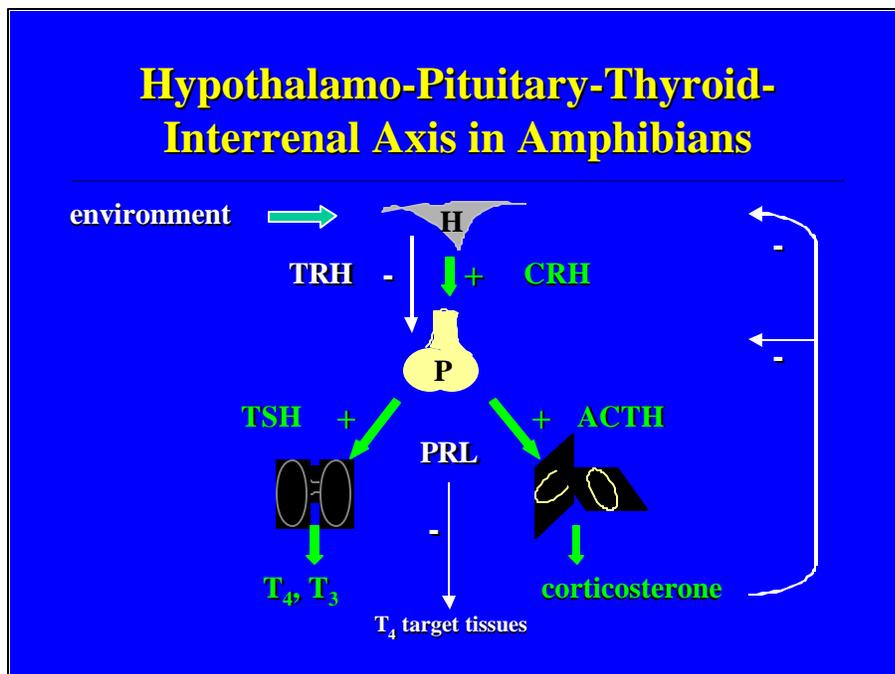
During the tadpole phase of development, environmental cues are received by the hypothalamus and translated into stimulatory or inhibitory signals to the pituitary. The roles of hypothalamic hormones regulating pituitary function in tadpoles differ from their roles in mammals. In tadpoles TRH (thyroid releasing hormone) is part of a thyroid inhibiting pathway. TRH stimulates the pituitary to produce PRL (prolactin) which probably decreases levels of thyroid receptor in target tissues, thereby antagonizing thyroid hormone action.

The stimulatory hypothalamic hormone is CRH (corticotropin releasing hormone) which stimulates pituitary release of both TSH (thyroid stimulating hormone) and ACTH (adrenocorticotropic hormone). TSH stimulates thyroid hormone synthesis and release from the thyroid gland. As in other vertebrates, the thyroid hormones are T_4 (L-thyroxine) and T_3 (triiodothyronine). Most TH is secreted as T_4 and converted to T_3 in target tissues. ACTH stimulates corticosterone production in the interrenal glands (the fish and amphibian equivalent of the adrenal glands). Corticosterone enhances the action of TH. Both TH and CORT have negative feedback effects on the pituitary and hypothalamus.

Figure 2.
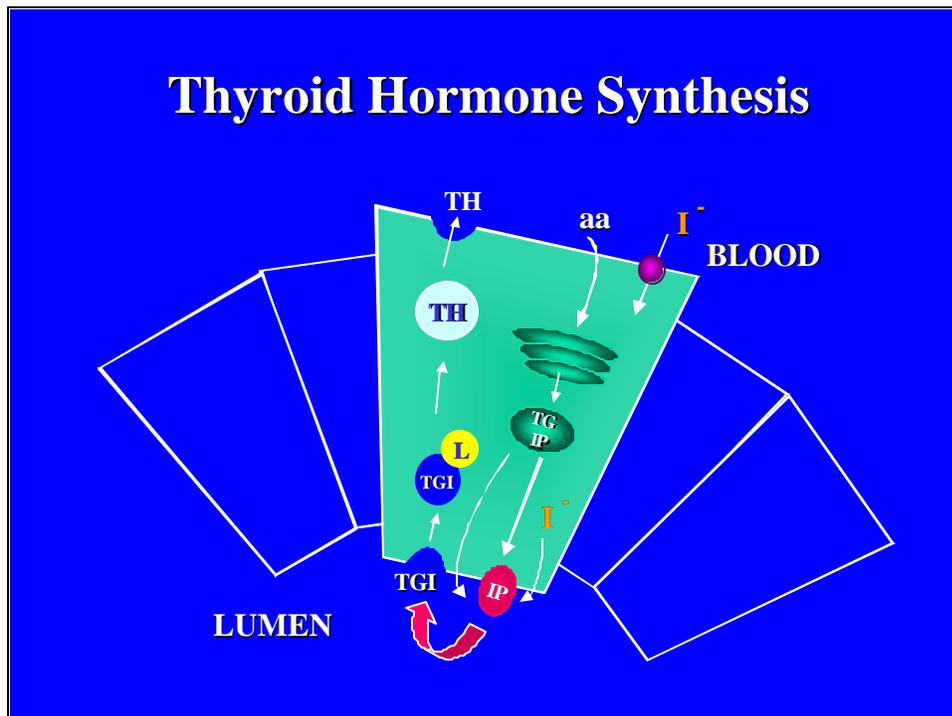
In amphibians and fish, as in all vertebrates, thyroid hormone synthesis occurs in thyroid follicles. The thyroid follicles of fish are scattered throughout the pharyngeal region, but in tadpoles are organized into a discrete gland (Norris, 1997).

Figure 2 represents the epithelial cells of the thyroid follicle, with the basolateral membrane in contact with the circulation and the apical membrane in contact with the colloid in the follicle lumen. Iodine (I^-) and amino acids (aa) are taken up from the circulation at the basolateral membrane. The amino acids are used to synthesize thyroglobulin (TG), which is packaged with iodide into vesicles that are exported to the lumen via exocytosis. Iodoperoxidase (IP) is a

membrane-bound enzyme that iodinates the tyrosine residues in TG. Iodination occurs extracellularly in the lumen. Iodinated TG re-enters the cell by endocytosis and the endosome containing TG-I fuses with a lysosome. TG-I is enzymatically cleaved to release the thyroid hormones, T_3 and T_4. The thyroid hormones, mainly in the form of T_4, are secreted into the circulation by exocytosis (Eales, 1997; Norris, 1997).

Figure 3.
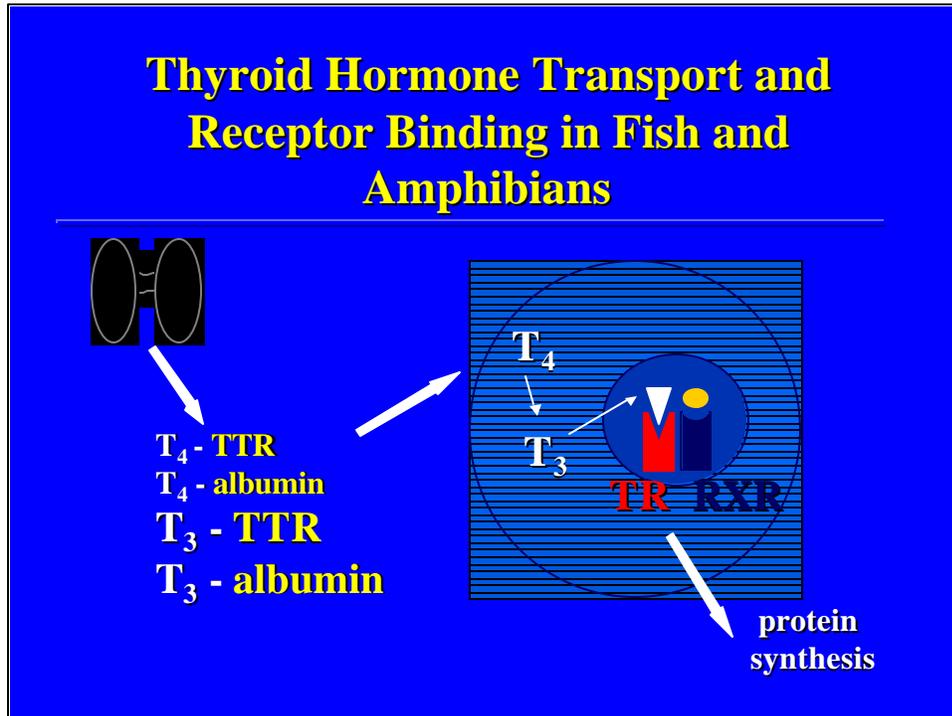
T_3 and T_4 are not very soluble in aqueous solution and are transported in the serum attached to thyroid binding proteins. These thyroid binding proteins are synthesized in the liver. The major thyroid hormone binding proteins in fish and amphibian plasma are transthyretin (TTR; also called prealbumin) and albumin. Both TTR and albumin have a higher affinity for T_3 than for T_4 (Larsson et al. 1985; Yamauchi et al. 1998; Santos and Power, 1999). In fish and amphibians, the major circulating thyroid hormone is T_3 (Eales and Brown, 1993; Denver, 1996; Kaltenbach, 1996). Unlike humans, ungulates (cattle, sheep, goats, pigs, water buffalo, and horses) and carnivores (dogs), fish and amphibians do not express thyroid binding globulin (TBG) (Larsson et al., 1985)

In all vertebrates, T_3 is the active form of TH and interacts with the nuclear thyroid receptor (TR) with higher affinity than T_4 (Tata, 1996; Norris, 1997). The current understanding of thyroid receptor interactions in amphibians is that TR and the retinoid X receptor (RXR) form heterodimers that bind to DNA response elements and act as transcription factors initiating protein synthesis. For the TR-RXR heterodimer to activate transcription, both T_3 and an RXR ligand, such as retinoic acid must bind to their receptors (Tata, 1996; Kakizawa et al., 1997; Wong and Shi, 1995; Zhang and Pfahl, 1993; Lazar, 1993).

Figure 4.

Thyroid Hormone Metabolism
Deiodination
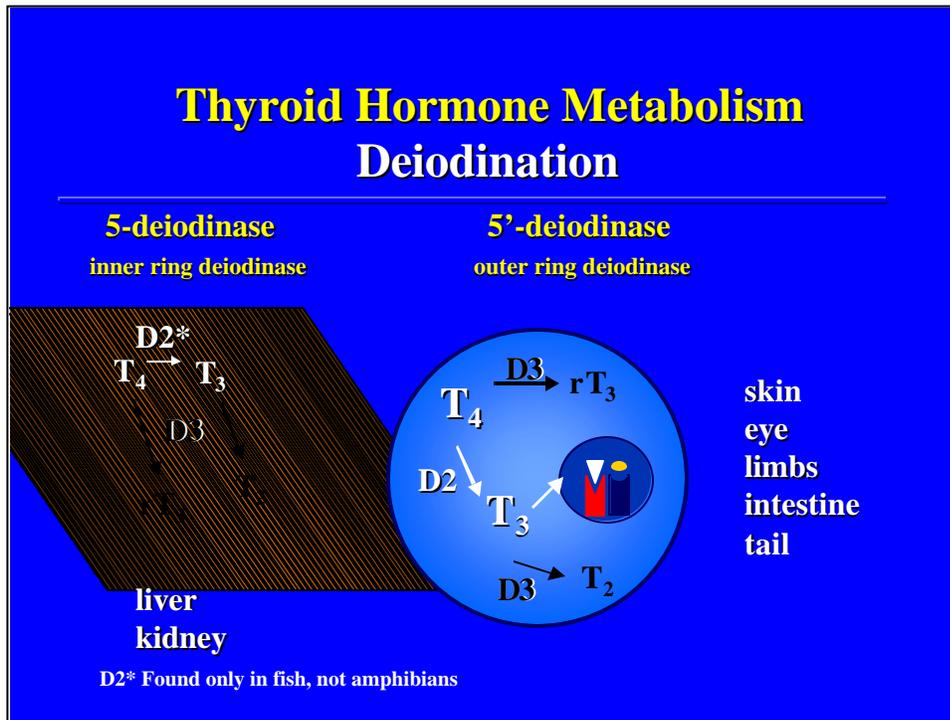
Activation of T_4 to T_3 occurs by deiodination in the peripheral tissues. Much of the T_4 to T_3 conversion in fish occurs in the liver (Eales and Brown, 1993). A variety of tissues in the tadpole, including the skin, eyes, limbs, intestine, and tail, are capable of deiodination (Galton et al, 1994).

Two classes of enzymes are responsible for deiodination. The outer ring or 5' deiodinases convert T_4 to the more active T_3. The inner ring or 5 deiodinases convert T_4 to the inactive rT_3 (reverse T_3) and T_3 to the inactive T_2 (Eales and Brown, 1993; Galton et al., 1994; McNabb and Freeman, 1990). The 5' deiodinases of amphibians and fishes are similar in function to the mammalian type II deiodinases, while the 5 deiodinases of amphibians and fish are similar to the mammalian type III deiodinases (Becker et al., 1997; Galton et al, 1994). Both inner and outer ring deiodination occur in the liver and kidney of fish (Eales and Brown, 1993) and inner ring deiodination occurs in all of the TH target tissues of the amphibian, including the skin, eye, limbs, intestine, and tail (Becker et al., 1997). However, outer ring deiodination does not occur in liver and kidney of tadpoles (Becker et al., 1997).

Figure 5.
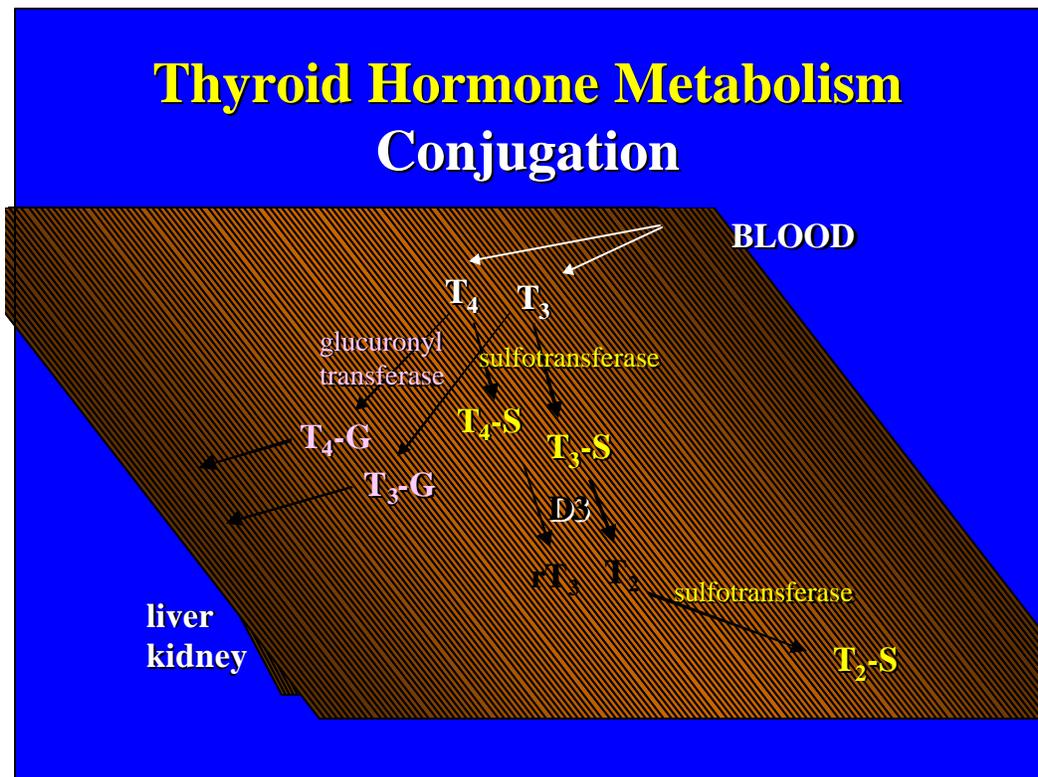
The major modes of thyroid hormone excretion in vertebrates are sulfation and glucuronidation by glucuronyl (uridine diphosphoglucuronyltransferase, UDPGT) and sulfotransferases in the liver with subsequent excretion in the bile (Eales, 1997).   In mammals, sulfated T_3 and T_4 are further degraded by the type I inner ring deiodinase and iodine can thus be recycled to the thyroid (Eales, 1997).   These recycling pathways have not yet been described for fish and amphibians.

In fish, the major route of TH excretion is via bile and urine, but also by secretion from the gills (Eales and Brown, 1993).

Evolution of thyroid function in vertebrates
To summarize the comparative aspects of thyroid function in vertebrates, I have mapped the components of thyroid function onto a phylogeny of the chordates (Figure 6). The more ancient groups are on the left of the diagram and the more recent groups are on the right. The bars on the lower axis represent the first appearance of a particular characteristic. The Urochordates include sea squirts and tunicates, the Cephalochordates include the lancelet (amphioxus). Together, these two groups are the Protochordates.

Figure 6.
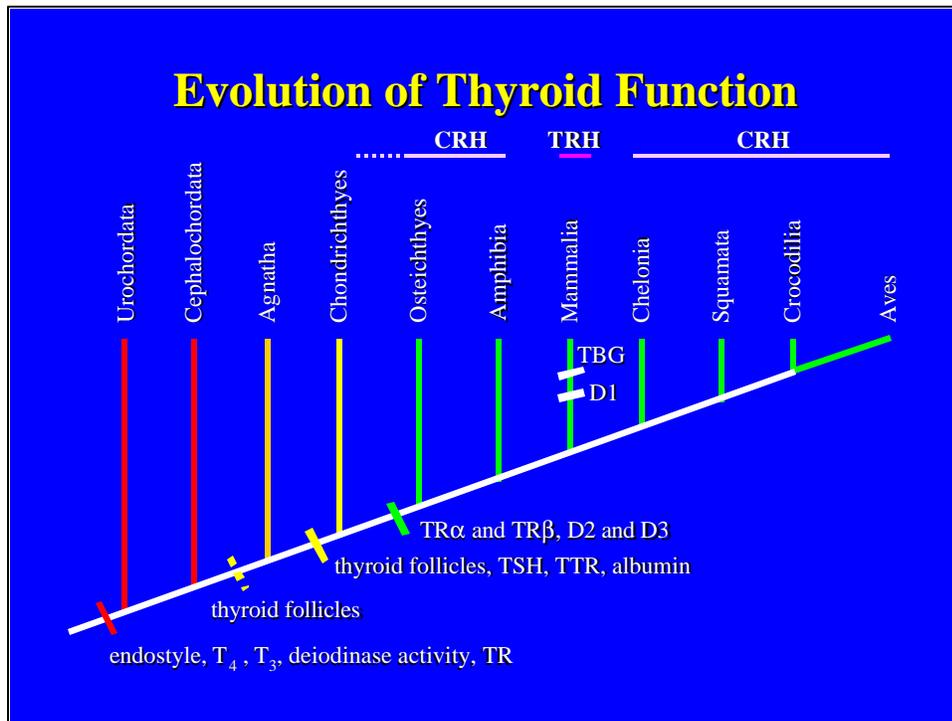
This phylogenetic map highlights evolutionary antiquity of thyroid hormone production. Sea squirts (urochordates) and amphioxus (cephalochordate) synthesize T_4 and T_3, although not in a thyroid gland, but in an organ called the endostyle. Because the endostyle has peroxidase, deiodinase and TH binding activity, it is considered homologous to the vertebrate thyroid gland (Ogasawara et al., 1999; Eales, 1997). TH are not secreted into the circulation but into the pharynx and are absorbed in the gut (Eales, 1997).

Thyroid follicles first appear in adult lampreys (agnathans). This bar is dotted in the diagram because larval lampreys have an endostyle that transforms into thyroid follicles in the adult (Eales, 1997). However, the thyroid follicles are not regulated by the pituitary (Gorbman et al., 1983).

Pituitary regulation of the thyroid gland by thyroid stimulating hormone (TSH) first appears in the fishes. In all vertebrates except mammals, the hypothalamus stimulates TSH secretion via corticotropin releasing hormone (CRH) (Norris, 1997).

In fishes and amphibians, TTR and albumin are the plasma transport proteins, binding T_3 with greater affinity than T_4 (Santos and Power, 1999; Yamauchi et al., 1999; Richardson et al., 1994). Only some mammals transport TH bound to thyroid binding globulin (TBG) (Larsson et al., 1985; Richardson et al., 1994). Bony fishes and all later groups of vertebrates have two distinct TRs, each with two sub-types (Eales, 1997). We also know that all these groups have inner ring (5 deiodinase or type II-like [D2]) and outer ring (5' deiodinase or type III-like [D3]) deiodinases (McNabb and Freeman, 1990; Galton et al., 1994; Becker et al., 1997; Eales, 1997). Mammals have three deiodinase enzymes; type I deiodinase (D1) deiodinates both the inner and outer ring (McNabb and Freeman, 1990).

Mechanisms of thyroid disruption

Much of the work on disruption of thyroid physiology has focused on polychlorinated biphenyls (PCBs) because of their structural similarity to TH and because of their strong correlation with thyroid pathologies, particularly goiter (Leatherland, 1998).

PCBs do not appear to inhibit thyroid hormone synthesis, although flavonoids found in vegetables of the cabbage family do inhibit synthesis by inhibiting iodoperoxidase activity (Divi et al., 1997).

Brouwer and colleagues have shown that hydroxylated PCBs (OH-PCBs), common metabolites of the parent compounds, compete for binding to serum binding proteins and alter thyroid hormone transport (for review see Brouwer et al., 1998). The data presented in Figures 7, 8, and 9 come from competitive binding studies comparing the interactions of OH-PCBs, DDTs, and chloroacetanilides (acetochlor and alachlor) with serum binding proteins and nuclear receptors (Cheek et al., 1999a).

Figure 7.
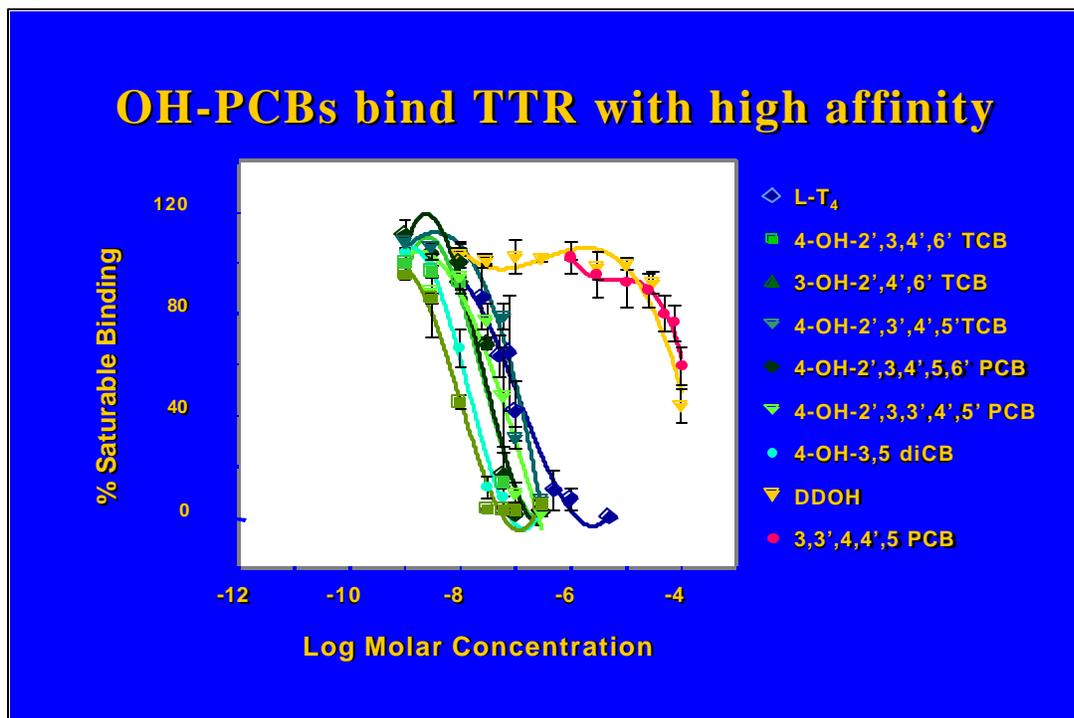
We found that OH-PCBs competed very effectively for binding to human TTR *in vitro*, having the same affinity for TTR as T_4, the native ligand. By contrast, DDOH, a metabolite of DDT, and parent PCB compounds did not bind TTR well.

Figure 8.
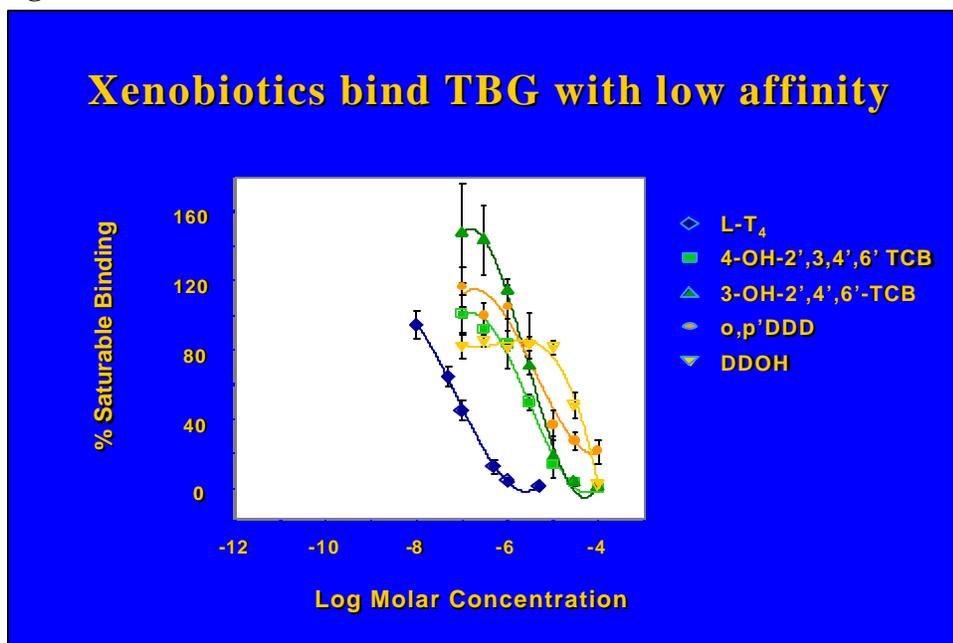
We also found that the same compounds that competed so effectively for TTR binding had very low affinity for TBG.

Figure 9.
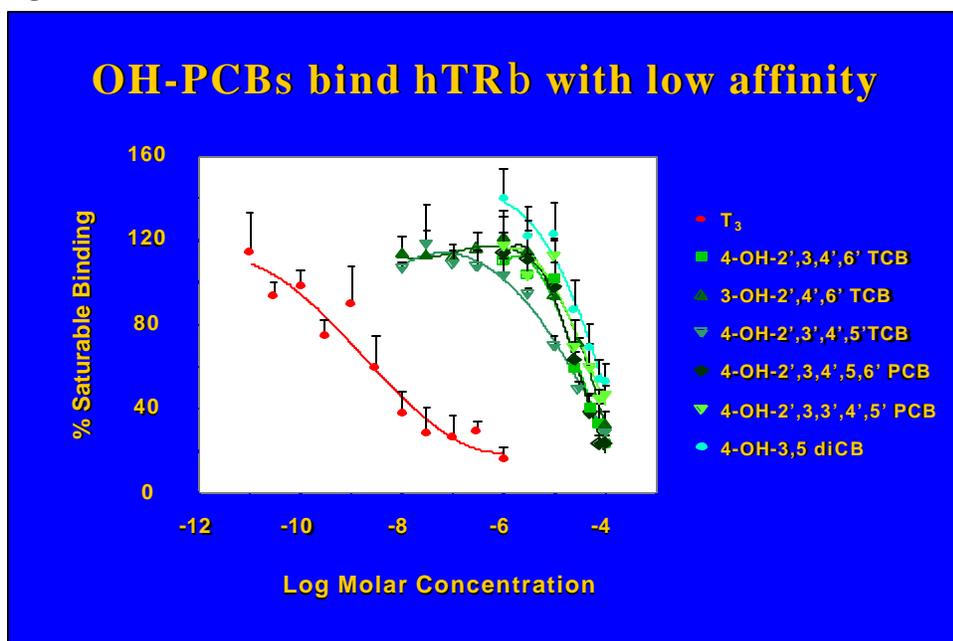
Similarly, none of the OH-PCBs or DDT metabolites bound to recombinant human TRβ1 with high affinity (IC$_{50}$ values were in the range of 10 - 100μM).

The high affinity of OH-PCBs for TTR, but not TBG or TR suggests that disruption of thyroid hormone transport is likely to be a major mechanism of thyroid disruption in many vertebrate species, since TTR is the major serum transport protein in most vertebrates (Larsson et al., 1985). The low affinity of all tested organochlorine compounds for recombinant TR suggests that receptor interaction may not be a major mode of thyroid disruption.

PCBs and their hydroxylated metabolites can also inhibit deiodinase and sulfotransferase activity, possibly leading to increases in circulating thyroid hormones (Schuur et al., 1998a,b; Brouwer et al., 1998). However, these xenobiotics also strongly upregulate uridine diphosphoglucuronyltransferase activity, enhancing both T_4 and T_3 glucuronidation and ultimately excretion via bile (Barter and Klaasen, 1992, 1994; Bastomsky, 1974; Brouwer et al., 1998; Morse et al., 1993; Van Birgelen et al., 1995).

Figure 10.
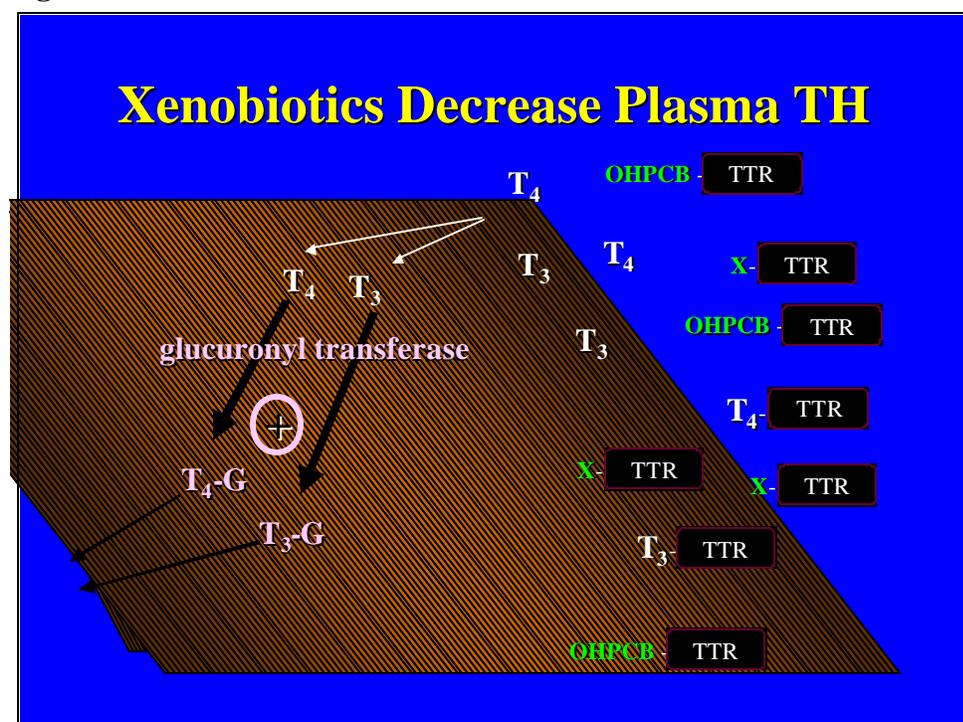
The consequence of these alterations in transport and degradation is often
a decrease in circulating levels of TH. The current hypothesis is that xenobiotic competition for transport protein binding sites displaces TH, making it available for glucuronide conjugation in the liver and subsequent excretion in the bile. These depressed levels of TH in circulation stimulates TSH secretion from the pituitary which stimulates thyroid gland hypertrophy. An animal may then have depressed plasma TH levels but an enlarged thyroid (Brouwer et al., 1998; Leatherland, 1998). These symptoms have been observed in salmonids in the Great Lakes exposed to PCB contamination (reviewed in Leatherland, 1993).

Consequences of thyroid disruption for amphibians.
Tadpole development can be divided into three broad stages: premetamorphosis, prometamorphosis, and metamorphic climax. Premetamorphosis is a period of general body and hindlimb growth. Prometamorphosis is the period of growth and differentiation when thyroid hormone levels are rapidly increasing. Metamorphic climax is a period of rapid differentiation from tadpole to frog. Thyroid and glucocorticoid hormones reach their highest levels at the beginning of metamorphic climax and massive tissue remodeling occurs. The beginning of metamorphic climax is marked by the emergence of the forelimbs and the end by resorption of the tail.

The duration of the tadpole stage in amphibians can change depending upon environmental conditions, but there is a minimum time which is determined by growth rate. Tadpoles must reach a minimum size before they will undergo metamorphosis (Wilbur and Collins, 1973). There is also a maximum time that depends on the duration of the pond. Species that develop in stable ponds may remain in the tadpole stage for as long as four years (bullfrogs), while species that develop in ephemeral pools on the desert floor may complete metamorphosis in as little as eight days (Western spadefoot toad). Environmental cues that affect the rate of development can include temperature, water level, tadpole density, food availability, and presence of predators (review: Denver, 1997).

Exogenous chemicals may also serve as environmental cues affecting the rate of metamorphosis. We exposed premetamorphic tadpoles to the chloracetanilide herbicide acetochlor (ACETO) alone or in combination with T_3 to test this hypothesis (Figure 11; Cheek et al., 1999b).

Figure 11.
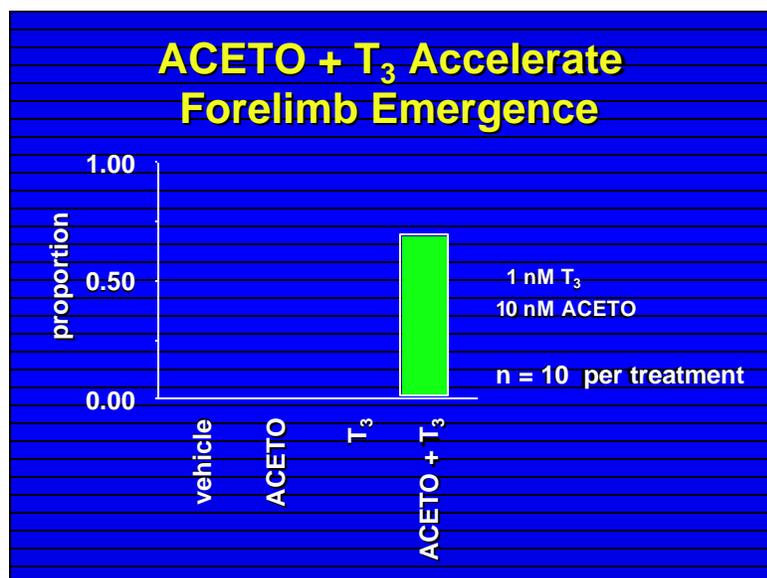
After seven days of exposure at 25°C, 66% of the tadpoles treated with T_3 and ACETO had undergone forelimb emergence (FLE), the beginning of metamorphic climax. None of the T_3 treated animals had emerged forelimbs. These results led us to ask whether ACETO was acting as a T_3 mimic by interacting with the TR. We "primed" tadpoles by first exposing them to T_3 for three days to upregulate TR expression, then exposed them to ACETO alone or in the presence

of T_3 for an additional seven days. ACETO treatment of primed tadpoles did not stimulate forelimb emergence, but T_3 and ACETO + T_3 treatment did. Twice as many tadpoles reached FLE when co-treated with T_3 and ACETO. In a longer term study (4 months) conducted at a lower temperature (21°C), we saw the same enhancement of T_3 action by ACETO (Cheek et al., 1999b).

What are the consequences of accelerated metamorphosis? In our study, tadpoles that reached FLE earlier tended to be smaller ($p = 0.29$). These results are consistent with those for other species of amphibians in which natural variation in time to metamorphosis has been observed and manipulated. In many species of amphibians, shorter times to metamorphosis mean smaller juvenile body size (Semlitsch et al., 1988; Smith, 1987). Small juvenile size is correlated with reduced survivorship and with smaller size at first reproduction, meaning fecundity of these individuals is reduced because smaller females cannot produce as many eggs (Semlitsch et al., 1988; Smith, 1987). Later age at first reproduction may mean reproducing as a two year old instead of as a one year old.

These individual consequences could be translated into population level consequences. Using the Lotka equation for population growth,

$$r = \frac{\ln(l_x \bullet m_x)}{x}$$

r	population growth rate
l_x	mean survival to reproductive age
m_x	mean number of offspring per individual
x	mean age at first reproduction

we can see that if smaller juveniles have decreased survival (l_x) then r will decrease. If smaller juveniles are smaller at first reproduction and therefore produce fewer offspring, m_x decreases and so does r, and if smaller juveniles reproduce later, x decreases and so does r.

Obviously, I have extrapolated from the currently available data on thyroid disruption in frogs to make this prediction. However, this equation for population growth applies to all organisms. It highlights the need for research to address not only the mechanisms of endocrine disruption in animals, but also to determine whether the consequences of endocrine disruption include altered survival, fecundity, and reproductive age. Only if we examine these variables in wild populations will we begin to understand the impact at the population level.

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