Potential Mechanisms of Thyroid Disruption in Humans: Interaction of Organochlorine Compounds with Thyroid Receptor, Transthyretin, and Thyroid-binding Globulin

Ann Oliver Cheek,1,2 Kelvin Kow,1 Jian Chen,1 and John A. McLachlan1,3

1Tulane/Xavier Center for Bioenvironmental Research; 2Department of Ecology, Evolution, and Organismal Biology; 3Department of Pharmacology, Tulane University, New Orleans, LA 70402 USA

Organochlorine compounds, particularly polychlorinated biphenyls (PCBs), alter serum thyroid hormone levels in humans. Hydroxylated organochlorines have relatively high affinities for the serum transport protein transthyretin, but the ability of these compounds to interact with the human thyroid receptor is unknown. Using a baculovirus expression system in insect cells (Sf9 cells), we produced recombinant human thyroid receptor β (hTRβ). In competitive binding experiments, the recombinant receptor had the expected relative affinity for thyroid hormones and their analogs. In competitive inhibition experiments with PCBs, hydroxylated PCBs (OH-PCBs), DDT and its metabolites, and several organochlorine herbicides, only the OH-PCBs competed for binding. The affinity of hTRβ for OH-PCBs was 10,000-fold lower (Kd = 20–50 μM) than its affinity for thyroid hormone (3,3',5-triiodothyronine, T3; Kd = 10 nM). Because their relative affinity for the receptor was low, we tested the ability of OH-PCBs to interact with the serum transport proteins—transthyretin and thyroid-binding globulin (TBG). With the exception of one compound, the OH-PCBs had the same affinity (Kd = 10–80 nM) for transthyretin as thyroid hormone (thyroxine; T4). Only two of the OH-PCBs bound TBG (Kd = 3–7 μM), but with a 100-fold lower affinity than T4. Hydroxylated PCBs have relatively low affinities for the human thyroid receptor in vitro, but they have a thyroid hormone-like affinity for the serum transport protein transthyretin. Based on these results, OH-PCBs in vivo are more likely to compete for binding to serum transport proteins than for binding to the thyroid receptor.

Key words: endocrine disruption, PCB, thyroid-binding globulin, thyroid receptor, transthyretin. Environ Health Perspect 107:273–278 (1999). [Online 9 March 1999] http://ehpnet1.niehs.nih.gov/docs/1999/107p273-278cheek/abstract.html

An important question in endocrine disruption is the mechanism by which a xenobiotic compound alters the action of endogenous hormones. One possible mechanism is direct interaction with the hormone receptor, either as an agonist or as an antagonist. In the case of thyroid hormone, a second important mechanism may be the ability of compounds to alter serum transport of thyroid hormones (T3). In nonmammalian vertebrates, the major transport protein is prealbumin (transthyretin); while some mammals, including humans, have a second binding protein, thyroid-binding globulin (TBG) (1). Assessing the relative affinity of the thyroid receptor and the serum transport proteins for xenobiotics should help clarify one of the mechanisms by which xenobiotics alter thyroid homeostasis.

Alterations in thyroid homeostasis by organochlorine compounds have been documented for many species, including humans. In most cases, exposure to organochlorine compounds is correlated with decreased serum levels of thyroid hormone, particularly thyroxine (T4). Exposure to polychlorinated biphenyls (PCBs) has been correlated with decreased serum T4 concentrations in rats (2–10) and humans (6,11,12). Evidence from rat studies indicates that PCB-induced decreases in serum T4 are the result of increased metabolism by uridine diphosphate glucuronyltransferase (UDPGT), a hepatic enzyme that glucuronidates T4 (5,13–15). Another class of organochlorines, the choroacetanilides acetochlor and alachlor, elevates UDPGT activity and concomitantly decreases serum T4 levels in rats (16,17). Acetochlor also alters thyroid hormone (3,3',5'-triiodothyronine; T3) action in amphibians, accelerating T3-induced metamorphosis (18). DDT and its metabolites alter serum T4 levels in birds (19) and humans (20). DDT also alters thyroid metabolism in rats by increasing hepatic UDPGT activity (21).

Because of their physiological effects and their structural resemblance to thyroid hormones (Fig. 1), several studies have investigated the ability of PCBs to bind to the serum transport proteins transthyretin (21,22) and TBG (22) and to the rat thyroid receptor (23). Transthyretin (TTR) and TBG have similar affinities for the natural ligand, T4 (50–90 nM) (22), but have different affinities for PCBs. Hydroxylated PCBs are potent ligands for TTR, having affinities in the 1 nM range, 50-fold greater than that of T4 (8,22,24). Few hydroxylated PCBs bind TBG (22) and few unmetabolized PCBs have strong affinities for either TTR or TBG (8,22,23).

Like the transport proteins, the rat thyroid receptor appears to have a higher affinity for hydroxylated versus parent PCBs (23).

Although studies of binding affinity suggest that organochlorine compounds may alter thyroid homeostasis by interacting with thyroid hormone transport proteins in humans and animals, little is known about the ability of organochlorines to interact with the thyroid receptor, particularly in humans. We examined the ability of PCBs, DDTs, choroacetanilides, and an isoprenoid to bind a recombinant human thyroid receptor (hTRβ1). To evaluate the relative significance of receptor versus transport protein binding for disrupting thyroid homeostasis, compounds that bound the receptor were also tested for binding to human transthyretin and TBG.

Methods

Chemicals

T3, T4, 3,3',5'-triiodothyroacetic acid (Triac), 3,3',5',5'-tetraiodothyroacetic acid (Tetraac), 3,3',5'-triiodo-l-thyronine (T3), 2,2-bis(p-chlorophenyl)-ethanol (DODOH), human TBG, and human transthyretin (prealbumin) were purchased from Sigma Chemical Co. (St. Louis, MO). The PCBs—3,3',4',4'-pentachlorobiphenyl (PCB 126), 3,3',4'-tetrachlorobiphenyl (PCB 77), 3,3',4',4'-pentachlorobiphenyl, 4-OH-3,3',4',4'-pentachlorobiphenyl, 4-OH-3,3',4',4'-pentachlorobiphenyl, and 4-OH-3,3',4',4'-pentachlorobiphenyl—were purchased from AccuStandard (New Haven, CT). The hydroxylated PCB, 4,4'-diOH-3,3',5,5'-tetrachlorobiphenyl, was purchased from

Address correspondence to A.O. Cheek, Department of Biology, Southeastern Louisiana University, SLU 10736, Room 205 Mead Hall, North Pine Extension, Hammond, LA 70402 USA.

Many thanks to P. Vonier for teaching techniques in cell culture and baculovirus expression and to A. Holstein for performing preliminary Western blot analysis. This study was supported by a grant from the National Institute of Environmental Health Sciences (ES-05207).

Received 21 September 1998; accepted 21 December 1998.
Articles • Cheek et al.

Ultra Scientific (North Kingstown, RI). The DDTs—1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2.2-trichloroethane (o,p'-DDT), 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2.2-dichloroethane (o,p'-DDE), 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT), and 2,2-bis(4-chlorophenyl)-1.1-dichloroethane (p,p'-DDE)—were purchased from Aldrich Chemical (Milwaukee, WI). All chemicals were dissolved in DMSO. DMSO did not exceed 0.01% in the binding assays.

Transfection
SP9 insect cells (pupal ovarian cells from the fall armyworm) were purchased from Invitrogen (Carlsbad, CA) and cultured at 27°C in complete Grace’s media (10% fetal bovine serum lactalbumin hydrolysate, tissue culture yeastolate, and glutamine; Invitrogen). A baculovirus phagemid containing the human thyroid receptor β1 cDNA was cloned into the multiple cloning site of the baculovirus phagmid pFastBac1 (Gibco BRL, Grand Island, NY) by DNA Technologies (Gaithersburg, MD). Confluent cells in a T-25 flask (Corning, Corning, NY) were rinsed with serum-free Grace’s media and transfected with 1 μg phagemid DNA and 2 μl lipofectin in 2 ml serum-free Grace’s media at 27°C. At the end of 5 hr, the transfection medium was replaced with complete Grace’s media. After 7 days, medium containing baculovirus was harvested and stored at 4°C. To produce recombinant hTRβ, confluent SP9 cells in a T-150 flask were incubated with 7 ml baculovirus-containing medium at 27°C for 1.5 hr. An additional 13 ml of Grace’s media was added and cells were cultured for 5 days. Virus-containing medium was harvested and cell extracts were prepared.

Preparation of Protein Extract
Protocols for preparing cell extracts were modified from Toscano (25), Bres and Eales (26), and Sullivan et al. (27). Transfected SP9 cells were scraped from the flask and centrifuged at 5,000 rpm for 5 min. The supernatant was harvested and stored at 4°C. The cell pellet was resuspended in 2.5 ml buffer A [10 mM Tris-HCl (pH 7.6), 10% glycerol, 3 mM MgCl2, 2 mM CaCl2, 5 mM dithiothreitol (DTT), 1 mM Pefabloc, 1 μg/ml aprotinin, and 20 μM leupeptin], incubated for 20 min on ice, and homogenized in a glass dounce. KCl was added to a concentration of 0.4 M and the homogenate was incubated on ice for 30 min, with shaking through a Pasteur pipet every 10 min. The homogenate was then centrifuged at 25,000 rpm for 15 min at 4°C. The supernatant (cell extract) was aliquotted and stored at -80°C until use.

Immunodetection of TR Protein
Cell extracts were heated (95°C) in sodium dodecyl sulfate (SDS) loading buffer and electrophoresed through 10% SDS-PAGE. Gels were electrophoretically transferred to polyvinylidene difluoride membranes (Sigma) at 25 V overnight. Membranes were rinsed in Tris-buffered saline (TBS; 10 mM Tris-HCl, pH 7.6, and 150 mM NaCl) and blocked in TBS supplemented with 3% bovine serum albumin, fraction V (Sigma). Membranes were incubated with a polyclonal antiserum to amino acids 62–82 of human TRβ1 (1:1250; Affinity BioReagents, Golden, CO) in TBS + 0.1% Tween-20 for 2 hr, rinsed 3 times in TBS, incubated for 1 hr with HRP-conjugated goat antirabbit IgG (Kirkegaard-Perry, Gaithersburg, MD), rinsed 4 times with TBST, and visualized with enhanced chemiluminescence (Amersham, Arlington Heights, IL).

TR Binding Assays
Saturation analysis. Cell extract containing hTRβ was added to assay buffer [10 mM Tris-HCl (pH 7.4), 10% glycerol, 5 mM DTT, and 0.5% CHAPS] to achieve a final concentration of 50 μg protein/ml in 100 μl total volume. Extracts were preincubated with 1,000-fold excess unlabeled T3 for 15 min at 21°C, then increasing concentrations of [125I]T3, T4, and T2 were added and incubated for an additional 60 min. The reaction was terminated on ice and unbound [125I]T3 was separated from bound [125I]T3 with the addition of hydroxyapatite (HAP) slurry (1:1 v/v in 0.1 M KCl, 10 mM Tris-HCl, pH 7.4). Extracts were incubated in HAP slurry for 40 min, with vortexing every 10 min. The slurry was centrifuged at 5,000 rpm for 3 min and buffer was aspirated. HAP pellets were then washed 3–4 times with 400 μl wash buffer [0.1 M KCl, 10 mM Tris-HCl (pH 7.4), 0.5% CHAPS], resuspended in 200 μl slurry buffer, and counted in ScintiVerse scintillation fluid (Fisher, Houston, TX).

Competitive inhibition experiments. Based on the saturation analysis, 2.5 nM [125I]T3 was used in competitive inhibition experiments. Nonsaturable binding was estimated by preincubating cell extracts with 10,000-fold molar excess unlabeled T3 for 15 min. Varying concentrations (10^-8–10^-4 M) of unlabeled competitors were also...
preincubated with cell extract before addition of $^{[125]}$I-T$_3$. All other conditions were as described above.

To characterize the specificity of the recombinant receptor, the known TR agonists T$_3$, T$_4$, Triac, and Tetrac, and the inactive T$_3$ metabolite, rT$_3$, were tested in competitive inhibition experiments. We selected organochlorine compounds for testing based on their reported ability to alter in vivo responses or to bind serum transport proteins. PCB mixtures and some specific PCBs decrease serum T$_4$ levels, but PCB 126 and PCB 77 markedly enhance spatial learning in rats exposed in utero, while only slightly altering serum T$_4$ (2). Because the enhancement of learning is similar to that in hyperthyroid rat pups, Schantz et al. (2) proposed that PCBs 126 and 77 might be thyroid receptor agonists. We examined the interaction of these two compounds with the human thyroid receptor. Because several OH-PCBs bind TTR as effectively as T$_3$ (22), we also tested the ability of OH-PCBs to bind the receptor. DDTs and chloroacetanilides alter serum T$_4$ levels and catabolism, but their ability to bind TR is unknown.

Thyroid-binding Protein and Transthyretin Binding Assays

Conditions for these binding assays were modified from Lans et al. (22). Briefly, purified TBG or TTR was added to 200 µl assay buffer (TR assay buffer discussed previously) for a final concentration of 30 nM. We used 55 nM L-T$_4$ containing 100,000 cpm $^{[125]}$I-L-T$_4$ to estimate total binding. Non-saturable binding was estimated by preincubating protein solutions with 100-fold molar excess unlabeled L-T$_4$ for 15 min. Varying concentrations of unlabeled competitors were also pre-incubated with the protein before the addition of $^{[125]}$I-L-T$_4$. All other conditions were as described for TR saturation analysis, except the volumes of HAP slurry and of slurry buffer for resuspension were doubled to account for the twofold larger assay volume.

Results

Immunodetection

Infected SF9 cells expressed a protein of approximately 51 kDa that cross-reacted with a polyclonal antibody specific to hTRβ1 (Fig. 2). This protein is similar in size to the 52–55 kDa proteins recombinantly expressed in Escherichia coli (28,29).

TR Binding

Saturation analysis. Preliminary experiments using 50, 100, 200, and 300 µg protein/ml indicated that minimal non-saturable binding (50% of total binding) occurred at 50 µg protein/ml. Similarly, varying incubation times (15, 30, 45, 60, 90, and 120 min) showed that a 60-min incubation period was sufficient for achieving equilibrium binding. Binding to recombinant hTRβ was saturable at 3 nM $^{[125]}$I-T$_3$ with a K$_D$ of 1.37 ± 0.24 nM (n = 4 experiments) and a B$_{max}$ of 0.30 ± 0.09 nM (Fig. 3). The binding affinity observed with recombinant hTRβ expressed in SF9 cells is similar to that reported for other recombinant thyroid receptors (28,29), but is lower than that reported for thyroid receptors extracted from tissues (approximately 0.1 nM) (30).

Competitive binding experiments. Thyroid hormones and their analog showed the expected order of affinity for recombinant hTRβ: Triac > T$_3$ > L-T$_4$ > Tetrac > DL-L-T$_4$ = Tetrac (Fig. 4 and Table 1). Of the xenobiotics tested, only the hydroxylated PCBs could inhibit 50% of $^{[125]}$I-T$_3$ binding to hTRβ (Fig. 5 and Table 1), although with 10,000-fold lower potency than L-T$_4$. The coplanar PCBs, PCB 77 and PCB 126, did not displace T$_3$ from the receptor, nor did o,p'-DDT, p,p'-DDD, acetochlor, or methoprene. Although some displacement (20%) was achieved by the highest concentrations (100 µM) of o,p'-DDD, o,p'-DDT, DDOH, and alachlor, none of these compounds could inhibit 50% of $^{[125]}$I-T$_3$ binding.

TTR and TBG Binding

The binding affinity of unlabeled L-T$_4$ was 62 ± 12 nM for TTR and 76 ± 15 nM for TBG. These affinities are in agreement with those reported by Lans et al. (22): 88–138 nM for TTR and 52–85 nM for TBG. All...
of the 4-hydroxylated PCBs bound TTR with affinities (10–140 nM) similar to that of the natural ligand L-T₄ (62 nM) (Fig. 6 and Table 1). Hydroxylation in the meta position appeared to abolish TTR binding (Table 1). PCB 126 bound TTR weakly, with a 1,000-fold lower affinity than L-T₄ and the 4-hydroxylated PCBs (Table 1 and Fig. 6). DDOH was the only DDT metabolite with weak affinity for TTR (approximately 100 μM).

Few of the xenobiotics competitively bound TBG. Two of the hydroxylated PCBs competed for TBG binding—4-OH-2',3,4',6'-tetrachlorobiphenyl and 3-OH-2',4',6'-trichlorobiphenyl (Fig. 7 and Table 1)—but with affinities 30–100-fold lower than L-T₄. Cl ions in the 2',4', and 6' positions appeared to facilitate binding to TBG. Addition of an extra Cl' at the 5 position seemed to abolish TBG binding, as 4-OH-2',3,4,5,6'-pentachlorobiphenyl did not compete for binding, whereas 4-OH-2',3,4',6'-tetrachlorobiphenyl did. None of the other hydroxylated PCBs bound TBG. α,α'-DDT and DDOH bound TBG, but with affinities 70–800-fold lower than L-T₄ (Fig. 7 and Table 1). Acetochlor, alachlor, and methoprene showed no affinity for TTR or TBG.

### Discussion

Of the four groups of compounds examined, only the hydroxylated PCBs bound to human TRβ1 with affinities ranging from 30–90 μM—affinities 10,000-fold lower than the natural ligand T₃. The hydroxylated PCBs had 1,000-fold greater affinities for TTR than for TR, making them competitors for the natural ligand L-T₄. Half of the hydroxylated PCBs tested had higher affinities for TTR than did T₄.

| Compound                               | TRβ | TTR | TBG |
|----------------------------------------|-----|-----|-----|
| T₃                                     |     |     |     |
| D₄-T₄                                  |     |     |     |
| Triac                                  |     |     |     |
| Tetrac                                 |     |     |     |
| 3-OH-2',4',6'-trichlorobiphenyl        |     | >100| 1.1 |
| 4-OH-3,5-dichlorobiphenyl              | 83.5| 0.016| >100|
| 4-OH-2',3',4',5'-tetrachlorobiphenyl   | 37.7| 0.089| >100|
| 4-OH-2',3',4',6'-tetrachlorobiphenyl   | 43.0| 0.033| >100|
| 4-OH-2',3',4',5'-pentachlorobiphenyl   | 67.2| 0.141| >100|
| 4-OH-2',3',4',5,6'-pentachlorobiphenyl | 36.5| 0.040| >100|
| 4',4'-diOH-3,3',5,5'-tetrachlorobiphenyl| 32.7| 0.011| >100|
| PCB 77                                 | >100| >100| >100|
| PCB 126                                | >100| 140 | >100|
| α,α'-DDD                               | >100| >100| >100|
| α,α'-DDD                               | >100| >100| >100|
| DDOH                                   | >100| 94  | >100|
| α,α'-DDE                               | >100| >100| >100|
| α,α'-DDE                               | >100| >100| >100|
| Acetochlor                             | >100| >100| >100|
| Alachlor                               | >100| >100| >100|
| Methoprene                             | >100| >100| >100|

Abbreviations: TRβ, thyroid receptor β; TTR, transthyretin; TBG, thyroid-binding globulin; T₃, 3,3',5-triiodothyronine; T₄, thyroxine; TTR, 3,3',5-triiodothyroacetic acid; Tetrac, 3,3',5,5'-tetrachloroacetic acid; α,α'-DDT, 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane; PCB 77, 3,3',4',4'-tetrachlorobiphenyl; PCB 126, 3,3',4',5'-pentachlorobiphenyl; α,α'-DDD, 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane; DDOH, 2,2'-bis(p-chlorophenyl)-vinyl ether; α,α'-DDE, 2,2'-bis(4-chlorophenyl)-1,1-dichloroethane; α,α'-DDT, 1,1-bis(4-chlorophenyl)-2,2-di-chloroethane.

Only two of the hydroxylated PCBs bound TBG—3-OH-2',4',6'-trichlorobiphenyl and 4-OH-2',3',4',6'-tetrachlorobiphenyl. In fact, 3-OH-2',4',6'-trichlorobiphenyl did not bind TTR, but had a 20-fold lower affinities than 3-OH-2',4',6'-tetrachlorobiphenyl for TBG binding. Table 1 shows the inhibition constants (± standard error, n = 3 experiments in duplicate) for thyroid hormones and environmental chemicals interacting with recombinant hTRβ, hTTR, and hTBG.

---

**Figure 4.** Competitive binding of known thyroid receptor (TR) agonists to recombinant human thyroid receptor β. Increasing concentrations of agonist competed with 2.5-nM ³²P]-3,3',5-triiodothyronine (T₃). Nonsaturable binding was estimated by incubation with 10,000-fold molar excess unlabeled T₃. n = 3 experiments performed in duplicate, except n = 5 experiments for T₄.

**Figure 5.** Competitive binding of hydroxylated polychlorinated biphenyls with recombinant human thyroid receptor β. Assay conditions are as described in Figure 4. n = 3 experiments performed in duplicate. Abbreviations: T₃, 3,3',5-triiodothyronine; CB, chlorinated biphenyl.
greater affinity for TBG than for TR. PCBs and especially their hydroxylated metabolites interact with multiple components of the thyroid system, enhancing hepatic metabolism of thyroid hormones (5,13-15), competing for transport via serum proteins (21), especially TTR (3,8,22-24), and competing for receptor binding (this study). The physiological result is alteration in serum thyroid hormone levels. Of the known mechanisms, interaction with the thyroid receptor is likely to be less important than competition for serum transport proteins and induction of hepatic metabolism of T₄. Given Kᵣ values of 30–90 μM for xenobiotics that interacted with the receptor, high concentrations on the order of 20 ppm (micrograms per gram) would have to be achieved in target tissues for hydroxylated PCBs to significantly alter T₄ binding to the receptor. Alternatively, the Kᵣ values for TTR suggest that a concentration of only 0.017 ppm would have to be achieved for hydroxylated PCBs to significantly alter thyroid hormone transport via TTR. Hydroxylated PCB concentrations on the order of 0.36 ppm have been measured in human serum (31). Based on these data, in vivo disruption of TTR binding is more likely than disruption of receptor binding.

Neither of the coplanar dioxinlike PCBs, PCB 77 and PCB 126, bound the TR. Schantz et al. (2) observed that these PCBs accelerated spatial learning in rat pups exposed in utero and during lactation, an effect observed in hyperthyroid neonatal rats. Because both coplanar PCBs caused slight decreases in serum T₃ and T₄ but accelerated spatial learning, Schantz et al. (2) suggested that these PCBs might directly activate the thyroid receptor. In this study, PCB 77 and PCB 126 did not bind the human TRβ1, so direct interaction with the receptor probably does not explain the thymimetic learning effect observed in the rats.

Previous work by McKinney et al. (23) indicated that two other coplanar PCBs, PCB 169 and PCB 80, bound to a rat nuclear extract with 100-fold lower affinities than T₄, whereas PCB 54, an ortho-substituted congener, did not bind at all. Although the present study did not examine the binding affinity of PCBs 169 and 80, reported differences in affinity for coplanar PCBs may be due to several factors. First, the current study used a protein extract of insect cells producing recombinant human TRβ1. Therefore, only a single TR isofrom was available to interact with compounds in competitive binding experiments. In contrast, the rat liver nuclear extract probably contained not only only TRβ1, but also TRα1 and TRα2 (30). Second, because only a single TR isofrom was present in recombinant cell extracts, only TR homodimers could form, while in rat liver nuclear extracts, retinoid X receptors (RXRs), the heterodimeric partners of TR, were probably also present (30,32). In vitro, TR-RXR heterodimers exhibit different affinities for ligands than do TR-TR homodimers, although both appear to form spontaneously in cells (30). Third, species-specific differences in TR affinity for coplanar PCBs may exist.

DDTs and chloroacetanilide herbicides cause hypothyroidlike effects in animals, decreasing serum T₄ (16,17,19-21). None of these compounds bound to the thyroid receptor, indicating that they are unlikely to disrupt the thyroid axis via receptor interaction. DDOH bound to TTR and to TBG, but with such low affinity that concentrations in serum are unlikely to be high enough to compete for T₄ binding. o,p′-DDD bound to TBG with a fairly low affinity (5 μM) and is unlikely to reach such high concentrations in serum because of environmental exposure. However, clinical treatment of adrenal carcinomas resulted in 100–600 μM doses of o,p′-DDD (20). One consequence of o,p′-DDD treatment was decreased serum T₄, purportedly due to direct competition with o,p′-DDD for TBG binding (20). Our results support that hypothesis. Neither of the chloroacetanilides acetochlor nor alachlor bound TTR or TBG, but they enhance hepatic metabolism of T₄ in rats (16,17). Alteration of metabolism is probably the major mechanism by which chloroacetanilides affect thyroid axis function.

Our results suggest that disruption of thyroid hormone transport is one of the
mechanisms by which organochlorine compounds alter thyroid homeostasis. In particular, hydroxylated PCBs compete effectively for T₄ binding to TTR, but few compounds compete for TBG binding, even at μM concentrations. TBG is found only in some mammals, including priates, unguulates (cattle, sheep, goats, pigs, water buffalo, and horses), and carnivores (dog), but not in rodents (rat) or lagoon-morphs (rabbit). Depending on species, TBG binds 60–90% of serum T₄. Interestingly, TBG deficiency in humans does not interfere with euthyroid status, suggesting that TTR is also important for T₄ transport in humans. TTR is a highly conserved TH binding protein in all vertebrate species, so disruption of thyroid hormone transport by hydroxylated PCBs could potentially occur in all vertebrates, not only in humans.

**References and Notes**

1. Larsson M, Pettersson T, Carlstrom A. Thyroid hormone binding in serum of 15 vertebrate species: isolation of thyroxine-binding globulin and prealbumin analogs. Gen Comp Endocrinol 58:360–375 (1985).
2. Schantz SL, Seo BW, Moshaghian J, Amin S. Developmental exposure to polychlorinated biphenyls or dioxin: do changes in thyroid function mediate effects on spatial learning? Am Zool 37:359–408 (1997).
3. Dannerud PO, Morse D, Klasson-Wehier E, Brouwer A. Binding of 3,3',4,4'-tetrachlorobiphenyl (CB-77) metabolite to fetal thyrotrypsin and effects on fetal thyroid hormone levels in mice. Toxicology 106:105–114 (1996).
4. Goldiey ES, Kehn LS, Lau C, Rehberg GL, Crafton KM. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol Appl Pharmacol 135:77–88 (1995).
5. Van Birgelen AP, Smit EA, Kampen IM, Groeneveld CN, Fase KM, Van der Kolk J, Poelger N, VandenBerg M, Koeman JH, Brouwer A. Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. Eur J Pharmacol 293:77–85 (1995).
6. Sauer PJ, Huisman M, Koopman-esseboom C, Morse DC, Prooije AESV, Berg KJVD, Tuisstra LGMT, Paauw CGVD, Boersma ER, Weisglas-Kuperus N, et al. Effects of polychlorinated biphenyls (PCBs) and dioxins on growth and development. Hum Exp Toxicol 13:900–906 (1994).
7. Morse DC, Wehler KE, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre-and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). Toxicol Appl Pharmacol 130:269–278 (1996).
8. Lans MC, Klasson-Wehier E, Willemens M, Meussen E, Safe S, Brouwer A. Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human thyroid hormone. Chem Biol Interact 88–9:21 (1993).
9. Gray LEJ, Ostby J, Marshall R, Andrews J. Reproductive and thyroid effects of low-level polychlorinated biphenyls (Aroclor 1254) exposure. Fundam Appl Toxicol 20:288–294 (1993).
10. Byrne JJ, Carbone JP, Hanson EA. Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polychlorinated biphenyl. Endocrinology 121:520–527 (1987).
11. Koopman-Esseboom C, Van Dongen J, Van Der M, Huisman M, Touwen BC, Boersma ER, Brouwer A, Sauer PJ, Weisglas-Kuperus N. Newborn infants diagnosed as neurologically abnormal with relation to PCB and dioxin exposure and their thyroid hormone status. Dev Med Child Neurol 39:785 (1997).
12. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lukeschopohl J, Van Der Paauw CGVD, Tuisstra LGMT, Brouwer A, Sauer PJ. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediat Res 36:468–473 (1994).
13. Beestings JB, van Engelen IJM, Karlis P, van der Hoek HJ, de Jong M, Doctor R, Krenning EP, Hennemann G, Brouwer A, Visser TJ. Thyroxine and 3,3',5'-triiodothyronine are glucuronidated in rat liver by different uridine diphosphate-glucuronosyltransferases. Endocrinology 128:741–746 (1991).
14. Barter RA, Klaassen CD. UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. Toxicol Appl Pharmacol 136:36–42 (1992).
15. Barter RA, Klaassen CD. Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. Toxicol Appl Pharmacol 129:8–19 (1997).
16. Wilson AG, Thake DC, Heydens WS, Brewer DW, Hotz KJ. Mode of action of thyroid tumor formation in the male Long-Evans rat administered high doses of alachlor. Fundam Appl Toxicol 33:16–23 (1996).
17. Ashby J, Kier L, Wilson AG, Green T, Lefevre PA, Tinwell H, Willis SA, Heydens WF, Clapp MJL. Evaluation of the potential carcinogenicity and genetic toxicity to humans of the herbicide alachlor. Hum Exp Toxicol 15:702–715 (1996).
18. Cheek AD, Ide CF, Bollinger JE, Rider CV, McLachlan JA. Alteration of leopard frog (Rana pipiens) metanmorphosis by the herbicide acetochlor. Arch Environ Contam Toxicol (in press).
19. Grässle B, Biessmann A. Effects of DDT, polychlorinated biphenyls and thouracil on circulating thyroid hormones, thyroid histology and eggshell quality in Japanese quail (Coturnix coturnix japonica). Chemical Biol Interact 42:371–377 (1982).
20. Marshall JS, Tompkins L. Effect of α,α-DDD and similar compounds on thyroid binding globulin. J Clin Endocrinol 28:386–392 (1968).
21. Bastomsky CH. Effects of a polychlorinated biphenyl mixture (Aroclor 1254) and DDT on bilirary thyroxine excretion in rats. Endocrinology 95:1150–1155 (1974).
22. Lans MC, Spieritz C, Brouwer A, Koeman JH. Different combination of thyroid binding to thyroxine and thyroxine-binding globulin by hydroxy-PCBs, PCDs, PCDDs. Eur J Pharmacol 270:129–136 (1994).
23. McKinney J, Fannin R, Jordan S, Chae K, Rickenbacher U, Pedersen L. Polychlorinated biphenyls and related compound interactions with specific binding sites for thyroxine in rat liver nuclear extracts. J Med Chem 80:79–86 (1987).
24. Rickenbacher U, McKinney JD, Oatley SJ, Blake CEF. Structurally specific binding of halogenated biphenyls to thyroxine transport protein. J Med Chem 29:641–648 (1986).
25. Toscano D. Personal communication.
26. Bres D, Eales J. Thyroid hormone binding to isolated trout (Salmo gairdneri) liver nuclei in vitro: binding affinity, capacity, and chemical specificity. Gen Comp Endocrinol 61:29–39 (1986).
27. Sullivan D, Darol D, Dickhoff W. Nuclear receptors for 3,3',5'-triiodothyronine in trout erythrocytes. Gen Comp Endocrinol 85:149–160 (1997).
28. Park J-B, Ashizawa K, Parkinson C, Cheng S-Y. One-step immunoaffinity purification of human 3T thyroid hormone receptor with DNA and hormone binding affinity. J Biochem Biophys Meth 27:95–103 (1993).
29. Falcone M, Miyamoto T, Ferro-Renoy F, Macchina E, DeGroot LJ. Antipeptide polyclonal antibodies specifically recognize each human thyroid hormone receptor isoform. Endocrinology 131:2419–2429 (1992).
30. Lazzar MA. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocrinol Rev 14:184–193 (1993).
31. Bergman A, Klasson-Wehier E, Kuroki H. Selective retention of hydroxylated PCB metabolites in blood: Environ Health Perspect 102:464–469 (1994).
32. Glass CK. Some new twists in the regulation of gene expression by thyroid hormone and retinoid acid receptors. J Endocrinol 150:349–357 (1996).