2009

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M. D Miller
K. M Crofton
D. C Rice
R. Thomas Zoeller, *University of Massachusetts - Amherst*

# Thyroid-Disrupting Chemicals: Interpreting Upstream Biomarkers of Adverse Outcomes

*Mark D. Miller,[1,2] Kevin M. Crofton,[3] Deborah C. Rice,[4] and R. Thomas Zoeller[5]*

[1]Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, California, USA; [2]Pediatric Environmental Health Specialty Unit, University of California at San Francisco, San Francisco, California, USA; [3]National Health and Environmental Effects Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [4]Maine Center for Disease Control, Augusta, Maine, USA; [5]Biology Department, Morrill Science Center, University of Massachusetts at Amherst, Amherst, Massachusetts, USA

**Background:** There is increasing evidence in humans and in experimental animals for a relationship between exposure to specific environmental chemicals and perturbations in levels of critically important thyroid hormones (THs). Identification and proper interpretation of these relationships are required for accurate assessment of risk to public health.

**Objectives:** We review the role of TH in nervous system development and specific outcomes in adults, the impact of xenobiotics on thyroid signaling, the relationship between adverse outcomes of thyroid disruption and upstream causal biomarkers, and the societal implications of perturbations in thyroid signaling by xenobiotic chemicals.

**Data sources:** We drew on an extensive body of epidemiologic, toxicologic, and mechanistic studies.

**Data synthesis:** THs are critical for normal nervous system development, and decreased maternal TH levels are associated with adverse neuropsychological development in children. In adult humans, increased thyroid-stimulating hormone is associated with increased blood pressure and poorer blood lipid profiles, both risk factors for cardiovascular disease and death. These effects of thyroid suppression are observed even within the "normal" range for the population. Environmental chemicals may affect thyroid homeostasis by a number of mechanisms, and multiple chemicals have been identified that interfere with thyroid function by each of the identified mechanisms.

**Conclusions:** Individuals are potentially vulnerable to adverse effects as a consequence of exposure to thyroid-disrupting chemicals. Any degree of thyroid disruption that affects TH levels on a population basis should be considered a biomarker of adverse outcomes, which may have important societal outcomes.

**Key words:** children's health, endocrine disruption, hazard identification, risk assessment, science policy, thyroid hormone, toxicologic assessments. *Environ Health Perspect* 117:1033–1041 (2009). doi:10.1289/ehp.0800247 available via *http://dx.doi.org/* [Online 12 February 2009]

Recent epidemiologic studies have demonstrated significant relationships between circulating levels of thyroid hormones (THs) and exposures to environmental chemicals (Blount et al. 2006; Boas et al. 2006; Longnecker et al. 2003; Steinmaus et al. 2007). In controlled animal studies, environmental chemicals have been shown to cause a reduction in serum TH levels, also supporting a causal association (Boas et al. 2006; Brucker-Davis 1998; DeVito et al. 1999; Zoeller 2007). In this article we review the role of THs in development and adult life, the impact of xenobiotics on thyroid status, the relationships between adverse outcomes of thyroid disruption and upstream causal biomarkers, and the societal implications of perturbations in THs by xenobiotic chemicals.

## The Role of THs in Development

THs include both thyroxine ($T_4$) and triiodothyronine ($T_3$). The independent regulation of circulating levels of these two forms of TH is complex, but in this review we refer generally to both forms as TH. THs are evolutionarily conserved molecules present in all extant vertebrates and some invertebrates (Heyland and Moroz 2005). Molecular signaling pathways regulated by these hormones affect development, energy balance, and metabolism in all taxonomic groups. For example, TH induces metamorphosis in the sand dollar (Heyland et al. 2004), flounder (Yamano et al. 1994), and frogs (Buchholz et al. 2005), and TH is essential for development in birds (McNabb 2006) and mammals (Zoeller and Rovet 2004). In humans, TH is important for normal development of brain (Bernal 2007; Oerbeck et al. 2007), lungs (Bizzarro and Gross 2004; van Tuyl et al. 2004), heart (Danzi et al. 2005; Grover et al. 2005; Stoykov et al. 2006), and other organs. Likewise, the mechanism(s) by which THs exert their actions through nuclear receptors that influence gene expression is highly conserved across the vertebrate taxa (Bertrand et al. 2004; Buchholz et al. 2006; Whitfield et al. 1999).

The regulation of serum TH levels and of TH action in various tissues involves a complex interplay of physiologic processes. Thyroid function depends on iodine uptake, TH synthesis and storage in the thyroid gland, stimulated release of hormone into and transport through the circulation, hypothalamic/pituitary control of TH synthesis, cellular TH transporters, tissue-specific TH deiodination, and degradation of THs by catabolic hepatic enzymes (Figure 1). Given the key role of TH for normal development and physiologic function in all vertebrates, it is important to identify environmental factors that may adversely affect thyroid function and/or TH signaling and to evaluate their ability to adversely affect public health (Brucker-Davis 1998). In addition, because of the highly conserved nature of TH chemistry, synthesis, signaling, and regulation, environmental factors that affect thyroid function or TH signaling in one species may well affect thyroid function or TH signaling in others—including humans.

***THs and nervous system development.*** It is becoming clear that, although somatic and brain growth retardation occur with severe TH insufficiency, moderate or even transient TH insufficiency can cause specific developmental defects in rodents (Auso et al. 2004; Crofton 2004; Crofton et al. 2000; Goldey et al. 1995a, 1995b; Goodman and Gilbert 2007; Morreale de Escobar 2003) and in humans (Haddow 2005; Haddow et al. 1999;

Address correspondence to M. Miller, Office of Environmental Health Hazard Assessment, 1515 Clay St., 16th Floor, Oakland, CA 94612 USA. Telephone: (510) 622-3159. Fax: (510) 622-3210. E-mail: mmiller@oehha.ca.gov

This review developed from conversations among the four authors as a result of a workshop sponsored by the California Environmental Protection Agency and the U.S. Environmental Protection Agency (EPA) in May 2007 to explore ways to integrate information from testing for upstream biochemical events when conducting hazard identification and risk assessment.

The information in this document has been funded wholly (or in part) by the U.S. EPA.

The manuscript has been reviewed following the policy of the National Health and Environmental Effects Research Laboratory, U.S. EPA, and was approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The views expressed by M.D.M. are his and do not necessarily represent those of the Office of Environmental Health Hazard Assessment, the California Environmental Protection Agency, or the State of California.

The authors declare they have no competing financial interests.

Received 2 October 2008; accepted 12 February 2009.

Kooistra et al. 2006; Oerbeck et al. 2003, 2007; Pop et al. 1999, 2003; Pop and Vulsma 2005). Small differences (~25%) in point-estimates of maternal $T_4$ during the early fetal period are associated with adverse outcomes (e.g., reduced IQ scores), even though these deficits do not constitute clinical hypothyroidism (Haddow et al. 2002; Morreale de Escobar et al. 2000). However, in a hallmark study by Bongers-Schokking et al. (2000), the Mental Development Index of children with congenital hypothyroidism was affected by the age of onset of treatment, rather than the serum free $T_4$ concentration after treatment. Thus, the degree of TH insufficiency is not the only variable affecting human development; the duration of the insufficiency and the developmental timing of the insufficiency are also important and may vary by species, presenting a challenge for hazard assessment.

Experimental work in animals provides strong support for the hypothesis that moderate TH insufficiency can alter development in rodents. Integrating data over a series of studies, a decrease in serum total $T_4$ by 50% during the critical period for cochlear development was associated with a permanent hearing loss in adult offspring (Crofton 2004). Auso et al. (2004) found that less than a 30% decrease in serum total $T_4$ in dams, for only 3 days, was associated with structural abnormalities in the brains of their offspring. An average decrease in serum total $T_4$ of only 28% in 2-week-old pups given low doses of propylthiouracil was associated with marked reduction in cell density of the corpus callosum (Sharlin et al. 2008). Gilbert and Sui (2008) found that a 28% reduction in circulating levels of $T_4$ in rat dams produced significant adverse effects on synaptic function of the adult offspring despite no detected change in serum $T_4$ levels in the pups after birth. Thus, these experimental findings confirm what has been observed in humans: small, even transient, decreases in serum total $T_4$ are associated with altered brain development.

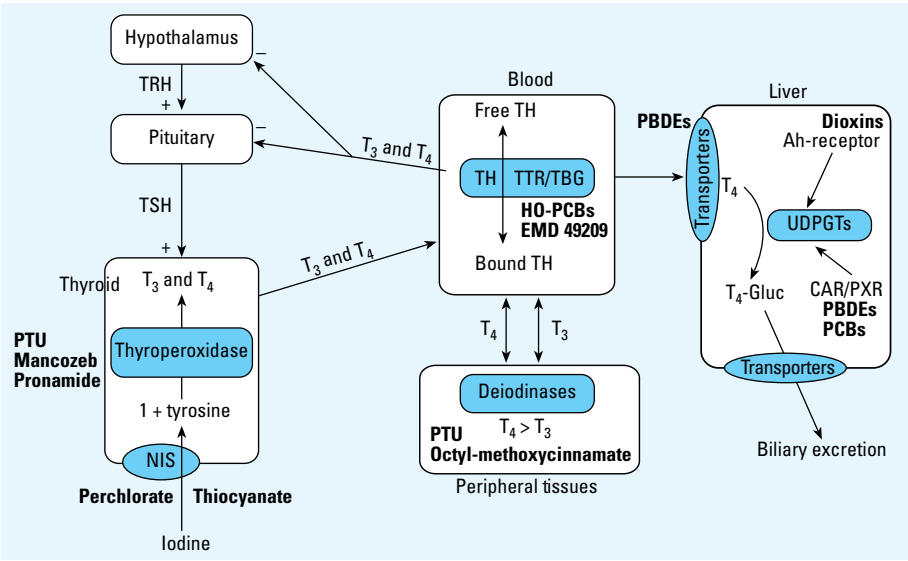


**Figure 1.** TH control pathways and sites of disruption by xenobiotic chemicals. Abbreviations: Gluc, glucose; HO-PCBs, hydroxyl-PCBs; NIS, sodium/iodide symporter; PBDE, polybrominated diphenyl ether; PTU, propylthiouracil; $T_4$-Gluc, $T_4$-glucuronide; TBG, thyroid-binding globulin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; TTR, transthyretin; UDPGT, uridine diphosphate glucuronyltransferase. Sites or processes where xenobiotics are known or hypothesized to act as TDCs are indicated in the boxes and ovals. Xenobiotics that block, inhibit, or up-regulate these processes are shown in bold (modified from Crofton 2008).

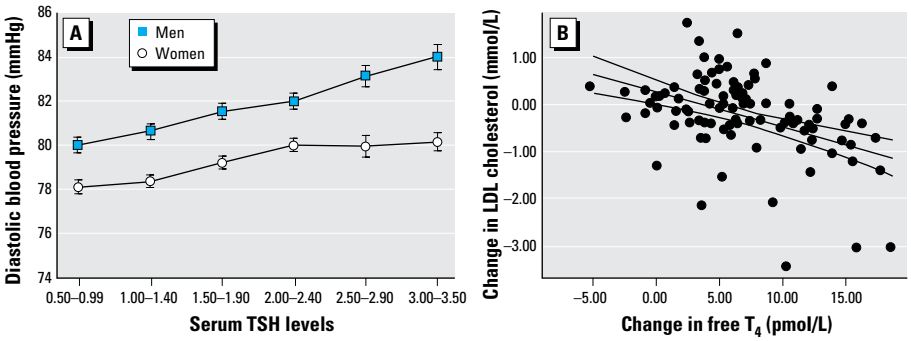


**Figure 2.** Population changes in diastolic blood pressure (*A*) and cholesterol (*B*) in relation to serum TSH or free $T_4$, respectively. (*A*) Diastolic blood pressure in men and women are significantly correlated with serum TSH within the normal reference range for TSH, indicating that as serum $T_4$ declines, diastolic blood pressure increases. (*B*) Serum cholesterol is negatively associated with serum free $T_4$. An increase in free $T_4$ by 5, 10, or 15 pmol/L would reduce LDL cholesterol by 0.13, 0.53, and 0.93 mmol/L, respectively. The data are redrawn with permission from Asvold (2007b; *A*) and from Razvi (2007; *B*) (Copyrights 2007, The Endocrine Society).

## TH Effects in Other Organ Systems and Adults

It is important to recognize that TH concentrations are correlated with adverse effects in organ systems other than the nervous system, including the cardiovascular system and control of serum lipids (Asvold et al. 2007a; Biondi et al. 2005; Osman et al. 2001), pulmonary system (Krude et al. 2002; Lei et al. 2003; Mendelson and Boggaram 1991), and kidney. Total cholesterol, low-density lipoproteins (LDL), non-high-density lipoproteins (non-HDL), and triglycerides increased linearly with increasing thyroid-stimulating hormone (TSH), and HDL decreased consistently with increasing TSH across normal reference ranges without evidence of any threshold effect (Asvold et al. 2007b). Similar trends in lipid profiles were identified across clinical categories from hypothyroid to euthyroid to hyperthyroid individuals (Canaris et al. 2000). Within the reference ranges for TSH, there was a linear positive association between TSH and both systolic and diastolic blood pressure (Asvold et al. 2007b) (Figure 2). Intimal medial thickness, a measure of atherosclerosis and predictive of coronary vascular disease and stroke, was inversely related to free $T_4$ after controlling for lipids, clinical factors, and thyroid autoantibodies (Dullaart et al. 2007). Some of these adverse effects were ameliorated by treatment with $T_4$. Not surprisingly, deficits in thyroid homeostasis were associated with cardiovascular risk in multiple epidemiologic studies. A meta-analysis of 14 epidemiologic studies (Rodondi et al. 2006) found an overall increase in risk of coronary heart disease of > 65% in those with subclinical hypothyroidism (elevation in TSH with normal $T_4$). A higher relative risk was noted in those studies that adjusted for most cardiovascular risk factors, suggesting that confounding was not responsible for these effects. Treatment with L-$T_4$ of patients with subclinical hypothyroidism resulted in improvements in cardiovascular risk factors, including total cholesterol and endothelial function (flow-mediated dilatation) (Razvi et al. 2007). Michalopoulou et al. (1998) found that treatment with $T_4$ of hypercholesterolemic individuals who have "high normal" TSH values significantly reduced both total

and LDL cholesterol, additionally supporting a causal association. In addition, environmental exposure to the thyroid-disrupting chemical (TDC) polychlorinated biphenyls (PCBs) had an inverse association with $T_3$ in men (Meeker et al. 2007) and was associated with both unfavorable lipid profiles and self-reported cardiovascular disease in men and women (Goncharov et al. 2008). Therefore, epidemiologic as well as mechanistic and therapeutic evidence substantiates the concern that TDCs may adversely affect cardiovascular risk in humans by reducing serum $T_4$.

## Impact of Xenobiotics on TH Signaling

TDCs are broadly defined as xenobiotics that interfere with TH signaling. These can include chemicals that alter the structure or function of the thyroid gland (e.g., perchlorate and methimazole), alter binding of hormones to thyroid receptors (e.g., bisphenol A, PCBs, and polybrominated diphenyl ethers), or alter regulatory enzymes associated with TH synthesis (e.g., propylthiouracil) (Crofton et al. 2005). A number of extrathyroidal mechanisms affect TH levels by altering binding to hormone transport proteins (e.g., hydroxyl-PCBs), hepatic clearance (e.g., PCBs, triclosan), inhibition of deiodination to $T_3$ (e.g., FD&C red dye number 3), and receptor agonism/antagonism (e.g., tetrabromobisphenol A). The downstream consequences of these effects are to alter TH-directed transcription either directly or via changes in circulating or tissue concentrations of THs. Several uncertainties complicate basic risk assessment approaches when assessing the hazards of TDCs. These include defining the biomarkers used for assessing hazard, defining the magnitude of change in the biomarker(s) that reliably predict downstream adverse outcomes, intraspecies extrapolation that is hampered by a lack of mechanistic and dose response data, and predicting the effects of real life exposures to low-level mixtures of xenobiotics that contain components that individually have vastly different kinetic and dynamic properties.

Several specific chemicals were shown to bind to TH receptors (TRs) (Zoeller 2005, 2007). This has important implications because there is good evidence that different effects of TH in the developing brain are mediated by different TR isoforms (Bernal 2007). There are two different classes of TRs (TRα and TRβ), and different chemicals can selectively interact with various isoforms. Thus, these chemicals will likely produce a mosaic of effects on TH signaling in the developing brain and may do so without affecting circulating levels of TH. It also may be challenging to develop high-throughput *in vitro* screens for TR binding because many of these screens use only the ligand-binding domain of the receptor, and there is some evidence that environmental chemicals can bind to an allosteric site on the DNA binding domain of the TR (Miyazaki et al. 2008).

The variety of mechanisms by which TDCs alter TH signaling (Table 1) provide a number of biomarkers that could be used in assessing hazard. These include molecular targets, which could be chemical-class specific, and downstream consequences, such as serum TH concentrations, brain morphology or biochemistry, or behavior. These changes may be either directly or indirectly related to TH action (Figure 3). Accurately and thoroughly assessing the health risks of thyroid disruption by environmental xenobiotics will require an improved understanding of how divergent mechanisms alter the relationship between serum THs and consequent adverse impacts on health.

The most commonly used biomarker of effect for TDC exposure is serum total $T_4$ concentrations (DeVito et al. 1999; Zoeller et al. 2007). Although TSH is a well-accepted biomarker for hypothyroidism, a number of xenobiotics alter circulating TH levels but do not change TSH (DeVito et al. 1999). Therefore, it is central to risk assessment to understand the relationship between perturbations in circulating concentrations of $T_4$ and adverse effects. In addition, it is important to test the hypothesis that changes in circulating concentrations of $T_4$ represent a common pathway by which adverse outcomes are produced. This hypothesis is consistent with the accepted role of circulating concentrations of $T_4$ in defining thyroid disease (Brabant et al. 2006). Many kinds of adverse effects are associated with either TH excess or insufficiency, depending on the timing, severity, and duration of the perturbation. Although the pattern of effects may differ, changes in serum TH are predictive of downstream adverse outcomes.

Upstream biomarkers of TDC exposure are predictive of adverse effects if the mechanisms of action are well characterized. Mechanism 1 in Figure 4 illustrates this point: alterations in circulating THs during development are predictive of adverse neurodevelopmental outcomes. This concept has been

**Table 1.** Classes, mechanisms of action, and effects of TDCs on TH homeostasis.

| Class | Mechanism | Effect on THs | Chemical | References |
|---|---|---|---|---|
| Iodine transport | Competition/block of sodium/iodide symporter | Decreased thyroidal synthesis of $T_3$ and $T_4$ | Perchlorate, chlorate, bromated nitrates, thiocyanate | Tonacchera et al. 2004; Van Sande et al. 2003; Wolff 1998 |
| Synthesis inhibitors | Inhibition of thyroid peroxidase | Decreased thyroidal synthesis of $T_3$ and $T_4$ | Methimazole, propylthiourea, amitrole mancozeb, soy isoflavones, benzophenone 2,1-methyl-3-propyl-imidazole-2-thione | Biegel et al. 1995; Capen 1997; Doerge and Sheehan 2002; Hurley 1998; Schmutzler et al. 2007 |
| Transport disruption | Altered binding to serum transport proteins | Unknown | Hydroxyl-PCBs, EMD 49209, pentachlorophenol | Lans et al. 1993; Schroder-van der Elst et al. 1997; van den Berg 1990 |
| Enhanced hepatic catabolism | Up-regulation of glucuronylsyltransferases or sulfotransferases (via CAR/PXR or AhR) | Increased biliary elimination of $T_3$, $T_4$ | Acetochlor, phenobarbital, 3-methylcolanthrene, PCBs, 1-methyl-3-propyl-imidazole-2-thione | Biegel et al. 1995; Brucker-Davis 1998; Hood and Klaassen 2000; Hurley 1998; Liu and Klaassen 1996 |
| Enhanced cellular transport | Up-regulation of organic anion-transporting polypeptides or MCT transporters via CAR/PXR or AhR | Increased biliary elimination of $T_3$, $T_4$ | 1,4-Bis[2-(3,5-dichloropyridyloxy)] benzene, PCN, TCDD, rifampicin, phenobarbital, oltipraz | Guo et al. 2002; Jigorel et al. 2006; Petrick and Klaassen 2007; Staudinger et al. 2001 |
| Sulfotransferases | Inhibition of sulfotransferases | Decrease sulfation of THs | Hydroxy-PCBs, triclosan, pentachlorophenol | Schuur et al. 1998; Wang et al. 2004; Wang and James 2006 |
| Deiodinases | Inhibition or up-regulation of deiodinases | Decreased peripheral synthesis of $T_3$ | FD&C red dye no. 3, propylthiouracil, PCB, octylmethoxycinnamate | Capen 1998; Klammer et al. 2007; Morse et al. 1993; Visser et al. 1979 |
| TR agonists and antagonists | Direct or indirect alterations in TR–$T_3$ response element binding | Altered activation of TH-dependent gene transcription | Tetrabromobisphenol A, bisphenol A, hydroxy-PCBs | Gauger et al. 2004; Kitamura et al. 2005; Moriyama et al. 2002 |

Abbreviations: Ahr, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; FD&C red dye no. 3, Food, Drug and Cosmetics red dye no. 3; PCN, pregnenolone-16a-carbonitrile; PXR, pregnane X receptor. Modified from Crofton (2008).

known for decades and is the basis for newborn TH screening (Rose et al. 2006). These adverse consequences are well documented in animals for xenobiotics that alter circulating levels of TH (Crofton and Zoeller 2005; Zoeller and Crofton 2005).

## Cross-Species Extrapolation

Although interspecies extrapolation of adverse effects of TDCs requires careful consideration, there are many situations in which the effects of a chemical in one species are similar to those in another, including in humans. For example, perchlorate competitively inhibits iodine uptake into the thyroid gland, with subsequent decreases in TH synthesis and declines in circulating TH concentrations (Wolff 1998). The kinetics for perchlorate inhibition of iodine uptake in humans and rats are extremely similar [U.S. Environmental Protection Agency (EPA) 2002], indicating the homologous nature of the initial toxic event. However, species differences in the relationship between changes in serum total $T_4$ and downstream adverse effects, perhaps mediated by differences in kinetics such as tissue TH concentrations and the sensitivity of specific developmental outcomes to low $T_4$, cannot be ruled out at this time (National Research Council 2005).

For some TDCs, there may be little data to support cross-species extrapolation (Crofton 2004). Both *in vivo* and *in vitro* studies suggest that PCBs activate the pregnane X receptor (PXR) in rodents, which leads to up-regulation of hepatic catabolic enzymes and subsequent declines in circulating concentrations of $T_4$ (Schuetz et al. 1998). The steroid X receptor (SXR) is the human equivalent for rodent PXR (Blumberg et al. 1998), and there are species differences between PXR and SXR: Rodent PXR is activated by pregnenolone-16α-carbonitrile (PCN), but not by rifampicin, whereas human SXR is activated by rifampicin but not by PCN (Kliewer et al. 2002). In addition, *in vitro* data suggest that high concentrations of PCB-153 act as an antagonist at the human SXR (Tabb et al. 2004). As well, species differences in circulatory transport proteins (e.g., transthyretin and thyroid-binding globulin) complicate extrapolation from animals to humans (Capen 1997; Hill et al. 1998). Thus, species differences in the expression or structure of specific functional proteins (e.g., receptors and enzymes) may at times affect the toxicity of specific compounds in different species.

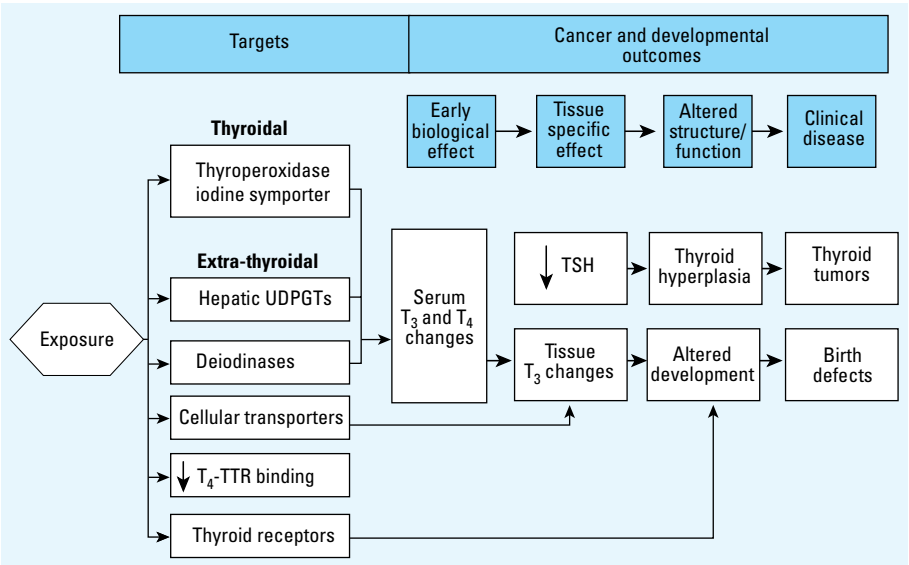


**Figure 3.** A combined mode-of-action model for the effects of TDCs on cancer and developmental outcomes. Abbreviations: TTR, transthyretin; UDPGT, uridine diphosphate glucuronyltransferase. Mixture models are needed to better predict effects of mixtures containing xenobiotics that affect multiple targets with common downstream effects (modified from Crofton and Zoeller 2005; U.S. EPA 2002).

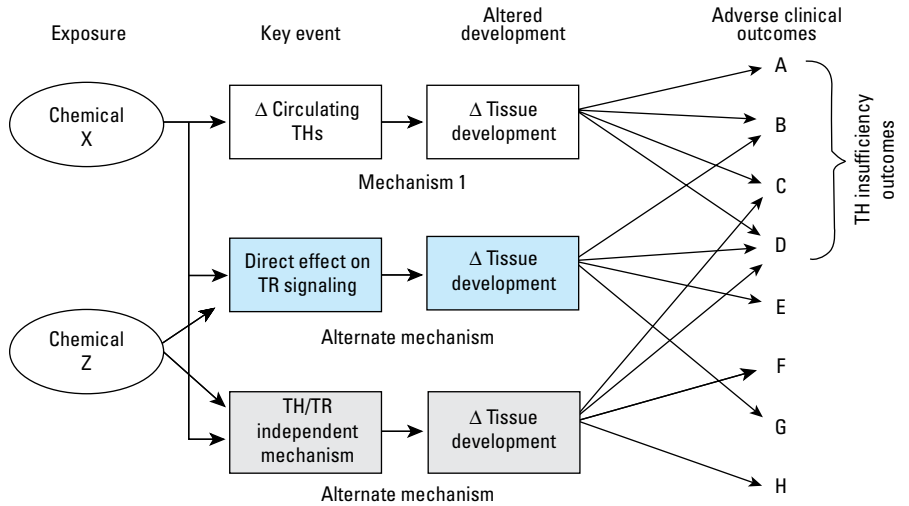


**Figure 4.** Diagnostic relationships between upstream biomarkers and adverse outcomes.

## Mixtures

Evaluating the potential for additive or synergistic (i.e., greater than additive) effects resulting from exposure to mixtures or environmental xenobiotics presents challenges for the assessment of endocrine disruptors (Daston et al. 2003). Additivity for mixtures of chemicals with a similar target is now a default assumption for some classes of chemicals (U.S. EPA 2000). A variety of predictive models are available for use with mixtures of similarly acting chemicals (Feron and Groten 2002; Kroes et al. 2005; Mumtaz et al. 1993; Teuschler 2007; U.S. EPA 2000). For example, the toxic equivalents methodology predicts the cumulative effects of aryl hydrocarbon receptor (AhR) agonists using dose addition (Haws et al. 2006; Van den Berg et al. 2006). However, these models may not predict effects of mixtures containing chemicals with multiple mechanisms of action (e.g., synthesis inhibitors, low dietary iodine, hepatic catabolism). The small number of studies reporting effects of mixtures of TDCs lack, either by study design or statistical approach, the ability to test for additivity (Desaulniers et al. 2003; Khan et al. 2005; McLanahan et al. 2007; Wade et al. 2002). The use of rigorous statistical models is critical for testing hypotheses of effect or dose addition and determining whether antagonism or synergism exists (Feron and Groten 2002; Hertzberg and Teuschler 2002; LeBlanc and Olmstead 2004).

Crofton et al. (2005) tested a mixture of 18 TDCs (dioxins, dibenzofurans, and PCBs) for effects on serum $T_4$. These chemicals were each known to decrease circulating concentrations of $T_4$ (Craft et al. 2002; Crofton et al. 2005; Khan and Hansen 2003; McLanahan et al. 2007). The mechanisms by which these chemicals alter THs involve up-regulation of hepatic catabolic enzymes (e.g., uridine diphosphate glucuronosyltransferases). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD),

dibenzofurans, and dioxin-like PCBs activate a network of phase II and III proteins via binding of the AhR (Schrenk 1998). The non-dioxin-like PCBs activate a slightly different set of enzymes (and possibly transporters) via binding to PXR and the constitutive androstane receptor (CAR) (Kretschmer and Baldwin 2005; Schuetz et al. 1998). These differences in mechanisms of action (i.e., AhR agonists and CAR/PXR agonists) suggest that dose addition theory would not predict the effects of the mixture. A "flexible single-chemical-required" method (Casey et al. 2004; Gennings et al. 2002) demonstrated no deviation from dose additivity at the lowest doses of the mixture but a greater-than-additive effect at the highest mixtures doses (Figure 5). At high doses the dose-additivity model underpredicted the empirical effects by 2- to 3-fold but worked well at lower doses typical of environmental exposures.

Future work is needed to improve the ability of mixtures models to account for the homeostatic processes that are activated by changes in both tissue and serum TH concentrations. The paucity of data in this area makes it difficult to determine whether these models will accurately predict changes in common downstream adverse outcomes after exposure to complex mixtures of chemicals that act on multiple upstream targets. Indeed, the effects of the complex mixtures will likely depend on the interaction of both kinetic and dynamic factors. Increasingly, it may become possible to identify interactions of chemicals in population-based biomonitoring databases. For example, sizable subpopulations for whom the relationship between perchlorate exposure and serum $T_4$ concentrations are modified by coexposure to thiocyanate, nutrition (iodide consumption), and behavior (smoking) have been identified using the National Health and Nutrition Examination Survey database (Blount et al. 2006; Steinmaus et al. 2007). Because additivity or synergy of TDCs with different mechanisms of action has been demonstrated, as noted above, a broad approach to cumulative risk that would account for these interactions seems appropriate. This is particularly true considering the limitations of current modeling methodologies.

## Causality

A critical issue affecting the interpretation of upstream events is the relationship between biomarkers captured in clinical or animal studies and specific adverse outcomes. Studies involving upstream biomarkers are most useful when these biomarkers have been causally linked to downstream adverse outcomes. For example, interpreting studies of perchlorate and $T_4$ are relatively straightforward because the only known toxic effect of perchlorate is interference with thyroid function (National Research Council 2005); thus, any effects of perchlorate on the nervous system are necessarily interpreted to be subsequent to a reduction in serum THs.

Difficulties can arise when attempting to predict changes in upstream biomarkers based on adverse outcomes. For example, if the adverse outcome(s) of a specific toxicant or mixture is caused by more than one mechanism, then individual downstream outcomes (i.e., "effects") are not diagnostic of upstream events, and causative links between a known exposure and outcome are difficult to discern. Figure 4 illustrates this by the alternative mechanisms activated by chemical X that may cause similar adverse outcomes. Indeed, some of these adverse outcomes may be caused by exposure to other chemicals (chemical Z). A key to using adverse outcomes in these cases is the use of patterns of outcomes that may be diagnostic.

PCBs offer a good example of the problems associated with inferring upstream changes in THs as the causative agent of downstream neurotoxic outcomes. PCBs produce changes in a number of behavioral domains in humans and animals (Rice 2000; Schantz et al. 2003). They also affect multiple neurochemical pathways (Kodavanti et al. 1993; Kodavanti and Ward 1998; Seegal 1996; Seegal et al. 1991) in addition to TH (Crofton and Zoeller 2005). Although changes in THs during development predict specific behavioral changes, effects of PCBs on some specific tasks in animals or outcomes in epidemiologic studies may not necessarily be attributable to changes in THs.

Another example of the difficulty in linking serum TH to adverse outcomes is provided by the recent observation in humans of an abnormal TH profile in boys with a genetic mutation in the $T_3$-specific transporter monocarboxylate anion transporter 8 (*MCT8*). In all cases, serum $T_3$ is elevated, but serum $T_4$, free $T_4$, and TSH may be low, normal, or elevated (Jansen et al. 2007). Thus, the elevated serum $T_3$ appears to be a biomarker of the MCT8 mutation among the patients evaluated, although it is not the only mechanism by which $T_3$ can become elevated. In addition, all of the boys evaluated presented with severe psychomotor deficits, but it is unlikely that the elevated serum $T_3$ itself was the root cause of their condition. Thus, environmental factors that influence $T_3$ transport through MCT8 may represent a situation in which the profile of serum TH hormones is perturbed in ways that are not immediately recognizable as due to an endocrine disruptor, but may signal that adverse effects occur through a mechanism that interferes with TH signaling.

Recognition of the role of "critical windows of exposure" in characterizing causal relationships between toxicant effects on serum THs and downstream adverse effects is critical. Specifically, the role of TH in brain development changes as development proceeds (Zoeller and Rovet 2004). Therefore, to establish a causal role of toxicant-induced low TH in the mechanism of neurotoxicity, it is important to show that $T_4$ replacement can reverse the effects of toxicant. However, it is important to be cognizant of the relevant "windows" of vulnerability in the design of these experiments. For example, the impact of TH disruption on the development of auditory function in rats correlates well with circulating $T_4$ levels during the second postnatal week (Crofton 2004). This is entirely consistent with the known role of THs in auditory development (Uziel et al. 1981), the critical postnatal ontogeny of auditory function (Rubel 1978), and the pharmacokinetics of the chemicals tested (Crofton and Zoeller 2005). In addition, this correlation establishes a prognostic power of early postnatal $T_4$ for adverse consequence of developmental exposure to TDCs in rats (Crofton 2004). An understanding of the role of THs in development, coupled with hormone level measurement during the critical window, allows the establishment of a developmental mode of action that assigns a key causative role to TH disruption in the adverse outcome (Figure 4).

Studies designed to test for associations between toxicant exposures and circulating levels of TH in humans require careful consideration of confounding variables. For example, blood levels of TH vary among individuals (Andersen et al. 2002, 2003), which will affect the number of samples required for such a study to be sufficiently powered to identify associations of interest. In the case of newborn TH levels, a number of maternal, infant, and delivery factors influence TH levels in cord blood and in infant serum (Herbstman et al. 2008), and these must be

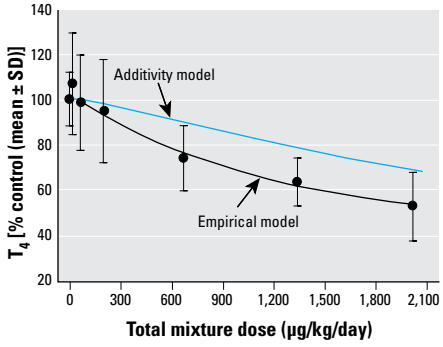


**Figure 5.** The predicted and empirical effects of a mixture of dioxins, furans, and PCBs on serum total $T_4$ in rats. Predicted outcomes (additivity model) were generated using a single chemical-required additivity model. Empirical results (empirical model) showed a small but significant departure from dose additivity at the three highest mixture doses, whereas the remaining lower mixture doses were not significantly different than that predicted by additivity (modified from Crofton et al. 2005).

carefully considered when attempting to identify associations between toxicant exposures and serum TH levels. A good recent example is that of Herbstman et al. (2008), who showed that PCB measures in cord blood were associated with circulating levels of TH only in those babies born via an unassisted vaginal delivery. Thus, these confounding variables may explain the studies in which PCB body burden has not been found to be associated with THs.

## Sensitive Populations

There may be individuals within the general population who are more at risk than others (i.e., sensitive subpopulations). For example, because pregnancy causes an increased demand on the thyroid gland, pregnant women may be particularly sensitive to specific kinds of toxicants that produce an additional burden on the thyroid gland, such as perchlorate, or chemicals that activate liver metabolism of $T_4$. Women in general appear to be more sensitive to the adverse effects of perchlorate (Blount et al. 2006), although it is not clear why. An estimated 7.3% of the U.S. population either have self-reported hypothyroidism or take thyroid medication, and three-quarters of these are women (Aoki et al. 2007). More than 17% of those > 12 years of age report taking medications known to alter TH levels (e.g., estrogen, lithium, androgens). Those 50–79 and ≥ 80 years of age have a 2-fold and 5-fold increased risk of hypothyroidism, respectively, compared with those 12–49 years of age (Aoki et al. 2007). These are examples of large subpopulations at risk with any additional exposures that affect thyroid homeostasis.

The set-point around which THs are regulated is very individualistic (Andersen et al. 2002, 2003), and differences between individuals in their set-point is largely determined by genetics (Hansen et al. 2004). Epidemiologic studies have identified elevated risk of cardiovascular disease in patients with subclinical hypothyroidism, characterized by elevated TSH with normal $T_4$. Many studies identify that TDCs are associated with decreases in $T_4$ but not elevations in TSH. However, the low level of interference with thyroid homeostasis seen in subclinical hypothyroidism and with TDCs may be equivalent, suggesting that elevated risk of cardiovascular disease should be considered possible from exposure to TDCs. The variance in serum $T_3$, $T_4$, and TSH in individuals is about half of the range of population variance, known as the "reference range," as shown for $T_4$ in Figure 6 (Andersen et al. 2002). Therefore, a value within standard "normals" is not necessarily normal for the individual, and an elevated TSH (which responds with a logarithmically amplified variation to minor changes in $T_3$ and $T_4$) should be interpreted as indicating that serum $T_3$ and $T_4$ levels are not normal for the individual (Andersen et al. 2002). Thus, it is highly likely that unidentified subpopulations exist that have particular sensitivity to thyroid disruption. The ability of epidemiologic studies to identify associations between thyroid disruptors and cardiovascular (or other) outcomes may be diminished as a result of failure to recognize risk in individuals who may have $T_4$ levels in the normal population range but below their own normal individual range. Therefore, any exposure that would result in altered TH homeostasis in a population should be considered an adverse effect.

## Societal Burden

The burden to society of even small changes in function should not be dismissed or underestimated. The consequences of developmental lead exposure provide an informative example of the effects of a small shift in the IQ of a population. Lead exposure has been widespread in the United States, although blood lead concentrations decreased from a mean toddler blood lead of 15 µg/dL to < 2 µg/dL over the past four decades with the introduction of nonleaded gasoline and other measures (Centers for Disease Control and Prevention 2007). A mean toddler blood lead of 15 µg/dL would be expected to decrease population IQ by ≥ 5 points (Lanphear et al. 2005). Although the consequences of a 5-point decrease in an individual's IQ may be difficult to discern, the impact of this 5% shift at the tails results in a 57% national increase in those classified as mentally retarded (IQ < 70) and a concomitant decrease in individuals considered gifted (IQ > 130) (Schettler 2001; Weiss 1997).

Small decrements in maternal $T_4$ or free $T_4$ during the first trimester are associated with impaired neuropsychological development in the child (Haddow 2005; Haddow et al. 1999; Oerbeck et al. 2003, 2007; Pop et al. 1999, 2003; Pop and Vulsma 2005). However, children born to women with moderately low TH identified in these studies largely fall within the lower portion of the normal range for measures of neuropsychological function. Although they have lower IQ as a population, their individual IQ is in the normal range (Haddow 2005; Haddow et al. 1999).

The cardiovascular consequences of disruption of thyroid homeostasis also potentially affect a large portion of the adult population. As noted above, there is a linear association between TSH (including through the normal reference range) and both blood pressure and cholesterol (Asvold et al. 2007a, 2007b). The magnitude of these changes associated with changes in THs would be considered to confer minimal risk to an individual, even though the individual risk of myocardial infarction (MI) and death from MI increases linearly for increased systolic and diastolic blood pressure (U.S. EPA 1985) and serum cholesterol (Rose 1981) (Figure 7). There is an important distinction that needs to be recognized, however: the difference between individual (relative) risk and population-attributable risk. Typically, the medical community assigns specific values for blood pressure and cholesterol as "high" or "borderline" to advise individuals on individual

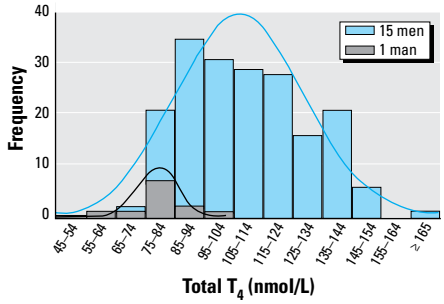


**Figure 6.** Individual versus population reference range for $T_4$: the distribution of 12 monthly measurements for 15 men compared with one individual. The distribution width for the individual is approximately one-half that of the group [adapted from Andersen et al. (2002); copyright 2002, The Endocrine Society].

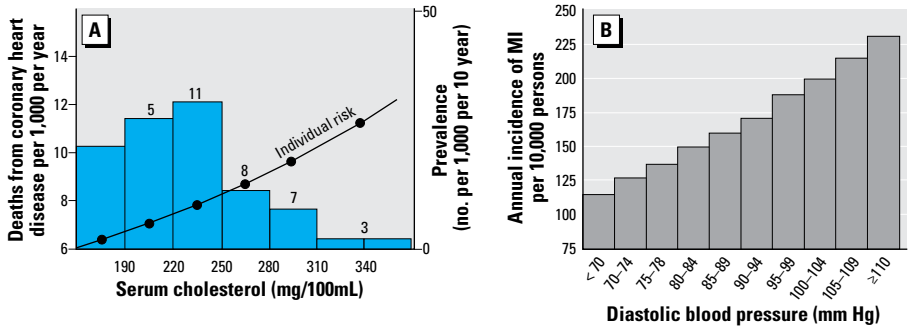


**Figure 7.** Individual risk and mortality associated with MI. (*A*) Individual risk and prevalence for MI associated with increased serum cholesterol levels. The number above each bar represents estimate of attributable deaths per 1,000 per 10 years. Note that individual risk increases linearly (including within the range of values considered normal) but that most deaths attributable to increased cholesterol levels occur in the lower range, because this represents a greater proportion of the population (adapted from Rose 1981; with permission from the BMJ Publishing Group). (*B*) Death from MI associated with increased diastolic blood pressure in males 45–74 (age-adjusted rate) (adapted from U.S. EPA 1985).

health risk. However, as illustrated in Figure 7, most of the morbidity in the population as a whole is associated with lower rather than higher levels, because a higher percentage of the population falls within the low to moderate range (Rose 1985; Rose and Day 1990).

The population-attributable risk can be used to monetize the societal burden of exposure to chemicals that affect thyroid function. For example, the U.S. EPA estimated the effects of lead, which is associated with increases in both systolic and diastolic blood pressure, on cardiovascular function (U.S. EPA 1985). The monetary burden of lost IQ associated with lead or methylmercury exposure has also been estimated at billions of dollars per year (Landrigan and Garg 2002; Trasande et al. 2006). Similar estimations could be made for the burden of exposure to chemicals that decrease THs and result in IQ deficits or increased incidence of cardiovascular disease. It is important to recognize that these outcomes are not only relevant if "abnormal" (e.g., mental retardation, clinically defined high blood pressure, or high cholesterol) but also relevant to outcomes in the "normal" range. Therefore, it is extremely important not to confuse the goal of minimizing population risk with arguments focused on individual relative risk.

## Conclusions

Two conclusions follow from the recognition that thyroid dysfunction affects multiple end points and that population-attributable risk is greater at levels associated with lower individual risk. First, from fetal life through old age, people are potentially vulnerable to adverse health effects as a consequence of exposure to TDCs. Second, any degree of thyroid disruption that lowers TH levels on a population basis should be considered a biomarker of increased risk of adverse outcomes. Because TH insufficiency in both humans and experimental animals results in serious neurodevelopmental and cardiovascular effects with large societal costs, chemicals with the ability to affect thyroid homeostasis should be carefully evaluated for potential population impacts. Finally, considering the complexity of the regulatory mechanisms affecting TH signaling and the variety of known TDCs that affect the thyroid system at different points of regulation, it will be essential to incorporate new information in human risk assessment strategies as it becomes available.

### References


Andersen S, Bruun NH, Pedersen KM, Laurberg P. 2003. Biologic variation is important for interpretation of thyroid function tests. Thyroid 13(11):1069–1078.

Andersen S, Pedersen KM, Bruun NH, Laurberg P. 2002. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. J Clin Endocrinol Metab 87(3):1068–1072.

Aoki Y, Belin RM, Clickner R, Jeffries R, Phillips L, Mahaffey KR. 2007. Serum TSH and total T(4) in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999–2002). Thyroid 17(12):1211–1223.

Asvold BO, Bjoro T, Nilsen TI, Vatten LJ. 2007a. Association between blood pressure and serum thyroid-stimulating hormone concentration within the reference range: a population-based study. J Clin Endocrinol Metab 92(3):841–845.

Asvold BO, Vatten LJ, Nilsen TI, Bjoro T. 2007b. The association between TSH within the reference range and serum lipid concentrations in a population-based study. The HUNT Study. Eur J Endocrinol 156(2):181–186.

Auso E, Lavado-Autric R, Cuevas E, Escobar Del Rey F, Morreale De Escobar G, Berbel P. 2004. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 145(9):4037–4047.

Bernal J. 2007. Thyroid hormone receptors in brain development and function. Nat Clin Pract Endocrinol Metab 3(3):249–259.

Bertrand S, Brunet FG, Escriva H, Parmentier G, Laudet V, Robinson-Rechavi M. 2004. Evolutionary genomics of nuclear receptors: from twenty-five ancestral genes to derived endocrine systems. Mol Biol Evol 21(10):1923–1937.

Biegel LB, Cook JC, O'Connor JC, Aschiero M, Arduengo AJ, Slone TW. 1995. Subchronic toxicity study in rats with 1-methyl-3-propylimidazole-2-thione (PTI): effects on the thyroid. Fundam Appl Toxicol 27(2):185–194.

Biondi B, Palmieri EA, Klain M, Schlumberger M, Filetti S, Lombardi G. 2005. Subclinical hyperthyroidism: clinical features and treatment options. Eur J Endocrinol 152(1):1–9.

Bizzarro MJ, Gross I. 2004. Effects of hormones on fetal lung development. Obstet Gynecol Clin North Am 31(4):949–961, xii.

Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL. 2006. Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. Environ Health Perspect 114:1865–1871.

Blumberg B, Sabbagh W Jr, Juguilon H, Bolado J Jr, van Meter CM, Ong ES, et al. 1998. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. Genes Dev 12(20):3195–3205.

Boas M, Feldt-Rasmussen U, Skakkebaek NE, Main KM. 2006. Environmental chemicals and thyroid function. Eur J Endocrinol 154(5):599–611.

Bongers-Schokking JJ, Koot HM, Wiersma D, Verkerk PH, de Muinck Keizer-Schrama SM. 2000. Influence of timing and dose of thyroid hormone replacement on development in infants with congenital hypothyroidism. J Pediatr 136(3):292–297.

Brabant G, Beck-Peccoz P, Jarzab B, Laurberg P, Orgiazzi J, Szabolcs I, et al. 2006. Is there a need to redefine the upper normal limit of TSH? Eur J Endocrinol 154(5):633–637.

Brucker-Davis F. 1998. Effects of environmental synthetic chemicals on thyroid function. Thyroid 8(9):827–856.

Buchholz DR, Paul BD, Fu L, Shi YB. 2006. Molecular and developmental analyses of thyroid hormone receptor function in Xenopus laevis, the African clawed frog. Gen Comp Endocrinol 145(1):1–19.

Buchholz DR, Paul BD, Shi YB. 2005. Gene-specific changes in promoter occupancy by thyroid hormone receptor during frog metamorphosis. Implications for developmental gene regulation. J Biol Chem 280(50):41222–41228.

Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. 2000. The Colorado Thyroid Disease Prevalence Study. Arch Int Med 160(4):526–534.

Capen CC. 1998 Correlation of mechanistic data and histopathology in the evaluation of selected toxic endpoints of the endocrine system. Toxicol Lett 102–103:405–409.

Capen CC. 1997. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicol Pathol 25(1):39–48.

Casey M, Gennings C, Carter WH, Moser VC, Simmons JE. 2004. Detecting interaction(s) and assessing the impact of component subsets in a chemical mixture using fixed-ratio mixture ray designs. J Agric Biol Environ Stat 9(3):339–361.

Centers for Disease Control and Prevention. 2007. Interpreting and managing blood lead levels below 10 µg/dL in children and reducing childhood exposures to lead: recommendations of CDC's Advisory Committee on Childhood Lead Poisoning Prevention. MMWR Recomm Rep 56(RR-8):1–16.

Craft ES, DeVito MJ, Crofton KM. 2002. Comparative responsiveness of hypothyroxinemia and hepatic enzyme induction in Long-Evans rats versus C57BL/6J mice exposed to TCDD-like and phenobarbital-like polychlorinated biphenyl congeners. Toxicol Sci 68(2):372–380.

Crofton KM. 2004. Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. Risk Anal 24(6):1665–1671.

Crofton KM. 2008. Thyroid disrupting chemicals: mechanisms and mixtures. Int J Androl 31(2):209–223.

Crofton KM, Craft ES, Hedge JM, Gennings C, Simmons JE, Carchman RA, et al. 2005. Thyroid-hormone-disrupting chemicals: evidence for dose-dependent additivity or synergism. Environ Health Perspect 113:1549–1554.

Crofton KM, Ding D, Padich R, Taylor M, Henderson D. 2000. Hearing loss following exposure during development to polychlorinated biphenyls: a cochlear site of action. Hear Res 144(1–2):196–204.

Crofton KM, Zoeller RT. 2005. Mode of action: neurotoxicity induced by thyroid hormone disruption during development—hearing loss resulting from exposure to PHAHs. Crit Rev Toxicol 35(8–9):757–769.

Danzi S, Dubon P, Klein I. 2005. Effect of serum triiodothyronine on regulation of cardiac gene expression: role of histone acetylation. Am J Physiol Heart Circ Physiol 289(4):H1506–H1511.

Daston GP, Cook JC, Kavlock RJ. 2003. Uncertainties for endocrine disrupters: our view on progress. Toxicol Sci 74:245–252.

Desaulniers D, Leingartner K, Musicki B, Yagminas A, Xiao GH, Cole J, et al. 2003. Effects of postnatal exposure to mixtures of non-ortho-PCBs, PCDDs, and PCDFs in prepubertal female rats. Toxicol Sci 75(2):468–480.

DeVito M, Biegel L, Brouwer A, Brown S, Brucker-Davis F, Cheek AO, et al. 1999. Screening methods for thyroid hormone disruptors. Environ Health Perspect 107:407–415.

Doerge DR, Sheehan DM. 2002. Goitrogenic and estrogenic activity of soy isoflavones. Environ Health Perspect 110(suppl 3):349–353.

Dullaart RP, de Vries R, Roozendaal C, Kobold AC, Sluiter WJ. 2007. Carotid artery intima media thickness is inversely related to serum free thyroxine in euthyroid subjects. Clin Endocrinol (Oxf) 67(5):668–673.

Feron VJ, Groten JP. 2002. Toxicological evaluation of chemical mixtures. Food Chem Toxicol 40(6):825–839.

Gauger KJ, Kato Y, Haraguchi K, Lehmler HJ, Robertson LW, Bansal R, et al. 2004. Polychlorinated biphenyls (PCBs) exert thyroid hormone-like effects in the fetal rat brain but do not bind to thyroid hormone receptors. Environ Health Perspect 112:516–523.

Gennings C, Carter WH Jr, Campain JA, Bae D, Yang RSH. 2002. Statitical analysis of interactive cytotoxicity in human epidermal keratinocytes following exposure to a mixture of four metals. J Agric Biol Environ Sci 7(1):58–73.

Gilbert ME, Sui L. 2008. Developmental exposure to perchlorate alters synaptic transmission in hippocampus of the adult rat. Environ Health Perspect 116:752–760.

Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. 1995a. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol Appl Pharmacol 135(1):77–88.

Goldey ES, Kehn LS, Rehnberg GL, Crofton KM. 1995b. Effects of developmental hypothyroidism on auditory and motor function in the rat. Toxicol Appl Pharmacol 135(1):67–76.

Goncharov A, Haase RF, Santiago-Rivera A, Morse G, McCaffrey RJ, Rej R, et al. 2008. High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a Native American population. Environ Res 106(2):226–239.

Goodman JH, Gilbert ME. 2007. Modest thyroid hormone insufficiency during development induces a cellular malformation in the corpus callosum: a model of cortical dysplasia. Endocrinology 148(6):2593–2597.

Grover GJ, Mellstrom K, Malm J. 2005. Development of the thyroid hormone receptor beta-subtype agonist KB-141: a strategy for body weight reduction and lipid lowering with minimal cardiac side effects. Cardiovasc Drug Rev 23(2):33–148.

Guo GL, Choudhuri S, Klaassen CD. 2002. Induction profile of rat organic anion transporting polypeptide 2 (OATP2) by prototypical drug-metabolizing enzyme inducers that activate gene expression through ligand-activated transcription factor pathways. J Pharmacol Exp Ther 300(1):206–212.

Haddow JE. 2005. Subclinical hypothyroidism and pregnancy outcomes. Obstet Gynecol 106(1):198–199.

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341(8):549–555.

Haddow JE, Palomaki GE, Williams J. 2002. Thyroid-stimulating-hormone concentrations and risk of hypothyroidism. Lancet 360(9350):2081–2082.

Hansen PS, Brix TH, Sorensen TI, Kyvik KO, Hegedus L. 2004. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. J Clin Endocrinol Metab 89(3):1181–1187.

Haws LC, Su SH, Harris M, Devito MJ, Walker NJ, Farland WH, et al. 2006. Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. Toxicol Sci 89(1):4–30.

Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, et al. 2008. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. Environ Health Perspect 116:1376–1382.

Hertzberg RC, Teuschler LK. 2002. Evaluating quantitative formulas for dose-response assessment of chemical mixtures. Environ Health Perspect 110(suppl 6):965–970.

Heyland A, Moroz LL. 2005. Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. J Exp Biol 208(pt 23):4355–4361.

Heyland A, Reitzel AM, Hodin J. 2004. Thyroid hormones determine developmental mode in sand dollars (Echinodermata: Echinoidea). Evol Dev 6(6):382–392.

Hill RN, Crisp TM, Hurley PM, Rosenthal SL, Singh DV. 1998. Risk assessment of thyroid follicular cell tumors. Environ Health Perspect 106:447–457.

Hood A, Klaassen CD. 2000. Differential effects of microsomal enzyme inducers on in vitro thyroxine ($T_4$) and triiodothyronine ($T_3$) glucuronidation. Toxicol Sci 55(1):78–84.

Hurley PM. 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ Health Perspect 106:437–445.

Jansen J, Friesema EC, Kester MH, Milici C, Reeser M, Gruters A, et al. 2007. Functional analysis of monocarboxylate transporter 8 mutations identified in patients with X-linked psychomotor retardation and elevated serum triiodothyronine. J Clin Endocrinol Metab 92(6):2378–2381.

Jigorel E, Le Vee M, Boursier-Neyret C, Parmentier Y, Fardel O. 2006. Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes. Drug Metab Dispos 34(10):1756–1763.

Khan MA, Fenton SE, Swank AE, Hester SD, Williams A, Wolf DC. 2005. A mixture of ammonium perchlorate and sodium chlorate enhances alterations of the pituitary-thyroid axis caused by the individual chemicals in adult male F344 rats. Toxicol Pathol 33(7):776–783.

Khan MA, Hansen LG. 2003. Ortho-substituted polychlorinated biphenyl (PCB) congeners (95 or 101) decrease pituitary response to thyrotropin releasing hormone. Toxicol Lett 144(2):173–182.

Kitamura S, Suzuki T, Sanoh S, Kohta R, Jinno N, Sugihara K, et al. 2005. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. Toxicol Sci 84(2):249–259.

Klammer H, Schlecht C, Wuttke W, Gotthardt I, Kohrle J, Jarry H. 2007. Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. Toxicology 238(2–3):192–199.

Kliewer SA, Goodwin B, Willson TM. 2002. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev 23(5):687–702.

Kodavanti PR, Mundy WR, Tilson HA, Harry GJ. 1993. Effects of selected neuroactive chemicals on calcium transporting systems in rat cerebellum and on survival of cerebellar granule cells. Fundam Appl Toxicol 21(3):308–316.

Kodavanti PR, Ward TR. 1998. Interactive effects of environmentally relevant polychlorinated biphenyls and dioxins on [3H]phorbol ester binding in rat cerebellar granule cells. Environ Health Perspect 106:479–486.

Kooistra L, Crawford S, van Baar AL, Brouwers EP, Pop VJ. 2006. Neonatal effects of maternal hypothyroxinemia during early pregnancy. Pediatrics 117(1):161–167.

Kretschmer XC, Baldwin WS. 2005. CAR and PXR: xenosensors of endocrine disrupters? Chem Biol Interact 155(3):111–128.

Kroes R, Kleiner J, Renwick A. 2005. The threshold of toxicological concern concept in risk assessment. Toxicol Sci 86(2):226–230.

Krude H, Schutz B, Biebermann H, von Moers A, Schnabel D, Neitzel H, et al. 2002. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. J Clin Invest 109(4):475–480.

Landrigan PJ, Garg A. 2002. Chronic effects of toxic environmental exposures on children's health. J Toxicol 40(4):449–456.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. 2005. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113:894–899.

Lans MC, Klasson-Wehler E, Willemsen M, Meussen E, Safe S Brouwer A. 1993. Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. Chem Biol Interact 88(1):7–21.

LeBlanc GA, Olmstead AW. 2004. Correspondence: evaluating the toxicity of chemical mixtures. Environ Health Perspect 112:A729–A730.

Lei J, Nowbar S, Mariash CN, Ingbar DH. 2003. Thyroid hormone stimulates Na-K-ATPase activity and its plasma membrane insertion in rat alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 285(3):L762–L772.

Liu L, Klaassen CD. 1996. Regulation of hepatic sulfotransferases by steroidal chemicals in rats. Drug Metab Dispos 24(8):854–858.

Longnecker MP, Wolff MS, Gladen BC, Brock JW, Grandjean P, Jacobson JL, et al. 2003. Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. Environ Health Perspect 111:65–70.

McLanahan ED, Campbell JL Jr, Ferguson DC, Harmon B, Hedge JM, Crofton KM, et al. 2007. Low-dose effects of ammonium perchlorate on the hypothalamic-pituitary-thyroid axis of adult male rats pretreated with PCB126. Toxicol Sci 97(2):308–317.

McNabb FM. 2006. Avian thyroid development and adaptive plasticity. Gen Comp Endocrinol 147(2):93–101.

Meeker JD, Altshul L, Hauser R. 2007. Serum PCBs, *p,p*'-DDE and HCB predict thyroid hormone levels in men. Environ Res 104(2):296–304.

Mendelson CR, Boggaram V. 1991. Hormonal control of the surfactant system in fetal lung. Annu Rev Physiol 53:415–440.

Michalopoulou G, Alevizaki M, Piperingos G, Mitsibounas D, Mantzos E, Adamopoulos P, et al. 1998. High serum cholesterol levels in persons with "high-normal" TSH levels: should one extend the definition of subclinical hypothyroidism? Eur J Endocrinol 138:141–145.

Miyazaki W, Iwasaki T, Takeshita A, Tohyama C, Koibuchi N. 2008. Identification of the functional domain of thyroid hormone receptor responsible for polychlorinated biphenyl-mediated suppression of its action *in vitro*. Environ Health Perspect 116:1231–1236.

Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, et al. 2002. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metab 87(11):5185–5190.

Morreale de Escobar G. 2003. Maternal hypothyroxinemia versus hypothyroidism and potential neurodevelopmental. Ann Endocrinol (Paris) 64(1):51–52.

Morreale de Escobar G, Obregon MJ, Escobar del Rey F. 2000. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia. J Clin Endocrinol Metab 85(11):3975–3987.

Morse DC, Groen D, Veerman M, van Amerongen CJ, Koeter HB, Smits van Prooije AE, et al. 1993. Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. Toxicol Appl Pharmacol 122(1):27–33.

Mumtaz MM, Sipes IG, Clewell HJ, Yang RS. 1993. Risk assessment of chemical mixtures: biologic and toxicologic issues. Fundam Appl Toxicol 21(3):258–269.

National Research Council. 2005. Health Implications of Perchlorate Ingestion. Washington, DC:National Academies Press.

Oerbeck B, Reinvang I, Sundet K, Heyerdahl S. 2007. Young adults with severe congenital hypothyroidism: cognitive event related potentials (ERPs) and the significance of an early start of thyroxine treatment. Scand J Psychol 48(1):61–67.

Oerbeck B, Sundet K, Kase BF, Heyerdahl S. 2003. Congenital hypothyroidism: influence of disease severity and L-thyroxine treatment on intellectual, motor, and school-associated outcomes in young adults. Pediatrics 112(4):923–930.

Osman F, Gammage MD, Franklyn JA. 2001. Thyroid disease and its treatment: short-term and long-term cardiovascular consequences. Curr Opin Pharmacol 1(6):626–631.

Petrick JS, Klaassen CD. 2007. Importance of hepatic induction of constitutive androstane receptor and other transcription factors that regulate xenobiotic metabolism and transport. Drug Metab Dispos 35(10):1806–1815.

Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. 2003. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. Clin Endocrinol (Oxf) 59(3):282–288.

Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, et al. 1999. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin Endocrinol (Oxf) 50(2):149–155.

Pop VJ, Vulsma T. 2005. Maternal hypothyroxinaemia during (early) gestation. Lancet 365(9471):1604–1606.

Razvi S, Ingoe L, Keeka G, Oates C, McMillan C, Weaver JU. 2007. The beneficial effect of L-thyroxine on cardiovascular risk factors, endothelial function and quality of life in subclinical hypothyroidism: randomised, crossover trial. J Clin Endocrinol Metab 92:1715–1723.

Rice DC. 2000. Identification of functional domains affected by developmental exposure to methylmercury: Faroe Islands and related studies. Neurotoxicology 21(6):1039–1044.

Rodondi N, Aujesky D, Vittinghoff E, Cornuz J, Bauer DC. 2006. Subclinical hypothyroidism and the risk of coronary heart disease: a meta-analysis. Am J Med 119(7):541–551.

Rose G. 1981. Strategy of prevention: lessons from cardiovascular disease. Br Med J (Clin Res Ed) 282(6279):1847–1851.

Rose G. 1985. Sick individuals and sick populations. Int J Epidemiol 14(1):32–38.

Rose G, Day S. 1990. The population mean predicts the number of deviant individuals. Br Med J (Clin Res Ed) 301(6759):1031–1034.

Rose SR, Brown RS, Foley T, Kaplowitz PB, Kaye CI, Sundararajan S, et al. 2006. Update of newborn screening and therapy for congenital hypothyroidism. Pediatrics 117(6):2290–2303.

Rubel EW. 1978. Ontogeny of structure and function in the vertebrate auditory system. In: Handbook of Sensory Physiology, Vol 9 (Jacobson M, ed). Berlin: Springer-Verlag, 135–237.

Schantz SL, Widholm JJ, Rice DC. 2003. Effects of PCB exposure on neuropsychological function in children. Environ Health Perspect 111:357–576.

Schettler T. 2001. Toxic threats to neurologic development of children. Environ Health Perspect 109(suppl 6):813–816.

Schmutzler C, Bacinski A, Gotthardt I, Huhne K, Ambrugger P, Klammer H, et al. 2007. The ultraviolet filter benzophenone 2 interferes with the thyroid hormone axis in rats and is a potent in vitro inhibitor of human recombinant thyroid peroxidase. Endocrinology 148(6):2835–2844.

Schrenk D. 1998. Impact of dioxin-type induction of drug-metabolizing enzymes on the metabolism of endo- and xenobiotics. Biochem Pharmacol 55(8):1155–1162.

Schroder-van der Elst JP, van der Heide D, Rokos H, Kohrle J, Morreale de Escobar G. 1997. Different tissue distribution, elimination, and kinetics of thyroxine and its conformational analog, the synthetic flavonoid EMD 49209 in the rat. Endocrinology 138(1):79–84.

Schuetz EG, Brimer C, Schuetz JD. 1998. Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the CYP3A23 hormone response element. Mol Pharmacol 54(6):1113–1117.

Schuur AG, Legger FF, van Meeteren ME, Moonen MJ, van Leeuwen-Bol I, Bergman A, et al. 1998. In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. Chem Res Toxicol 11:1075–1081.

Seegal RF. 1996. Epidemiological and laboratory evidence of PCB-induced neurotoxicity. Crit Rev Toxicol 26:709–737.

Seegal RF, Bush B, Brosch KO. 1991. Sub-chronic exposure of the adult rat to Aroclor 1254 yields regionally-specific changes in central dopaminergic function. Neurotoxicology 12:55–65.

Sharlin DS, Tighe D, Gilbert ME, Zoeller RT. 2008. The balance between oligodendrocyte and astrocyte production in major white matter tracts is linearly related to serum total thyroxine. Endocrinology 149(5):2527–2536.

Staudinger J, Liu Y, Madan A, Habeebu S, Klaassen CD. 2001. Coordinate regulation of xenobiotic and bile acid homeostasis by pregnane X receptor. Drug Metab Dispos 29(11):1467–1472.

Steinmaus C, Miller MD, Howd R. 2007. Impact of smoking and

thiocyanate on perchlorate and thyroid hormone associations in the 2001–2002 National Health and Nutrition Examination Survey. Environ Health Perspect 115:1333–1338.

Stoykov I, Zandieh-Doulabi B, Moorman AF, Christoffels V, Wiersinga WM, Bakker O. 2006. Expression pattern and ontogenesis of thyroid hormone receptor isoforms in the mouse heart. J Endocrinol 189(2):231–245.

Tabb MM, Kholodovych V, Grun F, Zhou C, Welsh WJ, Blumberg B. 2004. Highly chlorinated PCBs inhibit the human xenobiotic response mediated by the steroid and xenobiotic receptor (SXR). Environ Health Perspect 112:163–169.

Teuschler LK. 2007. Deciding which chemical mixtures risk assessment methods work best for what mixtures. Toxicol Appl Pharmacol 223(2):139–147.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, et al. 2004. Relative potencies and additivity of perchlorate, thiocyanate, nitrite, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. Thyroid 14(12):1012–1019.

Trasande L, Schechter C, Haynes KA, Landrigan PJ. 2006. Applying cost analyses to drive policy that protects children: mercury as a case study. Ann NY Acad Sci 1076:911–923.

U.S. EPA. 1985. Costs and Benefits of Reducing Lead in Gasoline. EPA-230-05-85-006. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. EPA/630/R-00/002. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2002. Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization. External Review Draft. NCEA-1-0503. Washington, DC:U.S. Environmental Protection Agency, National Center for Environmental Assessment, Office of Research and Development.

Uziel A, Gabrion J, Ohresser M, Legrand C. 1981. Effects of hypothyroidism on the structural development of the organ of Corti in the rat. Acta Otolaryngol 92(5–6):469–480.

van den Berg KJ. 1990. Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin. Chem Biol Interact 76(1):63–75.

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, et al. 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93(2):223–241.

Van Sande J, Massart C, Beauwens R, Schoutens A, Costagliola S, Dumont JE, et al. 2003. Anion selectivity by the sodium iodide symporter. Endocrinology 144:247–252.

van Tuyl M, Blommaart PE, de Boer PA, Wert SE, Ruijter JM, Islam S, et al. 2004. Prenatal exposure to thyroid hormone is necessary for normal postnatal development of murine heart and lungs. Dev Biol 272(1):104–117.

Visser TJ, van Overmeeren E, Fekkes D, Docter R, Hennemann G. 1979. Inhibition of iodothyronine 5′-deiodinase by thioureylenes; structure—activity relationship. FEBS Lett 103(2):314–318.

Wade MG, Parent S, Finnson KW, Foster W, Younglai E, McMahon A, et al. 2002. Thyroid toxicity due to subchronic exposure to a complex mixture of 16 organochlorines, lead, and cadmium. Toxicol Sci 67(2):207–218.

Wang LQ, Falany CN, James MO. 2004. Triclosan as a substrate and inhibitor of 3′-phosphoadenosine 5′-phosphosulfate-sulfotransferase and UDP-glucuronosyl transferase in human liver fractions. Drug Metab Dispos 32(10):1162–1169.

Wang LQ, James MO. 2006. Inhibition of sulfotransferases by xenobiotics. Curr Drug Metab 7(1):83–104.

Weiss B. 1997. Endocrine disruptors and sexually dimorphic behaviors: a question of heads and tails. Neurotoxicology 18(2):581–586.

Whitfield GK, Jurutka PW, Haussler CA, Haussler MR. 1999. Steroid hormone receptors: evolution, ligands, and molecular basis of biologic function. J Cell Biochem (Suppl) 32–33:110–122.

Wolff J. 1998. Perchlorate and the thyroid gland. Pharmacol Rev 50(1):89–105.

Yamano K, Araki K, Sekikawa K, Inui Y. 1994. Cloning of thyroid hormone receptor genes expressed in metamorphosing flounder. Dev Genet 15(4):378–382.

Zoeller RT. 2005. Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemical? Mol Cell Endocrinol 242(1–2):10–15.

Zoeller RT. 2007. Environmental chemicals impacting the thyroid: targets and consequences. Thyroid 17(9):811–817.

Zoeller RT, Crofton KM. 2005. Mode of action: developmental thyroid hormone insufficiency—neurological abnormalities resulting from exposure to propylthiouracil. Crit Rev Toxicol 35(8–9):771–781.

Zoeller RT, Rovet J. 2004. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J Neuroendocrinol 16(10):809–818.

Zoeller RT, Tyl RW, Tan SW. 2007. Current and potential rodent screens and tests for thyroid toxicants. Crit Rev Toxicol 37(1):55–95.