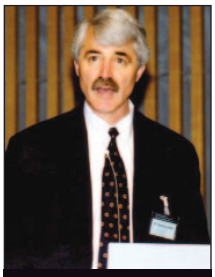
Guest Editorial

Thyroid Toxicology and Brain Development: Should We Think Differently?


R. Thomas Zoeller

Thyroid hormone (TH) is essential for normal brain development. The simplicity of this statement, however, dramatically understates the complexity of the issues confronting us as we develop ways to identify factors in the environment that affect TH signaling in the developing brain, and methods of estimating the potential risk to human health of exposures to these factors. There are many known thyroid toxicants (Brucker-Davis 1998), and each has been identified solely by its ability to reduce circulating TH levels. Yet, we do not know the extent to which TH levels must decline before brain development is compromised. In addition, if there are chemicals in the environment that directly interfere with TH action on their receptors but do not affect TH levels in the blood, they cannot be identified as thyroid toxicants by the current screening methods. These two issues have been the focus of several recent reports, and the implications of their findings may change the way we think about thyroid toxicology.

Few experimental studies have modeled the effect of subtle TH insufficiency on brain development, but one group has begun to investigate this issue because undiagnosed maternal hypothyroxinemia may be prevalent in the general population. Lavado-Autric et al. (2003) reported that subtle TH insufficiency in the pregnant rat disrupts the migration of neurons in the fetal cortex and hippocampus, leading to the presence of neurons in aberrant locations of the adult offspring's brain and "blurring" cortical layers. Thus, the developing brain is more sensitive to maternal TH insufficiency than originally believed.

The paucity of experimental studies designed to identify the most sensitive end points of TH action in the developing brain shows that there are no well characterized end points of TH action that can be immediately recruited into toxicologic studies. However, new studies using genetic models of TH insufficiency are providing new insight that will help remedy this problem. Thyroid hormone receptors (TRs) are nuclear proteins that regulate gene expression; there are two types of TRs—α and β—and there are several isoforms of each of these two types (Zhang and Lazar 2000). These TR isoforms exhibit selective spatial and temporal patterns of expression in the developing brain, and recent work employing genetic lines carrying targeted deletions of specific TRs is revealing that specific TR isoforms mediate TH actions on specific developmental events. For example, migration of cerebellar granule cells is affected by TH acting on TRα, whereas development of the cerebellar Purkinje cells is dependent upon TH acting on both TRα and TRβ (Morte et al. 2002).

These studies map the time and place of TH action during brain development, and the specificity with which TR isoforms mediate TH actions on specific developmental events. In turn, this information will permit the development of end points of thyroid toxicity in the developing brain. The combination of different end points of thyroid toxicity, reflecting actions mediated through different receptors, will likely provide strong evidence of the specificity of toxicant effects on TH signaling in the developing brain much the same way that the uterotrophic or Hershberger assays provide information about the specificity of toxicant effects on sex steroid signaling.

Identification of specific end points of TH action in the developing brain will also allow us to test whether environmental factors can exert effects on TR function. Moriyama et al. (2002) reported that bisphenol A (BPA) can bind to the TR; as little as 10 μM can act as an indirect antagonist. Specifically, BPA can inhibit the ability of TH to regulate gene expression by inhibiting the release of the corepressor N-CoR from the TR. A second group independently reported that polyhalogenated derivatives of BPA, tetrabromo- and tetrachloro-BPA, can bind to the TR with higher affinity than BPA and can act as TR agonists *in vitro* (Kitamura et al. 2002). These studies represent the first evidence that there are chemicals in the environment that may interfere with TH signaling by acting directly on the TR, rather than by inhibiting function of the thyroid gland.

Currently, strategies for identifying thyroid toxicants only include assays of thyroid function—serum hormone levels and thyroid histopathology (Daston et al. 2003). These strategies will not identify chemicals, such as BPA, that interfere with TH signaling without reducing hormone levels. The variety of chemicals that could act in this manner may be more extensive than is generally believed. For example, Iwasaki et al. (2002) recently reported that some hydroxylated polychlorinated biphenyls can interfere with TH-induced gene expression by the same indirect antagonism as shown for BPA. In addition, if environmental chemicals can selectively affect a particular TR isoform, they will exert effects on brain development that are not consistent with hypothyroidism per se, but will instead produce a mosaic of effects that would be impossible to interpret without understanding the complexity of TH action on brain development.

Because TH is essential for normal brain development, we must incorporate new insights about the temporal and spatial complexity of TH action on the developing brain into strategies to identify thyroid toxicants. Moreover, it is essential to empirically determine the degree to which the developing brain is sensitive to TH insufficiency.

R. Thomas Zoeller
Biology Department
Morrill Science Center
University of Massachusetts Amherst
Amherst, Massachusetts
E-mail: tzoeller@bio.umass.edu

R. Thomas Zoeller's research is focused on understanding the role of thyroid hormone in brain development, with a special emphasis on the fetal cerebral cortex, and on identifying the mechanisms by which environmental chemicals may interfere with thyroid hormone signaling.

References

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

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

Iwasaki T, Miyazaki W, Takeshita A, Kuroda Y, Koibuchi N. 2002. Polychlorinated biphenyls suppress thyroid hormone-induced transactivation. Biochem Biophys Res Commun 299:384–388.

Kitamura S, Jinno N, Ohta S, Kuroki H, Fujimoto N. 2002. Thyroid hormonal activity of the flame retardants tetrabromobisphenol A and tetrachlorobisphenol A. Biochem Biophys Res Commun 293:554–559.

Lavado-Autric R, Auso E, Garcia-Velasco JV, Arufe Mdel C, Escobar del Rey F, Berbel P, et al. 2003. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 111:1073–1082.

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

Morte B, Manzano J, Scanlan T, Vennstrom B, Bernal J. 2002. Deletion of the thyroid hormone receptor α1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Proc Natl Acad Sci USA 99:3985–3989.

Zhang J, Lazar MA. 2000. The mechanism of action of thyroid hormones. Annu Rev Physiol 62:439–466.

Starr T. 2003. Significant issues raised by meta-analyses of cancer mortality and dioxin exposure. Environ Health Perspect 111:1443–1447.

Swaen GM, Meijers JM. 1988. Influence of design characteristics on the outcome of retrospective cohort studies. Br J Ind Med 45(9):624–629.

Willman T. 2003. Stealth merger: drug companies and government medical research—some of the NIH's top scientists are also collecting paychecks and stock options from biomedical firms. Los Angeles Times (7 December). Available: http://www.latimes.com/news/nationworld/nation/la-na-nih7dec07.story [accessed 16 January 2004].

Conflicts of Interest: Gibson's Response

I applaud Tweedale's effort to ferret out and expose true conflicts of interest in regard to health research. However, he is barking up the wrong tree when he suggests that conflict of interest was present in my study of perceived treatment efficacy for therapies for multiple chemical sensitivity (MCS) (Gibson et al. 2003). Although I admit to having a strong interest in and caring deeply about issues relating to my topic of research, I do not think that this distinguishes me from any other researcher.

My funding was from the Chemical Injury Information Network (CIIN), a nonprofit organization that works to provide education and advocacy regarding MCS. Neither the CIIN nor I have any vested financial interest whatsoever in any of the treatments researched. My purpose for the study was to examine resource allocation for and efficacy of treatments for a currently delegitimized condition. The CIIN has an interest in gathering and providing information to those with MCS about this issue, but the organization neither advocates nor benefits from the use or sale of any particular treatment. Tweedale's suggestion that the CIIN may gain financially from "seeing MCS declared a prevalent hazard" seems inappropriate, given the study's focus on treatment and not prevalence.

Finally, I clearly disagree with Tweedale's suggestion that even funding from the National Institutes of Health (NIH) is suspect because the NIH has an interest in health. Nonprofit organizations have been funding health research for decades and, in my mind, having an "interest in health" is in no way synonymous with a financial vested interest.

The author declares she has no competing financial interests.

Pamela Gibson
James Madison University
Harrisonburg, Virginia
E-mail: gibsonpr@jmu.edu

References

Gibson PR, Elms AN-M, Ruding LA. 2003. Perceived treatment efficacy for conventional and alternative therapies reported by persons with multiple chemical sensitivity. Environ Health Perspect 111:1498–1504.

Editors' note: *Because the phrase "conflict of interests" can be interpreted so broadly that almost everyone could be considered to have a conflict, we modified our policy for declaring conflicts of interest in December 2003 (Environ Health Perspect 111:A900–905).* EHP *now requires authors to declare "competing financial interests." The new policy is available on the web in our updated Instructions to Authors (**http://ehp.niehs.nih.gov/docs/admin/edpolicy.html**); the new downloadable form is also available (**http://ehp.niehs.nih.gov/cfi.pdf**).*

Because EHP *is not in the position to confirm the accuracy of disclosure statements made by our authors, we hold authors responsible for providing accurate information.* EHP *authors can expect scrutiny of their statements by our readers and by the authors' own employers. We welcome letters to the editor that address alleged inaccuracies of declarations of competing financial interests.*

Six Modern Plagues

In his generally positive review of my book, *Six Modern Plagues and How We Are Causing Them* (Walters 2003), Donald S. Burke (2004) neglected to mention that *Six Modern Plagues* goes out of its way to differentiate between fact and theory. I stated, for example, that the basic mechanism of the emergence of human immunodeficiency virus "is still unproven," that "there is some evidence" for *Salmonella* drug resistance being acquired from fish farms in Asia, that the widely accepted belief that mad cow disease originated from scrapie in sheep is "still just a hypothesis," and that "perhaps" an infected person first introduced West Nile virus into the United States. However, Burke's blanket assertion in the review that "an infected arriving human could not have been the origin of the West Nile epidemic in Queens, New York" confuses fact with theory, indeed. This may be his informed opinion, but it is far from scientifically established fact.

The author receives royalties fom the publication and distribution of this book.

Mark Jerome Walters
University of South Florida
St. Petersburg, Florida
E-mail: mjw@bayflash.stpt.usf.edu

Reference

Burke DS. 2004. Book review: Six Modern Plagues and How We Are Causing Them. Environ Health Perspect 112:A66.

Walters MJ. 2003. Six Modern Plagues and How We Are Causing Them. Washington, DC:Island Press.

Hypothyroxinemia, Iodine Deficiency, and Subtle Changes in Migration and Cytoarchitecture

In the Guest Editorial in the September issue of *EHP*, Zoeller (2003) commented on an article by Lavado-Autric et al. (2003). Lavado-Autric et al. (2003) used the term "hypothyroxinemia" in this article to indicate that thyroxine (T_4) or free T_4 concentrations are low compared with values usually found at the same stage of pregnancy in normal women with adequate iodine intake, with or without the presence of clinical or subclinical hypothyroidism (when thyroid-stimulating hormone is above normal values). This is important because, in many instances, women in iodine-deficient populations are hypothyroxinemic; these women are not clinically hypothyroidal because they have normal or above-normal levels of circulating 3,5,3′-triiodothyronine (T_3) that can be supplied to the tissues.

It is important to note that the rats in the study were treated drastically by Lavado-Autric et al. (2003). The dams were first fed a diet with a low iodine content (LID) for 10 days and given an incredibly high amount of a goitrogen—1% perchlorate ($KClO_4$)—in the drinking water to lower the initial content of iodine-containing compounds in the thyroid gland; the 1% $KClO_4$ was then withdrawn. After dividing the rats into three groups, Lavado-Autric et al. (2003) treated one group with LID containing potassium iodide (LID-plus-KI) to ensure a normal iodine intake (approximately 10 μg iodine/day), the second group with LID alone (LID-1), and the third group with LID containing 0.005% $KClO_4$ (LID-2). This third treatment was used to further decrease thyroid uptake of the small amounts of iodine contained in the LID itself and in the supplements given to the rats throughout pregnancy and lactation to prevent nutritional deficiencies other than iodine.

In his editorial, Zoeller (2003) stated the following:

> Lavado-Autric et al. (2003) reported that subtle TH [thyroid hormone] insufficiency in the pregnant rat disrupts the migration of neurons in the fetal cortex and hippocampus.…

This was not subtle TH insufficiency. In fact, Lavado-Autric et al. (2003) stated that

> T_4 values in the LID-1 dams were well below normal (< 10% of the values of LID-plus-KI dams), and T_3 values remained normal. In LID-2 dams, however, T_3 values decreased, though much less markedly than T_4. Despite the decrease in T_3 values, the reproductive performance of these animals was normal, as was the postnatal growth of the pups at [postnatal day 40].

As a result,

> the subtle changes in cytoarchitectonic organization found in the progeny of both LID-1 and LID-2 dams indicate that the normal process of brain maturation … [is] likely to be impaired.

The author declares he has no competing financial interests.

Offie P. Soldin
Lombardi Cancer Center
Georgetown University Medical Center
Washington, DC
E-mail: os35@georgetown.edu

References

Lavado-Autric R, Auso A, Garcia-Velasco JV, Arufe Mdel C, Escobar del Rey F, Berbel P, et al. 2003. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 111(7):1073–1082.

Zoeller RT. 2003. Thyroid toxicology and brain development: should we think differently? [Editorial]. Environ Health Perspect 111:A628.

Hypothyroxinemia: Zoeller's Response

Soldin addresses an important issue that was not fully developed in my editorial (Zoeller 2003a), specifically, the description of thyroid status in experimental animals designed to model human conditions. There are two separate issues in this regard. The first is that the language describing thyroid status is well defined for humans but not for experimental animals. Clinical assays for the various hormones of the pituitary–thyroid axis are standardized (and calibrated) across clinical chemistry laboratories, and reference ranges have been published for various subgroups of the population (e.g., Adams et al. 1995; Singh et al. 2003; Wiersinga 2001). Therefore, terms such as "hypothyroxinemia" and "subclinical (or mild) hypothyroidism" have very specific definitions for humans. In contrast, experimental studies are internally controlled (i.e., using control groups), and there are no reference ranges or assays that are calibrated across research laboratories performing these assays. Therefore, caution is needed when applying terms such as "subclinical hypothyroidism" to experimental animals.

The second, and more important, issue is that the vast majority of research focused on identifying the role of thyroid hormone (TH) in brain development has modeled severe hypothyroidism (reviewed by Schwartz 1983). Perhaps for this reason, the "clinical" symptoms of severe hypothyroidism in animals, including reduction in litter size, body weight, and brain size, and a delay in developmental landmarks such as tooth eruption and eye opening, have come to be viewed as cardinal developmental effects of TH insufficiency. Therefore, by association, if these "clinical" signs are not observed, the implication is that there would be no other effects on brain development. In large part, the work by Lavado-Autric et al. (2003) was testing whether "subtle" (my term) hypothyroidism could affect brain development (Zoeller 2003b).

By "subtle," I meant that maternal thyroxine (T_4) was reduced to a level below that of control animals but that overt effects on litter size, body weight, and other characteristics were not observed. Soldin is correct that the initial treatment of young adult female rats described by Lavado-Autric et al. (2003) was not subtle. Subgroups of these animals were treated for 10 days with a low iodine diet plus 1% potassium perchlorate in their drinking water. However, the animals were then taken off perchlorate treatment and placed on specifically designed diets for 3 months before being mated. Thus, the article by Lavado-Autric et al. is not about perchlorate treatment; it is about the sensitivity of the developing brain to TH insufficiency and the developmental timing of this vulnerability. The fact remains that there are no experimental studies designed to determine what might be considered a no effect level for maternal or neonatal TH insufficiency on brain development. However, this will be an important issue to clarify as we consider the significance of maternal hypothyroxinemia or the effects of thyroid toxicants on brain development.

The author declares he has no competing financial interests.

R. Thomas Zoeller
University of Massachusetts
Amherst, Massachusetts
E-mail: tzoeller@bio.umass.edu

References

Adams LM, Emery JR, Clark SJ, Carlton EI, Nelson JC. 1995. Reference ranges for newer thyroid function tests in premature infants. J Pediatr 126(1):122–127.

Lavado-Autric R, Auso E, Garcia-Velasco JV, Arufe Mdel C, Escobar del Rey F, Berbel P, et al. 2003. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 111(7):1073–1082.

Schwartz HL. 1983. Effect of thyroid hormone on growth and development. In: Molecular Basis of Thyroid Hormone Action (Oppenheimer JH, Samuels HH, eds). New York:Academic Press, 413–444.

Singh PK, Parvin CA, Gronowski AM. 2003. Establishment of reference intervals for markers of fetal thyroid status in amniotic fluid. J Clin Endocrinol Metab 88(9):4175–4179.

Wiersinga WM. 2001. Thyroid hormone replacement therapy. Horm Res 56(suppl 1):74–81.

Zoeller RT. 2003a. Thyroid toxicology and brain development: should we think differently? [Editorial]. Environ Health Perspect 111:A628.

Zoeller RT. 2003b. Transplacental thyroxine and fetal brain development. J Clin Invest 111(7):954–957.

Chemical Safety Requires Local Government Action

In "REACHing for Chemical Safety," Brown (2003) described the European Union's proposed Registration, Evaluation, and Authorisation of Chemicals (REACH) legislation and the shocking ineffectiveness of the laws currently governing toxic chemicals. Brown (2003) also revealed the depressing extent to which U.S. environmental officials, who are supposed to be advocates for the public and the environment, have instead fallen in line with the Bush administration's pro-business policies. Much-needed reform of U.S. policy on chemicals seems remote at best, but glimmers of hope, and possibly the future, exist outside the Washington, DC, Beltway. Last summer, San Francisco, California, became the first government jurisdiction in the United States to adopt the precautionary principle as a controlling environmental policy. San Francisco has also passed a resolution supporting a strong REACH in Europe, which would clearly benefit the people of California and elsewhere in the United States by promoting a safer global chemicals industry. Local communities and states can and must take environmental protection into their own hands; Californians are showing how bridges to Europe can help bypass the federal government altogether. Official San Francisco websites provide further information on the San Francisco ordinance on the Precautionary Principle Ordinance (SF Environment 2003) and the REACH Resolution (City and County of San Francisco Board of Supervisors 2003).

The author declares he has no competing financial interests.

Joseph H. Guth
Center for Environmental Health
Oakland, California
E-mail: jguth@cehca.org

References

Brown VJ. 2003. REACHing for chemical safety. Environ Health Perspect 111: A766–A769.

SF Environment. 2003. SF Precautionary Principle Ordinance. Available: http://sfgov.org/sfenvironment/aboutus/policy/legislation/precaution_principle.htm [accessed 3 March 2004].

City and County of San Francisco Board of Supervisors. 2003. Supporting European Union Efforts to Regulate Hazardous Chemicals. Resolution 0715-03. Available: http://www.sfgov.org/site/uploadedfiles/bdsupvrs/resolutions03/r0715-03.pdf [accessed 3 March 2004].