

Thyroid Toxicants: Assessing Reproductive Health Effects

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A thyroid toxicant workshop sponsored by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction convened on 28–29 April 2003 in Alexandria, Virginia. The purpose of this workshop was to examine and discuss chemical-induced thyroid dysfunction in experimental animals and the relevance of reproductive and developmental effects observed for prediction of adverse effects in humans. Presentations highlighted and compared reproductive and developmental effects of thyroid hormones in humans and rodents. Rodent models of thyroid system dysfunction were presented. Animal testing protocols were reviewed, taking into account protocol designs that allow extrapolation to possible human health effects. Potential screening methods to assess toxicant-induced thyroid dysfunction were outlined, and postnatal bioassays of thyroid-related effects were discussed. *Key words:* chemical screening, chemical testing, developmental, perinatal exposure, reproductive, thyroid gland, thyroid hormones, toxicology. *Environ Health Perspect* 112:363–368 (2004). doi:10.1289/ehp.6637 available via <http://dx.doi.org/> [Online 12 November 2003]

Normal thyroid hormone blood levels are essential for growth and development of tissues and for the maintenance of tissue and organ function. Changes in thyroid hormone levels can adversely affect fertility, pregnancy outcome, and postnatal development in humans and animals, with major effects on growth, hearing, mental acuity, and reproductive system development and function in the offspring (Becks and Burrow 1991; Drury et al. 1984). Hypothyroidism and hyperthyroidism have been associated with increased risk of adverse pregnancy outcomes in humans (Becks and Burrow 1991; Drury et al. 1984). The effects of pregnancy and the environment—whether dietary (e.g., low iodine), hormonal, or from synthetic chemical/drug exposures [e.g., propylthiouracil (PTU)]—on thyroid status have been observed for some time. A recent article in *Environmental Health Perspectives* (Howdeshell 2002) identified 116 synthetic chemicals that have been reported to affect production, transport, or metabolism of thyroid hormones. Some of these chemicals adversely affect thyroid function by more than one mechanism.

Although thyroid hormones can affect prenatal and postnatal development, determining thyroid status is not a routine practice in standard reproductive and developmental toxicity bioassays. Screening for thyroid disruption does not occur primarily because there is no definitive, easily measurable biologic end point apart from triiodothyronine (T₃), tetraiodothyronine (T₄), and thyroid-stimulating hormone (TSH) serum concentrations and thyroid gland weight and histology. However, separation of the thyroid gland from surrounding tissue and accurate weighing of the rodent thyroid gland is technically difficult, especially in pups, and is often done

after fixation of tissue. Thyroid hormone assays often require larger blood volumes than are obtainable from rodent pups. Furthermore, the human relevance of thyroid effects observed from chemical testing in rodents is not fully understood. In a recent presentation (Ambroso et al. 2003), the need for a logical approach for addressing thyroid effects in toxicity studies was emphasized and a decision tree for assessing thyroid status during pre-clinical drug development was presented. At a recent international conference on the thyroid gland and brain development titled “Thyroid Hormone and Brain Development: Translating Molecular Mechanisms to Population Risk,” held 23–25 September 2002 at the National Institute of Environmental Health Sciences (Research Triangle Park, NC), the extent to which children are affected by undiagnosed thyroid dysfunction and how to test for it were discussed. It was agreed the effects of thyroid toxicants are not well studied and the relative sensitivities of various end points are not well characterized. Subsequently, the National Toxicology Program’s Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) convened this workshop to focus on the reproductive health effects of exposures to thyroid toxicants.

At this workshop, the comparative physiology of rodent and human thyroid systems was presented. Discussions focused on the relevance of thyroid-related adverse reproductive and developmental outcomes observed in rodents for predicting health risks in humans. Possible inclusion of thyroid end points in routine developmental and reproductive toxicity tests led to consideration of two questions. First, when testing chemicals of unknown thyroid toxicity, what end points could be included in the protocols to provide evidence

that thyroid development or function is adversely affected? Traditional end points include thyroid gland histopathology and weight and blood levels of T₄, T₃, and TSH. However, measurement of these end points in the neonatal rodent is difficult. Second, if the test chemical is known to affect thyroid development or function, how can developmental or reproductive toxicity testing protocols be refined to identify effects resulting from thyroid dysfunction? Protocol factors considered important include timing and duration of exposure, timing of tissue collection to determine thyroid status, monitoring of end points sensitive to alterations in thyroid status, and test chemical dose selection. To have a common academic framework for workshop participants, a detailed background document was prepared comparing normal and abnormal thyroid function in rodents and humans, examining similarities and differences among these species, and discussing effects of thyroid hormone disruption by four thyroid-active chemicals—PTU, methimazole (MTZ), sulfamethazine (SMZ), and phenobarbital (PB).

Comparison of Thyroid Function and Development in Rodents and Humans

The first session of this workshop provided an overview of the similarities and differences between the human and rodent thyroid systems (Table 1) and presented two rodent models of human thyroid dysfunction.

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Thyroid hormone production and function.

The production and function of thyroid hormones are similar in rodents and humans and are controlled through physiologic feedback mechanisms. Thyroid hormones modulate lipid, carbohydrate, and protein metabolism and oxygen consumption by cells (Larsen and Ingbar 1992; Martini 1998). There are, however, some important species differences in transport and metabolism.

In rodents and humans, follicles in the normal thyroid gland are filled with thyroglobulin, a glycoprotein that is one of the starting molecules for thyroid hormone synthesis. Epithelial cells of the thyroid follicle, which have a sodium iodide symporter, take up iodide from the blood. Once inside the thyroid epithelial cell, iodide is transported to the apical plasma membrane, and thyroid peroxidase, an integral membrane enzyme, catalyzes sequential reactions in thyroid hormone production. Thyroid peroxidase first oxidizes iodide to iodine and then iodates tyrosines on thyroglobulin to produce monoiodotyrosine and diiodotyrosine. Thyroid peroxidase finally links two iodinated tyrosines to produce T_4 and T_3 .

Gene expression studies have identified sodium iodide symporter transcripts in the thyroid, stomach, salivary glands, lacrimal glands, mammary glands, and ovaries of mice, rats, and humans. However, there are significant differences in gene expression of the symporter among species. Human symporter expression is observed in the heart, adrenal gland, and thymus, whereas no expression is observed in these tissues in mice or rats (Perron et al. 2001). In most species, including rodents and humans, TSH is the major regulator of sodium iodide symporter gene expression in the thyroid gland.

Thyroid hormones are released from the thyroid gland into the blood, where they may bind to one of three binding proteins, thyroxine-binding globulin, albumin, and transthyretin. The chemical nature of these liver-derived binding proteins and the distribution of thyroid hormones to these proteins vary considerably among animal species. In humans, thyroxine-binding globulin is the major binding protein; in rodents, albumin is the major binding protein (Kaneko 1989).

The serum half-life of T_4 and T_3 in normal human adults is 5–9 days and 1 day, respectively. Comparatively, the serum half-life of T_4 and T_3 in rats is 0.5–1 and 0.25 days, respectively. The basis for the difference in half-lives is not completely understood. It is proposed that lack of high-affinity thyroid-hormone-binding proteins, that is, thyroxine-binding globulin, in the adult rat plays a role, leading to increased availability of T_4 for metabolism and elimination, for example, deiodination (Döhler et al. 1979). The higher rate of thyroid hormone production in rodents is proposed to

play a role in species differences in follicle morphology (Hill et al. 1998).

The thyroid gland in rats and humans produces 10–30 times more T_4 than T_3 , the latter of which is the primary biologically active form. T_3 released by the thyroid gland accounts for approximately one-tenth of the T_3 in peripheral tissues. The balance of T_3 is produced from T_4 through deiodinase activity. Approximately 80% and 60% of T_3 production in humans and rats, respectively, are from extrathyroidal deiodinase activity (Bianco et al. 2002). Type I and type II deiodinases convert T_4 to T_3 , and type III inactivates T_3 and T_4 to 3,3'-diiodothyronine (T_2) and 3,3',5'-triiodothyronine (reverse T_3 , or rT_3), respectively (Table 2). In the adult human and rat, tissues with high levels of T_3 are the brain, pituitary gland, thyroid gland, liver, and kidney. The type and amount of deiodinase activity at the cellular level can vary with the stage of development, tissue type, species, diet, and hormonal state (Table 2). For example, the fetal rat produces high levels of type III deiodinase, but the

adult rat produces very low levels (Köhrle 1999). Type I deiodinase is located in the cell membrane, and enzyme activity is decreased by fasting and increased by increased thyroid hormone. Type II deiodinase is located within the cell on the endoplasmic reticulum and can undergo rapid degradation with increases in thyroid hormone.

Thyroid system development. Development of the thyroid system is similar in humans and rodents, but there is a difference in the stage of thyroid development at birth. Rats are born with a less developed thyroid system. Function of the hypothalamic–pituitary–thyroid axis begins at birth and is complete by 4 weeks of age. Comparatively, in humans the hypothalamic–pituitary–thyroid axis develops prenatally—from the 4th to 5th weeks through the 30th and 35th weeks of gestation—and as in the rat, full maturation of thyroid system function is complete by 4 weeks after birth (Fisher and Klein 1981).

Overall, studies indicate that the role of thyroid hormones in development of reproductive

Table 1. Comparison of the thyroid system in humans and rodents.

Parameter	Human	Rat	Mouse
Half-life of T_4	5–9 days	0.5–1 days	0.5–0.75 days
Half-life of T_3	1 day	0.25 day	0.45 day
High-affinity thyroxine-binding globulin	Present	Absent	Absent
Primary serum-binding protein	Thyroxine-binding globulin	Albumin	Albumin
Sex differences in serum TSH levels	No difference	Adult males > adult females	Adult males > adult females
Amount of T_4 required in absence of functional thyroid gland	2.2 $\mu\text{g}/\text{kg}$ body weight/day	20 $\mu\text{g}/\text{kg}$ body weight/day	Information not found
Development of the fetal pituitary–thyroid axis	TSH and T_3 secretion increases between 18 and 20 weeks gestation Becomes functional late in the 1st and early 2nd trimester Maturation appears complete by 4th postnatal week	Thyroid hormone synthesis and TSH-secreting cells appear by gestational day 17 Becomes functional in late gestation and postnatally Maturation appears complete by 4th postnatal week	Information not found

Table 2. Properties of deiodinases present in laboratory animals and humans.

Property	Deiodinase type I (5 and 5' deiodinase activity)	Deiodinase type II (5' deiodinase activity)	Deiodinase type III (5 deiodinase activity)
Limiting K_m	0.5 μM	1–2 nM	5–20 μM
Reaction catalyzed	T_4 to rT_3 or T_3	T_4 to T_3	T_4 to rT_3 T_3 to T_2
Inhibitors	Thiouracils, iopanoate, halogenated aromates, propranolol	Iopanoate, flavonoids, high T_4 levels, rT_3	Iopanoate, flavonoids
Tissue distribution	CNS, euthyroid pituitary gland, thyroid gland, kidney, liver, skeletal muscle, placenta	CNS, pituitary gland, thyroid gland, brown adipose tissue, placenta	Almost all tissues except pituitary gland, thyroid gland, kidney, liver; high in rat fetal tissue
Hyperthyroidism	Increase	Decrease	Increase

Data from Bianco et al. (2002), Kelly (2000), and Köhrle (1999).

structure and function is similar in humans and rodents of both sexes. In human and rodent males, thyroid hormones regulate testis development through promotion of Sertoli cell differentiation. The effect is proposed to occur through activation of thyroid receptor (TR)- α 1 in both species (Jannini et al. 1995). Thyroid receptors function as hormone-activated transcription factors that modulate gene expression. Thyroid receptors bind DNA in the absence of thyroid hormone, usually leading to transcriptional repression. Thyroid hormone binding leads to a conformational change in thyroid receptor and subsequent transcriptional activation of genes controlled by thyroid hormone.

In human and rodent females, thyroid hormones modulate hormone-induced transcription pathways and factors to affect hormonal status and reproduction (Krassas 2000). Additionally, alterations in thyroid status in humans and rodents during pregnancy appear similar. Interestingly, thyroid hormones seem to play a significant role in male but not female reproductive tract development in humans and rodents (Jannini et al. 1995; Krassas 2000). Despite the general similarities in the role of thyroid hormones on reproduction and reproductive tract development in these species, the exact roles these hormones play—for example, mechanisms of action and interactions with other hormones—have not been fully evaluated.

Pregnancy. Pregnancy increases iodine clearance by the kidney. Therefore, low serum iodine levels can occur during pregnancy if the diet is deficient or marginally sufficient in iodine. Subclinical hypothyroidism can be detected by measurement of serum TSH. In this condition, slightly higher TSH serum levels are observed, although T_3 and T_4 levels are within normal range and there are no clinical signs of hypothyroidism. Thyroid gland size correlates to TSH serum levels and increases in size during pregnancy by 10–20%. TSH serum levels and thyroid gland histology are more sensitive end points of thyroid status than are T_3 and T_4 serum levels.

Deiodinases present in the human placenta rapidly metabolize maternal T_4 to T_3 for use by the fetus. However, a significant amount of T_4 is still transferred to the fetus (Chan and Kilby 2000). The placenta is freely permeable to iodide and thyrotropin-releasing hormone (TRH) but not to TSH. It is proposed that maternal TRH may have a role in regulating fetal thyroid function during fetal development of the hypothalamic–pituitary–thyroid system. Thyroid receptor isoforms are present in the placenta, and expression of receptor protein increases with fetal age. However, the role of these receptor isoforms and the contribution of maternal hormones to the development of the fetal thyroid system are unclear (Braverman and Utiger 2000; Chan and Kilby 2000).

Thyroid dysfunction. Hyper- and hypothyroidism-induced alterations in the reproductive system, such as altered levels of leuteinizing hormone (LH) and follicle stimulating hormone, altered steroid levels and steroid ratios, decreased sperm count, and decreased libido, are observed in adult male laboratory animals and humans (Bourget et al. 1987; Jannini et al. 1995). Hypothyroidism produces differing effects in juvenile male rats and humans. Prepubertal hypothyroidism is associated with precocious sexual development (enlargement of the testes without virilization) in humans and absence of libido and ejaculate in rats (Jannini et al. 1995; Longcope 2000).

Hypothyroidism in adult female humans and rats is associated with altered menstrual and estrous cycles and interference with gestation, usually during the first trimester (humans) or first half (rodents) of pregnancy (Fisher and Brown 2000; Krassas 2000). In humans, increased abortions and stillbirths are noted, whereas in rodents, increased resorptions, stillbirths, and reduced litter sizes are observed (Fisher and Brown 2000; Krassas 2000). Fetal hypothyroidism in female rats alters reproductive tract development, but a similar effect is not seen in female humans. Hypothyroidism in the prepubertal period is associated with delayed sexual maturity in female rats and humans. Paradoxically, in some cases, “precocious puberty” is associated with hypothyroidism in female humans (Krassas 2000). Hyperthyroidism in adult female humans and rats is associated with altered levels of LH. Results of studies of the effects of hyperthyroidism in prepubertal female humans and rats are conflicting. Some studies indicate that hyperthyroidism in humans and rats leads to early onset of menses and puberty, whereas other studies indicate hyperthyroidism either delays estrus (rats) or does not affect onset of menses (humans).

Animal models of thyroid disease. The relevance of animal models for predicting human health risks was discussed. Because of differences in the thyroid system physiology and differences in adverse effects seen in humans and animals with altered thyroid systems, some current models may not be highly predictive of human health effects. Models of thyroid cancer in rats do not correlate with human risks because of the differences between rodent and human thyroid systems. For example, a sequela for chemical-induced thyroid hypertrophy in rats can be thyroid cancer, whereas in humans thyroid hypertrophy leads to noncancerous goiter. In humans, only ionizing radiation has been shown to cause thyroid cancer (Hill et al. 1998). Therefore, there is a continuing and pressing need to develop and validate animal models as good predictors of human health effects. Two presentations discussed below note different animal models and

their usefulness in predicting human health risks and hazards.

Thyroid epithelial cell differentiation and proliferation depend on several transcription factors, including thyroid transcription factors TTF1, TTF2, and PAX8. TTF1 and PAX8 are implicated in proliferation and survival of thyroid cell precursors, and TTF2 plays a role in migration and proliferation of thyroid precursors. Mutant TTF1 proteins do not bind to DNA and thus do not interfere with the activity of wild-type proteins present in the cell. Heterozygous TTF1 knockout mice have increased levels of TSH and decreased function in locomotor (rotarod) tests. Humans heterozygous for mutant TTF1 display dyskinesia, respiratory dysfunction, and elevated concentrations of serum TSH. The neurologic effects and high TSH blood levels suggest that these heterozygous knockout mice may provide a model to assess the effects of alterations in TTF1 structure and expression in humans (Pohlenz et al. 2002). In this study, TTF2 alterations were shown to produce similar adverse effects in humans and mice, including severe hypothyroidism due to thyroid agenesis or ectopy, cleft palate, and spiky hair. Interestingly, PAX8 defects produce variable effects in humans, including no effect, thyroid gland hypoplasia, or ectopic thyroid gland. Similar defects in mice lead to a lack of thyroid follicle development (Pohlenz et al. 2002). Overall, these studies suggest that mouse models of thyroid transcription factor alterations may provide suitable models to assess the physiologic effects of similar alterations in humans.

The next group of models presented mimicked thyroid hormone resistance, which is clinically defined as exhibiting elevated serum levels of free T_4 and T_3 , normal or slightly elevated levels of serum TSH, no signs of thyroid toxicosis, and goiter (Weiss and Refetoff 2000). Thyroid hormone resistance may or may not be associated with mutations in thyroid receptor, specifically in the TR- β gene. Studies of thyroid resistance using mouse models allow identification of tissue-specific effects, testing of therapeutic agents, and evaluation of genetic factors modulating phenotype. Current mouse models of thyroid resistance include knockout and transgenic mice. Comparisons of specific characteristics—for example, TSH and T_4 levels, hearing, vision, and growth—between humans lacking the TR- β receptor and TR- β knockout mice show that deletion of the receptor leads to similar effects, except growth, in both species. Another model shows that removal of receptor co-activators also leads to thyroid resistance and suggests there are thyroid-receptor-specific interactions *in vivo*.

In vitro assays. *In vitro* screening tests (People for the Ethical Treatment of Animals. Unpublished data) have been developed to identify chemicals that may be hormonally

active *in vivo*. Validation of these assays requires not only a validation of methodology but also an understanding of how well and in what respects these assays predict effects observed in life. Currently, these assays are used in research to answer specific mechanistic questions and have not been validated for use as a chemical screening tool. Studies of the biochemistry of thyroid receptors are very limited, making it difficult to translate *in vitro* results to *in vivo* effects. However, determination of xenobiotic binding affinity to thyroid receptors and effects on transcription yield important information for the testing process. Therefore, thyroid receptor binding and gene reporter assays could be helpful in identifying chemicals that affect thyroid hormone metabolism. Induction of liver enzymes important in xenobiotic and thyroid hormone metabolism—for example, uridine diphosphatase glucuronosyl transferases—can be measured *in vitro* in hepatocytes cultured with the test chemical. However, more detailed studies would have to be performed in experimental animals to determine whether chemicals that induce uridine diphosphatase glucuronosyl transferases affect the thyroid gland or pituitary gland, and the physiologic consequence(s) of these effects.

The role of thyroid-hormone-binding serum proteins in the availability of thyroid hormone to target tissues is not completely understood. Therefore, the relevance of results from *in vitro* assays for determination of chemical displacement of thyroid hormone from serum binding proteins may be difficult to interpret. At present, target-cell-specific effects and embryonic developmental assays are not sufficiently refined to provide reliable information on *in vivo* thyroid system effects.

Reproductive Effects of Selected Thyroid-Active Chemicals in Rodents and Humans

Presentations and discussions in this session focused on comparing the effects induced in rodents and humans by four thyroid-active drugs. MTZ and PTU are drugs typically prescribed to people with hyperthyroidism, and both drugs are used during pregnancy. SMZ is widely used as an antimicrobial agent in human and veterinary medicine. These chemicals inhibit thyroid peroxidase within the thyroid gland, and PTU also inhibits monoiodinase, leading to an inhibition of thyroid hormone synthesis. PB, an anticonvulsant drug, induces hepatic enzymes and increases metabolism and excretion of thyroid hormones.

In the adult male rat there is minimal effect of thyroid hormone variations on fertility; the primary thyroid hormone effect occurs during testicular development. Physical and

chemical-induced hypothyroidism in rats increases the period of Sertoli cell proliferation and differentiation compared with euthyroid rats, leading to an increase in testicular size. Administration of drugs such as PTU and MTZ to rats produces hypothyroidism and increases testicular size. Rats provided 0.1% PTU in drinking water from birth to postnatal day 25 were hypothyroid, had greater testicular size and higher sperm counts, and were 15–20% smaller by weight than were control animals. In rats, increases in testicular size are seen at doses as low as 0.0004% PTU and 0.025% MTZ in drinking water.

The exact mechanism of action of PTU and MTZ on testis development is unclear, but one proposed mechanism discussed at the meeting was through stimulation of cyclin-dependent kinase (CDK) inhibitors (Holsberger 2003). Increased Sertoli cell proliferation, similar to that seen in hypothyroid animals, was observed in p27^{KIP1} knockout mice. It is hypothesized (Holsberger 2003) that T₃ affects Sertoli cell maturation by regulating the expression of p27^{KIP1} which affects cell cycle control. Immunohistochemical studies show that p27 expression is low in rapidly proliferating cells, such as neonatal Sertoli cells or Sertoli cell tumors, and in hypothyroid animals. Comparatively, p27 expression is increased when Sertoli cells are not proliferating or the animals are hyperthyroid (Buzzard 2003). In other cell types, T₃ induces p27, inhibiting CDK2 activity, phosphorylation of retinoblastoma protein, and inhibition of DNA synthesis. Thus, increases in p27 expression as a result of high levels of T₃ are proposed to enable the cell to exit from the cell cycle at the G₁ restriction point.

Similar to PTU and MTZ, SMZ inhibits thyroid peroxidase activity. Rodent studies have shown that SMZ at very high doses produces adverse reproductive effects associated with increases in serum TSH. SMZ appears to be a weak rodent male reproductive tract toxicant at doses that produce significant effects on the thyroid gland. Studies with structurally similar chemicals (sulfonamides, sulfasalazine) also show reproductive effects at high doses in rodents, but these effects are not associated with an alteration in thyroid status. Thyroid peroxidase in primates and humans is insensitive to inhibition by sulfonamide. However, a reproductive epidemiology study of sulfonamide factory workers (Prasad et al. 1996) showed an increased number of abortions and decreased numbers of live births. There are conflicting data on the effects of sulfasalazine on adult male fertility, but decreases in sperm motility are commonly observed with normal sperm counts (Marmor 1995). Because there is no evidence for thyroid peroxidase inhibition or other antithyroid effects in humans from sulfonamide treatment, these reproductive

effects apparently are not mediated by the thyroid gland. Human reproductive health effects from relevant exposures to SMZ are predicted to be minimal or none.

PB is a teratogen, with pharmacologic properties similar to those of other anticonvulsant drugs. The thyroid effects of PB do not appear to be related to direct effects on thyroid gland, but may be related to altered hepatic metabolizing capabilities. PB alters thyroid hormone homeostasis in rats, rarely alters thyroid hormone homeostasis in mice, and can influence thyroid hormone levels in humans. In rats, PB is associated with increased glucuronidation by the liver, decreased T₄ levels, decreased T₃ at high doses, and increased TSH levels. In humans, PB is associated with liver enzyme induction and decreases T₄ levels without affecting T₃ or TSH levels.

Protocol Factors and Human Relevance

The adequacy of existing rodent toxicity testing models for assessing human thyroid health hazards was discussed. Under the current testing guidelines, T₃, T₄, and TSH levels may be determined in subchronic and chronic toxicity studies if an effect is suspected. Thyroid gland weights and histopathology often are reported. Developmental and reproductive toxicity testing guidelines do not specify testing for thyroid status. However, evaluation of thyroid weight at necropsy is recommended in some testing protocols for reproductive toxicity. There is currently no consensus on the most appropriate study design or end points to use in assessing effects on the thyroid system. Therefore, dose, timing, duration, and life stage of exposure are not completely addressed with current rodent protocols. These are important issues to consider because late *in utero* human exposures equate with early postnatal exposures in the rodent, but postnatal exposures are not adequately addressed in rodent protocols. Early changes in thyroid status may result in reproductive effects in adulthood. Furthermore, current drug screening protocols are designed to examine development through maturation of the offspring exposed *in utero*, but there is no requirement for transgenerational studies. Therefore, chemicals that affect reproductive performance via the thyroid or other targets in the offspring of treated animals would not be identified with current testing protocols.

The rat is the experimental animal most often used to identify chemical hazards. Compared with rabbits and mice, rats have a lower incidence of spontaneous malformations and are more able to maintain a normal pregnancy under testing conditions. Furthermore, the adult rat is an adequate size for obtaining multiple serum samples for total T₃, total T₄, and TSH determinations. Blood tests (spot tests) for thyroid hormone levels requiring

much smaller blood volumes are being developed. For some mechanistic studies, the mouse would be the species of choice, because transgenic, gene deletion, gene transfer, and targeted mutation mouse strains are available. RNA-mediated interference of gene expression (Carmell et al. 2003; Couzin 2002) also offers a promising method for studying the effects of altered levels of thyroid system proteins.

There are periods throughout neonatal development when changes in thyroid status can profoundly affect development of the organism. For a comprehensive protocol design, the timing and duration of exposures during periods of greatest vulnerability for the developmental end points studied need to be determined and taken into account, as well as appropriate end points for the life stage examined. Assessing thyroid hormone effects solely through serum chemistry has limitations. For example, the sensitivity of thyroid-hormone-mediated central nervous system events and other thyroid-sensitive end points to rodent and human serum thyroid hormone levels need careful assessment and chemical validation. Because the thyroid system is designed to maintain homeostasis within the organism, thyroid hormone serum levels may reflect physiologic changes only for a limited time relatively soon after exposure. Other manifestations of exposure, such as impaired reproductive capacity, may be evident only at later life stages.

A comprehensive screening protocol design should include end points that guide the direction of subsequent toxicity testing. Therefore, end points screening for thyroid effects need the flexibility to be included in current testing protocols and the ability to separate mild thyroid effects from other toxic effects. It was proposed that end points to assess thyroid status of the dam and offspring could be included in the two-generation reproductive toxicity study design. Incorporated into this study protocol would be screening of the progeny for thyroid-related effects. For example, affected pups may have low birth weights or a low rate of weight gain. If pups are exhibiting failure to thrive, the fetal thyroid gland could be examined to determine whether it is affected. If there is a need for further assessment of thyroid status, thyroid hormone levels in blood could be determined in the pregnant dam before parturition and then in the dam and pups at times after parturition. Depending on the protocol, the low blood volume of pups may limit postnatal serum thyroid hormone assessment. Thyroid gland tissue would be taken for histopathology evaluation. It was noted that in the rodent, thyroid gland histopathology is a more sensitive indicator of thyroid status than T_3 or T_4 serum hormone values. Hypertrophy of follicular cells in the thyroid gland can be observed with normal thyroid hormone values because

of a compensatory response by the rodent thyroid system. Ideally, toxicokinetic studies on the test chemical could be obtained before the start of the study, to determine appropriate time points for blood and tissue collection. Concentrations of the chemical in the dam and fetus could also be determined from blood samples.

Standard, as well as novel, end points for postnatal measurement of thyroid-related effects were discussed. Postnatal neurotoxicity assessments of rat pups could include acoustic startle response, which has a good rodent-human cross-species correlation. Body temperature regulation in newborn animals depends on thyroid hormones. Therefore, temperature regulation and/or shivering responses in pups are other end points that could be assessed. The above tests could be appended to existing reproductive toxicity protocols. Tissue assays that assess more specific neurologic end points of development were also discussed. In the cerebellum, migration and proliferation of developing granule cells are regulated at least partly by thyroid hormones. Therefore, assessment of external granule layer (EGL) migration, mitosis, and apoptosis in the cerebellum (assessed at postnatal days 5–8) may provide a well-defined developmental end point. Thyroid hormones also regulate rat retinogenesis. Mitotic indices in treatment groups can be determined in the retina during the perinatal period and compared with control groups. These assays would provide a means to identify a possible thyroid toxicant through a specific early developmental effect. Although no single one of these end points would clearly define a thyroid effect, effects on multiple end points may provide a good indication of thyroid dysfunction and lead to more definitive studies.

Summary

The relevance of thyroid-related reproductive and developmental effects in rodents for predicting similar effects in humans was summarized and discussed. Important similarities and differences between the rat and human were noted (Table 1), and this complex subject was addressed in detail in a background thyroid document prepared for this workshop. There was general agreement that to predict human health outcomes, a comparison of the magnitude of thyroid dysfunction resulting in adverse effects in various organs/tissues and between rodents and humans is needed.

The adequacy of existing rodent toxicity testing models for assessing human thyroid health hazards was discussed. Currently, developmental and reproductive toxicity testing guidelines do not specify testing for thyroid status. However, evaluation of thyroid gland weight is recommended in some reproductive protocols. For subchronic and chronic toxicity studies, thyroid gland weight

and histopathology may be assessed at necropsy, and evaluation of serum levels of T_3 , T_4 , and TSH is recommended if an effect is suspected. Current chemical testing methods do not adequately screen for thyroid perturbations that may affect reproduction and development of offspring. Therefore, it was proposed at this workshop that an assessment of thyroid status be included within reproductive and developmental toxicity testing protocols.

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