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# Topic 3.7

# Evaluation of thyroid function in neonatal and adult rats: The neglected endocrine mode of action*

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*Abstract*: Although known to regulate growth and development, cellular metabolism, the use of oxygen, and basal metabolic rate, thyroid hormones have been only minimally evaluated in neonatal rodents at critical times of development. Despite some modulation of metabolic rate by other hormones, such as testosterone, growth hormone, and norepinephrine, 3,5,3′-triiodothyronine (T3) and 3,5,3′,5′-tetraiodothyronine (T4) are the most important metabolic rate modulators. Endpoints used for thyroid function assessment in neonatal and adult rats include thyroid-stimulating hormone (TSH), T3, and T4 levels and histopathology. In rodents, decreased serum levels of T3 and T4 and increased serum TSH levels, with sustained release of TSH and resultant follicular cell hypertrophy/hyperplasia, are typical hormonal and histopathological findings attributable to compounds altering thyroid function. Hypothyroidism early in the neonatal period can affect reproductive endpoints in both male and female rats, with the critical period of exposure being the first two weeks postnatal. Hypothyroidism has been shown to reduce gonadotrophin levels and delay pubertal spermatogenesis in male rats and to block gonadotropin-induced first ovulation in immature female rats by decreasing FSH and luteinizing hormone (LH) serum concentrations. Inclusion of evaluations of TSH, T3, and T4 assays in multigeneration and developmental neurotoxicity protocols may assist in risk assessments.

## INTRODUCTION

Since the 1920s, hypothyroidism in infants has been noted as a cause of mental retardation. This endemic hypothyroidism resulted from absence of sufficient dietary iodine in the diet. Since that time, multiple causes of infant hypothyroidism have been identified. In order of frequency, these include endemic hypothyroidism (1:7), thyroid dysgenesis (1:4000), thyroid dyshormonogenesis (1:30 000), transient hypothyroidism (1:40 000), and anomalies of the hypothalamic-pituitary axis (1:100 000) [1].

In humans, hypothyroidism can affect brain function and eventually produce histopathological findings [2]. Permanent damage may be prevented by treatment with thyroid hormones immediately after birth, and, due to recent concern for loss of potential due to severe hypothyroidism at birth, treatment is often initiated in utero in suspected cases derived from ultrasound studies showing goiters and a maternal history of Graves' disease [2,3].

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## THYROID GLAND AND ITS FUNCTIONS

Histologically, the thyroid gland primarily consists of spherical follicles that surround colloid. In addition to these components, parafollicular cells are present between the thyroid follicles and secrete calcitonin [4].

As noted by Farwell and Braveman [5], the main function of the thyroid is to take iodine, generally from food, convert it to iodide, and uptake the iodide into the thyroid gland via the sodium-iodide symporter (NIS) system, which is stimulated by thyroid-stimulating hormone (TSH). Iodide is oxidized by thyroid peroxidase and yields the amino acid residues monoiodotyrosyl and diiodotyrosyl. These amino acid residues of thyroglobulin, a glycoprotein made by the cuboidal follicular cells [4], make up and are stored in the colloid. Two diiodotyrosyls, or one monoiodotyrosyl and one diiodotyrosyl, are combined via an oxidative reaction to form thyroxine [3,5,3′,5′-tetraiodothyronine (T4)] and 3,5,3′-triiodothyronine (T3). Approximately 80 % of the hormones secreted by the thyroid are T4; 99 % of secreted T4 is bound to plasma proteins [6]. After release into the bloodstream, T3 and T4 bind to albumin and prealbumin (transporting proteins) via noncovalent bonds and are distributed to peripheral tissues [7].

Thyroid hormones are transported throughout the body in the bloodstream by an acidic glycoprotein, thyroxine-binding globulin (TBG). This bound transport prevents the thyroid hormones from being metabolized and excreted. As shown on Fig. 1, once these hormones reach the peripheral tissues, T4 is converted to the active form of T3 and binds to the intracellular nuclear receptor, which, in turn, stimulates gene transcription [5,8]. The remaining T4, or free T4, is biologically active, exerts negative feedback (inhibition) on pituitary TSH secretion, and is capable of entering cells [9]. Once free T4 enters the cells, it is deiodinated to form T3 or rT3. T3 is generally being produced during normal circumstances, while reverse T3 (rT3) is generally produced during times of illness, starvation, or excessive endogenous catabolism [9].

Metabolism of T4 and T3 primarily occurs in the liver. However, local metabolism by target tissues, such as the brain, can also occur. T4 is metabolized in the liver by removal of the 5′ iodide, which yields T3, or by removal of the 5 iodide, which yields inactive T3 (rT3) [5]. Once in the liver, T3 and T4 are combined with glucuronic and sulfuric acids via the phenolic hydroxyl group and then are ex-

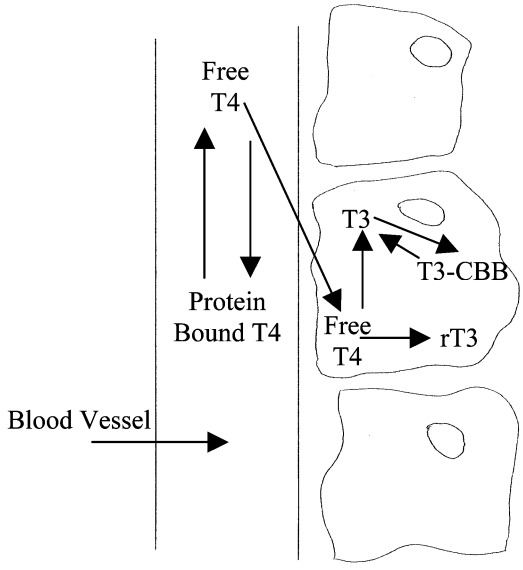


**Fig. 1** Cellular metabolism of T3 and T4 in the peripheral tissues (adapted from ref. [8]).

creted in the bile [5]. Thyroid hormones can also be metabolized locally by target tissues such as the brain [5].

The thyroid gland operates via a negative feedback mechanism and is regulated by the pituitary gland, which, in turn, is regulated by the hypothalamus gland [10]. As shown on Fig. 2, the hypothalamus gland releases thyroid-stimulating hormone releasing factor (TSHRF), which stimulates the pituitary gland to release TSH and subsequently stimulates the thyroid gland to release T3 and T4 into the bloodstream. When T3 and T4 levels are too low or too high, the hypothalamus and pituitary glands function to regulate production [10].

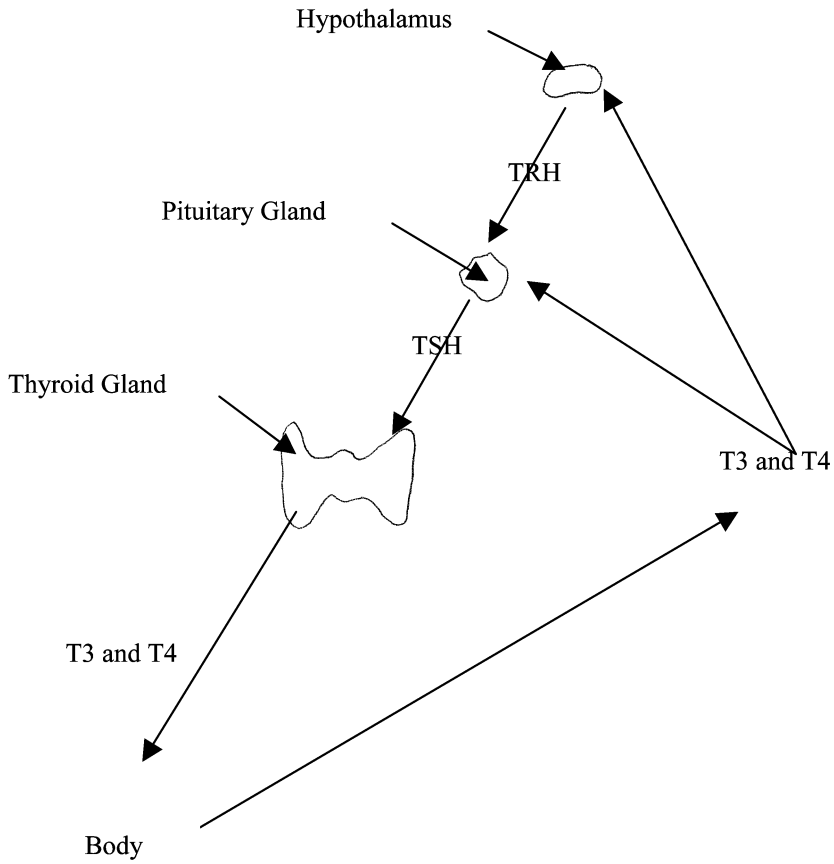


**Fig. 2** Negative feedback mechanism for the thyroid gland (adapted from ref. [10]).

## ROLE OF THE THYROID IN FETAL DEVELOPMENT

Porterfield and Hendrich [11] describe brain development relative to thyroid function in rats as divided into three phases. Phase I occurs on days 0 to 17 of gestation; during this phase, the fetus is solely dependent on maternal thyroid function. Phase II occurs from day 17 of gestation until birth, with the fetus dependent on maternal and fetal thyroid function (radioiodine levels can be detected in the thyroid follicle at day 17 of gestation; by day 20 of gestation, fetal rats can produce thyroid hormones). Phase III occurs postnatally, with the neonatal rat totally dependent on its own thyroid function.

Neuro-endocrine development interactions occur in utero in humans but during the first 2 to 3 weeks postnatal in rats [12,13]. At 10 days postpartum, the rat brain is equivalent in development to the human brain at birth [12]. Conversely, the human brain at 5 to 6 months of gestation is equivalent to the rat brain at birth [13]. Neurogenesis occurs in rats from day 12 of gestation through birth, at which

time cerebral neurogenesis is generally complete [14–16]. Gliogenesis is initiated at birth in rats and continues until adulthood, with the highest amount of glyosis occurring between days 10 to 45 postpartum [15]. Myelinogenesis and development of neuronal processes in rats occurs from birth to 30 days postpartum [14]. In contrast, neurogenesis in humans is generally completed by the seventh month of pregnancy, with gliogenesis initiated in utero [17,18].

Thyroid hormones are critical for normal neural development in both rats and humans. As demonstrated by Eayrs [19], thyroid deficiency requires early remediation for normal development in the rat, as in humans. The degree of cerebral impairment produced in rats was inversely related to the age at thyroidectomy. Thyroidectomy of weanling rats on or after day 25 postpartum did not adversely affect neural development. Withholding T3 and T4 hormone treatment until after day 24 postpartum resulted in irreversible effects on neural development, including a decrease in the number of cerebral vascular connections [20] and alterations of the negative feedback system [21].

In addition to adverse effects on brain development resulting from hypothyroidism, brain development can be affected by excess thyroid hormones. As noted by Best, Duncan, and Best [22], administration of excessive levels of thyroid hormones to neonatal rats over the first 2 weeks postnatal permanently impaired growth and resulted in a resetting of the activity level of the thyroid axis, with permanent reduction of pituitary and plasma TSH concentrations [21,23].

Most experimental data regarding the thyroid's effect on fetal development has been obtained in rodent and bovine models (rats and sheep, respectively) [24]. When interpreting this experimental data and correlating it to human data, it is important to compare the developmental stages of each species because thyroid hormones play a critical role in fetal growth, tissue differentiation, and metabolism. This role of thyroid hormones can be demonstrated by removing the thyroid gland from the fetus before birth. Such studies can be complicated to conduct and interpret because removal of the thyroid gland from the fetus as early as possible is not only difficult but confounded by the fetus' ability to utilize maternal T3 and T4 for growth.

The circulating half-life of maternal thyroid hormones is 12 to 24 h; the half-life of tissue-stored thyroid hormones is greater then 24 h, and a pool of protein-bound thyroid hormones is present that can last for several days [2]. Although iodine freely crosses the placenta and occurs at similar concentrations in the mother and fetus, not all chemicals and hormones are transported equally across the placenta. T4 and T3 cross the placenta but occur at higher concentration in the mother. TSH does not cross the placenta. TSHRF occurs at higher concentrations in the fetus than the mother [11].

### Growth

Hopkins and Thorburn [24] clearly demonstrated the in utero effects of thyroid hormones on growth in a thyroidectomized lamb model. Removal of the fetal thyroid between days 81 to 96 of gestation resulted in a malformed lamb with short limbs and a birth weight only 33 % of the normal body weight of a newborn lamb. In Long–Evans rats, postnatal daily subcutaneous administration of propylthiouracil (PTU), an agent used to decrease thyroid function, from birth until day 24 or 30 postpartum caused growth impairment, including reduced body weight and retarded brain growth [25].

### Bone development

Thyroid hormones have been demonstrated to affect bone development in fetal rats, including delays in ossification centers, reduced longitudinal bone growth, and retarded development of the bones of the skull [2].

### Sexual maturation

Puberty is the stage between the juvenile and adult stages when secondary sex characteristics and fertility develop. Puberty begins in the hypothalamic-pituitary-gonadal (HPG) axis [26].

#### *Males*

Sexual maturation in male rats is considered to occur in four phases: (1) neonatal, days 1 to 7 postpartum; (2) infantile, days 8 to 21 postpartum; (3) juvenile, days 22 to 35 postpartum; and (4) peripubertal, days 36 to 55/60 postpartum [27,28]. Additional landmarks of sexual maturation in male rats include testicular descent, at approximately day 15 postpartum [28], and preputial separation, at approximately day 45 postpartum [28,29].

The thyroid plays an essential role in male sexual maturation. Induced hypothyroidism, both chemically and via thyroidectomy, of male rats results in atrophy of the testes and secondary sex glands; reduced gonadotrophin, prolactin, and testosterone levels; and inhibition of gametogenesis and interstitial cell development [30,31]. In addition, T3 receptors have been recognized in Sertoli cells, a finding suggestive of T3's having a role in Sertoli cell function [32].

#### *Females*

Puberty in female rats is considered to occur at vaginal patency, which is present between days 30 to 37 postpartum [29,33] and is strain-dependent. Vaginal patency occurs approximately 8 days after late follicular growth resulting from the first ovulation [34].

Adverse affects of hypothyroidism on puberty in female rats include changes in folliculogenesis, formation of corpora lutea, irregular estrous cycles, and ovarian atrophy [6].

## RELEVANCE OF EVALUATING POTENTIAL EFFECTS OF ENDOCRINE DISRUPTORS ON THYROID FUNCTION

Xenobiotics may affect thyroid function by altering the biosynthesis, secretion, absorption, or metabolism of the thyroid hormones. Such agents are generally categorized as thioamides, aminoheterocyclic compounds, or substituted phenols [35]. Many pharmaceutical agents alter peripheral thyroid function, including calcium channel blockers, steroids, retinoids, chlorinated hydrocarbons, and polyhalogenated biphenyls [36]. Table 1 lists some of the agents that have been identified as altering thyroid function in rats and/or humans.

Because the thyroid has regulatory activity on essentially all tissues, thyroid function should be considered when determining the risk potential of an agent. Thus, the U.S. Environmental Protection Agency's (USEPA's) Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) included evaluation of thyroid function in its two-tiered screening and testing program for estrogenic, androgenic, and thyroid effects. Tier 1 tests, designed to be extremely sensitive and to minimize false negatives. These tests include a diverse range of organisms used to evaluate all types of metabolism and to detect all modes of action of the endocrine endpoints within a range of taxonomic groups that incorporate diverse endpoints. Proposed Tier 1 in vivo screens, including evaluation of thyroid function, are the rodent 20-day pubertal female assay, the 14-day intact adult male with thyroid assay, and the 20-day thyroid/pubertal assay. The Tier 2 screen, including thyroid evaluation, is the two-generation mammalian reproduction and development study. These EDSTAC testing guidelines are currently undergoing an extensive review and validation process by the Endocrine Disruptor Methods Validation Subcommittee (EDVMS).

**Table 1** Agents producing thyroid alterations in rats [7,48,49].

| | |
|---|---|
| **Environmental chemicals** | |
| Propylthiouracil (PTU) | Aroclor 1242 |
| Extracts of subsurface soil | Perchlorates |
| with 2.5 % PCBs | Brominated flame retardants |
| | Nitrofen |
| **Pharmaceuticals decreasing TSH function** | |
| Dopamine | Octreotide |
| Glucocorticoids | |
| **Pharmaceuticals altering thyroid hormone secretion** | |
| ***Reduced thyroid hormone secretion*** | |
| Lithium | Amiodarone |
| Iodide | Aminoglutethimide |
| ***Increased thyroid hormone secretion*** | |
| Iodide Amiodarone | |
| **Pharmaceuticals reducing T4 absorption** | |
| Colestipol | |
| Cholestyramine | Ferrous sulfate |
| Aluminum hydroxide | Sucralfate |
| **Pharmaceuticals altering T4 and T3 transport in serum** | |
| ***Increased serum TBG concentration*** | |
| Estrogens | Methadone |
| Tamoxifen | Mitotane |
| Heroin | Fluorouracil |
| ***Reduced serum thyroxine binding globulin concentration*** | |
| Androgens | Slow-release nicotinic acid |
| Anabolic seroides | Glucocorticoids |
| ***Displacement from protein binding sites*** | |
| Furosemide | Mefenamic acid |
| Fenclofanac | Salicylates |
| **Pharmaceuticals altering T4 and T3 metabolism** | |
| ***Increased hepatic metabolism*** | |
| Phenobarbital | Phenotoin |
| Rifampin | Carbamazepine |
| ***Decreased T4 5′-deiodinase activity*** | |
| Propylthiouracil | Beta-adrenergic-antagonist drugs |
| Amiodarone | Glucocorticoids |
| **Cytokines** | |
| Interferon alpha | Interleukin-2 |

## THYROID FUNCTION TESTS

Weights and histological evaluations of the thyroid are classically required for evaluation of thyroid function. Additional evaluations include biomarkers demonstrating effects on metabolism, thyroid function, and blood levels of these hormones.

In addition, interspecies differences in protein binding capabilities, metabolism, production, and sex-related differences should be considered. For example, TBG is present in humans but essentially absent in rats. The half-life of both T4 and T3 is remarkably longer in humans than in rats. Production of both T4 and TSH is much higher in rats than in humans, and rats also show a sex-related difference, with TSH levels in female rats approximately twice that observed in male rats. In addition to the sex-related differences, multiple other factors are also known to affect thyroid hormone levels in rats. These include common factors such as strain, sex, and age, and, in females, estrous cycling, circadian rhythms,

room temperature, stress associated blood collection techniques or animal handling, and the relative activity of the animal. Serum thyroid hormone radioimmunoassay (RIA) tests are currently available for measurement of T4, free T4, T3, free T3, rT3, and TSH concentrations.

Distinct differences exist between male and female rats with regard to TSH, T3, and T4 levels. TSH and T3 occur at higher concentrations in female rats than in male rats, while T4 occurs at higher concentrations in male rats than in female rats. Cycling female rats have a cycle-associated biorhythm for TSH, which peaks during proestrus. In rats, T4 has predominate binding to albumin, with lower amounts bound to prealbumin and postalbumin, and T3 bound only to albumin. This is because rats do not have TBG, which is the principal transport protein for T4 in man [37].

The first test developed to measure thyroid hormone levels was serum iodine concentration [9]. Thyroid function is now usually measured by comparing baseline serum thyroid hormone concentrations with levels attained after exposure to a xenobiotic.

Because most T4 and free T4 are present in the bloodstream, the easiest and least expensive way to assess thyroid function is to determine serum T4 concentration, the sum of T4 and free T4. This is now generally accomplished in the laboratory by use of RIA kits. RIA tests provide an evaluation of the competitive binding of free T4 with an isotope-labeled T4 to IgG molecules in the antiserum [9].

An alternate method for determining thyroid hormone levels is equilibrium dialysis, which is considered to be the "gold standard". However, the complexities of this method and its expense have eliminated it from common use since the development of the more sensitive RIA kits. Serum measurements of T3 and free T3 are not recommended because most T3 is stored in cells.

RIA kits developed for human use can be used across species to measure T4 and T3 levels. However, RIA kits for measuring TSH levels must be specific to the species tested, because human, rat, mouse, and rabbit TSH are not equivalent.

Manson et al. [38] published control thyroid hormone levels for Long–Evans sometimes hyphenated hooded pregnant female rats and their fetuses in a study in which Nitrofen, an herbicide similar in structure to thyroid hormones, significantly altered thyroid function. Amerlex RIA kits (Amersham Corporation, Arlington Heights, Illinois) were used for evaluation of plasma T3 and T4 levels, and a rat TSH assay kit, provided by the National Institutes of Health's Rat Pituitary Hormone Distribution Program (National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases [NIADDK]) was used for evaluation of plasma TSH. Further description of the methodology used by Manson et al. [38] follows.

Thyroidectomized nonpregnant female rats were assigned to an untreated (control) group or administered T4 or nitrofen orally (gavage) for 2 weeks, after which, blood samples were collected by cardiac puncture. As shown in Table 2, T4 levels obtained from control nonpregnant female rats (5.10 μg/100 ml ± 0.61) [38] were similar to those obtained by Lu and Staples [39] in this strain of rats (6.2 μg/100 ml). Treatment with 15 or 30 mg/kg/day of nitrofen suppressed T4 plasma levels and increased TSH levels, as compared to the sham-operated rats which were the expected findings for a negative feedback system.

**Table 2** Control data for T3, T4, and TSH levels in Long–Evans hooded nonpregnant female rats and DG 22 fetuses as maternal peak level [42].

| | Nonpregnant female rats | DG 22 fetuses |
|---|---|---|
| N | 6 | 5 (litters) |
| T3 levels (ug/100 ml) | 0.47 ± 0.06 | NA |
| T4 levels (ug/100 ml) | 5.10 ± 0.61 | 4.45 ± 0.55 |
| TSH levels (ng/ml) | 125 ± 26 | 365 ± 60 |

NA = Not available

TSH levels in pregnant rats were studied by obtaining a baseline blood sample by cardiac puncture on day of gestation (DG) 11 (sperm observed = DG 1) and subsequent blood samples 6 h after oral (gavage) treatment of the rats with corn oil (control), or nitrofen and on DGs 12, 14, 17, and 22. Blood samples for T3 and T4 evaluations were obtained via the tail vein from nonanesthetized pregnant rats before treatment with corn oil or nitrofen, at 2, 4, 6, and 8 h post-treatment on DGs 11, 12, 13, 14, 18, 20, and 22. DG 22 fetal blood samples were obtained via a neck incision, using heparin-coated capillary tubes. Fetal blood was pooled by litter for analysis for T3 and T4 levels.

The results of these studies by Manson et al. [38] demonstrated that, in Long–Evans hooded female rats, maternal TSH levels increased from DGs 11 to 17 and peaked at DG 22, while T4 levels were reduced from DGs 1 to 21 (data not shown), replicating results described by Fukuda et al., in which nitrofen treatment of Sprague–Dawley rats reduced plasma T4 levels in the maternal rats and in their fetuses [40]. Values for the nonpregnant female rats and DG 22 fetuses at the maternal peak level are presented in Table 2 [38].

Studies by Gray et al. [41,42] demonstrated that nitrofen also affects thyroid function in CD-1 mice. In a study in which nitrofen was administered to pregnant CD-1 mice on days 7 to 17 of gestation, oral (gavage) dosages of 6.25 to 100 mg/kg/day reduced the weight of the Harderian gland, and the Harderian glands were absent or visibly reduced in size in groups given 25, 50, and 100 mg/kg/day. Dosages off 12.5 to 100 mg/kg/day retarded pup growth rates, and groups given 150 and 200 mg/kg/day dosages had no surviving pups by day 3 postpartum. In groups with surviving pups, lung and liver weights were reduced (≤6.25 mg/kg/day), seminal vesicle weights were reduced (≤12.5 mg/kg/day) and testes weights were reduced (100 mg/kg/day). Puberty was delayed in the offspring in the 50 and 100 mg/kg/day dosage groups [41]. In the study by Gray in which mice were administered 500 and 1000 mg/kg/day of nitrofen for 3 consecutive days, statistically significant reductions in serum T4 levels in both treatment groups were observed. Control values for serum T3 and T4 levels for these mice were 43.2 ng/dl and 2.15 ng/dl, respectively.

## THYROID HORMONES AND THEIR DEVELOPMENT IN RATS

Little information regarding age-related differences in thyroid hormone levels in rats is provided in the literature.

In a study of Sprague–Dawley male rats, serum TSH levels generally increased on days 30 to 50 postpartum, with a subsequent decrease by day 60 postpartum [43], as shown on Fig. 3. Trends for T3 and T4 levels in neonatal male rats were not noted. In Sprague–Dawley female rats, TSH levels were reported to have an initial peak during the first 2 weeks postnatal [44] with a second elevation on days 40 and 50 postnatal, followed by a decline on days 60 to 80 postnatal [43]. T3 and T4 serum levels are reported to be low at birth in female rats, with the T4 level increasing on days 4 through 16 postpartum, and the T3 level increasing on days 10 to 30 postpartum [44,45], as shown on Fig. 4.

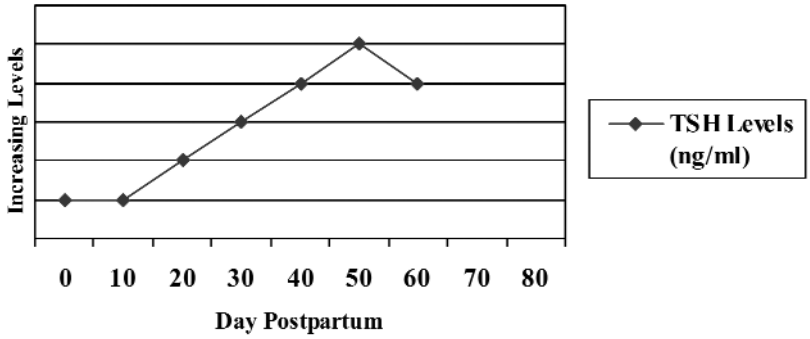


**Fig. 3** Serum TSH levels obtained from Sprague–Dawley male rats following birth (adapted from ref. [43]).

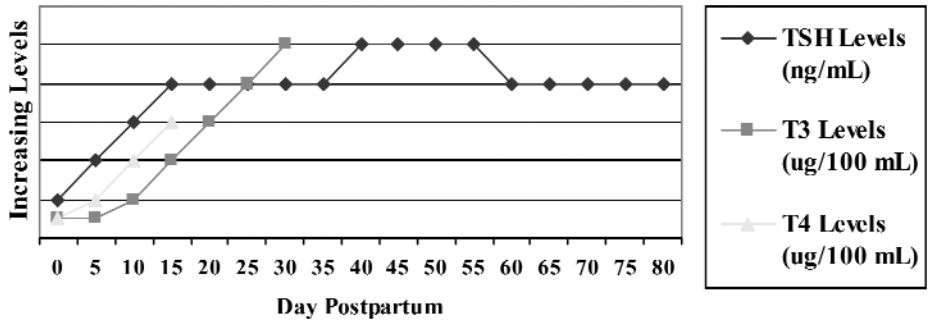


**Fig. 4** Serum TSH, T3, and T4 levels obtained from female Sprague–Dawley rats following birth (adapted from refs. [44,45]).

## METHODS

The thyroid hormone values that are the subject of this publication are control values for Charles River Crl:CD®BR VAF/Plus® (Sprague–Dawley) rats used in several different EPA define- (GLP-) compliant studies [46,47]. These include a two-generation study (Study A), a hormone evaluation study (Study B), and a neurobehavioral developmental toxicity study (Study C). The vehicle (control article) used in each of these studies was reverse osmosis processed deionized water (R.O. water); in each study, the test substance was provided to the rats in the drinking water. All blood samples were obtained from the inferior vena cava; obtained serum was allocated into three vials, immediately frozen on dry ice, and subsequently analyzed for serum T3, T4, and TSH levels, using either RIA kits from Diagnostic Product Corporation (T3 and T4) or a double antibody RIA procedure developed by Analytics Inc., Gaithersburg, Maryland. The thyroid/parathyroid glands were weighed after fixation in neutral buffered 10 % formalin for at least 48 h. Additional pertinent information regarding these study designs is presented in Table 2.

### Study A

At weaning on lactation day (DL) 21, 30 rats per sex per dosage group were randomly selected for continued analysis of potential behavioral changes. This cohort was not evaluated for thyroid function. Of the remaining pups, at least three pups/sex/litter were necropsied and examined for gross lesions; blood samples were pooled by sex and litter. After fixation in neutral buffered 10 % formalin, the thyroids/parathyroids were weighed, trimmed, and retained for histopathological evaluation.

### Study B

This study had three subsets: (1) DG 21 fetuses; (2) DL 10 pups; and (3) DL 22 pups. Fetuses or pups were weighed and examined for sex and gross external alterations. Serum was pooled by litter (DG 21 fetuses and DL 10 pups) and by sex and litter (DL 22 pups). The thyroid and brain were retained from each subset for morphometric and histopathological evaluations. Table 3 describes additional methodology pertaining to the fetal evaluations.

**Table 3** Summary of study designs in which thyroid function was evaluated in adult Sprague–Dawley rats.

| Parameter | Study A[a] | Study B[a] | Study C[a] |
|---|---|---|---|
| N | 30/sex/group | 15/16 per group | 25/group |
| Treatment period | 70 days prior to cohabitation until sacrifice | 14 days prior to cohabitation until sacrifice | DG 0 until DL 10 |
| Method of sacrifice | Exsanguination following $CO_2$ anesthetization | Exsanguination following $CO_2$ anesthetization | $CO_2$ asphyxiation |
| Day of sacrifice | DL 21 | DG 21, DL 10 or DL 22 | DL 10 |

[a]See following text for fetal analyses.
DG—Day of gestation.
DL—Day of lactation.

### Study C

All pups not selected for continued observation were sacrificed via carbon dioxide asphyxiation and necropsied on day 5 postpartum (DP 5). Blood samples were pooled by litter. The thyroids were fixed in situ in neutral buffered 10 % formalin before histological examination. On DPs 90 to 92, blood was obtained from the inferior vena cava of one F1 generation rat per sex per litter in each dosage group after sacrifice, and serum samples were evaluated for thyroid hormone levels.

## RESULTS

Data obtained from control animals are presented in Tables 4 through 7. In each study, statistically significant, dosage-dependent effects on thyroid function were observed; only hormone levels will be described. These effects were detected by serum analyses of T3, T4, and TSH levels made using RIA kits. Study A (York et al.) [48] demonstrated that exposure of rats to ammonium perchlorate in the drinking water at doses of 0.3, 3.0, and 30.0 mg/kg/day significantly increased relative thyroid weights in the female rats in each ammonium perchlorate-exposed group and in the male rats in the 3.0 and 30.0 mg/kg/day dosage groups. Dosage-dependent increases in thyroid hypertrophy and hyperplasia were also evident, and statistically significant differences in TSH, T3, and T4 levels were present in the 30 mg/kg/day dosage group. Further information regarding the results obtained for studies B and C are proprietary and not currently available.

Table 4 shows that control T3, T4, and TSH levels obtained from adult male rats in two studies were comparable, although the younger male rats (21–23 weeks, 147–161 days of age) had slightly lower T4 levels and slightly higher T3 and TSH levels than the older male rats (26 weeks, 182 days, of age).

**Table 4** Summary of subsets in Study B in which thyroid function was evaluated in Sprague–Dawley fetal and neonatal rats.

| Subset | 1 | 2 | | 3 | |
|---|---|---|---|---|---|
| Day sacrificed | DG 21 | DL 5 | DL 10 | DL 5 | DL 22 |
| N | 16 (T3 = 8) | 12–16 | 16 | 15 (T3 = 11) | 15/sex |
| Method of sacrifice | Beuthanasia®-D special[a] (histo.) Decapitation (thyroid hormone) | Exsanguination following $CO_2$ anesthetization | | $CO_2$ asphyxiation | |
| Blood sampling | Decapitation | Cardiac puncture | | Cardiac puncture | |
| Sample pooling | By litter | By litter | By litter | By litter | By litter and sex |

[a]Manufactured by Schering-Plough Animal Health, Union, New Jersey.

Table 5 shows control data for adult female rats on DG 21, DL 10, or DLs 21/22. Generally, T3 levels were increased on DG 21 and DL 10, as compared with DLs 21/22 values. TSH values were generally decreased on DLs 21/22, as compared with DG 21 or DL 10 values. T4 values were similar on DG 21 and DLs 21/22 and increased on DL 10.

**Table 5** Control data for T3, T4 and TSH levels in Sprague–Dawley adult male rats.

| Study | A | A |
|---|---|---|
| Age | 21–23 wks | 26 wks |
| N | 30 | 29 |
| T3 Levels (ng/dl)[a] | 82.5 ± 8.7 | 72.5 ± 11.2 |
| T4 Levels (ug/dl)[a] | 3.8 ± 0.5 | 4.6 ± 0.5 |
| TSH Levels (ng/ml)[a] | 2.5 ± 1.0 | 1.5 ± 1.0 |

[a]Data provided as means and standard deviations.

Table 6 shows control data for fetuses on DG 21 and for pups on DLs 5 and 10. Table 7 shows control data for male and female pups on DLs 21/22. T3 and T4 levels tended to increase from DG 21 to DL 10, while TSH levels were highest on DG 21 and lowest on DL 5. T3 and T4 levels on DLs 21/22 were generally increased, as compared to those obtained from fetuses on DGs 21 or pups on DLs 5 and 10. TSH levels were generally decreased for male and female pups on DLs 21/22, as compared to fetal values on DGs 21 or values for pups on DLs 5 and 10.

**Table 6** Control data for T3, T4, and TSH levels in Sprague–Dawley adult female rats.

| Study | B | B | C | A | A | B |
|---|---|---|---|---|---|---|
| DG/DL | DG 21 | DL 10 | DL 10 | DL 21 | DL 21 | DL 22 |
| Age | 16 wks | 18 wks | 16 wks | 23–24 wks | 20–22 wks | 19 wks |
| N | 16 (T3 = 15) | 16 | 3 | 30 | 30 | 15 |
| T3 Levels (ng/dl)[a] | 99.7 ± 6.0 | 99.9 ± 10.9 | 79.0 ± 2.2 | 57.8 ± 28.2 | 61.5 ± 25.2 | 120.3 ± 12.4 |
| T4 Levels (ug/dl)[a] | 2.3 ± 0.1 | 4.0 ± 0.4 | 3.0 ± 0.1 | 2.1 ± 0.7 | 2.2 ± 1.0 | 3.5 ± 0.4 |
| TSH Levels (ng/ml)[a] | 6.0 ± 0.6 | 5.1 ± 0.5 | 5.0 ± 0.3 | 2.0 ± 0.9 | 1.6 ± 1.0 | 6.9 ± 0.7 |

[a]Data provided as means and standard deviations.

**Table 7** Control data for T3, T4, and TSH levels in Sprague–Dawley fetuses.

| Study | B | B | B |
|---|---|---|---|
| DG/DL | DG 21 | DL 5 | DL 10 |
| M/F | M/F | M/F | M/F |
| N | 16 (T3 = 8) | [a] | 16 |
| T3 Levels (ng/dl)[b] | 23.4 ± 3.3 | 62.1 ± 6.6 (15) | 79.5 ± 6.6 |
| T4 Levels (ug/dl)[b] | 1.6 ± 0.2 | 2.1 ± 0.2 (12) | 3.7 ± 0.4 |
| TSH Levels (ng/ml)[b] | 7.2 ± 0.7 | 4.9 ± 0.5 (16) | 6.6 ± 0.7 |

[a]N value listed in parentheses following the value.
[b]Data provided as means and standard deviations.

**Table 8** Control data for T3, T4, and TSH levels in day 21/22 postpartum Sprague–Dawley pups.

| Study | A | A | B | A | A | B |
|---|---|---|---|---|---|---|
| Male/female | Male | Male | Male | Female | Female | Female |
| N | 27 | 20 | 15 | 28 | 20 | 15 |
| T3 Levels (ng/dl)[a] | 105.9 ± 10.0 | 106.3 ± 18.3 | 191.0 ± 20.0 | 106.0 ± 13.1 | 108.4 ± 21.1 | 169.3 ± 15.6 |
| T4 Levels (ug/dl)[a] | 4.4 ± 1.0 | 3.2 ± 0.8 | 4.0 ± 0.5 | 4.3 ± 1.0 | 3.4 ± 0.7 | 3.6 ± 0.4 |
| TSH Levels (ng/ml)[a] | 1.2 ± 0.4 | 0.8 ± 0.2 | 3.6 ± 0.4 | 1.1 ± 0.5 | 0.9 ± 0.3 | 5.3 ± 0.5 |

[a]Data provided as means and standard deviations.

Follicular cell hypertrophy/hyperplasia are typical hormonal and histopathological findings attributable to agents altering thyroid function. These findings occurred in the treated groups in these studies, as described previously. All control instances of these findings were minimal, mild, or moderate (data not shown). No control rats had marked hypertrophy and hyperplasia of the follicular epithelium.

Control data obtained for the two generations of adult Sprague–Dawley male rats were similar and within the ranges previously identified for Sprague–Dawley male rats.

## DISCUSSION

In adult female rats, T3 levels during gestation and the early period of lactation were generally above the range observed for the adult male rats, as would be expected, based on the sex-related difference. T3 levels decreased as the lactation period continued. T4 values were generally lower than the range observed for the male rats. TSH levels were relatively variable in the female rats but were generally higher than the values for the male rats.

Male and female fetuses and pups showed the expected increase in T3 values with age, reaching maximum levels that were higher than adult levels by day 21 postnatal. T4 levels showed a similar pattern of gradual increase. As would be expected, TSH levels decreased as the rats aged, and were lower than adult levels by day 21 postnatal.

## CONCLUSION

Minimal historical experience exists in evaluation of thyroid hormone levels in pregnant rats and their offspring in regulatory compliant studies. Although relatively small in number, the results obtained for CRL Sprague–Dawley rats in USEPA-design multigeneration and developmental neurotoxicity studies conducted at our laboratory indicate that the use of RIA kits for analyses for serum TSH, T4, and T3 levels in fetal, neonatal, juvenile, and adult rats provides a relatively consistent, sensitive, and appropriate biomarker for detecting functional changes in the thyroid.

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