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## APPROVAL PACKAGE FOR:

## APPLICATION NUMBER
## NDA 21-402

# Pharmacology Review(s)

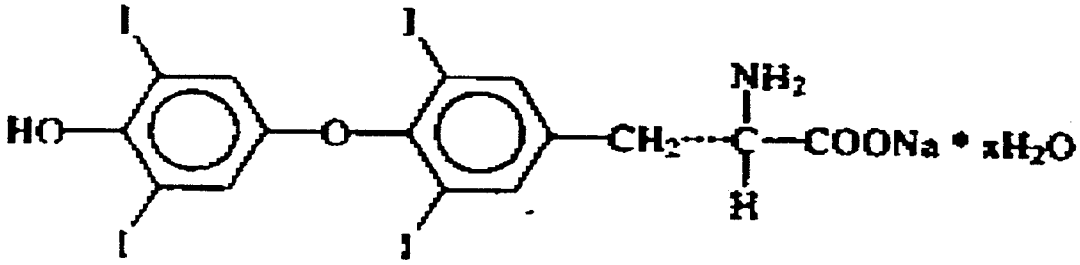
# *PHARMACOLOGY/TOXICOLOGY COVER SHEET*

NDA 21-402
Review number: 1
Sequence number/date/type of submission: original, 8/02/01
Information to sponsor: Yes ( ) No ( X)
Sponsor and/or agent: Abbott Laboratories; Abbott Park, Ill
Manufacturer for drug substance : same

Reviewer name: Karen Davis-Bruno; Ph.D.
Division name: DMEDP
HFD #: 510
Review completion date: 4/23/02

Drug:

Trade name: Synthroid
Generic name (list alphabetically): levothyroxine sodium
Code name: T4; thyroxine
Chemical name: 3,5, 3',5'-tetraiodothyronine sodium
Molecular formula/molecular weight: 798.86 g/mol; $C_{15}H_{10}I_4NNaO_4 \bullet H_2O$
Structure:




Relevant INDs/NDAs/DMFs: IND — NDAs: 21-116, — 21-210, 21-292, 21-301
Drug class: synthetic thyroid hormone
Indication: replacement or supplemental therapy for hypothyroidism

Clinical formulation: tablets of 25, 50, 75, 88, 100, 112, 125, 137, 150 ,175, 200 300 mcg
Inactive ingredients acacia, confectioner's sugar, lactose, magnesium stearate, providone, talc and color additives to distinguish tablet strength —

[ — — ]

Route of administration: oral
Proposed use: see indication
Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

## *Executive Summary*

I. Recommendations

A. Recommendation on Approvability-Pharmacology/Toxicology recommends approval

B. Recommendation for Nonclinical Studies-Levothyroxine has been marketed extensively for years as a tablet and injectable form. The replacement indication for naturally occurring thyroid hormone indicates little safety concern. Under a Federal Register notice of August 14, 1997 (Volume 62, Number 157) current products were considered mislabeled as of August 2001 in the absence of an approved NDA and removed from the market. The prior lack of stability and batch to batch variability in these products as a class were the impetus for this legislation. Potential problems may arise from inappropriate dosing or manufacturing impurities/degradation products. The sponsor has performed a one month rat toxicity study to evaluate potential impurities/degradants in an expired drug lot compared to a newly manufactured lot. Prior experience suggests that proper monitoring can minimize the associated safety risk.

C. Recommendations on Labeling- the draft labeling represents class labeling and is adequate

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: The toxicity profile of levothyroxine has been well documented in published literature and represents an extension of its pharmacologic activity.

B. Pharmacologic Activity: Levothyroxine is indicated as replacement therapy for hypothyroidism it is a synthetic form of thyroxine. Thyroid hormones (thyroxine, triiodothyronine) maintain metabolic homeostasis by interaction at both nuclear and non-nuclear receptors. Circulating levels of thyroid hromones are controlled by feedback inhibition to the hypothalamic-pituitary axis in response to TSH which is secreted from the anterior pituitary in response to thyrotropin releasing hormone.

C. Nonclinical Safety Issues Relevant to Clinical Use: none provided proper individualized replacement dosing is performed with adequate monitoring and appropriate product stability is demosntrated.

III. Administrative

A. Reviewer signature: _____

## *TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW*

# *PHARMACOLOGY/TOXICOLOGY REVIEW*

## I. PHARMACOLOGY:

Primary pharmacodynamics: The thyroid hormones thyroxine (3,5, 3',5'-tetraiodothyronine or T4) and triiodothyronine (3, 3',5'-triiodothyronine or T3) are crucial for development. They act via stimulation of growth hormone synthesis. In adults thyroid hormones maintain metabolic homeostasis. Circulating levels of thyroid hormones are controlled by feedback inhibition to the hypothalamic-pituitary axis in response to TSH, which is secreted from the anterior pituitary in response to thyrotropin-releasing hormone.

Mechanism of action: Thyroid hormones exert their functions by interaction at both nuclear and non-nuclear receptors. Triiodothyronine binds to high affinity nuclear receptors at specific promoter regions of specific genes. Levothyroxine binds to the same nuclear receptors but at a much lower affinity. T3 is at least 5X more potent than levothyroxine (T4). Receptor independent interactions include increased calcium uptake, increased opening of sodium channels and changes in cytoskeletal structure. The complexity of thyroid hormone actions is evident by the coordination of gene activation actions with that of other hormone systems such as glucocorticoids, growth hormone and IGF-1. Thyroid hormones have effects on growth and development, brain development, calorigenesis, thermogenesis, bone development and cardiovascular development and function.

Drug activity related to proposed indication: Levothyroxine is indicated for replacement therapy in hypothyroidism.

Secondary pharmacodynamics: Levothyroxine has the potential to increase bone formation and resorption. Sprague-Dawley rats, 50 days old were castrated and given IP levothyroxine at 200 µg/kg for 3 weeks which resulted in decreased BMD in the femur by 10% without an effect on vertebrae. The effect of hypothyroidism (thyroidectomy) and excess levothyroxine (60-80 µg/kg/day) on serum calcium concentrations was investigated in Wistar rats for 35 days. Thyroidectomy resulted in a 30% decrease in serum calcium whereas levothyroxine treatment of of thyroidectomized rats increased serum calcium by 43%. This is consistent with the effects of levothyroxine on femur bone mineral density. The effect of prolonged hypo- and hyperthyroidism have been investigated in the rat. At the end of 20 weeks treatment with 200 µg/kg/day of levothyroxine, femur BMD was decreased ~6% compared to controls. Failure to observe large effects on BMD was not due to a lack of increase in serum concentrations of T3 or levothyroxine as these hormones increased by 70% and 75% respectively and serum TSH was reduced by 53%. Messenger RNA for femur osteocalcin, osteopontin and alkaline phosphatase and tartrate resistant acid phosphatase were all increased by 2-3 fold. These factors were not increased in the lumbar vertebra examined.

## II. SAFETY PHARMACOLOGY:

**Cardiovascular effects:** Thyroid hormones can effect the expression of genes encoding isoforms of the heavy chains of myosin. T3 increases the expression of α gene and decreases the expression of β-gene. T3 up regulates the gene encoding for a myosin calcium ATPase needed

for myocardial contraction. Both T3 and T4 caused cardiac enlargement in a variety of species. In rat, cardiac enlargement was due to hypertrophy6. Tachycardia along with myocardial round cell infiltration and muscle cell necrosis were observed.

Levothyroxine and triiodothyronine have been shown to increase connexin 43 protein and mRNA which correlates with an increase in gap junctions. Based on the cardiac arrhythmia and excitability that may occur due to excess cardiac gap junctions this may be an explanation for the atrial arrhythmogenic activity of thyroid hormones.

## III. PHARMACOKINETICS/TOXICOKINETICS:

**Absorption:** Levothyroxine is absorbed from the gastrointestinal tract and is highly protein bound in the blood. Bioavailability was assessed by determining the fecal and urine content of radioactivity following oral or IP administration of $^{131}$I-levothyroxine to male rats (0.014-0.042 µg). Assuming 100% of radioactivity was absorbed following IP administration ~60% bioavailability was achieved with oral administration. From 20-50% of injected radioactivity for levothyroxine and 20-40% triiodothyronine was absorbed from the rat intestine. The greatest absorption of levothyroxine occurred in the cecum, colon and rectum. The overall absorption of triiodothyronine tended to be lower and less segment dependent .

**Distribution:** Intravenously administered triiodothyronine has greater blood brain barrier penetrability (1.7% at 6 h postdose) than levothyroxine (0.6% at 6 h post dose). The time to reach equilibrium was 90 minutes for triiodothyronine compared to 300 minutes for levothyroxine. Similarly following intrathecal administration; brain triiodothyronine was 2% versus administered levothyroxine of 5.6% in serum. Transthyretin (thyroid hormone prealbumin) is a thyroid hormone carrier protein produced in the choroid plexus and liver. Levothyroxine has a higher affinity for transthyretin than triiodothyronine. Levothyroxine is also extensively bound to albumin with less than 0.1% of plasma levels existing free.

The largest extrathyroidal pool of levothyroxine and triiodothyronine was the intestine accounting for 18.1% and 33.1% respectively. The liver and kidney contained 8.72% and 1.01% of the total extrathyroidal pool of levothyroxine respectively and 8% and 2.3% respectively of the total extra thyroidal pool of triiodothyronine. Whole blood contained 31.2% levothyroxine and 3.6% of the triiodothyronine extrathyroidal pool.

The contribution of local conversion of levothyroxine to triiodothyronine in maintaining local tissue concentrations was significant in the CNS (66% cerebral cortex), liver, thyroid, lymph node, thymus (19%), prostate, testis (29%), anterior pituitary and brown adipose fat (27%) of euthyroid rats. The contribution of local conversion of levothyroxine to triiodothyronine in hypothyroid rats was 75% for the cerebral cortex, 31% thymus, 43% testes, 65% brown adipose and 15% liver. The concentration of triiodothyronine decreased in all the tissues in hypothyroid rats but the greatest decrease was in the liver suggesting that the conversion of levothyroxine to triiodothyronine is important in maintaining tissue levels of the later especially in hepatic tissue.

**Metabolism:** Metabolism by sequential mondeiodination in the periphery accounts for 80% of circulating triiodothyronine with the remainder secreted from the thyroid gland. The major site of conversion of thyroxine to triiodothyronine outside of the thyroid is the liver which allows for a homeostatic balance between the two hormones when levothyroxine is administered to hypothyroid patients. Most peripheral tissues utilize triiodothyronine derived from circulating

hormone with the exception of the brain and pituitary where local generation of triiodothyronine is the major source.

The major route of metabolism for levothyroxine apart from deiodinative degradation is by glucuronidation, with biliary excretion being the primary route of elimination of both conjugated and nonconjugated levothyroxine. 21.3% of levothyroxine is converted to triiodothyronine via deiodinase activity in the periphery (liver, kidney, thyroid). It has been suggested that in diseases of the thyroid involving hyperproliferation, hypervascularity and hyperrsecretion such as Graves disease, the contribution of the deiodinase activity of the thyroid may be significant in the generation of peripheral triiodothyronine tissue concentrations. Small amounts of deaminated metabolites; tetraiodothyroacetic acid and triiodothyroacetic acid were found in intestine. Following triiodothyronine infusion small amounts of triiodothyroacetic acid and diiodothyroacetic acid were found in the intestine, only a trace amount of residual triiodothyroacetic acid was found. Both levothyroxine and triiodothyronine can be glucuronidated , however this is not a major metabolic pathway for triiodothyronine.

**Excretion:** In euthyroid rats approximately equal amounts of $^{131}I$ was recovered in stool and urine. Significant fecal excretion following IP administration of radiolabeled levothyroxine was indicative of biliary excretion of parent or an iodine containing metabolite. The levothyroxine glucuronide was excreted in bile. Studies suggest that glucuronidation of levothyroxine is saturable and non-conjugated levothyroxine can be excreted without conjugation.

**PK/TK conclusions:** Plasma and tissue levels of levothyroxine and triiodothyronine are controlled by metabolic, excretory and tissue uptake processes.

## IV. GENERAL TOXICOLOGY:

A one-month bridging study from the current manufacturing process to expired lots of previously manufactured levothyroxine was performed. Synthroid has been extensively used in humans.

**One Month Oral Safety Study of Levothyroxine Sodium Formulations in Sprague-Dawley Rats**

**Key study findings:**

- Findings in levothyroxine treatment groups (160 mcg/kg/day; 944 mcg/M$^2$ current or expired lots) given at 5X the maximum recommended daily human dose (300 mcg, 185 mg/M$^2$ ) adjusted for body surface area were similar. In the expired lot 3/10 males had focal microcalculus in the collecting tubules of the papilla compared to 1/10 in the newer lot. The expired lot treated group had 2/10 rats with testicular degeneration which accounts for the epididymal sperm effects seen.

- ——— was present at —— in the non-expired lot and —— in the end of shelf life material.

**Study no: TA01-166**
**Volume #, and page #:** N00BP, vol 1. Pg. 2

**Conducting laboratory and location:** Abbott
**Date of study initiation:** 11/12/01-3/14/02
**GLP compliance: yes**
**QA report:** yes ( X ) no ( )
**Drug, lot # and % purity:** Lot 0000265189; expired lot 3000076
**Formulation/vehicle:** 0.2% hydroxypropyl methylcellulose tablets dissolved in oral suspension

**Methods** (unique aspects):
**Dosing:**

Species/strain: Sprague Dawley rat
#/sex/group or time point (main study): 10
Satellite groups used for toxicokinetics: 5
Age: 6 weeks
Weight: 148-231 g
Doses in administered units: 0, 60, 160 mcg/kg/day
Route, form, volume, and infusion rate: —— , 10 ml/kg

**Observations and times:**

Clinical signs: before and after daily dosing detailed observations of physical condition and behavior were recorded 1-2 h post dose about 3X weekly
Body weights: 2x weekly
Food consumption: weekly
Ophthalmoscopy: pretreatment and at study termination
EKG: not performed
Hematology: via abdominal vein at study termination
Clinical chemistry: via abdominal vein at study termination
Urinalysis: Day 21 male and Day 22 female under fasted (also no water) for 4h
Gross pathology: at necropsy
Organs weighed: at necropsy see histopath list
Histopathology: at necropsy see histopath list
Toxicokinetics: orbital plexus of $CO_2/O_2$ anesthetized satellite rats at 1, 2, 3, 6, 24h post dose on Day 28.

**Results:**

Mortality: none
Clinical signs: unremarkable
Body weights: unremarkable
Food consumption: Increased food consumption in all drug treated females and males dosed with 160 mcg/kg/day from non-expired tablets. This is an expected pharmacologic effect of the drug. However it is interesting that the expired drug treated groups did not show this effect despite reported potency of 296 mcg/tablet compared to 299.7 mcg/tablet of the nonexpired lot. Potency was determined at study initiation.
Ophthalmoscopy: unremarkable
Hematology: Mean reticulocyte count for males given 160 mcg/kg/day (233±28, 241±29 10E3/µl, expired, non-expired respectively) were statistically higher than the corresponding controls (172±15 10E3/µl). The sponsor considers these within an acceptable normal range

Clinical chemistry: An expected decrease in TSH levels for both sexes at all drug dose levels. A corresponding increase in thyroid colloid accumulation was observed in drug treated rats compared to controls. Both T3 and T4 levels were statistically significantly increased in all drug treatment groups as expected.

| | 0 | | 60 µg/kg/day | | 160 µg/kg/day expired lot | | 160 µg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| BUN (mg/dl) | 15.4±2.1 | 16±2.5 | 15±1.5 | 13.8*±1.5 | 14.9±2.2 | 12.2*±1.7 | 13.1±2.1 | 13.5*±1.5 |
| Creatinine (mg/dl) | 0.49±0.06 | 0.5±0.05 | 0.47±0.05 | 0.46±0.05 | 0.46±0.05 | 0.42*±0.04 | 0.45±0.05 | 0.44*±0.05 |
| Na (mmol/l) | 146.7±1.3 | 145.1±1.8 | 147.5±0.9 | 146.2±1 | 148±1.7 | 145.8±1.4 | 149.1±1.1 | 147.2±2.3 |

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# Table 14. Hormone Levels - Males

| Treatment Group | Statistic | Serum T3 (ng/ml) | Serum T4 (µg/dl) | Serum TSH (ng/ml) |
|---|---|---|---|---|
| T0: 0 µg LT4/kg | Mean | 0.313 | 2.703 | 2.069 |
| | SD | 0.098 | 1.045 | 0.745 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| T1: 60 µg LT4/kg Expired | Mean | 0.258 | 8.063 | 0.614 |
| | SD | 0.022 | 1.099 | 0.386 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| T2: 160 µg LT4/kg Expired | Mean | 0.706 | 11.241 | 0.280 |
| | SD | 0.155 | 2.322 | 0.070 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| T3: 160 µg LT4/kg Non-expired | Mean | 0.916 | 11.096 | 0.265 |
| | SD | 0.271 | 1.052 | 0.062 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| Results of Statistical Analysis: T2 vs. T3 | | NS | NS | NS |
| T0 vs. T1, T2, and T3 | | 0<2,3 | 0<1,3,2 | 3,2,1<0 |

Notes: (1) Serum T3; 3, 3', 5'-triiodothyronine; Serum T4: levothyroxine; Serum TSH: thyroid-stimulating hormone.
(2) Expired refers to Lot 3000076; Non-expired refers to Lot 0000265189.
(3) The symbols "0", "1", "2", and "3" have been used as abbreviations for treatment groups T0, T1, T2, and T3 in the presentation of the Results of Statistical Analysis.

# Table 15. Hormone Levels - Females

| Treatment Group | Statistic | Serum T3 (ng/ml) | Serum T4 (µg/dl) | Serum TSH (ng/ml) |
|---|---|---|---|---|
| T0: 0 µg LT4/kg | Mean | 0.285 | 1.016 | 1.055 |
| | SD | 0.064 | 0.051 | 0.452 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| T1: 80 µg LT4/kg Expired | Mean | 0.265 | 4.165 | 0.316 |
| | SD | 0.048 | 0.985 | 0.064 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| T2: 160 µg LT4/kg Expired | Mean | 0.526 | 5.936 | 0.229 |
| | SD | 0.141 | 1.886 | 0.069 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| T3: 160 µg LT4/kg Non-expired | Mean | 0.482 | 5.271 | 0.300 |
| | SD | 0.169 | 1.055 | 0.276 |
| | Min | | | |
| | Max | | | |
| | N | 10 | 10 | 10 |
| Results of Statistical Analysis: T2 vs. T3 | | NS | NS | NS |
| T0 vs. T1, T2, and T3 | | 0<3,2 | 0<i,3,2 | 2,3,1<0 |

Notes: (1) Serum T3; 3, 3', 5'-triiodothyronine; Serum T4: levothyroxine; Serum TSH: thyroid-stimulating hormone.
(2) Expired refers to Lot 3000076; Non-expired refers to Lot 0000265189.
(3) The symbols "0", "1", "2", and "3" have been used as abbreviations for treatment groups T0, T1, T2, and T3 in the presentation of the Results of Statistical Analysis.

Urinalysis: unremarkable

Organ weights: Increased heart weight in both 160 mcg/kg/day groups attributed to cardiac hypertrophy in response to thyroxine. Increased liver, spleen and kidney weight in both 160 mcg/kg/day groups without histopathology correlates.

| Weight | 0 | | 60 µg/kg/day | | 160 µg/kg/day expired lot | | 160 µg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| % Relative to Body | M | F | M | F | M | F | M | F |
| Heart | 0.39 | 0.43 | 0.40 | 0.42 | 0.48* | 0.48 | 0.47* | 0.48 |
| Kidney | 0.78 | 0.81 | 0.82 | 0.81 | 0.92* | 0.89* | 0.94* | 0.91* |
| Spleen | 0.78 | 0.48 | 0.69 | 0.53 | 1.02 | 0.66* | 1.09* | 0.56 |
| Liver | 3.2 | 3.1 | 3.1 | 3.0 | 3.4 | 3.6* | 3.4 | 3.5* |

Gross pathology: unremarkable

| Histopathology | 0 | | 60 µg/kg/day | | 160 µg/kg/day expired lot | | 160 µg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| **Epididymis** Aspermia bilateral | | | | | 1/10 | | | |
| Cellular debris bilateral | | | | | 1/10 | | | |
| Sperm granuloma focal | | | | | 1/10 | | | |
| **Esophagus** muscle inflamm focal | | | | | | | | 1/10 |
| **Eye** inflamm. Post. Chamber, subacute, unilateral | | | | | | | 1/10 | |
| Retinopathy focal unilateral | | | | | | | 1/10 | |
| Retinal rosettes unilateral focal | | | | | | | | 1/10 |
| **Heart** degen/necrosis subepic. Focal | | | | | 1/10 | | | |
| Inflamm chronic focal | | | | | | | 1/10 | |
| Mononuclear infiltration auricle focal | | | | | 1/10 | | | |
| **Kidney** dilation bilateral | | | | | 1/10 | | | |
| Chronic interstitial inflamm. Focal | | 1/10 | | | | 2/10 | | 2/10 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Inflamm. Pelvis chronic | | | | | | | 1/10 | |
| Microcalculus collecting tubule papilla focal | | | | | 3/10 | | 1/10 | |
| **Liver** necrosis multifocal | | | | | | | 1/10 | |
| Vacuolation multifocal | 3/10 | | | | 2/10 | | 3/10 | |
| **Lung** interstitial chronic focal inflamm | | | | | | | 1/10 | |
| **Lymph node** mesenteric/thoracic lymphocytic hyperplasia | | | | | 1/10 | | | |
| **Pancreas** infiltration mononuclear microfocus | | | | | | | 1/10 | |
| Focal atrophy | | | | | | 1/10 | | |
| **Peyer's patch/GALT** focal mineraliz. | | | | | | 2/10 | | |
| **Prostate** neutroph. Inflamm. Chronic acitve | | | | | 1/10 | | | |
| **Thyroid** attenuation, follicular cell diffuse | | | 5/10 | 8/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| Colloid increased diffuse | | | 5/10 | 8/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| Ultimobrachial cyst | | 2/10 | | 6/10 | | 5/10 | | 5/10 |
| Ectopic thymus | | 1/10 | | | | | | 2/10 |
| **Urinary bladder** inflamm. Chronic active | | | | | 1/10 | | | |
| **Thymus** cysts | | | | | | | | 1/10 |
| Hemorrhage microfocus | | | | | | 1/10 | | 1/10 |

Minimal to mild colloid accumulation in the thyroid present in all drug treated groups. The accumulation of excessive colloid was attributed to the pharmacologic effect of levothyroxine. Histopathological correlates to liver, kidney, spleen or heart weights (including hypertrophy)

were not observed according to the sponsor. There are heart (focal degeneration/necrosis, chronic inflammation, mononuclear infiltration), kidney (dilation, inflammation, microcalculus), and liver (necrosis, vacuolation) histopathology which is similar in the 160 mg/kg/day treatment groups. Focal microcalculus in the collecting tubules of the papilla is present in 3/10 rats given expired lot compared to 1/10 in the 160 mg/kg/day group. Splenic histopathology correlates were not observed. The non-expired lot group had some ocular findings (inflammation posterior chamber, retinopathy) that were absent in the expired lot dosed group. The expired lot group had two rats with epididymal sperm histopathology (aspermia, cell debris, granuloma) which was not present in the non-expired lot. One rat in the 160 mcg/kg/day expired group had bilateral testicular degeneration. A second rat in this group had minimal multifocal bilateral testicular degeneration. A control male had unilateral, but complete atrophy of testes.

The mean ± standard deviation pharmacokinetic values for levothyroxine (T4) for the four SYNTHROID® dose groups are presented in the following table.

**Levothyroxine (T4) Mean ± SD Pharmacokinetic Parameters**

| | Dose (µg/kg/day) | | | |
|---|---|---|---|---|
| Pharmacokinetic Parameter | 0 | 60 Expired | 160 Expired | 160 Non-Expired |
| | Male Rats | | | |
| $T_{max}$ (h) | 1.2 ± 0.4 | 4.8 ± 1.6 | 5.4 ± 1.3 | 6.0 ± 0.0 |
| $C_{max}$ (µg/dL) | 2.87 ± 0.52 | 12.4 ± 1.2 | 22.7 ± 3.7 | 22.2 ± 1.9 |
| $AUC_{24}$ (µg•h/dL) | 54.8 ± 12.2 | 222.3 ± 23.1 | 399.2 ± 44.2 | 414.1 ± 43.3 |
| | Female Rats | | | |
| $T_{max}$ (h) | 1.7 ± 0.6 | 4.6 ± 1.9 | 2.8 ± 0.4 | 4.2 ± 1.6 |
| $C_{max}$ (µg/dL) | 1.79 ± 0.74 | 6.50 ± 1.96 | 14.6 ± 4.5 | 11.9 ± 5.8 |
| $AUC_{24}$ (µg•h/dL) | 24.2 ± 9.3 | 116.7 ± 29.7 | 221.7 ± 80.3 | 195.4 ± 92.6 |

The mean ± standard deviation pharmacokinetic values for triiodothyronine (T3) for the four dose groups are presented in the following table.

**Triiodothyronine (T3) Mean ± SD Pharmacokinetic Parameters**

| | Dose (µg/kg/day) | | | |
|---|---|---|---|---|
| Pharmacokinetic Parameter | 0 | 60 Expired | 160 Expired | 160 Non-Expired |
| | Male Rats | | | |
| $T_{max}$ (h) | 14.8 ± 12.6 | 5.8 ± 10.2 | 5.0 ± 2.2 | 6.0 ± 0.0 |
| $C_{max}$ (ng/mL) | 0.343 ± 0.080 | 0.383 ± 0.112 | 1.11 ± 0.48 | 1.28 ± 0.22 |
| $AUC_{24}$ (ng•h/mL) | 4.08 ± 1.36 | 6.90 ± 2.18 | 22.5 ± 9.6 | 27.3 ± 4.3 |
| | Female Rats | | | |
| $T_{max}$ (h) | 1.0 ± 0.0 | 1.3 ± 0.5 | 2.4 ± 0.9 | 4.4 ± 2.3 |
| $C_{max}$ (ng/mL) | 0.408 ± 0.150 | 0.362 ± 0.091 | 0.737 ± 0.266 | 0.841 ± 0.119 |
| $AUC_{24}$ (ng•h/mL) | 4.60 ± 1.17 | 8.71 | 14.1 ± 5.3 | 17.4 ± 3.5 |

Exposure was lower for females compared to male rats. Levothyroxine and triiodothyronine exposures were similar after administration of 160 mcg/kg/day of the expired and non-expired lots for 28 days.

| Impurity Identity | Lot 0000265189 (non-expired) | | | | | Lot 3000076 (expired) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Peak Area | Rat Nominal Dosage | | Human TDI[a] | | Peak Area | Rat Nominal Dosage | | Human TDI | |
| | %[b] | µg/kg[c] | µg/m²[d] | µg/m²[e] | MOE[f] | % | µg/kg[g] | µg/m² | µg/m² | MOE |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

a. TDI: Total Daily Intake (Human).
b. Peak area % based on theoretical 300 µg levothyroxine sodium (LT4)/tablet.
c. Nominal dosage calculated as follows: [(peak area % ÷100) x (300 µg theoretical potency/tablet÷ — µg actual potency Lot 0000265189/tablet)] x 160 µg LT4/kg dosage (high-dose group).
d. Assumes a 150 g rat with a 0.025 m² surface area (conversion factor 5.9).
e. Assumes a 300 ug daily LT4 dose administered to a 60 kg human with a 1.6 m² surface area (conversion factor 37).
f. MOE: Margin of Exposure [rat nominal dosage (µg/m²)÷TDI, calculated before rounding].
g. Nominal dosage calculated as follows: [(average peak area % ÷100) x (300 µg theoretical potency/tablet÷ — µg actual potency/tablet Lot 3000076)] x 160 µg LT4/kg dosage (high-dose group).
h. No value reported.
i. Value reported for only one time point; used available value instead of average value.
j. ND: None Detected.

With the exception of ——— , no impurity and/or degradation product was present in excess of 1% in either tablet formulation. ——— was present at —— in the expired tablets. Based on a daily human dose of 300 µg LT4/day, the total daily intake of ——— from the expired tablet would be —— On a surface area basis, a 60 kg human would receive ——— On a dose per surface area basis, the high dose groups in the present study received approximately five times the maximum human

**Summary of individual study findings:** Findings in the levothyroxine treatment groups (expired lot, non-expired; 160 mg/kg/day) were similar. In the expired lot 3/10 males had focal microcalculus in the collecting tubules of the papilla compared to 1/10 in the newer lot. The expired lot treated group had 2/10 rats with testicular degeneration which accounts for the epididymal sperm effects seen.

Many of the findings were attributable to the exaggerated pharmacological activity: increased TSH levels, diffuse follicular cell attenuation, diffuse increased colloid formation, increased heart weight and hypertrophy, increased kidney weight with histopathology of dilation/inflammation/microcalculus formation. Interestingly body weight was unremarkable although increased food consumption was observed in the non-expired lot group.

[redacted] was present at [redacted] in the non-expired lot and [redacted] in the end of shelf life material.

**Toxicology summary:** see Sponsor's tabular summary of published toxicity data for levothyroxine and triiodothyronine.

In a 6 week SC toxicity study levothyroxine was administered to male Wistar rats at 0.5 mcg/kg/day for two weeks and twice weekly thereafter, survival was 83%. Rats had a 14% body weight deficit, relative heart weight increased 35%, kidney 21% and adrenal weight increased 32%. In a second study levothyroxine was administered in drinking water to male Sprague-Dawley rats at 850 mcg/kg/day for either 250 or 410 days. Time dependent degenerative changes including: glomerular capillary basement membrane thickening, hyalinization of tufts, thickening of tufts, adhesion of tufts to Bowmans capsule, thickening and fraying of Bowmans capsule, atrophy of convoluted tubules with thickening and wrinkling of the peri-tubular basement membranes and medial thickening of arteries and interstitial fibrosis were observed. Absolute and relative kidney, heart and adrenal weight increased. Body weight was reduced by 15% with an increase in food and water consumption attributed to the expected hyper-caloric state associated with an increased metabolic rate. Serum BUN was increased 5X in rats treated for 410 days. Hypercholesteremia was also observed.

Chronic administration of levothyroxine to Sprague-Dawley rats via drinking water at 850 µg/kg/day for 410 days resulted in degnerative changes being more advanced in kidneys of treated rats. These changes consisted of glomerular capillary basement membrane thickening and fraying of Bowman's capsule, atrophy of convoluted tubules and thickening and wrinkling of the peritubular basement membranes and medial thickening of arteries and interstitial fibrosis. Absolute and relative kidney weight, heart weight and adrenal weights were increased by levothyroxine treatment with the effect being larger in the older rats at the longest time of treatment (410 days treatment with sacrificed at day 600) where the absolute kidney weight was increased by 37%, heart weight 14% and absolute adrenal weight by 39%. Body weight was –15% in these levothyroxine treated rats. Serum BUN levels were elevated 5 fold in these rats as was a 51% increase in oxygen consumption/kg/h. Treatment with levothyroxine and triiodothyronine resulted in the expected thermogenic effect of increased body temperature of 1.5 °C. Liver malondialdehyde concentrations were elevated by 27% indicating a modest increase in liver peroxidation. Likewise xanthine oxidase and xanthine dehydrogenase were increased by ~15% while liver glutathione concentrations fell by 34%. These data indicate that the

hyperthyroid state is associated with an increased in oxidative free radical generation that could contribute to toxicity levels if protective glutathione mechanisms are overcome.

Levothyroxine can simultaneously increased erythroid production (RBC 11%, RBC mass 18%) and decrease thrombocytopoiesis (-43% platelets, -42% platelet mass).

Sodium ipodate is used for cholecystograms and has been successful in treatment of acute levothyroxine overdose. The mechanism involves inhibition of enzymatic conversion of levothyroxine to triiodothyronine by shunting the levothyroxine into reverse T3. It would be most useful given shortly after levothyroxine ingestion prior to a toxic accumulation of the more biologically active triiodothyrronine.

**Toxicology conclusions:**

Levothyroxine and triiodothyronine are endogenous hormones produced by the thyroid gland. They are required for physiological development and maintenance. Their effects are exerted through gene activation resulting in an increased expression of proteins and through non-genetic mechanisms. The toxicity of these hormones is a continuation of their pharmacological activities.

**Histopathology Inventory for NDA #21-402**

| Study | TA01 -166 |
|---|---|
| Species | Rat |
| Adrenals | √* |
| Aorta | √ |
| Bone Marrow smear | √ |
| Bone (femur) | √ |
| Brain | √* |
| Cecum | √ |
| Cervix | √ |
| Colon | √ |
| Duodenum | √ |
| Epididymis | √* |
| Esophagus | √ |
| Eye | √ |
| Fallopian tube | |
| Gall bladder | |
| Gross lesions | √ |
| Harderian gland | |
| Heart | √* |
| Ileum | √ |
| Injection site | |
| Jejunum | √ |
| Kidneys | √* |
| Lachrymal gland | |
| Larynx | √ |
| Liver | √* |
| Lungs | √ |
| Lymph nodes, cervical | √ |
| Lymph nodes thoracic | √ |
| Lymph nodes, mesenteric | √ |
| Mammary Gland | √ |
| Nasal cavity | |
| Optic nerves | √ |
| Ovaries | √* |
| Pancreas | √ |

| | |
|---|---|
| Parathyroid | √* |
| Peripheral nerve | |
| Pharynx | |
| Pituitary | √* |
| Prostate | √* |
| Rectum | |
| Salivary gland | √ |
| Sciatic nerve | √ |
| Seminal vesicles | |
| Skeletal muscle | √ |
| Skin | √ |
| Spinal cord | √ |
| Spleen | √* |
| Sternum | |
| Stomach | √ |
| Testes | √* |
| Thymus | √* |
| Thyroid | √* |
| Tongue | √ |
| Trachea | √ |
| Urinary bladder | √ |
| Uterus | √* |
| Vagina | √ |
| Zymbal gland | |
| Standard List | |
| Peyer's patch/GALT | √ |

X, histopathology performed
*, organ weight obtained

## V. GENETIC TOXICOLOGY:

Levothyroxine was tested in a mouse micronucleus assay in dw/dw mice which exhibit features of pan hypopituitarism . Mice received SC, 5 days/week 3-4 or 30-40 µg/kg/day for 1, 4, or 13 weeks. In this strain 3-4 µg/kg/day produces a euthyroid condition while the higher dose produces hyperthyroidism. The number of observed micronuclei did not change from control suggesting that levothyroxine was not clastogenic in this assay.

## VI. CARCINOGENICITY: N/A

## II. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

**Reproductive and developmental toxicology summary:** Levothyroxine does not adversely effect reproductive performance. Female Wistar rats given 100 µg/kg/day for six days prior to estrous and mated with untreated males had increased corpus lutea (22%), increased implantation (21%) and litter size (17%). However a fertility-early embryo development study in Sprague-Dawley female rats given 30, 60, 120, 240, 480, 960 µg/kg/day on the day of estrous through Day 20 of lactation resulted in decreased pup and dam survival such that none survived ≥240 µg/kg/day. In another study in female Wistar rats given SC 0.25 or 1 mg/kg/day levothyroxine for 8-10 days prior to mating prolactin was increased 2 and 5 fold compared to 24 fold elevation in controls GD20. Serum progesterone decreased by 41%, 53%, 84% in 0.25, 1mg/kg/day and controls respectively on GD21. Growth hormone levels decreased by –57%, -90% and –61% at these doses respectively. Estradiol concentrations were unaffected and corticosterone concentrations rose by 48% and 70% in the treated groups by GD 20. Treatment with levothyroxine produced directional changes in hormone and receptors on GD 20 similar to those in control rats. These change in circulating hormone and receptor concentration in late gestation may be partially responsible for the earlier onset of parturition and difficulty in lactation encountered in pregnant rats treated with high doses of levothyroxine. Effects on receptors are also observed e.g. mammary gland estradiol receptors decreased by 52% and 48% at 0.25 and 1 mg/kg/day; levothyroxine and progesterone receptors decreased by 45% at both doses and by 57% in the controls, prolactin receptors increased by 40% at both levothyroxine doses on GD21. Uterus estadiol receptors increased by 470% and 269% whereas progesterone receptors increased by 275%, 293% and 200% in the 0.25, 1 mg/kg/day and control groups GD 21. In liver estradiol receptors were decreased by 60% in controls. Growth hormone receptors were decreased by 40% at 1 mg/kg/day levothyroxine on GD21 and the control. Prolactin receptors were decreased by 27% at the 0.25 mg/kg/day group and 60% at 1 mg/kg/day and control groups respectively.

A high frequency of mental retardation, hearing defects and neurological disorders (cretinism) occurred in geographic areas with a high frequency of endemic goiter which implicated the need for thyroid hormone production in normal brain development. Mental retardation in human hypothyroid neonates is variable and not always related to the extent of hypothyroidism in the neonate. Breast feeding may modulate the degree of mental deficits in infants by secretion of thyroid hormones into milk. The human thyroid is able to synthesize some iodothyronines by GW 10-12 but it does not appear functional until mid gestation. The bulk of brain growth occurs post-natal continuing until the third year. In rats behavioral deficits can be induced by hypothyroidism initiated from gestation day 18 to lactation day 15. This time period roughly corresponds to the human last trimester through the first 2-3 years of life. The effects produced are suggestive of neuronal maturation (dendrite formation and arborization rather than the number of neurons). This suggests that decreases in brain inter-neuronal conductivity may be an important factor in the mental retardation and neuronal deficits observed in cretinism. Conversely, excess triiodothyronine exposure (5 mcg/day for rat for 3 days) to newborns leads to permanent impairment of learning ability. Studies in a mutant autosomal recessive mouse (hyt/hyt) born with a hypoplastic thyroid demonstrate that elevated levothyroxine levels in the stomach of pups weaned by thyroid hormone supplemented dams results in an age dependent myelination that can be transferred to the neonate via lactation. Similarly levothyroxine treatment of human hypothyroid newborns during the early neonatal period was associated with improved intellectual capacity, whereas a poor prognosis when

treatment was initiated after 4 months. Reports of human cretinism demonstrate mental retardation, neurologic deficits and adverse effects of growth and development in general with hypothyroidism.
Thyroid hormones have effects on growth and development, brain development, calorigenesis, thermogenesis, bone development and cardiovascular development and function.

Levothyroxine given at 1 mg/kg/day for 2-3 days during neonatal life in Sprague-Dawley rats resulted in earlier eye opening increased locomotor activity and earlier appearance of cortical EEG and acoustical startle. When administered 16-18 days of age, levothyroxine appeared to facilitate learned shock avoidance compared to controls. However administration at 35-45 days postnatal resulted in deficits in maze learning. Passive avoidance in the post weaning period was impaired in the levothyroxine treated pups as was body weight gain. Maze learning showed an apparent persistent deficit in levothyroxine treated rats to at least post-natal days 180-195. At sacrifice at 235 days relative brain weights were 90% of control values.

The mating of levothyroxine neonate treated females with untreated males resulted in a higher pup mortality rate of 8% versus 2% for controls. Despite reductions in litter size, the F1 males derived from treated females had body weights at weaning that were 20% lower than controls. A similar effect was not observed in the F1 females. The F1 offspring at PND5 had a 54% reduction in pituitary TSH content/gland relative to controls.

Some effects of neonatal levothyroxine treatment persisted into the F2 generation. Pituitary weight and ovary weights were diminished in females and thyroid weight was increased in both sexes. Vaginal opening was accelerated, as was the appearance of first estrus. Final body weight of the F2 males whose grandmothers had been treated as neonates was reduced by 10%. The goiterogenic activity of propylthiouracil appeared to be increased in the F2 generation derived from levothyroxine treated grandmothers.

Thyroid hormones have effects on cardiac microvasculature (see safety pharmacology). Triiodothyronine given to neonatal rats starting on PND2 was given SC every other day at 200 µg/kg/day. An additional group received 0.05% propylthiouracil in mothers' drinking water with treatment continued to PND 12-28. At PND80 heart rate and serum triiodothyronine concentrations for both groups (hypo- and hyperthyroid) were similar to control indicating a return to an euthyroid state. Both hyper and hypthyroid rats sacrificed at PND12 had 19% deficits in weight gain and by PND 28 hyperthyroid rats were similar to controls while the hypothyroid group were markedly weight deficient (33% of control weight). Weight gain deficits were present in both hyper (-27%) and hypothyroid (-46%) groups by PND 28, which continued, to PND80. Left ventricular relative weight was effected by treatment. At PND12 a decreased of 20% was noted in hypothyroid rats. At PND28, left ventricular weight was increased by 34% in the hyperthyroid rats and decreased by 10% in the hypothyroid rat by PND80, left ventricular mass was slightly elevated being 10% and 14% above controls for the hyper and hypothyroid groups respectively. Cardiac capillary density and myocyte density (# capillaries or myocytes per area) were effected in the hypothyroid rats at PND28 by +20%. Left ventricular arteriolar density (# arterioles/volume) was decreased by 40% at PND12 and 19% by PND 28 in the hypothyroid rats. These values returned to normal by PND80.

Levothyroxine has been detected in the breast milk of cows, rats, rabbits and humans. Studies in lactating mice revealed physiologically meaningful amounts of thyroid hormones (desiccated thyroid) can be transferred from thyroid deficient mice to neonates via lactation. Levothyroxine and triiodothyronine cross the placenta efficiently and local conversion of levothyroxine to triodothyronine is substantial since the plasma increase of maternal or fetal

triiodothyronine was the same irrespective of the hormone given to the doe (rabbit). The physiological relevance of thyroid hormone placental transfer was established by the finding of 87% decrease in fetal liver glycogen content following levothyroxine administration to the does and a 38% decrease following triiodothyronine. Fetal liver glycogen decrease was greater when the does were treated with levothyroxine than with triiodothyronine the resulting increase in fetal plasma glucose was comparable (2 fold) for both maternal treatment groups.

## VIII. SPECIAL TOXICOLOGY STUDIES: N/A

## IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

**Conclusions:** Levothyroxine is an endogenous hormone produced by the thyroid gland and is required for physiological development and maintenance. These effects are exerted through gene activation resulting in an icnreased expression of proteins and through non-genetic mechanisms. The toxicities associated with levothyroxine are extensions of its pharmacologic activity. A significant body of clinical experience has been developed with levothyroxine as it has been marketed for many years.

**General Toxicology Issues:** none the toxicity of levothyroxine has been well documented in published literature

**Recommendations: approval**

**Labeling with basis for findings: The draft label is adequate and represents class labeling.**

## X. APPENDIX/ATTACHMENTS:

**Addendum to review: Sponsor's tabular summary of published toxicity data for levothyroxine and triiodothyronine**

## Table 5.3.1: Tabular Summary of Published Toxicity Data for Levothyroxine and Triiodothyronine

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| **Neonatal Toxicity** | | | | | |
| Rat-Sprague Dawley[99] | 8-10, sex unstated | ip | Levothyroxine 1 mg/kg/day | Post-natal days 2, 3, 4 | Earlier eye opening, locomotion, EEG, & acoustical startle response. Decreased body weight gain. |
| Rat-Holtzman[100] | 15-17, sex unstated | ip | Levothyroxine 27 µg/pup/day | Post-natal days 1, 2, 3, 5, 7, 9, 11, 13 observed until age 147 days | Earlier eye opening, locomotion, and sexual maturity. Decreased body weight gain to at least 147 days of age. |
| Rat-Holtzman[101] | 6-17, sex unstated | sc | Levothyroxine 2 µg/gm | Post-natal days 2-5 observed to age 235 days | Earlier eye opening, righting reflex, swimming ability, grasp reflex. Decreased passive avoidance response and maze learning to at least 185 days of age. Decreased body weight gain to 235 days of age. |
| Rat-Sprague Dawley[102] | 29, sex unstated | unstated | Levothyroxine 0.4 µg/gm | Post-natal days 1-12 observed to age 105 days | Earlier onset of acoustical invoked brain stem neuronal response. Increased amplitude and decrease latency. Differences no longer found at 65 days of age. |
| **Bone** | | | | | |
| Rat-Sprague Dawley[103] | 4-6, sex unstated | ip | Levothyroxine 30 µg/pup | Post-natal days 2, 3, & 4 & then every 2-3 days for 5 additional doses observed to age 42 days | Relative weights of the thyroid and pituitary decreased. TSH content of pituitary decreased. |
| Rat-Sprague Dawley[104] | number unstated, males & female neonates and mothers | sc | Levothyroxine 135-150 µg/pup 135-150 µg/mother | Post-natal days 1-10 to pups & mothers observed to age 273 days | Earlier eye opening, persistent weight gain deficit, persistent decrease in relative weight of pituitary and testes, increased mortality and decreased liter size in offspring of female levothyroxine treated neonates. $F_1$, earlier eye opening, and decreased pituitary content at 5 days of age. In the $F_2$ generation from levothyroxine treated grandmothers pituitary and ovary weight increased in females and thyroid weight increased in both sexes. Earlier vaginal opening and earlier first estrous, decreased body weight gain in males and increased goitrogenic activity in both sexes in response to propylthiouracil occurred. |

## Table 5.3.1: Tabular Summary of Published Toxicity Data for Levothyroxine and Triiodothyronine (Cont.)

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Sprague Dawley[105] | 4-6, males and females | sc, $T_3$ Neonates via drinking water propylthiouracil mothers via drinking water | Triiodothyronine Neonates 200 µg/kg. Propylthiouracil Separate group of mothers 0.05% in the drinking water | Triiodothyronine every other day, post-natal days 2-12 or 2-28, mothers post-natal days 2-12 or 2-28 obser. to age 80 days | In triiodothyronine treated neonate rats increased heart rate, earlier eye opening and decreased body weight gain. In propylthiouracil treated rats offspring decreased heart rate, delayed eye opening, decreased body weight gain. Serum triiodothyronine levels increased in hyperthyroid and decreased by hypothyroid treatments. At 80 days serum levels of triiodothyronine treated at euthyroid level. Weight gain deficits in hyper- and hypothyroid neonate rats persisted to 80 day sacrifice with larger effect in hypothyroid group. Left and right relative ventricular heart weight decreased in hypothyroid rats at 12 and 28 days and increased in hyperthyroid rats at 28 days. At 80 days left and right relative ventricular heart weight increased in both previously hyper- and hypothyroid groups. Capillary density and myocyte density was decreased at 28 days in the hypothyroid group, returning to normal at 80 days of age. Arteriolar density was decreased in hypothyroid rats at 12 and 28 days and returned to normal at 80 days of age. Arteriolar external diameter, internal diameter and wall thickness was unaffected. Arteriolar length was decreased in 28 day old hypothyroid rats and increased in 28 day old hyperthyroid rats. Left ventricular arteriolar density was not affected. The number of capillaries/arteriole was increased at 12 and 28 days in the hypothyroid rats, returning to normal at 80 days. |
| Rat-Sprague Dawley[106] | 10, males | ip | levothyroxine 200 ug/kg/day | 3 weeks | Bone mineral density decreased by 10% in femur. No effect in lumbar vertebrae 4 and 5 |
| Rat-Wistar[107] | 10, males | oral via drinking water | levothyroxine 60-80 µg/kg/day | 35 days | Serum calcium increased by 31% in normal rats. Thyroidectomy decreased serum calcium by 30% and levothyroxine treatment of thyroidectomized rats increased serum calcium by 43% relative to thyroidectomy alone. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Wistar[108] | 6, males oral via | drinking water | Levothyroxine 200 µg/kg/day or Propylthiouracil 0.10% | 12 weeks | Propylthiouracil group (hypothyroid) body weight 48% of controls. Cancellous bone volume unaffected in levothyroxine group (hyperthyroid), increased 58% in hypothyroid rats. Total osteoid surface decreased by 93% in hypothyroid rats. Mineral apposition rate decreased 49% in hypothyroid rats and increased 60% in hyperthyroid rats. Percent of mineralized bone surface decreased 95% in hypothyroid rats and increased 20% in hyperthyroid rats. |
| Rat-Sprague Dawley[109] | 10, males | ip | Levothyroxine 200 µg/kg/day | 20 weeks | Serum concentrations of levothyroxine and triiodothyronine increased by 75% and 70%, respectively, serum TSH concentration reduced by 53%. Femur messenger RNA for osteopontin, osteocalcin, alkaline phosphatase and tartrate resistant acid phosphatase all increased 1.8 -2.8 fold. No increases occurred in the lumbar vertebrae. Bone density was reduced 5.7% in the femur. No effect occurred in lumbar vertebrae. |
| Rat-Wistar[110] | 6-9, females | ip | Triiodothyronine 50 µg/kg/day 100 µg/kg/day Triiodothyroxine 100 µg/kg/day | 2 weeks 4 weeks | After 2 weeks of treatment with 100 ng/kg/day dry femur weight and ash weight decreased by about 5.6% and bone mineral density was decreased in the spine and tibia by 3.3% to 3.5%. Serum concentrations of pyridinoline and deoxypyridoline, two markers for bone resorption, were increased maximally from 2.5 to 15 fold at the 50 or 100 µg/kg/day doses given for 7 days. Larger effects occurred on dry bone weight, ash weight and bone mineral density in femur, lumbar spine and tibia when 100 µg/kg/day was given for 4 weeks compared to treatment for 2 weeks. |
| Rat-Sprague Dawley[115] | 19 day fetuses No. & sex unstated | *in vitro* bone culture | Triiodothyronine $1x10^{-6}$M to $1x10^{-11}$M | 8 day incubations | Increased calcium loss into medium at $1x10^{-10}$M & higher. Calcium loss not affected by a cyclooxygenase inhibitor or an IL-1 antagonist. Decreased by a inhibitor of DNA synthesis. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Wistar[116] | male, No. unstated | ip | Levothyroxine 200 µg/kg/day | 3 days | Ten day old rats had cessation of body weight gain. Relative kidney weight increased by 39%. Plasma concentrations of beta alanine, aspartic acid, glutamic acid, ornithine, asparagine, leucine, taurine, serine, alanine, and glutamine were increased. These changes correlated with increased tubular reabsorption. No changes were found in 55-day-old rats. |
| Rat-Sprague Dawley[117] | 10-12, males | sc | Levothyroxine 50 µg/kg/day | 7 or 20 days in non- HgCl intoxicated rats or 20 days in HgCl intoxicated rats | Levothyroxine treatment for 7 days did not affect the renal concentrations of alkaline phosphatase, acid phosphatase, ATPase or leucine aminopeptidase. After 20 days of treatment, the renal concentrations of ATPase and acid phosphatase were increased. HgCl intoxication decreased the renal concentrations of alkaline phosphatase, acid phosphatase, leucine aminopeptidase, and increased the concentration of renal ATPase. The co-administration of levothyroxine helped to restore the activity of alkaline phosphatase and leucine aminopeptidase. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Wistar[118] | number unstated, male & female | ip | Triiodothyronine 200 µg/kg/day | 3 days alone or in cisplatin intoxicated rats | Urine volume/kg was decreased 55% in 55-day-old cisplatin treated rats with or without triiodothyroonine treatment. Triiodothyronine alone had no effect on this parameter. In both 10 & 55 day old rats cisplatin increased urinary protein concentration by about 3 fold. Triiodothyronine co-administration lead to a further 30% to 50% increase. Triiodothyronine alone did not effect this parameter. In 10-day rats, cisplatin doubled serum BUN concentrations. Triiodothyronine administered alone to 10 day old rats or co-administered with cisplatin had no effect. In 55 day old rats cisplatin administration resulted a 4 fold increase in serum BUN that was increased to an 11 fold elevation by the co-administration of triiodothyronine. Both reduced and oxidized renal glutathione concentrations were slightly increased by triiodothyronine in 10 and 55 day old rats. The serum and renal levels of platinum in cisplatin treated 10 day old rats was less than that found in 55 day old rats. Co-administration of triiodothyronine decreased the serum and renal platinum concentrations 5 hours post the cisplatin dosage in both 10 day and 55 day old rats. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Wistar[119] | 15, males | sc | Levothyroxine 1 mg/kg | Weeks 1 & 2 daily dosing Weeks 3-6 twice weekly dosing. Given alone or in combination with l-epinephrine or dl-metanephrine | Levothyroxine treated rats had a 14% weight gain deficit. L-epinephrine produced a greater weight gain deficit but co-administration of levothyroxine was without effect. dl-metanephrine alone produced a 15% increase in weight gain. Co-dosing of levothyroxine caused a 16.5% weight gain deficit. Both l-epinephrine and Dl-metanephrine caused increased relative heart weight that was further increased by the co-administration levothyroxine. Relative kidney weight was increased in the l-epinephrine treated rats and further increased upon co-administration of levothyroxine. Metanephrine did not increase relative kidney weight but an increase occurred with the co-administration of levothyroxine. Levothyroxine treatment alone produced an increase in relative adrenal weight. Survival rate was reduced from 100% in the controls to 30% in the rats co-administered levothyroxine and l-epinephrine. Serum cholesterol was decreased 9% by levothyroxine, increased 29% by l-epinephrine and further increased to 71% by the co-administration of levothyroxine. Similar effects occurred with dl-metanephrine. Levothyroxine given in combination with l-epinephrine increased serum total lipids by 37% and decreased serum glucose by 28%. Lipid accumulation in aortic walls of the dl-metanephrine rats was decreased by the co-administration of levothyroxine. Cholesterol deposit was present in 80% of the dl-metanephrine rats but in none of the rats administered levothyroxine alone or in combination with dl-metanephrine. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Sprague Dawley[120] | 5-12, males | oral via drinking water | Levothyroxine 850 µg/kg/day | 250 or 410 days | The kidneys of rats treated for 250 days did not differ grossly from controls. Treatment for 410 days resulted in gross kidney changes of yellow color, enlarged granular surface, honeycombed parenchyma with cystically dilated ducts. Microscopically, levothyroxine mediated kidney changes consisted of glomerular capillary basement membrane thickening, hyalinization of tufts, thickening of tufts, adhesion of tufts to Bowman's capsule, thickening and fraying of Bowman's capsule, atrophy of convoluting tubules with thickening and wrinkling of the peritubular basement membranes, and medial thickening of arteries and interstitial fibrosis. These changes were more advanced in the longer term treatment group. Absolute and relative kidney, heart, and adrenal weight were all increased by levothyroxine treatment with larger effects occurring in the more prolonged treatment group. Food consumption and water consumption were increased by levothyroxine. After 225 days of levothyroxine treatment oxygen consumption/kg was increased by 51%. Serum BUN concentrations were increased 5 fold after 410 days of treatment with levothyroxine. |
| Rat-Sprague Dawley[122] | male, number unstated | ip | Triiodothyronine 1 part: Levothyroxine 4 parts 300 µg/kg/day | 3 days | Body temperature rose 1.5°C. Lipid peroxidation as measured by liver malondialdehyde concentrations increased 27%. The activity of both xanthine oxidase and xanthine dehydrogenase increased by 15% and liver total glutathione decreased by 34%. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Mice-C3H[122] | 4-10 sex unstated | sc | Levothyroxine and d-isomer of levothyroxine 25-200 µg/mouse/day | 2, 4, 6, 8, or 14 days | Doses of 25 to 200 µg/mouse/day of either levothyroxine or its d-isomer produced decreases in thrombocytopoiesis. A plateau dose response effect was achieved at the 25 ug/mouse/day dose when given for 2 days. At the 25 µg/mouse/day dose of levothyroxine, thrombocytopoiesis was decreased by 30% after 2 days of treatment and 60% after 6 days of treatment with no further decreases occurring after 8 or 14 days of treatment. Platelet counts on average were decreased by 15%, 30%, and 42.5% after 4, 6, and 8 days of treatment with 25 ug/mouse/day of levothyroxine. Megakaryocyte numbers and size decreased after 4 days of treatment with 25 ug/mouse/day of levothyroxine. Treatment with 25 µg/mouse/day of levothyroxine produced time dependent increases in reticulocyte count, RBC and percent hematocrit. |
| **Reproduction and Developmental Toxicity** | | | | | |
| Rat-Wistar[123] | 18, pregnant females | parenteral | Levothyroxine 30 µg/rat/day | six days prior to pro-estrous and mating | Twenty two percent increase in corpus lutea, 21% increase in implantations, and a 17% increase in liter size. |
| Rat-Sprague Dawley[124] | 6, pregnant females | sc | Levothyroxine 30, 60, 120, 240, 480, 960 µg/kg/day | starting day of estrous through Day 20 of lactation | No dams treated with 240 µg/kg/day or higher survived lactation and fewer dams at all lower doses survived lactation. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Wistar[125] | 8-12, pregnant females | sc | Levothyroxine 0.25 or 1 mg/kg/day | starting 8-10 days prior to mating through Day 20 of gestation. Controls sacrificed gestation Day 20. An untreated group allowed to liter on Days 21 | Serum prolactin increased 2-5 fold at the low and high doses, respectively compared to gestation Day 20 controls. Serum progesterone decreased 41%, 53% , and 84%, respectively in the low, high and gestation Day 21 groups compared gestation Day 20 controls. Serum growth hormone fell by 57%, 90%, and 61%, respectively for the low, high and gestation Day 21 group compared to the gestation Day 20 controls. Serum corticosterone increased by 48% and 70% in the low and high dose compared to the gestation Day 20 controls. Compared to the gestation Day 20 coontrols estradiol mammary receptors decreased at the low and high dose by 52% and 48%, progesterone receptors decreased at the low and high dose by 45% and by 57% in the gestation Day 21 untreated group. Prolactin receptors increased by 40% at the low, high and gestation Day 21 untreated groups. In the uterus estradiol receptors increased at the low and high levothyroxine doses by 470% and 269%, respectively. Progesterone receptors increased by 275%, 293%, and 200%, respectively, in the low, high, and gestation Day 21 untreated group. In the liver compared to the gestation Day 20 controls growth hormone receptors fell 40% in the high dose and gestation Day 21 untreated groups. Prolactin receptors decreased at the low dose by 27% and by 60% in the high and gestation Day 21 untreated groups. Liver mRNA concentrations for prolactin and growth hormone decreased correlated with the decreases in receptors for these hormones. |
| Mouse-Ajax[126] | 14-16, pregnant females | im | Levothyroxine 100 µg/mouse | gestation days 10.5 and 11.5 | Levothyroxine treatment had no effect on spontaneous incidence of cleft lip/cleft palate in the presence or absence of administered cortisone. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Mouse-CI/FR & A/HeJ[127] | 4-13 CI/FR pregnant females 4-17 A/HeJ female pregnant females | sc | Levothyroxine 33-150 µg/mouse | gestation days 9 and 10 | The spontaneous incidence of cleft lip unchanged by levothyroxine treatment in either strain. In CI/FR strain fetal death selectively occurring in cleft lipped fetuses of dams treated with levothyroxine. |
| Mouse-A/WySn[128] | 7-16, pregnant females | sc | levothyroxine 900 µg/mouse | gestation day 7, 8, 9, 10 or 12 | The spontaneous incidence of cleft lip was not increased by levothyroxine treatment but fetal mortality among the cleft lipped fetuses was increased. The oral administration of levothyroxine did not increase fetal mortality. |
| | | oral | Levothyroxine 300 µg/mouse | gestation day 9, 10, or 11 | |
| Mouse-C57BL/6n[129] | 8-31, pregnant females | oral | Triiodothyronine1 20, 240, or 480 µg/kg/day alone or in combination with TCDD Levothyroxine 625, 1250 or 2500 µg/kg/day alone or in combination with TCDD Triiodothyroxine 10000 µg/kg/day in combination with 2500 µg/kg/day of levothyroxine | gestation days 10-13 | None of the levothyroxine or triiodothyronine treatments given alone or in combination with each other increased the incidence of cleft palate. All doses of triiodothyronine or levothyroxine given in combination with TCDD increased the incidence of cleft palate. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| **Mutagenicity/Clastogenicity** | | | | | |
| Mouse-dwarf dw/dw[130] | 2-8, males and females | sc | Levothyroxine 0.1 or 1.0 µg/mouse/day | 5 days/week: low dose 13-16 weeks, high dose 4 or 13weeks | No increase in the amount of micronuclei in bone marrow smear erythrocytes. |
| **Miscellaneous** | | | | | |
| Rat-liver WB-F344 cells[131] | cell cultures | *In Vitro* | Triiodothyronine 1 or 100 nmolar Levothyroxine 1 or 100 nmolar | *In Vitro* incubations for 1 or 2 days | Increase in gap junctions with both concentrations of triiodothyronine and the 100 nm concentration of levothyroxine. |

| Species-strain Ref. No. [ ] | No./sex/group | Route | Doses | Duration | Results |
|---|---|---|---|---|---|
| Rat-Wistar[112] | 40, females | ip | Triiodothyronine 100 µg/kg/day | 50, 80 or 200 days | Body weight, serum calcium and phosphorous were not affected. Serum alkaline phosphatase elevated 12 fold at 18 days and 20 fold peak elevation at 35 days. Bone mineral density of calvaria, lumbar vertebrae, or femur unchanged at 50 or 80 days of treatment. Bone mineral density of calvarial bones decreased 22% after 200 days of treatment. Right left skull distance shortened by 4%, 10% and 15% after 50, 80, and 200 days of treatment, respectively. Histological differences observed at skull sagittal suture. The distance between bones sutured decreased by about 19% after 50 or 80 days. The distance from the inner to the mid fibreosseous junction increased 2-4 fold after 80 days of treatment. |
| **General Toxicity** | | | | | |
| Dog-Keeshond[113] | 1, male | oral | Levothyroxine 10 mg/kg | single accidental ingestion | One hundred & thirty three fold increase in serum levothyroxine concentrations at 3-9 hours postingestion with normalization at Day 25. Three & one half fold increase in serum triiodothyronine concentrations at 3 days postingestion. Increase in serum alanine transaminase (ALT) occurred on Day 6 and was approximately 7 fold normal value. The dog survived. |
| Dog-Strain Unstated[114] | 1, sex unstated | oral | Levothyroxine 2.4 g/dog | single accidental ingestion | On the day of ingestion; pulse rate 250 beats/minute, respiration rate, 120 breaths/minute, body temperature 41.3°C. The dog appeared blind. Died 12 hours after the ingestion. Thyroid congested and enlarged, kidneys congested, liver pale and stomach inflamed. |
| Dog-Beagle[115] | 3, males and 6, females | oral | Levothyroxine 0.5 mg/m$^2$ | 8 weeks | Serum levothyroxine levels rose only by about 40% after 4, 6, or 8 weeks of treatment and serum triiodothyronine concentrations were unchanged. There were no effects on electrocardiogram and echocardiogram measurements. |

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