CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202231Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**202231**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**August 30th 2010**
PRODUCT:	**Levothyroxine Sodium for Injection**
INTENDED CLINICAL POPULATION:	**Treatment for Myxedema Coma, (b) (4)**
SPONSOR:	**APP Pharmaceuticals, LLC**
DOCUMENTS REVIEWED:	**EDR**
REVIEW DIVISION:	**DMEP (HFD-510)**
PHARM/TOX REVIEWER:	**Miyun Tsai-Turton, PhD, MS**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, PhD**
DIVISION DIRECTOR:	**Mary Parks, MD**
PROJECT MANAGER:	**Linda Galgay**

Date of review submission to DARRTS: April 8th 2011

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Pharam/tox recommends approval of this application.

B. Recommendation for nonclinical studies

No further animal studies will be needed.

C. Recommendations on labeling

See pharm/tox comments on labeling on pages 61-62.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

APP Pharmaceuticals, LLC did not conduct any animal studies with regards to pharmacology, pharmacokinetics, or toxicology studies in support of their Levothyroxine Sodium for Injection. The sponsor based their nonclinical data on published literature through Medline and Toxnet databases. Currently, there are no other injectable levothyroxine products currently approved by the FDA. Most studies found in literature have different routes of exposure rather than IV, which is the intended route of exposure.

As similar to other oral levothyroxine products, the animal toxicity of levothyroxine is mostly associated with expected exaggerated levothyroxine pharmacology (i.e. hyperthyroidism - decreased body weight or increased heart rate) at high doses. Because there was no toxicokinetic information available in the literature, it is difficult to correlate thyroid hormone toxicity with exposure or maximal levothyroxine concentration or relate to human exposure. On the other hand, since this IV levothyroxine is intended to use in hospitals for an acute rescue (i.e. (b) (4) myxedema coma) (b) (4) any IV levothyroxine-related adverse effects (i.e. hyperthyroidism) can be easily monitored or reversed. Also, due to severe hypothyroid states of these patients, the risk of having these hyperthyroidism-associated adverse effects would be small, even if it is at a much higher IV dose of levothyroxine when compared to oral levothyroxine which is used to maintain thyroid hormone homeostasis.

There were several impurities identified in the drug substance/product. Since they are all within acceptable limits, no animal study is needed to further characterize these impurities.

In summary, the sponsor is relying on published literature to support this IV levothyroxine product. Published literature seems to reveal a similar profile, regarding to pharmacokinetic (no apparent difference in AUC between an injectable and oral product) and toxicity (hyperthyroidism-associated adverse effects). In addition, the sponsor is relying on physicochemical characterization of identity as there is an absence of bridging animal toxicology data. Based on CMC review, both drug substance (referenced to DMF (b) (4) and drug products (e.g. impurities levels are within ICHQ3) are consistent with the monograph. Therefore, there is no safety nonclinical concern at this time.

B. Pharmacologic activity

Levothyroxine Sodium for Injection is a synthetic T_4, which is identical to that produced in the human thyroid gland. The function of thyroid hormone has been well-characterized in human.

C. Nonclinical safety issues relevant to clinical use

The sponsor did not have any bridging animal data with their IV levothyroxine product. Therefore, it is not possible to directly identify any potential nonclinical safety issue that is specifically related to this product.

Based on animal data from literature with levothyroxine and information from other approved oral levothyroxine products, there would be no safety issue except an exaggerated pharmacological effect of levothyroxine (i.e. hyperthyroidism) when it is overcompensated. Since this product will be used as hospital rescue therapy, overcompensation should not be problematic. This Levothyroxine Sodium for Injection by APP Pharmaceuticals will be the first injectable levothyroxine on the market.

It seems that both IV and oral levothyroxine have very similar profiles (i.e. no major differences in AUC and hyperthyroidism-related toxicity). However, with no bridging animal data on this IV levothyroxine product, similarity of products would be determined by physicochemical means by CMC. The CMC has communicated that the characterization of both drug substance and drug product are consistent with the monograph.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

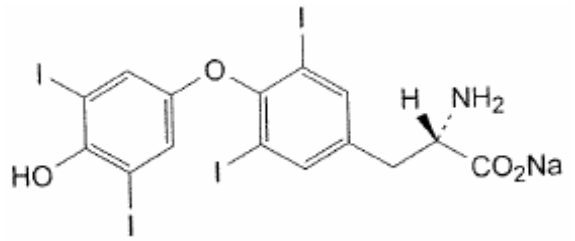
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 202231
Review number: 1
Sequence number/date/type of submission: 000/August 30th, 2010/Original 505(b)(2)
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: APP Pharmaceuticals, LLC
Manufacturer for drug substance: (b) (4)

Reviewer name: Miyun Tsai-Turton, PhD, MS
Division name: DMEP
HFD #: 510
Review completion date: 04/08/2011

Drug:

Trade name: --
Generic name: Levothyroxine Sodium for Injection
Code name(s): 506107 (100 mcg), 24710 (200 mcg), and 24810 (500 mcg)
Chemical name: L-tyrosine, O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-, monosodium salt
CAS registry number: 55-03-8
Molecular formula/molecular weight: $C_{15}H_{10}I_4NNaO_4$ and 798.85
Structure:



Relevant INDs/NDAs/DMFs:

- IND (b) (4)
- NDA 21-116: Levothroid (tablet)
- NDA 21-210: Unithroid (tablet)
- NDA 21-301: Levoxyl (tablet)
- NDA 21-342: LEVO-T (tablet) by Alara Pharm (approved on March 02')
- NDA 21-402: Synthroid (tablet)
- NDA21-924: Tirosint (capsule)
- DMF (b) (4) Levothyroxine sodium, USP

Drug class: Levothyroxine

Intended clinical population: [redacted] myxedema coma [redacted]

Clinical formulation: Levothyroxine Sodium, USP (100, 200, and 500 mcg/vial) with inactive ingredients listed below. The current dose recommendation, based on PK and clinical data, is an initial loading dose of levothyroxine between 300 to 500 mcg, followed by daily maintenance doses between 50 and 100 mcg until the patient can tolerate oral therapy. Note: During pre-IND meeting, the concern of [redacted]

APP's Levothyroxine Sodium for Injection Inactive Ingredients	Function of Inactive Ingredients
Dibasic Sodium Phosphate Heptahydrate, USP	[redacted]
Mannitol, USP	
Sodium Hydroxide, NF	
[redacted]	

1
2 [redacted]

Levothyroxine Sodium for Injection	Unit Dose	Exhibit Batches	Proposed Commercial Batch
Strength	100 mcg/vial		
Packaging Configuration	100 mcg in a 6.5–mL vial		
Product Code	506107		
Batch Size (L)	N/A	[redacted]	
Batch Size (vials)	N/A		
Ingredient	Ingredient Amount/mL	Ingredient Amount/Batch	Ingredient Amount/Batch
Levothyroxine Sodium, USP	100 mcg	[redacted]	
Dibasic Sodium Phosphate, Heptahydrate, USP	[redacted]		
Mannitol, USP			
Sodium Hydroxide, NF			
[redacted]			
Sodium Hydroxide, NF			
[redacted]			

1 Theoretical amount of Levothyroxine Sodium, USP required for the batch. [redacted]
2 [redacted]
3
4

Levothyroxine Sodium for Injection	Unit Dose	Exhibit Batches	Proposed Commercial Batch
Strength	200 mcg/vial		
Packaging Configuration	200 mcg in a 10–mL vial		
Product Code	24710		
Batch Size (L)	N/A	(b) (4)	
Batch Size (vials)	N/A		
Ingredient	Ingredient Amount/mL	Ingredient Amount/Batch	Ingredient Amount/Batch
Levothyroxine Sodium, USP	200 mcg	(b) (4)	
Dibasic Sodium Phosphate, Heptahydrate, USP	(b) (4)		
Mannitol, USP			
Sodium Hydroxide, NF			
(b) (4)			
Sodium Hydroxide, NF			
(b) (4)			

1 Theoretical amount of Levothyroxine Sodium, USP required for the batch. (b) (4)

2 (b) (4)

3

4

Levothyroxine Sodium for Injection	Unit Dose	Exhibit Batches	Proposed Commercial Batch
Strength	500 mcg/vial		
Packaging Configuration	500 mcg in a 10–mL vial		
Product Code	24810		
Batch Size (L)	N/A	(b) (4)	
Batch Size (vials)	N/A		
Ingredient	Ingredient Amount/mL	Ingredient Amount/Batch	Ingredient Amount/Batch
Levothyroxine Sodium, USP	500 mcg	(b) (4)	
Dibasic Sodium Phosphate, Heptahydrate, USP	(b) (4)		
Mannitol, USP			
Sodium Hydroxide, NF			
(b) (4)			
Sodium Hydroxide, NF			
(b) (4)			

1 Theoretical amount of Levothyroxine Sodium, USP required for the batch. (b) (4)

2 (b) (4)

3

4

Route of administration: (b) (4) IV

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

For (b)(2) applications:
Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 202231 are owned by APP Pharmaceuticals or are data for which APP Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 202231 that APP Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that APP Pharmaceuticals does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 202231. Note: the sponsor is relying published literature to support their drug product. However, two oral products (Synthroid® and Levothroid®) were mentioned in the labeling.

Studies reviewed within this submission: N/A

Studies not reviewed within this submission: N/A

2.6.2 PHARMACOLOGY

Levothyroxine sodium for injection acts like the endogenous T_4 produced naturally in the human thyroid gland.

MOA: T_4 is converted to T_3, the active metabolite in the liver and kidney. To increase solubility, the T_3 and T_4 attach to thyroid hormone binding proteins, which then transport and bind to thyroid hormone receptors in the cytoplasm and nucleus. Therefore, by acting as a replacement of natural T_4, symptoms of T_4 deficiency are relieved. The pharmacologic profile of levothyroxine (T_4) sodium is well established. This drug product is a synthetic T_4.

The primary functions of thyroid hormone, both T_3 and T_4, are to increase metabolism (i.e. protein synthesis), growth and development (i.e. growth hormone, bone growth, and neuronal maturation), and catecholamine effect (i.e. adrenaline). The sponsor provided a literature review, using Medline and Toxnet as two separate databases, to support the pharmacological profile (PD + safety pharm) of levothyroxine sodium for injection. These cited studies are non-GLP.

The most common finding with levothyroxine is hyperthyroidism, an exaggerated pharmacologic response (i.e. weight loss, increased food consumption, increased heart

rate, increased blood pressure), in thyroid-status compromised or uncompromised animals (i.e. mice, rats, guinea pigs, rabbits, or dogs) via different routes (i.e. intraperitoneal, subcutaneous, oral, or intravenous). Some CNS (i.e. changes in amygdale-related behavior in adult, spatial learning, and hippocampal morphology) and CV findings (i.e. cardiac hypertrophy) were linked with levothyroxine to animals. All in all, no new findings were identified which would impact on the safety of Levothyroxine Sodium for Injection.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Overview

Table 2.6.3-1 Pharmacology: Overview for Test Article: Levothyroxine

Type of study	Test System	Mode of Administration	Test Facility	Study Number	Location in CTD (File Path)
Primary Pharmacodynamics					
	Beagle dogs	p.o.	N/A	Panciera, et al. (1989)	Module 4.3
	Beagle dogs	p.o.	N/A	Panciera, et al. (1990)	Module 4.3
	English Coonhound dog	p.o./i.v.	N/A	Henik, and Dixon (2000)	Module 4.3
Secondary Pharmacodynamics					
	Sprague-Dawley rats and Wistar rats	i.p.	N/A	Sanchez, et al. (2009)	Module 4.3
	Wistar albino rats	i.p.	N/A	O Makay, et al. (2009a)	Module 4.3
	Wistar albino rats	i.p.	N/A	B Makay, et al. (2009b)	Module 4.3
	Wistar rats	s.c.	N/A	Pascual-Leone, et al. (1983)	Module 4.3
	Wistar rats/guinea pigs	s.c./i.p.	N/A	Bhatt and Makwana (2008)	Module 4.3
	Wistar rats	p.o.	N/A	Caria, et al. (2009)	Module 4.3
	NMRI mice	p.o.	N/A	Diepart et al., (2009)	Module 4.3
	New Zealand rabbits	p.o.	N/A	Nowosadzka, et al. (2009)	Module 4.3
	Unspecified dogs	p.o.	N/A	Gommeren, et al. (2009)	Module 4.3
	Mixed breed and quarter horse-type horses	p.o.	N/A	Frank, et al. (2005)	Module 4.3
	Mixed breed and	p.o.	N/A	Frank, et al. (2008)	Module 4.3

Table 2.6.3-1 Pharmacology: Overview for Test Article: Levothyroxine

Type of study	Test System	Mode of Administration	Test Facility	Study Number	Location in CTD (File Path)
	quarter horse-type horses				
	Wistar rats	i.p.	N/A	Bubber, et al (2009)	Module 4.3
Safety Pharmacology					
	Wistar rats	i.p.	N/A	Ruiz-Marcos, et al. (1994)	Module 4.3
	Wistar rats	i.p.	N/A	Bruno, et al. (2005)	Module 4.3
	Wistar rats	i.p.	N/A	Lenzen and Kucking (1982)	Module 4.3
	Wistar rats	i.p.	N/A	Szabo, et al. (1979)	Module 4.3
	Sprague-Dawley rats	i.p.	N/A	Yao and Eghbali (1992)	Module 4.3
	Sprague-Dawley rats	i.p.	N/A	Atkins, et al. (1983)	Module 4.3
	Sprague-Dawley rats	i.p.	N/A	Kuzman, et al. (2005)	Module 4.3
	Wistar rats	i.p.	N/A	Venditti, et al. (2009)	Module 4.3
	Wistar rats	s.c.	N/A	Yilmazer-Hanke, et al (2004)	Module 4.3
	Sprague-Dawley rats	Unspecified injection	N/A	Axelrod, et al. (1974)	Module 4.3
	Wistar rats	i.p.	N/A	Carneiro-Ramos, et al (2010)	Module 4.3
	Syrian hamsters	i.p.	N/A	Hegyvary, et al. (1979)	Module 4.3
	Sprague-Dawley rats	p.o.	N/A	Wang, et al (2010)	Module 4.3
	Guinea pigs	i.p.	N/A	Rackwitz, et al. (1975)	Module 4.3
	New Zealand white	i.p.	N/A	Yu, et al. (2009)	Module 4.3

Table 2.6.3-1 Pharmacology: Overview for Test Article: Levothyroxine

Type of study	Test System	Mode of Administration	Test Facility	Study Number	Location in CTD (File Path)
	rabbits				
Pharmacodynamic Drug Interactions					
	N/A	N/A	N/A	N/A	N/A

N/A = Not Applicable

Pharmacology

- Note: Articles in the table below were cited as Primary Pharmacodynamic studies by the sponsor.

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
Endocrine	Beagle dogs	p.o.	0.5 mg/m^2 of body surface twice daily for 8 weeks	5 male, 9 female	Suppression of the triiodothyronine (T_3) response to thyrotropin (TSH) was evident at treatment week 2, whereas the thyroxine (T_4) response was suppressed at Week 4 and remained suppressed for the duration of the study.	No	Panciera, et al. (1989)
Endocrine	Beagle dogs	p.o.	0.5 mg/m^2 of body surface twice daily, for 8 or 9 weeks	5 male, 9 female	In treated dogs, the volume density of thyrotropes decreased during thyroxine treatment and increased 6 weeks after cessation of treatment, compared to controls. The activity of the thyroid gland was decreased in dogs during thyroxine treatment. After cessation of thyroxine treatment, the thyroid gland had decreased colloid area, follicular area and epithelial volume density, and increased interstitial volume density, compared to controls	No	Panciera, et al. (1990)
Endocrine	English Coonhound dog	p.o./i.v.	4.0 µg/kg and 1.0 µg/kg, q12h	1 male, 0 female	IV administration of levothyroxine can be used successfully in combination with oral administration to restore normal metabolic function and assist in warming and thermoregulation,	No	Henik, and Dixon (2000)

- Note: Articles in the next two tables below were cited as Secondary Pharmacodynamic studies by the sponsor except Ruiz-Marcos, et al. (1994) which was cited as safety pharmacology study.

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					although dosages should be conservative to avoid adverse cardiovascular effects.		
Endocrine	Wistar rats	i.p.	1.7 µg/100 g for 3 days, 2.5 µg/100 g for 8 days, 5 µg/100 g for 3 days	Unspecified number of males and females	Growth hormone (GH) content of the adult neo-T_4 treated animals was significantly lower than that of the adult controls. Administration of different doses of T_4 to adult neo-T_4 rats did not alter these decreased pituitary GH levels.	No	Pascual-Leone, et al. (1983)
Endocrine	Wistar rats/guinea pigs	s.c. to rats/i.p. to guinea pigs	0.75 mg/kg every other day for 3 weeks to rats; 75 µg/kg every other day for 3 weeks to guinea pigs	0 males, 32 females	Verapamil and propranolol administered daily in the third week along with L-thyroxine to two separate groups of hyperthyroid animals reversed thyroxine-induced loss in body weight, reduction in serum TSH levels, and rise in body temperature. Compared to normal rats, L-thyroxine-treated animals showed a state of hyperglycemia, hyperinsulinemia, impaired glucose tolerance, and insulin resistance.	No	Bhatt and Makwana (2008)
Endocrine	Mixed breed and quarter horse-type horses	p.o.	24, 48, 72 or 96 mg per day for 8 weeks	0 males, 12 females	Plasma triglycerides, total cholesterol and very low density lipoprotein concentrations significantly decreased over time in treated horses. Insulin	No	Frank, et al. (2005)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					sensitivity significantly increased (> 2-fold) intreated horses, but glucose effectiveness and net insulin response were not affected.		
Endocrine	Mixed breed and quarter horse-type horses	p.o.	48 mg daily for 48 weeks	0 males, 6 females	During treatment, body weight decreased significantly from the pretreatment values. Compared with pretreatment findings, 1.8, 2.4 and 1.9 fold increases in mean insulin sensitivity were detected at weeks 16, 32 and 48, respectively.	No	Frank, et al. (2008)
Endocrine	Wistar rats	i.p	75 µg/100 g daily for 7 days	4 males, 0 females	Treatment for one week increased plasminogen activator activity significantly in rat kidneys compared with controls. There was significant decrease in total cholesterol and low density lipoprotein-cholesterol levels in the serum of the treated group resulting in the decrease of high density lipoprotein/low density lipoprotein and total cholesterol/cholesterol ratios. However, triglycerides and very low density lipoproteins showed significant higher activity in the serum of the treated group as compared to controls.	No	Bubber, et al (2009)
Endocrine	Wistar rats	i.p	1.50 µg/100 g	Unspecified	In contrast to the permanent	No	Ruiz-Marcos, et

- Note: Articles in the next eight tables were cited as Safety Pharmacology studies by the sponsor.

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
			daily for 20 days 1.75 µg/100 g daily for 20 days	number of males and females	alterations resulting from neonatal hypothyroidism, the effects of juvenile-onset hypothyroidism on the number and distribution of spines along the apical shaft of pyramidal neurons of the visual cortex appeared to be potentially reversible with adequate thyroxine therapy.		al. (1994)
Endocrine	Wistar rats	i.p	25 µg/100 g daily for 14 days	Unspecified number of males and females	Rats starting the treatment aged 5 days demonstrated a significant increase in adenosine diphosphate (ADP) and adenosine monophosphate (AMP) hydrolysis and increased fail-flick latency. In contrast, in the spinal cord from hyperthyroid rats aged 60 and 330 days old, the hydrolysis of adenosine triphosphate (ATP), ADP and AMP were significantly decreased.	No	Bruno, et al. (2005)
Endocrine	Wistar rats	i.p	0.05-2.0 µg/kg daily for 5 days; 20 mg/kg	Unspecified number of males, 0 females	Both L-thyroxine and D-thyroxine induced an inhibition of glucose-induced insulin secretion with comparable time- and dose-dependent characteristics. L-thyroxine was ten times more potent than D-thyroxine. While L-thyroxine and a ten times higher	No	Lenzen and Kucking (1982)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					dose of D-thyroxine had a similar potency in inducing hyperthermia and hypocholesterolemia, hyperglycemia in response to D-thyroxine was less pronounced than in response to L-thyroxine		
Endocrine	Wistar rats	i.p.	1 mg/kg daily for 14 days	50 males, 0 females	Wet and dry ventricle weights and the level of cardiac ribonucleic acid (RNA) and protein were augmented by thyroxine treatment. ATPase activity of cardiac myosin was stimulated as the Ca^{2+} concentration in the incubation medium increased. No difference was found in Ca^{2+} activation, salt sensitivity or ATPase activity of unreacted and sulphydryl-modified cardiac myosins from euthyroid or hyperthyroid groups.	No	Szabo, et al. (1979)
Endocrine	Sprague-Dawley rats	i.p.	10 µg/100 g daily for 1, 2, 3, 6, 12 or 14 days	100 males, 0 females	Northern blot analysis of RNA from total ventricular tissue showed that after 2 hours of treatment, the abundance of mRNA for pro α_2(I) collagen decreased by 53% ($p<0.05$) and reached the lowest level (60% decrease, $p<0.02$) at Day 1, remained diminished at Day 3 and then gradually returned toward	No	Yao and Eghbali (1992)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					normal levels. These findings suggest that cardiac fibroblasts and proteins originating from them are targets for thyroid hormone action in the myocardium and that this hormone plays a regulatory role on interstitial protein gene expression.		
Endocrine	Sprague-Dawley rats	i.p.	0.5 mg/kg daily for 7 days	Unspecified number of males, 0 females	Regression of cardiac hypertrophy with a decrease in receptor number to control values was seen as early as 2 days after stopping thyroxine. Cardiac norepinephrine concentrations had also returned to control values at this time. Displacement of bound [H^3] dihydroalprenolol by isoproterenol was not changed from control. Basal and isoproterenol stimulated adenyl cyclase activity was not changed by thyroxine administration or its cessation.	No	Atkins, et al. (1983)
Endocrine	Sprague-Dawley rats	i.p.	1 mg/100 g daily for 7 days	0 males, 33 females	Predictably, T_4 increased heart weight, left ventricular systolic pressure, and dP/dT. T_4 and propylithiouracil also had typical effects on expression of thyroid responsive genes such as α and β myosin heavy chain. T_4 treatment	No	Kuzman, et al. (2005)(1)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					caused phosphorylation of Akt and downstream signaling components such as GSK-3β, mammalian target of rapamycin (mTOR) and S6 kinase.		
Endocrine	Wistar rats	s.c.	7.5 µg daily for 12 days	39 males, 31 females	Thyroxine-treated pups presented an accelerated development including opening of eyes and snout elongation as typical signs of hyperthyroidism. Thyroxine-treated adult animals displayed a reduced anxiety in the motility box and elevated plus maze, a reduction in the number of corticotropin-releasing-factor immunoreactive (CRF-ir) neurons in the central nucleus of the amygdale, as well as an increase in the number of neuropeptide Y immunoreactive (NPY-ir) neurons and density of tyrosine hydroxylase immunoreactive (TH-ir) fibers inclucei of the basolateral complex of the amygdale. The findings suggested a shift toward an anxiolytic rather than anxiogenic distribution of peptidergeic neurons and fibers in the amygdale at adult age	No	Yilmazer-Hanke, et al (2004)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					following transient neonatal hyperthyroidism.		
Endocrine	Sprague-Dawley rats	Unspecified injection	1 µg/g daily for 14 days	Unspecified number of males and females	L-thyroxine had a small effect on mean blood pressure, a larger effect on heart rate, and caused a large increase in cardiac output. In companion experiments, D-and L-thyroxine were shown to be equipotent in stimulating atrial protein synthesis in vitro. The growth-promoting effects of both D-and L-thyroxine, therefore, seem largely direct and independent of the large increase in flow work produced by administration of the L-isomer in vivo.	No	Axelrod, et al. (1974)
Endocrine	Wistar rats	i.p.	0.100 mg/kg daily for 14 days	Unspecified number of males and females	Serum levels of T_3 and T_4 as well as hemodynamic parameters such as systolic blood pressure and heart rate, were significantly higher in the T_4-treated rats than in the control animals. Cardiac hypertrophy, given by the heart weight /body weight ratio, was considerably higher in rats treated with T_4 than in the control rats. Cardiac Angiotensin II levels were significantly increased as well as the Angiotensin II type 2 receptor	No	Carneiro-Ramso, et al (2010)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					expression in throid hormone-induced cardiac hypertrophy. Administration of an Angiotensin II type 2 receptor blocker was able to prevent cardiac mass gain and the Akt activation induced by thyroid hormone. The cardiac β_1 adrenergic receptor and adrenaline levels were significantly increased after T_4 treatment.		
Endocrine	Syrian hamsters	i.p.	15 µg, 30 µg, 50 µg or 100 µg daily for 4 weeks	102 males, 0 females	In contrast to normal controls, in myopathic hamsters administration of low doses of L-thyroxine for 4 weeks failed to induce tachycardia, increase the total activity of NaK-ATPase with the cells or improve contractility of the heart muscle. In addition, L-thyroxine treatment decreased the activity of adenyl cyclase in the myopathic and that of soluble protein kinases in both normal and myopathic hearts.	No	Hegyvary, et al. (1979)
Endocrine	Guinea pigs	i.p.	0.7 mg/kg, 0.35 mg/kg, 0.175 mg/kg or 0.007 mg/kg daily for 7 days	Unspecified number of males, 0 females	Thyroxine induced a dose-related decrease of creatine phosphate (CP) and a corresponding increase of inorganic phosphate (Pi) even in the lowest dose used in this study (0.07mg/kg) and became more pronounced with increased doses.	No	Rackwitz, et al. (1975)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					No remarkable changes of adeninenucleotides (ATP, ADP, AMP were observed. Glucose-6-phosphate (G-6-P) and fructose diphosphate (FDP) levels were markedly elevated in all dosages.		
Endocrine	Sprague-Dawley rats	p.o.	100 µg/kg daily for 2 or 4 weeks	Unspecified number of males, 0 females	Heart rate was significantly increased in the 2- and 4-week T_4-treated groups compared to controls. T_4-treated rats lost body mass compared with controls and absolute heart wet mass and ratio of heart mass to body mass increased significantly in T_4-treated rats compared to controls. The apoptotic rate was significantly increased in the 4-week T_4-treated group compared with controls. DNA laddering was detectable in the right and left ventricles of the 4-week T_4-treated group. The intrinsic heart rate of the isolated working heart was increased in both groups of T_4-treated rats.	No	Wang, et al (2010)
Endocrine	New Zealand white rabbits	i.p.	1 mg/kg daily for 2 weeks	Unspecified number of males and females	Atrial monophasic action potential at 90 repolarization ($AMAP_{90}$) and effective refractory period (AERP) were significantly shorter in the	No	Yu, et al. (2009)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					Thyroxine group than in the Control group. There were significant differences in the incidence of atrial arrhythmias between the two groups. The mean peak of L-type calcium current (I_{CaL}) densities at -10 mV was significantly higher in the Thyroxine group than in the Control group.		
Endocrine	Wistar rats	i.p.	10 µg/kg daily for 10 days	Unspecified number of males, 0 females	The metabolic response of homogenates and mitochondria indicated that all treatments increased the synthesis of respiratory chain components, whereas only the cold-induced mitochondrial proliferation. Determination of mRNA and protein expression of transcription factor activators, such as nuclear respiratory factor 1 (NRF-1) and nuclear respiratory factor 2 (NRF-2), and coactivators, such as proliferator-activated receptor γ (PPAR-γ) coactivator 1 (PGC-1), showed that mRNA levels, except PGC-1 ones, were not related to aerobic capacities. Conversely, a strong correlation was found	No	Venditti, et al. (2009)

- Note: Articles in the next four tables below were cited as Secondary Pharmacodynamic studies by the sponsor.

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					between cytochrome oxidase activity and PGC-1 or NRF-2 protein levels.		
Endocrine	Sprague-Dawley rats and Wistar rats	i.p.	10 µg daily for 1, 2 or 3 days	25 males, 0 females	Compared with vehicle-treated, euthyroid controls, animals made thyrotoxic by the i.p. administration of 10 µg L-T_4 daily for 1-3 days, showed dramatically increased accumulation of silver grains in the mediobasal hypothalamus and an approximately 80% increase in enzymatic activity. Pyroglutamyl peptidase II (PPII) inhibition in mediobasal hypothalamic explants increased thyrotropin-releasing hormone (TRH) secretion, whereas IP injection of a specific PPII inhibitor increased cold stress- and TRH-induced TSH levels in plasma.	No	Sanchez, et al. (2009)
Endocrine	New Zealand rabbits	p.o.	200 µg/kg daily for 4 days	21 males, 0 females	Significantly higher level of Sambucus nigra agglutinin (SNA) than Maackia amurensis agglutinin (MAA) linkage sialoglycoproteins in all evaluated fractions. The sialylation ratio was significantly lower ($p < 0.05$) in the nuclear fraction in case of hyperthyroidism	No	Nowosadzka, et al. (2009)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					(detected with both agglutinins). In contrast to the nuclear fraction the content of SNA-detected sialoglycoproteins was significantly reduced in the mitochondrial fraction of hyperthyroid hepatocytes ($p < 0.05$).		
Endocrine	Wister albino rats	i.p.	0.2 mg/kg daily for 21 days	42 males, 0 females	In both time periods, serum xanthine oxidase (XO), malondialdehyde (MDA), glutathione (GSH) and nitric oxide derivates (NO·x) levels were significantly increased after thyroid hormone induction ($p<0.05$). There was a remarkable decrease in both $T_3$and T_4 levels in group C after 21 days ($p<0.05$).	No	O Makay, et al. (2009)
Endocrine	Wistar albino rats	i.p.	0.2 mg/kg daily for 21 days	21 males, 0 females	MDA, GSH, NO•x, Interleukin 10 (IL-10), and (tumor necrosis factor alpha) TNFα levels increased after L-thyroxine induction. An inhibition of $T_3$and T_4 was detected, as a result of melatonin administration. MDA, GSH, and NO•x levels were also affected by melatonin.	No	B Makay (2009)
Endocrine	NMRI mice	p.o.	0.003% in drinking water daily for 21 days	Unspecified number of males and females	Tumor cells extracted from control mice consumed oxygen more slowly than tumor cells from hyperthyroid mice. The mean slopes significantly different from each other. The kinetics of the return of basal values was faster in hyperthyroid mice than in control mice	No	Diepart, et al. (2009)
Endocrine	Unspecified dogs	p.o.	20 µg/kg daily for 6 months	14 dogs; unspecified males and females	The average body weight of the dogs decreased from 35 ± 18 kg at baseline to 27 ± 14 kg at 6 months ($p<0.05$). All dogs remained normotensive throughout the study. Glomerular filtration rate (GFR) increased significantly with levothyroxine supplementation. Serum creatinine concentration decreased after treatment, with a significant difference between baseline and 1 month ($p=0.024$).	No	Gommeren, et al. (2009)
Endocrine	Wistar rats	Local microinjection	10-6 M, 0.4 µL/min	14 males, 0 females	Local T_4 administration promptly inhibited field excitatory postsynaptic potentials recorded in the dentate gyrus (DG) with stimulation of the medial perforant pathway, a result especially pronounced in hypothyroid rats. In separate *in vitro* experiments, the authors observed more subtle but statistically significant responses	No	Caria, et al. (2009)

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Table 2.6.3-2 Safety Pharmacology for Primary Pharmacodynamics, Secondary Pharmacodynamics and Safety Pharmacodynamics Test Article: Levothyroxine

Organ System Evaluated	Species/ Strain	Mode of Administration	Doses[a] (mg/kg)	Gender and Number Per Group	Noteworthy Findings	GLP Compliance	Study Number
					of hippocampal slices to treatment with the hormone. The results demonstrate that baseline firing rates of CA1 pyramidal cells were modestly reduced by pulse-perfusion with T_4. By contrast, administration of T_3 was often noted to have modest enhancing effects of CA1 cell firing rates in hippocampal slices from euthyroid animals. Moreover, and more reliably, robust firing rate increases induced by norepinephrine were amplified when preceded by treatment with T_3, whereas they were diminished by pretreatment with T_4.		

[a] Single dose unless otherwise noted.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Publications were extracted from Medline and Toxnet as two separate databases to support the PK/TK profile of levothyroxine sodium for injection.

Several analytical methods (i.e. radioimmunoassay or electrochemiluminescence immunoassays) were used to generate the ADME profile of levothyroxine.

Absorption (TK oral) data showed that the t_{max} was 3-7 hrs for tablet and solution oral formulations, and $t_{½}$ was approx. 8.6-14.6 hrs in dogs. Distribution (placental transfer) data indicated that minimal placental transfer of T_4 occurred in the rat during the last days of gestation. The metabolism data showed sodium salicylate increased the rate of T_4 disappearance from the blood and increased the update of T_4 by the isolated perfused rat livers, as well as increased rats of conjugation of T_4 with glucuronic acid. The elimination data showed there was the hepatobiliary clearance of T_4 by hepatic microsomal enzyme induction, and approx. 3% of total administration dose of T_4 was excreted into bile in rats. As similar to animals, the metabolism of T_4 occurs mostly in the human liver. T_4 can be conjugated with glucuronic and sulfuric acids and excreted in the bile. All in all, no new findings were identified which would impact on the safety of Levothyroxine Sodium for injection.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Overview

Table 2.6.5-1: Pharmacokinetics: Overview Test Article: Levothyroxine

Type of study	Test System	Mode of Administration	Test Facility	Study Number	Location in CTD (File Path)
Absorption					
	Mature, mixed-breed dogs	p.o.		Nachreiner, et al (1993)	Module 4.3
	Male hypothyroid beagle dogs	p.o.		Wood, et al (1990)	Module 4.3
	Male and female beagle dogs	p.o.		Le Traon, et al. (2008)	Module 4.3
Distribution					
	Virgin Sprague-Dawley rats	i.v.		Dussault and Coulombe (1980)	Module 4.3
Metabolism					
	Adult male Sprague-Dawley rats	i.v.		Flock and Owen (1965)	Module 4.3
Excretion					
	Adult male Sprague-Dawley rats	i.v.		Flock and Owen (1965)	Module 4.3
	Adult male Wistar and TR⁻ rats	gavage		Lecureux, et al. (2009)	Module 4.3
Pharmacokinetic Drug Interactions					
	Male Wistar rats	p.o. and i.v.		Jin, et al (2005)	Module 4.3
	Male rats (Upj:TUC(SD)spf)	gavage		Phillips, et al (1974)	Module 4.3
	Adult male Sprague-Dawley rats	i.v.		Flock and Owen (1965)	Module 4.3

Absorption

Note: single and repeated doses

Table 2.6.5 2: Pharmacokinetics: Absorption after a Single Dose for Test Article: Levothyroxine

	Study Number			
	Nachreiner, et al. (1993)	**Wood, et al. (1990)**	**Le Traon, et al. (2008)**	**Location in CTD (File Path)**
Species	Mixed-breed dogs	Beagle dogs	Beagle dogs	Module 4.3
Gender (M/F)/ No. of Animals	9 male, 3 female	6 male, 0 female	30 males and females; ratio unspecified	Module 4.3
Feeding Condition	Fasted	Not Specified	Fed, fasted	Module 4.3
Vehicle/Formulation	Tablets	Half-tablets	Tablet, oral solution, i.v. injection	Module 4.3
Method of Administration	p.o.	p.o.	p.o., i.v.	Module 4.3
Dose (mg/kg)	11, 22 or 44 µg/kg of body weight/day	100 µg	40 µg/kg body weight, 200 µg	Module 4.3
Sample [e.g., whole blood, plasma]	Whole blood, serum	Whole blood, serum	Whole blood, serum	Module 4.3
Analyte	T_4	T_4	T_4	Module 4.3
Assay	Radioimmunoassay	Radioimmunoassay	Electrochemiluminescence immunoassay	Module 4.3
		High Performance Liquid Chromatography (HPLC)	Radioimmunoassay	Module 4.3

Table 2.6.5 2: Pharmacokinetics: Absorption after a Single Dose for Test Article: Levothyroxine (continued)

	Study Number			
	Nachreiner, et al. (1993)	**Wood, et al. (1990)**	**Le Traon, et al. (2008)**	**Location in CTD (File Path)**
PK Parameters:	Mean residence time (MRT), area under the curve (AUC), AUC/dose, maximum concentration (C_{max}), minimum concentration (C_{min}), time of maximum concentration (T_{max}), half-life ($t_{1/2}$), mean steady state concentration (C_{ss})	AUC, C_{max}, T_{max}, observed absorption rate constant (k_a), elimination rate constant (k_{el})	C_{max}, T_{max}, $t_{1/2}$, AUC, MRT, Clearance (Cl), apparent volume of distribution (Vd)	Module 4.3
Additional Information:	Css was highest for dogs of the 44 µg/kg body weight once daily group. Clearance was faster for the 22- and 44 µg/kg body weight than for 11 µg/kg body weight	Average AUC_{0-8} for synthroid, brand A and brand B were 8.22, 6.32 and 8.70 ng·h/mL, respectively. C_{max} was 1.26, 1.07 and 1.36 ng/mL, respectively	The bioavailability of thyroxine (T4) following oral administration of the oral solution of L-T4 sodium to fasted dogs was 22 ± 5%. L-T4 absorption was significantly delayed and its rate and extent reduced when the oral solution of L-T4 sodium was administered with food. The relative bioavailability of L-T4 following oral administration to fed dogs was 55 ± 29%. The relative bioavailability of a single dose of the oral solution was 206% that of the same dose of the tablet formulation.	Module 4.3

Table 2.6.5-3: Pharmacokinetics: Absorption after Repeated Doses for Test Article: Levothyroxine

	Study Number			
	Nachreiner, et al. (1993)	**Wood, et al. (1990)**	**Le Traon, et al. (2008)**	**Location in CTD (File Path)**
Species	Mixed-breed dogs	Beagle dogs	Beagle dogs	Module 4.3
Gender (M/F)/No. of Animals	9 male, 3 female	6 male, 0 female	30 males and females; ratio unspecified	Module 4.3
Feeding Condition	Fasted	Not Specified	Fed, fasted	Module 4.3
Vehicle/Formulation	Tablets	Half-tablets	Tablet, oral solution, i.v. injection	Module 4.3
Method of Administration	p.o.	p.o.	p.o., i.v.	Module 4.3
Dose (mg/kg)	11, 22 or 44 µg/kg of body weight/day	100 µg	40 µg/kg body weight, 200 µg	Module 4.3
Sample [e.g., whole blood, plasma]	Whole blood, serum	Whole blood, serum	Whole blood, serum	Module 4.3
Analyte	T_4	T_4	T_4	Module 4.3
Assay	Radioimmunoassay	Radioimmunoassay	Electrochemiluminescence immunoassay	Module 4.3
		High Performance Liquid Chromatography (HPLC)	Radioimmunoassay	Module 4.3

Table 2.6.5-3: Pharmacokinetics: Absorption after Repeated Doses for Test Article: Levothyroxine (continued)

	Study Number (continued)			
	Nachreiner, et al. (1993)	**Wood, et al. (1990)**	**Le Traon, et al. (2008)**	**Location in CTD (File Path)**
PK Parameters:	Mean residence time (MRT), area under the curve (AUC), AUC/dose, maximum concentration (C_{max}), minimum concentration (C_{min}), time of maximum concentration (T_{max}), half-life ($t_{1/2}$), mean steady state concentration (C_{ss})	AUC, C_{max}, T_{max}, observed absorption rate constant (k_a), elimination rate constant (k_{el})	C_{max}, T_{max}, $t_{1/2}$, AUC, MRT, Clearance (Cl), apparent volume of distribution (Vd)	Module 4.3
Additional Information:	Css was lowest for the 11 µg/kg divided dose. Similarly, lower results for divided dose administration were found for Cmax and dosage-adjusted AUC, but the 22 µg/kg divided dose regimen gave higher results than did the 22 µg/kg single dose regimen.		C_{max}, T_{max} and AUC values calculated on day 14 were not significantly different from those observed on day 1. Although dogs received 400 µg of L-T4 as tablets vs. only 200 µg of L-T_4 of the oral solution, similar AUC values ($p < 0.05$) were observed over the 24 h post-treatment period.	Module 4.3

Distribution

Note: organ distribution, plasma protein binding, and PK in pregnant/nursing animals

Table 2.6.5-4: Pharmacokinetics: Organ Distribution Test Article: Levothyroxine

Species: Sprague-Dawley rats Gender (M/F)/No. of Animals: 6 female (pregnant), 0 male Feeding Condition: Fed Vehicle/Formulation: bolus solution Method of Administration: i.v. injection	Dose: 2 µCi Radionuclide: [^{125}I]T_4 Specific Activity: ~1000 µCi/µg Sampling Time: 16, 18 and 20 days of gestation	Study No.: Dussault and Coulumbe (1980) Location in CTD: Module 4.3

	Concentration (% dose/ml)					
Tissue/Organs	**T1 (16 days)**	**T2 (18 days)**	**T3 (20 days)**	**T(4)**	**T(5)**	**$t_{½}$**
Placenta	0.003 ± 0.001	0.009 ± 0.004	0.042 ± 0.002[1]			12.0 ± 0.86 h
Additional Information:						

[1] $p < 001$ versus day 18 values

Table 2.6.5-5: Pharmacokinetics: Plasma Protein Binding for Test Article: Levothyroxine

Study System: N/A
Target Entity, Test System, and Method: N/A

				Location in CTD
Species	**Concentration Tested**	**% Bound**	**Study No.**	**{Filepath}**
N/A	N/A	N/A	N/A	N/A
Additional Information: N/A				

N/A=not applicable

Table 2.6.5-6: Pharmacokinetics: Study in Pregnant or Nursing Animals for Test Article: Levothyroxine

Type of Study:	Placental Transfer	Study No.: Dussault and Coulumbe, (1980)
Species:	Sprague-Dawley rats	Location in CTD: Module 4.3
Gestation Day/Number of Animals:	16/6, 18/6, 20/6	
Vehicle/Formulation:	bolus solution	
Method of Administration:	i.v.	
Dose (mg/kg):	2 µCi	
Analyte:	T_4	
Assay:	Not Specified	

	Time (day)		
	16	**18**	**20**
Concentration/Amount (% of Dose)			
Dam serum	1.045 ± 0.161	0.946 ± 0.130	1.340 ± 0.120
Fetus serum	0.003 ± 0.001	0.009 ± 0.004	0.042 ± 0.002[1]

Additional Information: [1] $p < 0.001$ versus Day 18 values

Table 2.6.5-7: Pharmacokinetics: Other Distribution Study for Test Article: Levothyroxine

Study System:	Study No.: N/A
	Location in CTD: N/A

	Time (hr)				
	{Time}	{Time}	{Time}	{Time}	{Time}
N/A	N/A	N/A	N/A	N/A	N/A

Additional Information: N/A

N/A=not applicable

Metabolism

Note: in vivo and in vitro

Table 2.6.5-8: Pharmacokinetics: Metabolism *In Vivo* for Test Article: Levothyroxine

Gender (M/F)/Number of Animals:	All male/Not specified
Feeding Conditions:	Rockland rat diet
Vehicle/Formulation:	bolus solution
Method of Administration:	i.v. injection
Dose (mg/kg):	8.2-22.1 µCi/100 g
Radionuclide:	^{131}I T_4
Specific Activity:	

		Sampling Time	% of Compound in Sample						Location in CTD
Species	**Sample**	**or Period**	**Parent (T_4)**	**3,5,3' T_3**	**3,3',5' T_3**	**Tetrac**	**3,3' T_2**	**Study No.**	
Sprague-Dawley rats	Plasma	0-6 h						Flock and Owen (1965)	Module 4.3
	Urine	0-6 h							
	Bile	0-6 h	54.2 ± 3.2	4.5 ± 0.2	6.7 ± 2.5	8.3 ± 0.7	10.8 ± 1.8		
	Feces	N/A	N/A	N/A	N/A	N/A	N/A		
	Plasma	0-24 h							
	Urine	0-24 h							
	Bile	6-24 h	42.4 ± 2.8	3.1 ± 0.4	5.5 ± 1.9	6.4 ± 0.6	8.3 ± 1.4		
	Feces	N/A	N/A	N/A	N/A	N/A	N/A		

Additional Information: The multiple effects of salicylate on the exchange of T_4 between liver and blood and on the metabolism of T_4 in the liver were clearly seen when T_4 ^{131}I was administered to the rat one hour before the liver was removed for perfusion and T_4 ^{125}I and 50 or 75 mg of sodium salicylate were added to the perfusing blood before the liver was attached to the perfusion system. In a typical experiment, T_4 ^{131}I in the liver was decreased to 46% of the original level in one hour of perfusion and to 30% in five hours. There was 40% of the ^{131}I in the blood at one hour (most of it was in T_4) and 33% of the ^{131}I was in the blood at five hours (including 5% as iodide). About one third of the ^{131}I was excreted in the bile during the five-hour period. In similar experiments without salicylate, Gorman and co-workers found that T_4 ^{131}I present in the liver when the perfusion was begun decreased to about 20%of the dose in one hour, while the ^{131}I added to the blood by the liver reached a level of about 70%in the hour. About 15% of the dose of ^{131}I was excreted in the bile in five hours.

Table 2.6.5-9: Pharmacokinetics: Metabolism *In Vitro* for Test Article: Levothyroxine

Study System:				Study No.:N/A **Location in CTD:** N/A	
	Time (hr)				
	{Time}	**{Time}**	**{Time}**	**{Time}**	**{Time}**
Concentration	N/A	N/A	N/A	N/A	N/A
Compounds	N/A	N/A	N/A	N/A	N/A
Parent	N/A	N/A	N/A	N/A	N/A
M1	N/A	N/A	N/A	N/A	N/A
M2	N/A	N/A	N/A	N/A	N/A
Additional Information: N/A					

N/A=not applicable

Table 2.6.5-10: Pharmacokinetics: Possible Metabolic Pathways for Test Article: Levothyroxine

		Study No.: N/A **Location in CTD:** N/A
Type of Study:	N/A	
Method:	N/A	
Tabulated Results:	N/A	
Additional Information:		

N/A=not applicable

Table 2.6.5-11: Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes for Test Article: Levothyroxine

		Study No.: N/A **Location in CTD:** N/A
Type of Study:	N/A	
Method:	N/A	
Tabulated Results:	N/A	
Additional Information:		

N/A=not applicable

Excretion

Table 2.6.5-12: Pharmacokinetics: Excretion for Test Article: Levothyroxine

	Study No.					
	Flock and Owen (1965)					
Species	Sprague-Dawley rats					
Gender (M/F)/Number of Animals	All male/Not Specified					
Feeding Condition	Rockland rat diet					
Vehicle/Formulation	Bolus solution					
Method of Administration	i.v. injection					
Dose	8.2-22.1 µCi/100 g					
Analyte	T4					
Assay	Paper chromatography					
Location in CTD	Module 4.3					
Excretion Route[1]:	**Urine**	**Feces**	**Total**	**Urine**	**Feces**	**Total**
Time (hr)	6	N/A	6	N/A	N/A	N/A
0-T hr	10.3 ± 1.0	N/A	10.3 ± 1.0	N/A	N/A	N/A
Time (hr)	24	N/A	24	N/A	N/A	N/A
0-T hr	33.5 ±2.4	N/A	33.5 ±2.4	N/A	N/A	N/A

Additional Information: The urinary output of ^{131}I was somewhat higher when salicylate was administered than it was in untreated rats. Iodide accounted for 95% or more of the ^{131}I in urine of untreated rats. Small amounts of ^{131}I were found in plasma of rats treated with salicylate. Most of the ^{131}I in the plasma of the salicylate-treated rats was iodide, while about 74% of the plasma ^{131}I of normal rats was in T_4.

[1] when receiving salicylate 75 mg 1 hr before T_4

Table 2.6.5-13: Pharmacokinetics: Excretion into Bile for Test Article: Levothyroxine

	Study No.					
	Flock and Owen (1965)			Lecureux, et al. (2009)		
Species	Sprague-Dawley rats			Wistar and TR⁻ rats		
Gender (M/F)/Number of Animals	All male/Not Specified			All male/Not Specified		
Feeding Condition	Rockland rat diet			LabDiet Certified Rodent Diet 5002		
Vehicle/Formulation	Bolus solution			Bolus solution		
Method of Administration	i.v. injection			i.v. injection		
Dose	8.2-22.1 µCi/100 g			30 µCi/kg		
Analyte	T4			T4		
Assay	Paper chromatography			High performance liquid chromatography		
Location in CTD	Module 4.3			Module 4.3		
Excretion Route:	**Bile[1]**	**Feces**	**Total**	**Bile[1]**	**Feces**	**Total**
Time (hr)	6	N/A	6	1[3]	N/A	1[3]
0-T hr	23.6 ± 1.3	N/A	23.6 ± 1.3	3%, 0.85 mL/hr	N/A	3%, 0.85 mL/hr
Time (hr)	24	N/A	24	1[4]	N/A	1[4]
0-T hr	39.9 ± 2.2	N/A	39.9 ± 2.2	0.6%[5], 0.2 mL/hr	N/A	0.6%[5], 0.2 mL/hr

Additional Information:

[1] When 75 mg of sodium salicylate was administered by stomach tube, one hour before injection of T_4 ^{131}I to rats with biliary fistulas, 23.6% of the dose of ^{131}I was excreted in bile in 6 hours and 39.9% in 24 hours. The biliary output of the ^{131}I was much greater than in untreated rats, particularly during the first six-hour period. Most of the radioactivity of the bile was found in the glucuronide region of the paper chromatograms. Approximately two thirds of the ^{131}I in bile of untreated rats was found in this region, and about three fourths of that of salicylate treated rats. In bile from salicylate treated rats, only small amounts of the ^{131}I were found in T_4, tetrac, iodide, or the sulfate region.

[2] T_4-Glucuronidation (T_4-G) excreted into bile

[3] Wistar rats

[4] TR⁻ rats

[5] Reported as approximately 5 times lower than in Wistar rats

Drug-drug interactions

Table 2.6.5-14: Pharmacokinetics: Drug-Drug Interactions for Test Article: Levothyroxine

	Study No.: Jin et al (2005) Location in CTD: Module 4.3		
Type of Study:	Drug-Drug Interaction/Bioavailability		
Method:	loading dose of 8 µg/kg/day levothyroxine daily for three weeks followed by 100 µL p.o. or i.v. injection of 10mg/kg Cyclosporine A		
Tabulated Results:	**Pharmacokinetic Parameters of CyA After an i,v. or p.o. Administration of CyA (10 mg/kg) in Untreated and LTX-treated Rats (8 µg/kg/day, p.o. for 3 weeks)**		
	Parameter	**No Treatment**	**LTX-treatment**
	i.v. Administration		
	AUC (µg·h/mL)[1]	48.3 ± 4.4	39.7 ± 7.7
	MRT (h)[2]	5.74 ± 1.22	5.54 ± 1.92
	Vd_{ss} (L/kg)[3]	1.19 ± 0.32	1.39 ± 0.68
	CL_{tot} (mL/h/kg)[4]	207 ± 56	252 ± 52
	p.o. Administration		
	AUC (µg·h/mL)	13.8 ± 4.8	2.71 ± 0.78[5]
	Bioavailability (%)	28.6	6.8

Additional Information: The blood concentrations of CyA after i.v. administration in the levothyroxine group tended to be decreased compared with those of the control group, but the difference was not significant. After p.o. administration, the blood concentration of CyA in the levothyroxine group were significantly lower than those of the control group.

1. area under the blood concentration-time curve from zero to 24 h for CyA.
2. mean residence time from 0 to 24 h.
3. distribution volume at the steady-state
4. blood total clearance.
5. significantly different from no treatment at $p<0.01$.

Table 2.6.5-14: Pharmacokinetics: Drug-Drug Interactions for Test Article: Levothyroxine (continued)

Study No.: Phillips et al (1974) Location in CTD: Module 4.3

Type of Study: Drug-Drug Interaction/Absorption

Method: Male rats were dosed with either 71.5 or 214.5 mg/kg Colestipol by stomach tube, then immediately administered one of five different radioactive drugs (aspirin, L-thyroxine, phenobarbital, cortisone and sulfadiazine) also by stomach tube

Tabulated Results:

Kinetic Parameters for L-Thyroxine with Concurrent Administration of Colestipol or Cellulose Control

	Dose: 71.5 mg/kg		Dose: 214.5 mg/kg		
Parameters	**Control**	**Colestipol**	**Control**	**Colestipol**	**SD, %**
Model:					
K_A	0.176	0.221	0.169	0.270[2]	27.4
$A_{1/2}$	4.08	3.21	4.24	2.68[2]	27.9
K_E	0.028	0.028	0.023	0.027	29.7
$E_{1/2}$	26.5	27.1	32.5	28.0	29.8
Peak radioactivity	1,352	1,050[2]	1,476	1,081[2]	12.3
Area	72,650	54,150[1]	93,046	55,385[2]	25.6
Observed:					
Peak radioactivity	1,420	1,112[2]	1,564	1,153[2]	12.9
Area	58,222	43,375[2]	67,905	43,639[2]	15.6

Additional Information: Serum drug levels after oral administration of 0.029 mg/kg L-thyroxine significantly decreased with the low level o colestipol at 8, 12, 16, 24 and 48 hr. Model and observed peak radioactivities and AUCs were reduced significantly and to the same extent, 22 and 25% respectively; none of the other changes differed significantly from control values. At the high level of colestipol, the amount of radioactivity in the serum decreased significantly at all time intervals except at the 2 and 4 hour sampling periods. There was a significant increase in the rate of absorption (from 0.169 to 0.270 hr-1) and a decrease in the absorption half-life of the drug (from 4.24 to 2.68 hr). The rate of elimination and the elimination half-life did not vary significantly from the control values. Observed peak radioactivity and drug availability decreased significantly by 27 and 36%, respectively. Significant decreases of model values for these parameters were essentially the same as observed values.

[1] Significant difference between colestipol mean and mean of corresponding control at $p < 0.05$

[2] Significant difference between colestipol mean and mean of corresponding control at $p < 0.01$

K_A = rate of absorption; $A_{1/2}$ = absorption half-life; K_E = rate of elimination; $E_{1/2}$ = elimination half-life; SD = standard deviation

Table 2.6.5-14: Pharmacokinetics: Drug-Drug Interactions for Test Article: Levothyroxine (continued)

Study No.: Flock and Owen (1965) Location in CTD: Module 4.3

Type of Study: Drug-Drug Interaction/Metabolism

Method: Adult male Sprague-Dawley rats were given sodium salicylate by stomach tubce in doses of 12.5, 25, 50 and 75 mg/rat at intervals from 1 to 24 hr prior to injection of T_4. T_4, labeled with ^{131}I in the 3' or 5' position, was given by single injection into the internal saphenous vein in doses from 8.2 to 22.1 µCi/100 g. A continuous intravenous injection of physiologic saline (0.9%) was given at the rate of 1.25 ml/hr for 24 hr after injection of T_4.

Tabulated Results:

		^{131}I (% of dose), mean ± SE				
Rats (no.)	**Substance Given**	**In Bile**		**In Urine**		**In Plasma[1] 24 hr**
		0-6 hr	**0-24 hr**	**0-6 hr**	**0-24 hr**	
	Na salicylate, 75 mg					
	Hr before T_4					
14	1	23.6 ± 1.3	39.9 ± 2.2	10.3 ± 1.0	33.5 ± 2.4	0.8 ± 0.1
2	3	24.7, 29.0	42.3, 43.8	13.2, 14.7	27.6, 30.1	1.7, 1.6
6	6	16.7 ± 3.2	32.0 ± 2.9	9.8 ± 1.6	27.9 ± 2.4	1.2 ± 0.2
7	12	21.6 ± 1.3	35.2 ± 3.1	13.7 ± 2.5	39.9 ± 3.5	1.0 ± 0.1
5	24	18.8 ± 1.5	32.7 ± 3.9	8.3 ± 1.2	22.8 ± 2.6	2.3 ± 0.1

Additional Information: The bilary output of ^{131}I in rats treated with 75 mg of sodium salicylate was much greater than in untreated rats. The urinary output of ^{131}I was somewhat higher when salicylate was administered than it was in untreated rats. Most of the ^{131}I in the plasma of the salicylate-treated rats was iodide, while about 74% of the plasma ^{131}I of normal rats was in T_4. When rats were treated with salicylate at 6, 12 or 24 hours before injection of T_4, the increase in output of ^{131}I in bile was somewhat less than it was in those treated with salicylate one or three hours before injection of T_4.

[1] The plasma value was calculated on the basis that 3% of the body wt of the rat is plasma.

2.6.6 TOXICOLOGY

Publications were extracted from Medline and Toxnet as two separate databases to support the toxicological profile of levothyroxine sodium for injection. These cited studies included single/repeat doses of levothyroxine, and studies in pregnant animals and their offspring. These are non-GLP studies.

There are no animal studies provided. This is the first injectable levothyroxine product submitted for approval. The sponsor provided published literature (some oral animal studies). However, the sponsor did not make any reference to other approved oral products in this NDA submission.

- Repeat dose toxicity

Most toxicity seen in animals with levothyroxine is exaggerated pharmacological effect of T_4. These effects include decreased body weight, increased body temperature, cardiac hypertrophy (increased heart mass, heart rate, and ion channel changes), decreased bone mineral density, increased ALT and AST, and etc.

There was no study found via IV as a route of exposure.

There was one oral rodent study (Subudhi et al. 2008). The male rats were given 0.0012% in water daily for 30 days. The study focused on the role of vitamin E and curcumin on hyperthyroidism-induced mitochondrial oxygen consumption and oxidative damage to lipids and proteins of rat liver. The study suggested that both vitamin E and curcumin have a protective role against levothyroxine induced hepatic dysfunction (e.g. serum AST and ALT increases) and oxidative stress (lipid peroxidation). There was no NOAEL identified in this study.

There were few oral non-rodent studies. One study (Le Trano et al. 2009) focused on the role of levothyroxine in treating hypothyroidism. The dogs were given 12-40 µg/kg daily for 22 weeks. The study suggested that all hypothyroid dogs had rapid clinical and hormonal responses to oral T4 (liquid) supplementation. The oral dose of 40 µg/kg in dog correlates to 0.8 mg/m^2 exposure. There was no NOAEL identified in this study. Another dog study (Panciera et al. 1992) studied the effect of levothyroxine on echocardiographic and electrocardiographic measurement in euthyroid dogs. The dogs were given 0.5 mg/m2 (25 µg/mg) twice daily for 8 weeks. The results showed that levothyroxine did not affect all parameters measured (e.g. heart rate and EKG). There was no NOAEL identified in this study either.

In addition, no toxicokinetic data was found in the literature searches. Therefore, it is difficult to establish the correlation between thyroid hormone toxicity findings with exposure (e.g. AUC or Cmax) of levothyroxine.

- Reproductive and developmental toxicity

Excess levothyroxine could affect fetal and neonatal development (i.e. changes in lung maturation, growth stunting, and suppression of the fetal thyroid). High doses of levothyroxine could lead to neo-T4 syndrome, such as decreased body weight, decreased growth hormone, decreased TSH, and increased mortality and derangements in carbohydrate metabolism.

There was one IV pig study (Erenberg A and Rhodes 1980). The pigs were treated 50 µg thyroxine by bolus injection followed by a constant IV infusion of 100 µg T4/day for 72 hours during gestation. The results suggested that low dose of T4 to euthyroid ovine fetuses appeared to affect the mesenchymal portion of the lung and accelerate alveolarization. There was no NOAEL identified and no toxicokinetics data generated in this study. There was no NOAEL identified and no toxicokinetics data generated in this study.

There was one oral rodent study (Soerjodibroto et al.). The rats were given 0.1 µg of L-thyroxine daily for 28 days. The study focused on the effect of L-thyroxine on reproduction in protein-deficient rats. The study showed that physiological doses of thyroxine can modify intra-uterine growth (e.g. higher mean birth weight) in animals suffering from a long-term marginal protein deficiency. There was no NOAEL identified and no toxicokinetics data generated in this study either.

- Other toxicity (e.g. genotoxicity and carcinogenicity)

No genotoxicity and carcinogenicity studies were found in the published literature.

No local tolerance, antigenicity, immunotoxicity or toxicity studies on impurities/degradants were found in the published literature.

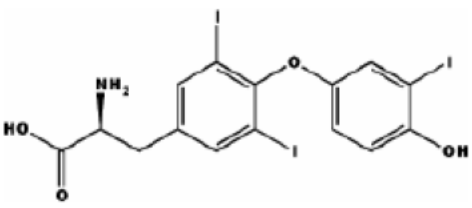
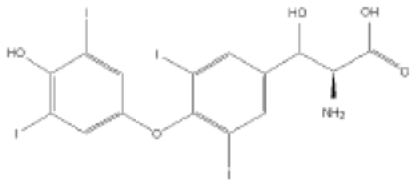
- Impurities

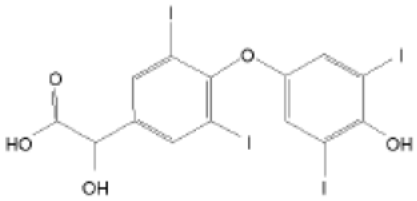
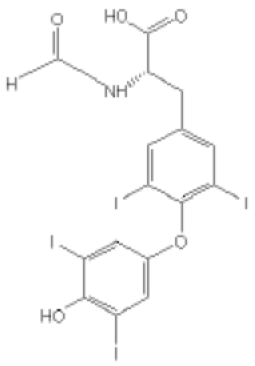
Drug substance: There are 9 impurities identified in the drug substance by the sponsor. There are apparently within acceptable limits. No animal studies were conducted to address any impurity and degradant issues.

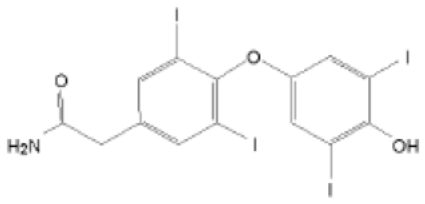
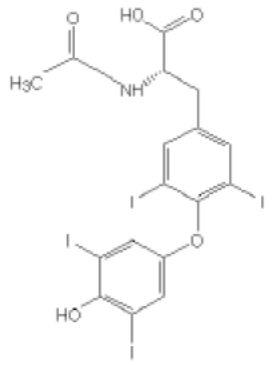
Table 5. Regulatory Specification for Levothyroxine Sodium, USP (effective August 1, 2008)

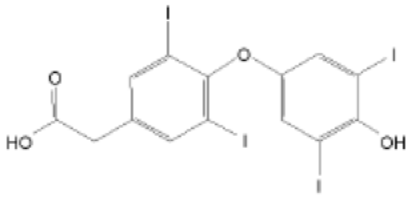
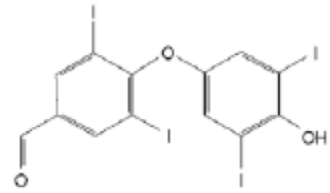
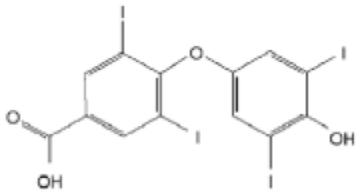
Test	Acceptance Criteria		Test Method[1]	Results Lot # 1001059
Related Compounds			USP	(b) (4)
A. Liothyronine	A. NMT 1.0%		10-08-01-6485	
B. B-Hydroxy-T4[3]	B. NMT 0.15%			
C. T4-Hydroxyacetic acid[4]	C. NMT 0.15%			
D. *N*-Formyl-T4[5] and T4-Acetamide[6]	D. NMT 0.15%			
E. *N*-Acetyl-T4[7]	E. NMT 0.20%			
F. T4-Acetic Acid[8]	F. NMT 0.15%			
G. T4-Aldehyde[9]	G. NMT 0.15%			
H T4-Benzoic Acid[10]	H. NMT 0.15%			
I. Individual Unspecified Impurity	I. NMT 0.10%			
J. Total Impurities	K. NMT 1.5%			
Microbial Bioburden	Total Aerobic Count	NMT 100 CFU/g	USP <61>	
	Total Yeast and Mold Count	NMT 100 CFU/g		
	Total Combined Bioburden	NMT 100 CFU/g		
Bacterial Endotoxin	NMT 22.3 EU/mg		USP <85>	

Impurities in Drug Substance

Name	Structure	Origin
Liothyronine		(b) (4)
β-Hydroxy-T4 *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-β-hydroxy-L-tyrosine		

Name	Structure
T4-Hydroxyacetic acid 2-hydroxy-2-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid	
N-Formyl-T4 *N*-Formyl-O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine	

		(b) (4)
T4-Acetamide 2-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetamide		
N-Acetyl-T4 *N*-Acetyl-*O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine		

Name	Structure	Origin (b) (4)
T4-Acetic Acid 2-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid		
T4-Aldehyde 4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde		
T4-Benzoic Acid 4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid		

Drug product: There were 9 batches tested. Based on Q3B(R2) for drug product, Levothyroxine Sodium for Injection, if the MDD (maximum daily dose) is 0.5 mg, the ICH identification and qualification thresholds are 1.0%.

The sponsor proposed limit on the levothyroxine assay (by HPLC) is (b) (4) which is a wider range than the (b) (4) range for oral levothyroxine products.

With regards to liothyronine (a metabolite of levothyroxine), all 9 batches showed similar results at (b) (4) There was no obvious increase over 6 months. The limit of

NMT (b) (4) is proposed to account for potential variability from the drug substance and finished product, and based on USP limits for the drug substance and drug product (USP limit for API: NMT 1.0% and USP limit for Tablets: NMT 2.0%). Since the sponsor indicated that liothyronine is a drug metabolite and this compound is an approved drug in the US, the proposed limit is not a safety issue.

With regards to all other individual impurities, all 9 batches showed similar results at (b) (4) There was no obvious increase over 6 months. The limit of NMT (b) (4) is proposed, according to the ICH identification threshold of 1.0%.

With regards to total impurities, all 9 batches showed similar results at (b) (4) There was no obvious growth over 6 months. A limit of NMT (b) (4) is proposed, by summing the impurity limits of liothyronine and all other individual impurities.

Note: The wider assay range for this injectable product was discussed at the filing meeting. The proposed assay range is acceptable since this is not an oral product and is intended to be used in the hospital setting, where patient serum levels will be measured. In addition, CMC has no issue with the specifications for these impurities.

Table 26. Summary of Stability Test Results

(b) (4)

2.6.7 TOXICOLOGY TABULATED SUMMARY

Overview

Table 2.6.7-1: Toxicology: Overview for Test Article: Levothyroxine

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) [a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Single-Dose Toxicity								
	Keeshond dog	p.o.	N/A	Up to 10.12 mg/kg	N/A	N/A	Hansen, et al (1992) [b]	Module 4.3
Repeat-Dose Toxicity								
	Wistar rats	p.o	Daily for 30 days	0.0012% in water	N/A	N/A	Subudhi, et al. (2008)	Module 4.3
	Sprague-Dawley rats	i.p	Daily for 20 weeks	20 µg/100 g	N/A	N/A	Suwanwalaikorn, et al. (1996)	Module 4.3
	Sprague-Dawley rats	i.p.	Daily for 10 days	0.5 mg/kg	N/A	N/A	Yu, et al. (1997)	Module 4.3
	Sprague-Dawley rats	i.p.	Daily for 3 weeks	20 µg/100 g	N/A	N/A	Ongphiphadhanakul, et al. (1992)	Module 4.3
	Sprague-Dawley rats	i.p.	Daily for 4 weeks	0.3 mg/kg	N/A	N/A	Mogulkoc and Baltaci (2006)	Module 4.3
	Wistar rats	s.c.	Every other day for 3 weeks	75 mg/kg	N/A	N/A	Bhatt, et al (2007)	Module 4.3
	Sprague-Dawley rats	i.p.	Daily for 7 days	0.25 mg/kg	N/A	N/A	Gao, et al. (2009)	Module 4.3
	Sprague-Dawley rats	s.c.	6 weeks	2.7 and 5.2 mg	N/A	N/A	Liu, et al. (2009)	Module 4.3
	Wistar rats	i.p.	10 days	10 mg/kg	N/A	N/A	Venditti, et al. (2009)	Module 4.3
	Guinea pigs and Sprague-Dawley rats	s.c.	Daily for 10 days	0.5 mg/kg	N/A	N/A	Dai, et al (2002)	Module 4.3

Table 2.6.7-1: Toxicology: Overview for Test Article: Levothyroxine (continued)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) [a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Repeat -Dose Toxicity								
	Beagle dogs	p.o.	Twice daily for 8 weeks	0.5 mg/m^2 of body surface area	N/A	N/A	Panciera, et al. (1992)	Module 4.3
	Various breed dogs	p.o	Daily for 22 weeks	12 µg/kg to 42 µg/kg	N/A	N/A	Le Traon, et al. (2009)	Module 4.3
	Short-haired cats	s.c	Daily for 42 days	25 µg/kg	N/A	N/A	Gallagher and Panciera (2009)	Module 4.3
	New Zealand rabbits	i.p.	Daily for 14 days	1 mg/kg	N/A	N/A	Yu, et al. (2009)	Module 4.3
	Mare horses	p.o.	Daily for 48 weeks	48 mg	N/A	N/A	Frank, et al. (2008)	Module 4.3
	Wistar rats	s.c.	Daily for 14 days	25 µg/100g	N/A	N/A	Cihan, et al. (2009)	Module 4.3
Genotoxicity								
	N/A							
Reproductive and Developmental Toxicity								
	rats	p.o.	Daily for 28 days	0.1 µg	N/A	N/A	Soerjodibroto, et al (1974)	Module 4.3
	Sprague-Dawley rats	i.p	Daily for 3 days or daily for 7 days	0.05 µg/g, 0.5 µg/g or 1.0 µg/g	N/A	N/A	Mutapcic, et al. (2005)	Module 4.3
	Sprague-Dawley-Holtzman rats	s.c.	Twice daily for 1 day	10 µg/100 g	N/A	N/A	Porterfield (1985)	Module 4.3
	Wistar rats	s.c.	Daily for 5 days	30 µg	N/A	N/A	Pascual-Leone, et al. (1976)	Module 4.3

Table 2.6.7-1: Toxicology: Overview for Test Article: Levothyroxine (continued)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) [a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Reproductive and Developmental Toxicity								
	Wistar rats	s.c.	Every other day for 8 days	37.5 µg	N/A	N/A	Escriva and Pascual-Leone (1984)	Module 4.3
	Sprague-Dawley rats	s.c.	Daily for 5 days	0.2 µg/g	N/A	N/A	Maeda, et al. (1986)	Module 4.3
	Sheep fetus	i.v.	Daily for 3 days	1-time 50 µg bolus; 100 µg infusion	N/A	N/A	Erenberg and Rhodes (1980)	Module 4.3
Local Tolerance								
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Other Toxicity Studies								
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

a Unless otherwise specified. For Repeat-Dose Toxicity, the highest No Observed Adverse Effect Level (NOAEL) is underlined.
b. In this study, a 6 year old male Keeshond was accidentally overdosed when it got into a bottle of thyroxine tablets intended for another dog.
N/A = Not Applicable

General toxicology

Table 2.6.7-5: Single-Dose Toxicity for Test Article: Levothyroxine

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and Number Per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study No.
Keeshond dog	p.o. (tablets)	Up to 10.12 mg/kg	1 Male, 0 Female	10.12 mg/kg	N/A	In this study, a 6 year old male Keeshond was accidentally overdosed when it got into a bottle of thyroxine tablets intended for another dog. At the time of admission the dog was alert and the only abnormality was bilateral hippos. Serum alanine tranaminase activity peaked on day 6 at 345 U/L (normal, 10 to 58 U/L).	Hansen, et al (1992)

N/A = Not Applicable

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
Wistar rats	p.o. solution	Daily for 30 days	0.0012% in water	Unspecified number of males, 0 female	N/A	L-thyroxine treatment resulted in significant increases in activities of Aspartate tansaminase (AspAT) and Alanine transaminase (AlaAT) in the hyperthyroid group than in the euthyroid group. Significant increase in mitochondrial lipid peroxidation was observed in L-thyroxine treated groups in comparison to euthyroid group.	Subudhi, et al. (2008)
Sprague-Dawley rats	i.p. solution	Daily for 20 weeks	20 µg/100 g	20 male, 0 female	N/A	Femoral bone mineral density (BMD) was significantly lowered in the L-thyroxine group compared to controls after 15, 18 and 20 weeks of treatment	Suwanwalaikorn, et al. (1996)
Sprague-Dawley rats	i.p. solution	Daily for 10 days	0.5 mg/kg	Unspecified number of either sex	N/A	The incidence of heart arrest was higher in hypertrophic hearts than that of normal heart ($p<0.05$). Risk of death was markedly worse in L-thyroxine-induced hypertrophic heart.	Yu, et al. (1997)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
Sprague-Dawley rats	i.p. solution	Daily for 3 weeks	20 µg/100 g	40 male, 0 female	N/A	Levothyroxine administration decreased femoral BMD in both orchidectomy and sham operated rats.	Ongphiphadhanakul, et al. (1992)
Sprague-Dawley rats	i.p. solution	Daily for 4 weeks	0.3 mg/kg	72 male, 0 female	N/A	Both basal and stimulated arginine vasopressin (AVP) levels significantly increased in hyperthyroidism group compared to control and sham hyperthyroid.	Mogulkoc and Baltaci (2006)
Wistar rats	s.c. solution	Every other day for 3 weeks	75 mg/kg	Unspecified number of females, 0 male	N/A	Fasting serum glucose and insulin levels in levothyroxine sodium-treated animals were significantly higher than in corresponding normal rats. After glucose load in the serum, glucose levels over a period of 120 min in levothyroxine sodium-treated animals were significantly higher than those in the corresponding normal group. The area under the curve for	Bhatt, et al (2007)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
Wistar rats (cont'd)						glucose (AUC_g) was also significantly greater in levothyroxine sodium-treated animals compared with normal animals.	
Sprague-Dawley rats	i.p. solution	7 days	0.25 mg/kg daily	Unspecified number of males, 0 females	N/A	Levothyroxine increased the heart weight index and left ventricular weight index of rats in the levothyroxine control group compared with the normal control group ($p < 0.01$). The average transverse area of a single myocardial cell in the levothyroxine control group was significantly larger than in the normal group ($p < 0.01$).	Gao, et al. (2009)
Sprague-Dawley rats	s.c. pellet	6 weeks	2.7 or 5.2 mg	32 males, 0 females	N/A	Hypothyroidism led to reductions in cardiac function, heart mass, body temperature and myocardial arterioles. High-dose thyroxine (T_4) prevented arteriolar loss and the development of hypothyroidism. Low-dose T_4 partially prevented the reduction in cardiac function but had minimal effects on arteriolar loss.	Liu, et al. (2009)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
Wistar rats	i.p. solution	Daily for 10 days	10 mg/kg	Unspecified number of males, 0 females	N/A	The heart weight increased differently in hyperthyroid rats so that such animals exhibited a heart/body weight ratio increased in comparison to the controls (2.17 ± 0.04). The ratio was significantly lower ($p<0.05$) in T_4-treated (2.50 ± 0.04) than in both groups of T_3-treated rats (3.18 ± 0.17 and 3.10 ± 0.06 for HT_3 and HT_3 + CE rats, respectively). Plasma levels of fT_3 were higher in both groups of T_3-treated rats, whereas fT_4 levels were higher in T_4-treated rats.	Venditti, et al. (2009)
Guinea pigs and Sprague-Dawley rats	i.p./s.c. solution[a]	Daily for 10 days	0.5 mg/kg	Unspecified number of either sex in both guinea pigs and rats	N/A	The mitochondrial Na+/K+-ATPase activity were stimulated markedly in chronic levothyroxine remodeled versus normal ($p <0.01$). The maximal current potency and density of the L-type calcium current ($I_{Ca,L}$) increased significantly in the levothyroxine – remodeled heart.	Dai, et al (2002)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
Beagle dogs	p.o. tablet	Twice daily for 8 weeks	0.5 mg/m^2 of body surface area	5 male, 9 female	N/A	Echocardiography and electrocardiogram (ECG) are not capable of detecting alterations in cardiovascular function due to thyroid hormone replacement therapy in euthyroid dogs, or cardiac changes did not occur, despite tissue evidence of exogenous thyroid hormone effects. These findings suggest that 8 weeks of replacement dose thyroid hormone supplementation fails to induce clinically detectable structural or functional cardiac changes, and that echocardiographic and ECG monitoring of dogs with unknown thyroid status undergoing levothyroxine administration is neither indicated nor useful.	Panciera, et al. (1992)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
Various breed dogs	p.o. solution	Daily for 22 weeks	12 µg/kg to 42 µg/kg	14 male, 21 female	N/A	From the visit when the correct dose was established, serum tT_4 concentrations measured 4-6 hours post-treatment increased significantly, compared with inclusion. Clinical signs of hypothyroidism improved or resolved in 91% of dogs after 4 weeks of L-T_4 treatment at 20 µg/kg once daily.	Le Traon, et al. (2009)
Short-haired cats	s.c. solution	Daily for 42 days	25 µg/kg	0 male, 15 female	N/A	There were no clinically relevant changes in complete blood counts (CBCs) for any group at any time period. In addition, there were no clinically relevant changes in blood urea nitrogen (BUN), glucose, calcium, albumin, globulins, total bilirubin, cholesterol, sodium, potassium, chloride, total Carbon dioxide (CO_2) or anion gap.	Gallagher and Panciera (2009)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
New Zealand rabbits	i.p. solution	Daily for 14 days	1 mg/kg	24 rabbits total, unspecified number of males and females	N/A	Atrial monophasic action potential at 90 repolarization ($AMAP_{90}$) and effective refractory period (AERP) were significantly shorter in the thyroxine group than in the control group. Sinus cycle length, atrial monophasic action potential at 90 repolarization, and atrial effective refractory period were significantly shorter in the thyroxine group. A significantly greater number of rabbits in the thyroxine group compared to the control group had atrial tachycardia and atrial fibrillation/flutter. The mean peak of L-type calcium current ($I_{ca,l}$) densities at -10 mV was significantly higher in the thyroxine group than in the control group.	Yu, et al. (2009)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
						Oral administration of L-thyroxine at a dosage of 48 mg/d for 48 weeks significantly increased serum tT_4 concentrations, suppressed thyroid hormone responses to thyrotropin releasing hormone (TRH) and induced weight loss but did not adversely affect general health or echocardiographic measurements in euthyroid adult horses	
Mixed-breed horses	p.o. solution	Daily for 48 weeks	48 mg	0 males, 6 females	N/A		Frank, et al. (2008)

Table 2.6.7-6: Repeat-Dose Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and Number Per Group	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No.
Wistar Rats	s.c. solution	Daily for 14 days	25 µg/100g	7 males, 0 females	N/A	The interval between para-chloroamphetamine (PCA) administration and first ejaculation was statistically significantly different between groups ($p = 0.001$). Interval values were significantly shorter in the hyperthyroid rats compared to the recovered rats and saline control group rats ($p < 0.05$). Seminal vesicle (SV) phasic contraction frequency was significantly higher in the hyperthyroid rat group compared with controls ($p = 0.047$). The number of SV phasic contractions for this group correlated with serum thyroid-stimulating hormone (TSH) values ($p = 0.012$, $r = -0.94$). The mean area under the curve (AUC) of bulbospongiosus (BS) muscle electromyography (EMG) activity was also significantly increased in the hyperthyroid group ($p = 0.0001$). After the recovery period for Group 2 rats, BS EMG activity returned to the control group level. AUC BS EMG values correlated with serum TSH values in hyperthyroid rats ($p = 0.016$, $f = -0.94$).	Cihan, et al. (2009)

[a] No Observed Adverse Effect Level.
N/A = Not Applicable

Reproductive toxicity

Table 2.6.7-11: Reproductive and Developmental Toxicity: Nonpivotal Studies for Test Article: Levothyroxine

Species/Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Doses (mg/kg)	Number per Group	Noteworthy Findings	Study No.
rats	p.o. supplement	Daily for 28 days	0.1 µg	149 males and females	Mean birth weight was increased from 4.9 g in the untreated groups (n=63) to 5.4 g in the thyroxine-treated group (n=86).	Soerjodibroto, et al (1974)
Sprague-Dawley rats	i.p. solution	Daily for 3 days or daily for 7 days	0.05 µg/g, 0.5 µg/g or 1.0 µg/g	220 of unspecified sex	Retinal vascular density was reduced in animals receiving daily 1 µg/g T_4 compared with saline controls after 3 days of T_4 and 7 days of T_4. However, retinal vascular density returned to normal after 3 days of treatment and 4 days of recovery. Vascularized retinal area was reduced in animals receiving 1 µg/g T_4 for 3 days followed by 3 days recover compared with saline controls.	Mutapcic, et al. (2005)
Sprague-Dawley-Holtzman rats	s.c. solution	Twice daily for 1 day	10 µg/100 g	Unspecified number of males and females	Maternal T_4 treatments resulted in suppression of the fetal/neonatal thyroid which was very apparent at 5 days of age. At 30 days post-partum, the thyroid controls system of the progeny of the T_4 and 3,5-dimethyl-3'-isopropyl-L-thyronine (DIMIT)-treated animals was still abnormal with low serum T_4 levels accompanied with normal serum TSH and T_3 levels. At 60 days of age serum T_4 levels remained low in the progeny of the T_4-treated animals and the TSH response to TRH was subnormal in both the progeny of the T_4-treated and the DIMIT-treated animals	Porterfield (1985)

Table 2.6.7-11: Reproductive and Developmental Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Doses (mg/kg)	Number per Group	Noteworthy Findings	Study No.
Wistar rats	s.c. solution	Daily for 5 days	30 µg	0 males, unspecified number of females	It was found that not only thyrotropin (TSH) but also growth hormone (GH) content of rats injected with very high doses of levothyroxine during the neonatal period was significantly lower than that of the saline-injected controls.	Pascual-Leone, et al. (1976)
Wistar rats	s.c. solution	Every other day for 8 days	37.5 µg	Unspecified number of males and females	High doses of levothyroxine in neonatal rats produced alterations in carbohydrate metabolism: hypoglycemia, hypoinsulinemia and increased levels of blood lactate, pyruvate and ketone bodies. T_4-treated and control rats were subjected to fasting. In contrast to controls where glycemia decreased to 60% of basal values, in 11-day-old neo-T_4 rats blood glucose levels decreased to only 90% of basal values.	Escriva and Pascual-Leone (1984)
Sprague-Dawley rats	s.c. solution	Daily for 5 days	0.2 µg/g	Unspecified number of males and females	The effects of levothyroxine, cortisol and diet decreased with the progression of weaning, and after the 20th postnatal day, the hormones had no effects of the postnatal development of either (facial and head) part of the skull. Moreover, these hormones played an additive role in the growth of facial part. On the other hand, continuous suckling from birth to the 30th postnatal day caused significant decrease in the ratio of the facial length to the head length.	Maeda, et al. (1986)

Table 2.6.7-11: Reproductive and Developmental Toxicity: Nonpivotal Studies for Test Article: Levothyroxine (continued)

Species/Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Doses (mg/kg)	Number per Group	Noteworthy Findings	Study No.
Sheep fetus	i.v. solution	Daily for 3 days	1-time 50 µg bolus; 100 µg infusion	12 unspecified males and females	The mean serum T_4 was significantly greater in the T_4-infused fetuses compared to the saline-infused fetuses throughout the 72-hour infusion period. Histologic examination of the saline-infused fetual lung tissue revealed areas of mature lung and areas of hypercellularity and thickened septae compatible with the canalicular stage of development. The T_4-infused fetal lung tissue was homogenous in its mature state of development. These results suggest that administration of low doses of T_4 to euthyroid ovine fetuses at 130 days gestation appears to affect the mesenchymal portion of the lung and accelerate alveolarization but not to accelerate surfactant maturation.	Erenberg and Rhodes (1980)

Table 2.6.7-12: Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development to Implantation for Test Article: Levothyroxine

Species/Strain: Rats	Duration of Dosing: M: Unspecified F: Unspecified			Study No.: Soerjodibroto, et al. (1974)	
				Study Title: The effect of an orally administered L-thyroxine supplement on reproduction in protein-deficient rats	
Initial Age: N/A	Day of Mating: N/A				
Date of First Dose: last third of gestation	Day of C-Section: N/A			Location in CTD: Module 4	
Special Features: N/A	Method of Administration: p.o.			GLP Compliance: No	
No Observed Adverse Effect Level: N/A	Vehicle/Formulation: supplement				
F0 Males: 0					
F0 Females: 21					
F1 Litters: 21					
Daily Dose (mg/kg):	**0 (Control)**	**0.1 µg L-thyroxine**			
Males	Unspecified	Unspecified			
Toxicokinetics: AUC (__)	N/A	N/A			
Number Evaluated	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Clinical Observations	Birth weight	Birth weight			
Necropsy Observations	N/A	N/A			
Body Weight	4.9 g	5.4 g			
Food Consumption (%)a	N/A	N/A			
Mean Number of Days Prior to Mating	N/A	N/A			
Number of Males that Mated	N/A	N/A			
Number of Fertile Males	N/A	N/A			
Females	Unspecified	Unspecified			
Toxicokinetics: AUC (__)	N/A	N/A			
Number Evaluated	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Clinical Observations	Lower birth weight	Higher birth weight			

Table 2.6.7-12: Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development to Implantation for Test Article: Levothyroxine (continued)

Species/Strain: Rats	Duration of Dosing: M: Unspecified F: Unspecified			Study No.: Soerjodibroto, et al. (1974)	
				Study Title: The effect of an orally administered L-thyroxine supplement on reproduction in protein-deficient rats	
Initial Age: N/A	Day of Mating: N/A				
Date of First Dose: last third of gestation	Day of C-Section: N/A			Location in CTD: Module 4	
Special Features: N/A	Method of Administration: p.o.			GLP Compliance: No	
No Observed Adverse Effect Level: N/A	Vehicle/Formulation: supplement				
F0 Males: 0					
F0 Females: 21					
F1 Litters: 21					
Necropsy Observations	N/A	N/A			
Premating Body Weight	166 g	174 g			
Gestation Body Weight (%)b	Unspecified	Unspecified			
Premating Food Consumption (%)b	Unspecified	Unspecified			
Gestation Food Consumption (%)b	Unspecified	Unspecified			
Mean Number of Estrous Cycles/14 days	Unspecified	Unspecified			
Mean Number of Days Prior to Mating	Unspecified	Unspecified			
Number of Females Sperm Positive	Unspecified	Unspecified			
Number of Pregnant Females	8	13			
Number Aborted or with Total Resorption of Litter	Unspecified	Unspecified			
Mean Number of Corpora Lutea	Unspecified	Unspecified			
Mean Number of Implantations	Unspecified	Unspecified			
Mean % Preimplantation Loss	Unspecified	Unspecified			
Mean Number of Live Conceptuses	Unspecified	Unspecified			
Mean Number of Resorptions	Unspecified	Unspecified			
Number of Dead Conceptuses	Unspecified	Unspecified			
Mean % Postimplantation Loss	Unspecified	Unspecified			

- * After 4 weeks of dosing. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[b] At end of premating or gestation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
N/A = Not Applicable

Table 2.6.7-13: Reproductive and Developmental Toxicity: Effects on Embryofetal Development for Test Article: Levothyroxine

Species/Strain: Sheep fetus	Duration of Dosing: M: F:			Study No.: Erenberg and Rhodes, (1980)	
Initial Age: N/A	Day of Mating: Unspecified			Study Title: The effect of thyroxine (T_4) on ovine fetal lung maturation	
Date of First Dose: 127 days of gestation (term ≈ 145-150 days)	Day of C-Section: Unspecified			Location in CTD: Module 4	
Special Features:	Method of Administration: IV			GLP Compliance: No	
No Observed Adverse Effect Level: N/A	Vehicle/Formulation: solution				
F0 Females: Unspecified					
F1 Litters: Unspecified					
Daily Dose (mg/kg):	**0 (Control)**	**50 µg one time followed by 100 µg per day levothyroxine**			
Dams/Dose	Unspecified	Unspecified			
Toxicokinetics: AUC	N/A	N/A			
Number of Pregnant	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Number Aborted or with Total Resorption of Litter	Unspecified	Unspecified			
Clinical Observations	Serum T_4, lung tissue protein and DNA concentration	Serum T_4, lung tissue protein and DNA concentration			
Necropsy Observations	N/A	N/A			
Body Weight (%)[a]	Unspecified	Unspecified			
Food Consumption (%)[a]	Unspecified	Unspecified			
Mean Number of Corpora Lutea	Unspecified	Unspecified			
Mean Number of Implantations	Unspecified	Unspecified			
Mean % Preimplantation Loss	Unspecified	Unspecified			
Litters	6	6			

Table 2.6.7-13: Reproductive and Developmental Toxicity: Effects on Embryofetal Development for Test Article: Levothyroxine (continued)

Species/Strain: Sheep fetus	Duration of Dosing: M: F:			Study No.: Erenberg and Rhodes, (1980)	
Initial Age: N/A	Day of Mating: Unspecified			Study Title: The effect of thyroxine (T_4) on ovine fetal lung maturation	
Date of First Dose: 127 days of gestation (term ≈ 145-150 days)	Day of C-Section: Unspecified			Location in CTD: Module 4	
Special Features:	Method of Administration: IV			GLP Compliance: No	
No Observed Adverse Effect Level: N/A	Vehicle/Formulation: solution				
F0 Females: Unspecified					
F1 Litters: Unspecified					
Number of Litters Evaluated	6	6			
Number of Live Fetuses	6	6			
Mean Number of Resorptions	Unspecified	Unspecified			
Mean Number of Litters with Dead Fetuses	Unspecified	Unspecified			
Mean % Postimplantation Lossa	Unspecified	Unspecified			
Mean Fetal Body Weight (%)[a]	Unspecified	Unspecified			
Fetal Sex Ratios	Unspecified	Unspecified			
Fetal Anomalies	Unspecified	Unspecified			
Gross External	Unspecified	Unspecified			
Visceral Anomalies	Unspecified	Unspecified			

G=Gestation day; -No noteworthy findings; +Mild ++Moderate +++Marked
*=p<0.05. **=p<0.01.
[a] At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
N/A = Not Applicable

Table 2.6.7-14: Reproductive and Developmental Toxicity: Effects on Pre- and Postnatal Development, Including Maternal Function for Test Article: Levothyroxine (Mutapcic, 2005)

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 3 days or 7 days	Study No.: Mutapcic, et al. (2005)
Initial Age: new-born	Day of Mating: N/A	Study Title: The effect of L-thyroxine supplementation on retinal vascular development in neonatal rats
Date of First Dose: day 1 of life	Method of Administration: i.p.	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: 22		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	0.05 µg/g	0.5 µg/g	1.0 µg/g
F0 Females	22	0	0	0
Toxicokinetics: AUC	N/A	N/A	N/A	N/A
Number of Pregnant	22	0	0	0
Number Died or Sacrificed Moribund	Unspecified	Unspecified	Unspecified	Unspecified
Number Aborted or with Total Resorption of Litter	Unspecified	Unspecified	Unspecified	Unspecified
Clinical Observations	N/A	N/A	N/A	N/A
Necropsy Observations	N/A	N/A	N/A	N/A
Gestation Body Weight (%)[a]	Unspecified	Unspecified	Unspecified	Unspecified
Lactation Body Weight (%)[a]	Unspecified	Unspecified	Unspecified	Unspecified
Gestation Food Consumption (%)[a]	Standard lab diet and water ad libitum	N/A	N/A	N/A
Lactation Food Consumption (%)[a]	Unspecified	Unspecified	Unspecified	Unspecified
Mean Duration of Gestation (days)	Unspecified	Unspecified	Unspecified	Unspecified
Abnormal Parturition	Unspecified	Unspecified	Unspecified	Unspecified
F1 Litters (preweaning)	Unspecified	Unspecified	Unspecified	Unspecified

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 3 days or 7 days	Study No.: Mutapcic, et al. (2005)
Initial Age: new-born	Day of Mating: N/A	Study Title: The effect of L-thyroxine supplementation on retinal vascular development in neonatal rats
Date of First Dose: day 1 of life	Method of Administration: i.p.	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: 22		
F1 Males: Unspecified		
F1Females: Unspecified		

Number of Litters Evaluated	Unspecified	Unspecified	Unspecified	Unspecified
Mean Number of Implantations	Unspecified	Unspecified	Unspecified	Unspecified
Mean Number of Pups/Litter	10	10	10	10
Mean Number of Liveborn Pups/Litter	Unspecified	Unspecified	Unspecified	Unspecified
Number of Litters with Stillborn Pups	Unspecified	Unspecified	Unspecified	Unspecified
Postnatal Survival to Day 4	19 (95%)	19 (95%)	18 (90%)	18 (90%)
Postnatal Survival to Weaning	Unspecified	Unspecified	Unspecified	Unspecified
Number of Total Litter Losses	Unspecified	Unspecified	Unspecified	Unspecified
Change in Pup Body Weights (g)[b]	Unspecified	Unspecified	Unspecified	Unspecified
Pup Sex Ratios	Unspecified	Unspecified	Unspecified	Unspecified
Pup Clinical Signs	Unspecified	Unspecified	Unspecified	Unspecified
Pup Necropsy Observations	Unspecified	Unspecified	Unspecified	Unspecified
F1 Males (postweaning)	Unspecified	Unspecified	Unspecified	Unspecified
Number Evaluated Postweaning Per Litter	Unspecified	Unspecified	Unspecified	Unspecified
Number Died or Sacrificed Moribund	Unspecified	Unspecified	Unspecified	Unspecified

Species/Strain: Sprague-Dawley rats Initial Age: new-born Date of First Dose: day 1 of life Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: 22 F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 3 days or 7 days Day of Mating: N/A Method of Administration: i.p. Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified		Study No.: Mutapcic, et al. (2005) Study Title: The effect of L-thyroxine supplementation on retinal vascular development in neonatal rats Location in CTD: Module 4 GLP Compliance: No	
Clinical Observations	Vascularized and total retinal area, retinal vessel density, serum thyroxine, TSH and IGF-1 levels	Vascularized and total retinal area, retinal vessel density, serum thyroxine, TSH and IGF-1 levels	Vascularized and total retinal area, retinal vessel density, serum thyroxine, TSH and IGF-1 levels	Vascularized and total retinal area, retinal vessel density, serum thyroxine, TSH and IGF-1 levels
Necropsy Observations	Unspecified	Unspecified	Unspecified	Unspecified
Body Weight Change (g)[c]	Unspecified	Unspecified	Unspecified	Unspecified
Food Consumption (%)[e]	Unspecified	Unspecified	Unspecified	Unspecified
Preputial Separation	Unspecified	Unspecified	Unspecified	Unspecified
Sensory Function	Unspecified	Unspecified	Unspecified	Unspecified
Motor Activity	Unspecified	Unspecified	Unspecified	Unspecified
Learning and Memory	Unspecified	Unspecified	Unspecified	Unspecified
Mean Number of Days Prior to Mating	Unspecified	Unspecified	Unspecified	Unspecified
Number of Males that Mated	Unspecified	Unspecified	Unspecified	Unspecified
Number of Fertile Males	Unspecified	Unspecified	Unspecified	Unspecified
F1 Females (postweaning)	Unspecified	Unspecified	Unspecified	Unspecified
Number Evaluated Postweaning	Unspecified	Unspecified	Unspecified	Unspecified
Number Died or Sacrificed Moribund	Unspecified	Unspecified	Unspecified	Unspecified
Clinical Observations	Unspecified	Unspecified	Unspecified	Unspecified
Necropsy Observations	Unspecified	Unspecified	Unspecified	Unspecified

Species/Strain: Sprague-Dawley rats Initial Age: new-born Date of First Dose: day 1 of life Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: 22 F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 3 days or 7 days Day of Mating: N/A Method of Administration: i.p. Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified		Study No.: Mutapcic, et al. (2005) Study Title: The effect of L-thyroxine supplementation on retinal vascular development in neonatal rats Location in CTD: Module 4 GLP Compliance: No	
Premating Body Weight Change (g)[c]	Unspecified	Unspecified	Unspecified	Unspecified
Gestation Body Weight Change (g)	Unspecified	Unspecified	Unspecified	Unspecified
Premating Food Consumption (%)[f]	Unspecified	Unspecified	Unspecified	Unspecified
Gestation Food Consumption (%)[f]	Unspecified	Unspecified	Unspecified	Unspecified
Mean Age of Vaginal Patency (days)	Unspecified	Unspecified	Unspecified	Unspecified
Sensory Function	Unspecified	Unspecified	Unspecified	Unspecified
Motor Activity	Unspecified	Unspecified	Unspecified	Unspecified
Learning and Memory	Unspecified	Unspecified	Unspecified	Unspecified
Mean Number of Days Prior to Mating	Unspecified	Unspecified	Unspecified	Unspecified
Number of Females Sperm Positive	Unspecified	Unspecified	Unspecified	Unspecified
Number of Pregnant Females	Unspecified	Unspecified	Unspecified	Unspecified
Mean Number of Corpora Lutea	Unspecified	Unspecified	Unspecified	Unspecified
Mean Number of Implantations	Unspecified	Unspecified	Unspecified	Unspecified
Mean % Preimplantation Loss	Unspecified	Unspecified	Unspecified	Unspecified
F2 Litters	N/A	N/A	N/A	N/A
Mean Number of Live Conceptuses/Litter	N/A	N/A	N/A	N/A
Mean Number of Resorptions	N/A	N/A	N/A	N/A
Number of Litter with Dead Conceptuses	N/A	N/A	N/A	N/A
Number of Dead Conceptuses	N/A	N/A	N/A	N/A
Mean % Postimplantation Loss	N/A	N/A	N/A	N/A

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 3 days or 7 days	Study No.: Mutapcic, et al. (2005)
Initial Age: new-born	Day of Mating: N/A	Study Title: The effect of L-thyroxine supplementation on retinal vascular development in neonatal rats
Date of First Dose: day 1 of life	Method of Administration: i.p.	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: 22		
F1 Males: Unspecified		
F1Females: Unspecified		

Fetal Body Weights (g)	N/A	N/A	N/A	N/A	N/A
Fetal Sex Ratios (% males)	N/A	N/A	N/A	N/A	N/A
Fetal Anomalies	N/A	N/A	N/A	N/A	N/A
F1 Females (postweaning)	N/A	N/A	N/A	N/A	N/A
Number Evaluated Postweaning	N/A	N/A	N/A	N/A	N/A
Number Died or Sacrificed Moribund	N/A	N/A	N/A	N/A	N/A
Clinical Observations	N/A	N/A	N/A	N/A	N/A
Necropsy Observations	N/A	N/A	N/A	N/A	N/A
Premating Body Weight Change (g)[d]	N/A	N/A	N/A	N/A	N/A
Gestation Body Weight Change (g)	N/A	N/A	N/A	N/A	N/A
Premating Food Consumption (%)[f]	N/A	N/A	N/A	N/A	N/A
Gestation Food Consumption (%)[d,f]	N/A	N/A	N/A	N/A	N/A
Mean Age of Vaginal Patency (days)	N/A	N/A	N/A	N/A	N/A
Sensory Function	N/A	N/A	N/A	N/A	N/A
Motor Activity	N/A	N/A	N/A	N/A	N/A
Learning and Memory	N/A	N/A	N/A	N/A	N/A
Number of Females Sperm Positive	N/A	N/A	N/A	N/A	N/A
Number of Pregnant Females	N/A	N/A	N/A	N/A	N/A
Mean Duration of Gestation	N/A	N/A	N/A	N/A	N/A
Abnormal Parturition	N/A	N/A	N/A	N/A	N/A

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 3 days or 7 days	Study No.: Mutapcic, et al. (2005)
Initial Age: new-born	Day of Mating: N/A	Study Title: The effect of L-thyroxine supplementation on retinal vascular development in neonatal rats
Date of First Dose: day 1 of life	Method of Administration: i.p.	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: 22		
F1 Males: Unspecified		
F1Females: Unspecified		

F2 Litters	N/A	N/A	N/A	N/A	N/A
Number of Litters Evaluated	N/A	N/A	N/A	N/A	N/A
Mean Number of Implantations	N/A	N/A	N/A	N/A	N/A
Mean Number of Pups/Litter	N/A	N/A	N/A	N/A	N/A
Mean Number of Liveborn Pups/Litter	N/A	N/A	N/A	N/A	N/A
Mean Number of Stillborn Pups/Litter	N/A	N/A	N/A	N/A	N/A
Postnatal Survival to Day 4	N/A	N/A	N/A	N/A	N/A
Postnatal Survival to Weaning	N/A	N/A	N/A	N/A	N/A
Change in Pup body Weights (g)[d]	N/A	N/A	N/A	N/A	N/A
Pup Sex Ratios	N/A	N/A	N/A	N/A	N/A
Pup Clinical Signs	N/A	N/A	N/A	N/A	N/A
Pup Necropsy Observations	N/A	N/A	N/A	N/A	N/A

G=Gestation day, L=Lactation day. -No noteworthy findings. +Mild ++Moderate +++Marked
*=p<0.05. **=p<0.01.
[a] At end of gestation or lactation. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[b] From birth to weaning.
[c] From weaning to mating.
[d] From birth to mating.
[e] At end of postweaning period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[f] At end of premating or gestation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
N/A = Not Applicable

Table 2.6.7-14: Reproductive and Developmental Toxicity: Effects on Pre- and Postnatal Development, Including Maternal Function for Test Article: Levothyroxine (Porterfield, 1985)

Species/Strain: Sprague-Dawley Holtzman rats	Duration of Dosing: 1 day	Study No.: Porterfield (1985)
Initial Age: Unspecified	Day of Mating:	Study Title: Prenatal exposure of the fetal rat to excessive L-thyroxine or 3,5-dimetyl-3'-isopropyl thyronine produces persistent changes in the thyroid control system
Date of First Dose: 21 days of gestation	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	10 µg/100 g L-thyroxine	2.5 µg/100 g DIMIT	N/A	N/A
F0 Females	Unspecified	Unspecified	Unspecified	N/A	N/A
Toxicokinetics: AUC	N/A	N/A	N/A	N/A	N/A
Number of Pregnant	Unspecified	Unspecified	Unspecified	N/A	N/A
Number Died or Sacrificed Moribund	Unspecified	Unspecified	Unspecified	N/A	N/A
Number Aborted or with Total Resorption of Litter	Unspecified	Unspecified	Unspecified	N/A	N/A
Clinical Observations	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	N/A	N/A
Necropsy Observations	N/A	N/A	N/A	N/A	N/A
Gestation Body Weight (%)[a]	Unspecified	Unspecified	Unspecified	N/A	N/A
Lactation Body Weight (%)[a]	Unspecified	Unspecified	Unspecified	N/A	N/A
Gestation Food Consumption (%)[a^]	Unspecified	Unspecified	Unspecified	N/A	N/A
Lactation Food Consumption (%)[a^]	Unspecified	Unspecified	Unspecified	N/A	N/A
Mean Duration of Gestation (days)	Unspecified	Unspecified	Unspecified	N/A	N/A
Abnormal Parturition	Unspecified	Unspecified	Unspecified	N/A	N/A

Species/Strain: Sprague-Dawley Holtzman rats	Duration of Dosing: 1 day	Study No.: Porterfield (1985)
Initial Age: Unspecified	Day of Mating:	Study Title: Prenatal exposure of the fetal rat to excessive L-thyroxine or 3,5-dimetyl-3'-isopropyl thyronine produces persistent changes in the thyroid control system
Date of First Dose: 21 days of gestation	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	10 µg/100 g L-thyroxine	2.5 µg/100 g DIMIT	N/A	N/A
F1 Litters (preweaning)	Unspecified	Unspecified	Unspecified	N/A	N/A
Number of Litters Evaluated	Unspecified	Unspecified	Unspecified	N/A	N/A
Mean Number of Implantations	Unspecified	Unspecified	Unspecified	N/A	N/A
Mean Number of Pups/Litter	12.1 ± 0.55	10.5 ± 1.19	12.6 ± 0.65	N/A	N/A
Mean Number of Liveborn Pups/Litter	Unspecified	Unspecified	Unspecified	N/A	N/A
Number of Litters with Stillborn Pups	Unspecified	Unspecified	Unspecified	N/A	N/A
Postnatal Survival to Day 4	0	0	0	N/A	N/A
Postnatal Survival to Weaning	0	0	0	N/A	N/A
Number of Total Litter Losses	Unspecified	Unspecified	Unspecified	N/A	N/A
Pup Body Weights (g)				N/A	N/A
22 day fetuses	5.85 ± 0.16	5.98 ± 0.06	5.44 ± 0.07	N/A	N/A
5 days postpartum	13.10 ± 0.48	11.63 ± 0.25	12.52 ± 0.27	N/A	N/A
30 days postpartum	M: 117.3 ± 3.7 F: 103.8 ± 4.5	M: 93.3 ± 9.3 F: 84.4 ± 7.6	M: 117.7 ± 4.7 F: 96.8 ± 2.6	N/A	N/A
60 days postpartum	M: 287 ± 7.4 F: 314 ± 4.5	M: 246 ± 11.1 F: 172 ± 15.3	M: 283 ± 8.6 F: 199 ± 8.1	N/A	N/A
Pup Sex Ratios				N/A	N/A

Species/Strain: Sprague-Dawley Holtzman rats	Duration of Dosing: 1 day			Study No.: Porterfield (1985)	
Initial Age: Unspecified	Day of Mating:			Study Title: Prenatal exposure of the fetal rat to excessive L-thyroxine or 3,5-dimetyl-3'-isopropyl thyronine produces persistent changes in the thyroid control system	
Date of First Dose: 21 days of gestation	Method of Administration: s.c. injection			Location in CTD: Module 4	
Special Features: N/A	Vehicle/Formulation: solution			GLP Compliance: No	
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: unspecified				
F0 Females: Unspecified					
F1 Males: Unspecified					
F1Females: Unspecified					
Daily Dose (mg/kg):	**0 (Control)**	**10 µg/100 g L-thyroxine**	**2.5 µg/100 g DIMIT**	N/A	N/A
30 days postpartum	12 male, 12 female	11 male, 11 female	14 male, 12 female	N/A	N/A
60 days postpartum	12 male, 12 female	12 male, 13 female	13 male, 13 female	N/A	N/A
Pup Clinical Signs	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	N/A	N/A
Pup Necropsy Observations	N/A	N/A	N/A	N/A	N/A
F1 Males (postweaning)	Unspecified	Unspecified	Unspecified	N/A	N/A
Number Evaluated Postweaning Per Litter	Unspecified	Unspecified	Unspecified	N/A	N/A
Number Died or Sacrificed Moribund	Unspecified	Unspecified	Unspecified	N/A	N/A
Clinical Observations	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	N/A	N/A
Necropsy Observations	N/A	N/A	N/A	N/A	N/A
Body Weight Change (g)[c]	Unspecified	Unspecified	Unspecified	N/A	N/A
Food Consumption (%)[e^]	Unspecified	Unspecified	Unspecified	N/A	N/A
Preputial Separation	Unspecified	Unspecified	Unspecified	N/A	N/A
Sensory Function	Unspecified	Unspecified	Unspecified	N/A	N/A

Species/Strain: Sprague-Dawley Holtzman rats	Duration of Dosing: 1 day			Study No.: Porterfield (1985)	
Initial Age: Unspecified	Day of Mating:			Study Title: Prenatal exposure of the fetal rat to excessive L-thyroxine or 3,5-dimetyl-3'-isopropyl thyronine produces persistent changes in the thyroid control system	
Date of First Dose: 21 days of gestation	Method of Administration: s.c. injection			Location in CTD: Module 4	
Special Features: N/A	Vehicle/Formulation: solution			GLP Compliance: No	
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: unspecified				
F0 Females: Unspecified					
F1 Males: Unspecified					
F1Females: Unspecified					
Daily Dose (mg/kg):	**0 (Control)**	**10 µg/100 g L-thyroxine**	**2.5 µg/100 g DIMIT**	N/A	N/A
Motor Activity	Unspecified	Unspecified	Unspecified	N/A	N/A
Learning and Memory	Unspecified	Unspecified	Unspecified	N/A	N/A
Mean Number of Days Prior to Mating	N/A	N/A	N/A	N/A	N/A
Number of Males that Mated	N/A	N/A	N/A	N/A	N/A
Number of Fertile Males	Unspecified	Unspecified	Unspecified	N/A	N/A
F1 Females (postweaning)	Unspecified	Unspecified	Unspecified	N/A	N/A
Number Evaluated Postweaning	Unspecified	Unspecified	Unspecified	N/A	N/A
Number Died or Sacrificed Moribund	Unspecified	Unspecified	Unspecified	N/A	N/A
Clinical Observations	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	Serum T_3 and T_4, serum TSH	N/A	N/A
Necropsy Observations	N/A	N/A	N/A	N/A	N/A
Premating Body Weight Change (g)[c]	N/A	N/A	N/A	N/A	N/A
Gestation Body Weight Change (g)	N/A	N/A	N/A	N/A	N/A
Premating Food Consumption (%)[f^]	N/A	N/A	N/A	N/A	N/A
Gestation Food Consumption (%)[f^]	N/A	N/A	N/A	N/A	N/A
Mean Age of Vaginal Patency (days)	N/A	N/A	N/A	N/A	N/A
Sensory Function	N/A	N/A	N/A	N/A	N/A

Species/Strain: Sprague-Dawley Holtzman rats	Duration of Dosing: 1 day	Study No.: Porterfield (1985)
Initial Age: Unspecified	Day of Mating:	Study Title: Prenatal exposure of the fetal rat to excessive L-thyroxine or 3,5-dimetyl-3'-isopropyl thyronine produces persistent changes in the thyroid control system
Date of First Dose: 21 days of gestation	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	10 µg/100 g L-thyroxine	2.5 µg/100 g DIMIT	N/A	N/A
Motor Activity	N/A	N/A	N/A	N/A	N/A
Learning and Memory	N/A	N/A	N/A	N/A	N/A
Mean Number of Days Prior to Mating	N/A	N/A	N/A	N/A	N/A
Number of Females Sperm Positive	N/A	N/A	N/A	N/A	N/A
Number of Pregnant Females	N/A	N/A	N/A	N/A	N/A
Mean Number of Corpora Lutea	N/A	N/A	N/A	N/A	N/A
Mean Number of Implantations	N/A	N/A	N/A	N/A	N/A
Mean % Preimplantation Loss	N/A	N/A	N/A	N/A	N/A
F2 Litters	N/A	N/A	N/A	N/A	N/A
Mean Number of Live Conceptuses/Litter	N/A	N/A	N/A	N/A	N/A
Mean Number of Resorptions	N/A	N/A	N/A	N/A	N/A
Number of Litter with Dead Conceptuses	N/A	N/A	N/A	N/A	N/A
Number of Dead Conceptuses	N/A	N/A	N/A	N/A	N/A
Mean % Postimplantation Loss	N/A	N/A	N/A	N/A	N/A
Fetal Body Weights (g)	N/A	N/A	N/A	N/A	N/A
Fetal Sex Ratios (% males)	N/A	N/A	N/A	N/A	N/A
Fetal Anomalies	N/A	N/A	N/A	N/A	N/A

Species/Strain: Sprague-Dawley Holtzman rats	Duration of Dosing: 1 day	Study No.: Porterfield (1985)
Initial Age: Unspecified	Day of Mating:	Study Title: Prenatal exposure of the fetal rat to excessive L-thyroxine or 3,5-dimetyl-3'-isopropyl thyronine produces persistent changes in the thyroid control system
Date of First Dose: 21 days of gestation	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	10 µg/100 g L-thyroxine	2.5 µg/100 g DIMIT	N/A	N/A
F1 Females (postweaning)	N/A	N/A	N/A	N/A	N/A
Number Evaluated Postweaning	N/A	N/A	N/A	N/A	N/A
Number Died or Sacrificed Moribund	N/A	N/A	N/A	N/A	N/A
Clinical Observations	N/A	N/A	N/A	N/A	N/A
Necropsy Observations	N/A	N/A	N/A	N/A	N/A
Premating Body Weight Change (g)[d]	N/A	N/A	N/A	N/A	N/A
Gestation Body Weight Change (g)	N/A	N/A	N/A	N/A	N/A
Premating Food Consumption (%)[f^]	N/A	N/A	N/A	N/A	N/A
Gestation Food Consumption (%)[d,f^]	N/A	N/A	N/A	N/A	N/A
Mean Age of Vaginal Patency (days)	N/A	N/A	N/A	N/A	N/A
Sensory Function	N/A	N/A	N/A	N/A	N/A
Motor Activity	N/A	N/A	N/A	N/A	N/A
Learning and Memory	N/A	N/A	N/A	N/A	N/A
Number of Females Sperm Positive	N/A	N/A	N/A	N/A	N/A
Number of Pregnant Females	N/A	N/A	N/A	N/A	N/A
Mean Duration of Gestation	N/A	N/A	N/A	N/A	N/A
Abnormal Parturition	N/A	N/A	N/A	N/A	N/A

Species/Strain: Sprague-Dawley Holtzman rats	Duration of Dosing: 1 day	Study No.: Porterfield (1985)
Initial Age: Unspecified	Day of Mating:	Study Title: Prenatal exposure of the fetal rat to excessive L-thyroxine or 3,5-dimetyl-3'-isopropyl thyronine produces persistent changes in the thyroid control system
Date of First Dose: 21 days of gestation	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	10 µg/100 g L-thyroxine	2.5 µg/100 g DIMIT	N/A	N/A
F2 Litters	N/A	N/A	N/A	N/A	N/A
Number of Litters Evaluated	N/A	N/A	N/A	N/A	N/A
Mean Number of Implantations	N/A	N/A	N/A	N/A	N/A
Mean Number of Pups/Litter	N/A	N/A	N/A	N/A	N/A
Mean Number of Liveborn Pups/Litter	N/A	N/A	N/A	N/A	N/A
Mean Number of Stillborn Pups/Litter	N/A	N/A	N/A	N/A	N/A
Postnatal Survival to Day 4	N/A	N/A	N/A	N/A	N/A
Postnatal Survival to Weaning	N/A	N/A	N/A	N/A	N/A
Change in Pup body Weights (g)[d]	N/A	N/A	N/A	N/A	N/A
Pup Sex Ratios	N/A	N/A	N/A	N/A	N/A
Pup Clinical Signs	N/A	N/A	N/A	N/A	N/A
Pup Necropsy Observations	N/A	N/A	N/A	N/A	N/A

G=Gestation day, L=Lactation day. -No noteworthy findings. +Mild ++Moderate +++Marked
*=p<0.05. **=p<0.01.
[c] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[a] At end of gestation or lactation. [b] From birth to weaning.
[c] From weaning to mating. [d] From birth to mating.
[e] At end of postweaning period. [f] At end of premating or gestation period.
N/A = Not Applicable

Table 2.6.7-14: Reproductive and Developmental Toxicity: Effects on Pre- and Postnatal Development, Including Maternal Function for Test Article: Levothyroxine (Pascual-Leone, 1976)

Species/Strain: Wistar rats	Duration of Dosing: 5 days	Study No.: Pascual-Leone, et al. (1976)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: Decreased pituitary growth hormone content in rats treated neonatally with high doses of L-thyroxine
Date of First Dose: Day 2, Day 3 or Day 6	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: 0		
F1Females: 8 per litter		

Daily Dose (mg/kg):	0 (Control)	30 µg T_4			
F0 Females	Unspecified	Unspecified			
Toxicokinetics: AUC	N/A	N/A			
Number of Pregnant	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Number Aborted or with Total Resorption of Litter	Unspecified	Unspecified			
Clinical Observations	Unspecified	Unspecified			
Necropsy Observations	Unspecified	Unspecified			
Gestation Body Weight (%)[a]	Unspecified	Unspecified			
Lactation Body Weight (%)[a]	Unspecified	Unspecified			
Gestation Food Consumption (%)[a]	Unspecified	Unspecified			
Lactation Food Consumption (%)[a]	Unspecified	Unspecified			
Mean Duration of Gestation (days)	Unspecified	Unspecified			
Abnormal Parturition	Unspecified	Unspecified			
F1 Litters (preweaning)	Unspecified	Unspecified			

Species/Strain: Wistar rats	Duration of Dosing: 5 days	Study No.: Pascual-Leone, et al. (1976)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: Decreased pituitary growth hormone content in rats treated neonatally with high doses of L-thyroxine
Date of First Dose: Day 2, Day 3 or Day 6	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: 0		
F1Females: 8 per litter		

Daily Dose (mg/kg):	0 (Control)	30 µg T_4			
Number of Litters Evaluated	Unspecified	Unspecified			
Mean Number of Implantations	Unspecified	Unspecified			
Mean Number of Pups/Litter	8	8			
Mean Number of Liveborn Pups/Litter	Unspecified	Unspecified			
Number of Litters with Stillborn Pups	Unspecified	Unspecified			
Postnatal Survival to Day 4	Unspecified	Unspecified			
Postnatal Survival to Weaning	Unspecified	Unspecified			
Number of Total Litter Losses	Unspecified	Unspecified			
Pup Body Weights (g)					
Administration on Day 2	28.8 ± 0.9	25.9 ± 0.9			
Administration on Day 6	32.1 ± 0.6	26.1 ± 1.1			
Pup Sex Ratios	0 male, 8 female (adjusted)	0 male, 8 female (adjusted)			
Pup Clinical Signs	TSH and GH, serum T_3 and T_4	TSH and GH, serum T_3 and T_4			
Pup Necropsy Observations	Unspecified	Unspecified			
F1 Males (postweaning)	0	0			
Number Evaluated Postweaning Per Litter	0	0			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			

Species/Strain: Wistar rats	Duration of Dosing: 5 days	Study No.: Pascual-Leone, et al. (1976)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: Decreased pituitary growth hormone content in rats treated neonatally with high doses of L-thyroxine
Date of First Dose: Day 2, Day 3 or Day 6	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: 0		
F1Females: 8 per litter		

Daily Dose (mg/kg):	0 (Control)	30 µg T_4			
Clinical Observations	N/A	N/A			
Necropsy Observations	N/A	N/A			
Body Weight Change (g)[c]	N/A	N/A			
Food Consumption (%)[g]	N/A	N/A			
Preputial Separation	N/A	N/A			
Sensory Function	N/A	N/A			
Motor Activity	N/A	N/A			
Learning and Memory	N/A	N/A			
Mean Number of Days Prior to Mating	N/A	N/A			
Number of Males that Mated	N/A	N/A			
Number of Fertile Males	N/A	N/A			
F1 Females (postweaning)	52	53			
Number Evaluated Postweaning	52	53			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Clinical Observations	TSH and GH, serum T_3 and T_4	TSH and GH, serum T_3 and T_4			
Necropsy Observations	Unspecified	Unspecified			
Premating Body Weight Change (g)[c]	Unspecified	Unspecified			
Gestation Body Weight Change (g)	Unspecified	Unspecified			

Species/Strain: Wistar rats	Duration of Dosing: 5 days	Study No.: Pascual-Leone, et al. (1976)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: Decreased pituitary growth hormone content in rats treated neonatally with high doses of L-thyroxine
Date of First Dose: Day 2, Day 3 or Day 6	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: 0		
F1Females: 8 per litter		

Daily Dose (mg/kg):	0 (Control)	30 µg T_4			
Premating Food Consumption (%)[f]	Unspecified	Unspecified			
Gestation Food Consumption (%)[f]	Unspecified	Unspecified			
Mean Age of Vaginal Patency (days)	N/A	N/A			
Sensory Function	N/A	N/A			
Motor Activity	N/A	N/A			
Learning and Memory	N/A	N/A			
Mean Number of Days Prior to Mating	N/A	N/A			
Number of Females Sperm Positive	N/A	N/A			
Number of Pregnant Females	N/A	N/A			
Mean Number of Corpora Lutea	N/A	N/A			
Mean Number of Implantations	N/A	N/A			
Mean % Preimplantation Loss	N/A	N/A			
F2 Litters	N/A	N/A			
Mean Number of Live Conceptuses/Litter	N/A	N/A			
Mean Number of Resorptions	N/A	N/A			
Number of Litter with Dead Conceptuses	N/A	N/A			
Number of Dead Conceptuses	N/A	N/A			
Mean % Postimplantation Loss	N/A	N/A			
Fetal Body Weights (g)	N/A	N/A			

Species/Strain: Wistar rats	Duration of Dosing: 5 days	Study No.: Pascual-Leone, et al. (1976)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: Decreased pituitary growth hormone content in rats treated neonatally with high doses of L-thyroxine
Date of First Dose: Day 2, Day 3 or Day 6	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: 0		
F1Females: 8 per litter		

Daily Dose (mg/kg):	0 (Control)	30 µg T_4			
Fetal Sex Ratios (% males)	N/A	N/A			
Fetal Anomalies	N/A	N/A			
F1 Females (postweaning)	N/A	N/A			
Number Evaluated Postweaning	N/A	N/A			
Number Died or Sacrificed Moribund	N/A	N/A			
Clinical Observations	N/A	N/A			
Necropsy Observations	N/A	N/A			
Premating Body Weight Change (g)[d]	N/A	N/A			
Gestation Body Weight Change (g)	N/A	N/A			
Premating Food Consumption (%)[f]	N/A	N/A			
Gestation Food Consumption (%)[d,f]	N/A	N/A			
Mean Age of Vaginal Patency (days)	N/A	N/A			
Sensory Function	N/A	N/A			
Motor Activity	N/A	N/A			
Learning and Memory	N/A	N/A			
Number of Females Sperm Positive	N/A	N/A			
Number of Pregnant Females	N/A	N/A			
Mean Duration of Gestation	N/A	N/A			
Abnormal Parturition	N/A	N/A			

Species/Strain: Wistar rats	Duration of Dosing: 5 days	Study No.: Pascual-Leone, et al. (1976)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: Decreased pituitary growth hormone content in rats treated neonatally with high doses of L-thyroxine
Date of First Dose: Day 2, Day 3 or Day 6	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: 0		
F1Females: 8 per litter		

Daily Dose (mg/kg):	0 (Control)	30 µg T_4			
F2 Litters	N/A	N/A			
Number of Litters Evaluated	N/A	N/A			
Mean Number of Implantations	N/A	N/A			
Mean Number of Pups/Litter	N/A	N/A			
Mean Number of Liveborn Pups/Litter	N/A	N/A			
Mean Number of Stillborn Pups/Litter	N/A	N/A			
Postnatal Survival to Day 4	N/A	N/A			
Postnatal Survival to Weaning	N/A	N/A			
Change in Pup body Weights (g)[d]	N/A	N/A			
Pup Sex Ratios	N/A	N/A			
Pup Clinical Signs	N/A	N/A			
Pup Necropsy Observations	N/A	N/A			

G=Gestation day, L=Lactation day. -No noteworthy findings. +Mild ++Moderate +++Marked
*=p<0.05. **=p<0.01.
[a] At end of gestation or lactation. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[b] From birth to weaning.
[c] From weaning to mating.
[d] From birth to mating.
[e] At end of postweaning period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

Species/Strain: Wistar rats	Duration of Dosing: 5 days	Study No.: Pascual-Leone, et al. (1976)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: Decreased pituitary growth hormone content in rats treated neonatally with high doses of L-thyroxine
Date of First Dose: Day 2, Day 3 or Day 6	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: 0		
F1Females: 8 per litter		

Daily Dose (mg/kg):	0 (Control)	30 µg T_4			

[f] At end of premating or gestation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
N/A = Not Applicable

Table 2.6.7-14: Reproductive and Developmental Toxicity: Effects on Pre- and Postnatal Development, Including Maternal Function for Test Article: Levothyroxine (Escriva and Pascual-Leone, 1984)

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified			Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No	
Daily Dose (mg/kg):	**0 (Control)**	**150 µg T_4 over 4 doses**			
F0 Females	Unspecified	Unspecified			
Toxicokinetics: AUC	N/A	N/A			
Number of Pregnant	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Number Aborted or with Total Resorption of Litter	Unspecified	Unspecified			
Clinical Observations	Glucose, lactate, pyruvate, insulin, corticosterone, plasma T_3 and T_4, liver mitochondrial α-glycerophosphate dehydrogenase.	Glucose, lactate, pyruvate, insulin, corticosterone, plasma T_3 and T_4, liver mitochondrial α-glycerophosphate dehydrogenase.			
Necropsy Observations	Unspecified	Unspecified			
Gestation Body Weight (%)[a]	Unspecified	Unspecified			
Lactation Body Weight (%)[a]	Unspecified	Unspecified			
Gestation Food Consumption (%)[a]	Unspecified	Unspecified			

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified			Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No	
Daily Dose (mg/kg):	**0 (Control)**	**150 µg T_4 over 4 doses**			
Lactation Food Consumption (%)[a]	Unspecified	Unspecified			
Mean Duration of Gestation (days)	Unspecified	Unspecified			
Abnormal Parturition	Unspecified	Unspecified			
F1 Litters (preweaning)	Unspecified	Unspecified			
Number of Litters Evaluated	Unspecified	Unspecified			
Mean Number of Implantations	Unspecified	Unspecified			
Mean Number of Pups/Litter	Unspecified	Unspecified			
Mean Number of Liveborn Pups/Litter	Unspecified	Unspecified			
Number of Litters with Stillborn Pups	Unspecified	Unspecified			
Postnatal Survival to Day 4	Unspecified	Unspecified			
Postnatal Survival to Weaning	Unspecified	Unspecified			
Number of Total Litter Losses	Unspecified	Unspecified			
Pup Body Weights (g)[b]					
2 days	6.5 ± 0.2	6.1 ± 0.3			
4 days	10.8 ± 0.5	9.3 ± 0.2			
8 days	17.4 ± 1.8	12.7 ± 2.1			
11 days	26.5 ± 2.5	17.8 ± 2.7			
14 days	28.9 ± 2.1	21.4 ± 1.9			
Pup Sex Ratios	Unspecified	Unspecified			

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified	Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No

Daily Dose (mg/kg):	0 (Control)	150 µg T_4 over 4 doses			
Pup Clinical Signs	Glucose, lactate, pyruvate, insulin, corticosterone, plasma T_3 and T_4, liver mitochondrial α-glycerophosphate dehydrogenase	Glucose, lactate, pyruvate, insulin, corticosterone, plasma T_3 and T_4, liver mitochondrial α-glycerophosphate dehydrogenase			
Pup Necropsy Observations	Unspecified	Unspecified			
F1 Males (postweaning)	Unspecified	Unspecified			
Number Evaluated Postweaning Per Litter	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Clinical Observations	Glucose, lactate, pyruvate, insulin, corticosterone, plasma T_3 and T_4, liver mitochondrial α-glycerophosphate dehydrogenase	Glucose, lactate, pyruvate, insulin, corticosterone, plasma T_3 and T_4, liver mitochondrial α-glycerophosphate dehydrogenase			
Necropsy Observations	Unspecified	Unspecified			

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified	Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No

Daily Dose (mg/kg):	0 (Control)	150 µg T_4 over 4 doses			
Body Weight Change (g)[c]	Unspecified	Unspecified			
Food Consumption (%)[g]	Unspecified	Unspecified			
Preputial Separation	Unspecified	Unspecified			
Sensory Function	Unspecified	Unspecified			
Motor Activity	Unspecified	Unspecified			
Learning and Memory	Unspecified	Unspecified			
Mean Number of Days Prior to Mating	Unspecified	Unspecified			
Number of Males that Mated	Unspecified	Unspecified			
Number of Fertile Males	Unspecified	Unspecified			
F1 Females (postweaning)	Unspecified	Unspecified			
Number Evaluated Postweaning	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Clinical Observations					
Necropsy Observations	Unspecified	Unspecified			
Premating Body Weight Change (g)[c]	Unspecified	Unspecified			
Gestation Body Weight Change (g)	Unspecified	Unspecified			
Premating Food Consumption (%)[f]	Unspecified	Unspecified			
Gestation Food Consumption (%)[f]	Unspecified	Unspecified			
Mean Age of Vaginal Patency (days)	Unspecified	Unspecified			

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified	Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No

Daily Dose (mg/kg):	0 (Control)	150 µg T_4 over 4 doses			
Sensory Function	Unspecified	Unspecified			
Motor Activity	Unspecified	Unspecified			
Learning and Memory	Unspecified	Unspecified			
Mean Number of Days Prior to Mating	N/A	N/A			
Number of Females Sperm Positive	N/A	N/A			
Number of Pregnant Females	N/A	N/A			
Mean Number of Corpora Lutea	N/A	N/A			
Mean Number of Implantations	N/A	N/A			
Mean % Preimplantation Loss	N/A	N/A			
F2 Litters	N/A	N/A			
Mean Number of Live Conceptuses/Litter	N/A	N/A			
Mean Number of Resorptions	N/A	N/A			
Number of Litter with Dead Conceptuses	N/A	N/A			
Number of Dead Conceptuses	N/A	N/A			
Mean % Postimplantation Loss	N/A	N/A			
Fetal Body Weights (g)	N/A	N/A			
Fetal Sex Ratios (% males)	N/A	N/A			
Fetal Anomalies	N/A	N/A			
F1 Females (postweaning)	N/A	N/A			

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified	Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No

Daily Dose (mg/kg):	0 (Control)	150 µg T_4 over 4 doses			
Number Evaluated Postweaning	N/A	N/A			
Number Died or Sacrificed Moribund	N/A	N/A			
Clinical Observations	N/A	N/A			
Necropsy Observations	N/A	N/A			
Premating Body Weight Change (g)[d]	N/A	N/A			
Gestation Body Weight Change (g)	N/A	N/A			
Premating Food Consumption (%)[f]	N/A	N/A			
Gestation Food Consumption (%)[d,f]	N/A	N/A			
Mean Age of Vaginal Patency (days)	N/A	N/A			
Sensory Function	N/A	N/A			
Motor Activity	N/A	N/A			
Learning and Memory	N/A	N/A			
Number of Females Sperm Positive	N/A	N/A			
Number of Pregnant Females	N/A	N/A			
Mean Duration of Gestation	N/A	N/A			
Abnormal Parturition	N/A	N/A			
F2 Litters	N/A	N/A			
Number of Litters Evaluated	N/A	N/A			
Mean Number of Implantations	N/A	N/A			

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified	Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No

Daily Dose (mg/kg):	0 (Control)	150 µg T_4 over 4 doses			
Mean Number of Pups/Litter	N/A	N/A			
Mean Number of Liveborn Pups/Litter	N/A	N/A			
Mean Number of Stillborn Pups/Litter	N/A	N/A			
Postnatal Survival to Day 4	N/A	N/A			
Postnatal Survival to Weaning	N/A	N/A			
Change in Pup body Weights (g)[d]	N/A	N/A			
Pup Sex Ratios	N/A	N/A			
Pup Clinical Signs	N/A	N/A			
Pup Necropsy Observations	N/A	N/A			

G=Gestation day, L=Lactation day. -No noteworthy findings. +Mild ++Moderate +++Marked
*=p<0.05. **=p<0.01.
[a] At end of gestation or lactation. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[b] From birth to weaning.
[c] From weaning to mating.
[d] From birth to mating.
[e] At end of postweaning period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[f] At end of premating or gestation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

Species/Strain: Wistar rats Initial Age: Unspecified Date of First Dose: Day 1 Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 8 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified	Study No.: Escriva and Pascual-Leone (1984) Study Title: Glucose homeostasis in fasted neonatal rats treated with high doses of L-thyroxine Location in CTD: Module 4 GLP Compliance: No

Daily Dose (mg/kg):	0 (Control)	150 µg T_4 over 4 doses			

N/A = Not Applicable

Table 2.6.7-14: Reproductive and Developmental Toxicity: Effects on Pre- and Postnatal Development, Including Maternal Function for Test Article: Levothyroxine (Maeda, 1986)

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 5 days	Study No.: Maeda, et al. (1986)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: L-thyroxine, cortisol and diet affect the postnatal development of the facial part of the skull in developing rats
Date of First Dose: Day 5, Day 10 or Day 15 after birth	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	0.2 µg/g L-thyroxine			
F0 Females	Unspecified	Unspecified			
Toxicokinetics: AUC	N/A	N/A			
Number of Pregnant	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Number Aborted or with Total Resorption of Litter	Unspecified	Unspecified			
Clinical Observations	Unspecified	Unspecified			
Necropsy Observations	Unspecified	Unspecified			
Gestation Body Weight (%)[a]	Unspecified	Unspecified			
Lactation Body Weight (%)[a]	Unspecified	Unspecified			
Gestation Food Consumption (%)[a]	Unspecified	Unspecified			
Lactation Food Consumption (%)[a]	Unspecified	Unspecified			
Mean Duration of Gestation (days)	Unspecified	Unspecified			
Abnormal Parturition	Unspecified	Unspecified			
F1 Litters (preweaning)	Unspecified	Unspecified			
Number of Litters Evaluated	Unspecified	Unspecified			

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 5 days	Study No.: Maeda, et al. (1986)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: L-thyroxine, cortisol and diet affect the postnatal development of the facial part of the skull in developing rats
Date of First Dose: Day 5, Day 10 or Day 15 after birth	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	0.2 µg/g L-thyroxine			
Mean Number of Implantations	Unspecified	Unspecified			
Mean Number of Pups/Litter	10	10			
Mean Number of Liveborn Pups/Litter	Unspecified	Unspecified			
Number of Litters with Stillborn Pups	Unspecified	Unspecified			
Postnatal Survival to Day 4	Unspecified	Unspecified			
Postnatal Survival to Weaning	Unspecified	Unspecified			
Number of Total Litter Losses	Unspecified	Unspecified			
Change in Pup Body Weights (g)[b]	Unspecified	Unspecified			
Pup Sex Ratios	Unspecified	Unspecified			
Pup Clinical Signs	Total length of skull, facial length	Total length of skull, facial length			
Pup Necropsy Observations	Unspecified	Unspecified			
F1 Males (postweaning)	Unspecified	Unspecified			
Number Evaluated Postweaning Per Litter	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Clinical Observations	Total length of skull, facial length	Total length of skull, facial length			

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 5 days	Study No.: Maeda, et al. (1986)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: L-thyroxine, cortisol and diet affect the postnatal development of the facial part of the skull in developing rats
Date of First Dose: Day 5, Day 10 or Day 15 after birth	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	0.2 µg/g L-thyroxine			
Necropsy Observations	Unspecified	Unspecified			
Body Weight Change (g)[c]	Unspecified	Unspecified			
Food Consumption (%)[e]	Unspecified	Unspecified			
Preputial Separation	Unspecified	Unspecified			
Sensory Function	Unspecified	Unspecified			
Motor Activity	Unspecified	Unspecified			
Learning and Memory	Unspecified	Unspecified			
Mean Number of Days Prior to Mating	N/A	N/A			
Number of Males that Mated	N/A	N/A			
Number of Fertile Males	N/A	N/A			
F1 Females (postweaning)	Unspecified	Unspecified			
Number Evaluated Postweaning	Unspecified	Unspecified			
Number Died or Sacrificed Moribund	Unspecified	Unspecified			
Clinical Observations	Total length of skull, facial length	Total length of skull, facial length			
Necropsy Observations	Unspecified	Unspecified			
Premating Body Weight Change (g)[c]	Unspecified	Unspecified			

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 5 days	Study No.: Maeda, et al. (1986)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: L-thyroxine, cortisol and diet affect the postnatal development of the facial part of the skull in developing rats
Date of First Dose: Day 5, Day 10 or Day 15 after birth	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	0.2 µg/g L-thyroxine			
Gestation Body Weight Change (g)	Unspecified	Unspecified			
Premating Food Consumption (%)[f]	Unspecified	Unspecified			
Gestation Food Consumption (%)[f]	Unspecified	Unspecified			
Mean Age of Vaginal Patency (days)	Unspecified	Unspecified			
Sensory Function	Unspecified	Unspecified			
Motor Activity	Unspecified	Unspecified			
Learning and Memory	Unspecified	Unspecified			
Mean Number of Days Prior to Mating	N/A	N/A			
Number of Females Sperm Positive	N/A	N/A			
Number of Pregnant Females	N/A	N/A			
Mean Number of Corpora Lutea	N/A	N/A			
Mean Number of Implantations	N/A	N/A			
Mean % Preimplantation Loss	N/A	N/A			
F2 Litters	N/A	N/A			
Mean Number of Live Conceptuses/Litter	N/A	N/A			
Mean Number of Resorptions	N/A	N/A			
Number of Litter with Dead Conceptuses	N/A	N/A			
Number of Dead Conceptuses	N/A	N/A			

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 5 days	Study No.: Maeda, et al. (1986)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: L-thyroxine, cortisol and diet affect the postnatal development of the facial part of the skull in developing rats
Date of First Dose: Day 5, Day 10 or Day 15 after birth	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	0.2 µg/g L-thyroxine			
Mean % Postimplantation Loss	N/A	N/A			
Fetal Body Weights (g)	N/A	N/A			
Fetal Sex Ratios (% males)	N/A	N/A			
Fetal Anomalies	N/A	N/A			
F1 Females (postweaning)	N/A	N/A			
Number Evaluated Postweaning	N/A	N/A			
Number Died or Sacrificed Moribund	N/A	N/A			
Clinical Observations	N/A	N/A			
Necropsy Observations	N/A	N/A			
Premating Body Weight Change (g)[d]	N/A	N/A			
Gestation Body Weight Change (g)	N/A	N/A			
Premating Food Consumption (%)[f]	N/A	N/A			
Gestation Food Consumption (%)[d,f]	N/A	N/A			
Mean Age of Vaginal Patency (days)	N/A	N/A			
Sensory Function	N/A	N/A			
Motor Activity	N/A	N/A			
Learning and Memory	N/A	N/A			
Number of Females Sperm Positive	N/A	N/A			

Species/Strain: Sprague-Dawley rats	Duration of Dosing: 5 days	Study No.: Maeda, et al. (1986)
Initial Age: Unspecified	Day of Mating: Unspecified	Study Title: L-thyroxine, cortisol and diet affect the postnatal development of the facial part of the skull in developing rats
Date of First Dose: Day 5, Day 10 or Day 15 after birth	Method of Administration: s.c. injection	Location in CTD: Module 4
Special Features: N/A	Vehicle/Formulation: solution	GLP Compliance: No
No Observed Adverse Effect Level: N/A	Litters Culled/Not Culled: Unspecified	
F0 Females: Unspecified		
F1 Males: Unspecified		
F1Females: Unspecified		

Daily Dose (mg/kg):	0 (Control)	0.2 µg/g L-thyroxine			
Number of Pregnant Females	N/A	N/A			
Mean Duration of Gestation	N/A	N/A			
Abnormal Parturition	N/A	N/A			
F2 Litters	N/A	N/A			
Number of Litters Evaluated	N/A	N/A			
Mean Number of Implantations	N/A	N/A			
Mean Number of Pups/Litter	N/A	N/A			
Mean Number of Liveborn Pups/Litter	N/A	N/A			
Mean Number of Stillborn Pups/Litter	N/A	N/A			
Postnatal Survival to Day 4	N/A	N/A			
Postnatal Survival to Weaning	N/A	N/A			
Change in Pup body Weights (g)[d]	N/A	N/A			
Pup Sex Ratios	N/A	N/A			
Pup Clinical Signs	N/A	N/A			
Pup Necropsy Observations	N/A	N/A			

Species/Strain: Sprague-Dawley rats Initial Age: Unspecified Date of First Dose: Day 5, Day 10 or Day 15 after birth Special Features: N/A No Observed Adverse Effect Level: N/A F0 Females: Unspecified F1 Males: Unspecified F1Females: Unspecified	Duration of Dosing: 5 days Day of Mating: Unspecified Method of Administration: s.c. injection Vehicle/Formulation: solution Litters Culled/Not Culled: Unspecified			Study No.: Maeda, et al. (1986) Study Title: L-thyroxine, cortisol and diet affect the postnatal development of the facial part of the skull in developing rats Location in CTD: Module 4 GLP Compliance: No	
Daily Dose (mg/kg):	**0 (Control)**	**0.2 µg/g L-thyroxine**			

G=Gestation day, L=Lactation day. -No noteworthy findings. +Mild ++Moderate +++Marked
*=p<0.05. **=p<0.01.
[a] At end of gestation or lactation. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
[b] From birth to weaning.
[c] From weaning to mating.
[d] From birth to mating.
[e] At end of postweaning period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

[f] At end of premating or gestation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
N/A = Not Applicable

OVERALL CONCLUSIONS AND RECOMMENDATIONS

APP Pharmaceuticals, LLC submitted this NDA 202231 as 505(b)(2) application for Levothyroxine Sodium for Injection (100, 200, and 500 mcg/vial) to treat myxedema coma. (b) (4) This was previously submitted as IND 101,385 and the pre-IND meeting was held with DMEP on March 2008. The drug product consists of the active ingredient, levothyroxine, and inactive ingredients such as dibasic sodium phosphate heptahydrate, mannitol, sodium hydroxide, (b) (4) (b) (4). The sponsor has based the efficacy and safety of their product on published literature. They did not conduct any animal studies (pharmacology, pharmacokinetic, or toxicology) in support of this drug product. All published literature cited were non-GLP studies.

- Pharmacology and safety pharmacology of IV levothyroxine

Published nonclinical pharmacology studies for myxedema coma (b) (4) (b) (4) are limited. Similar physiological responses (by repeated dose SC and IP administration) were observed in animals with normal thyroid activity and in those with hyperthyroidism. These responses include weight loss, increased food consumption, increased heart rates and etc. In patients with myxedema coma, these same physiological responses would be attenuated due to their severely hypothyroid states.

In addition, there were no standard safety pharmacology evaluations of levothyroxine in published literature. Nonetheless, the effects of levothyroxine on CV system (i.e. cardiac hypertrophy) and CNS system (i.e. changes in amygdale nuclei and its related behavior, spatial learning, and hippocampal morphology) have been noted. There findings are generally an exaggerated pharmacological activity of levothyroxine. Such adverse effects can be seen in clinical settings when overcompensated with levothyroxine. (b) (4)

Lastly, there was also no literature available with regards to IV levothyroxine-drug interactions. The sponsor based on the labeling information of Synthroid®, an approved oral levothyroxine product. However, since the levothyroxine sodium for injection would be administered acutely, the potential for drug-drug interactions would be less, compared to Synthroid®.

- Pharmacokinetics of IV levothyroxine

The bioavailability studies from the literature were performed in dogs via oral administration. However, Levothyroxine Sodium for Injection is intended to administer intravenously in humans. Therefore, any issues of oral bioavailability and absorption would not apply to this IV product. In addition, the $t_{1/2}$ ranged from 8.6 to 14.6 hrs in dogs. On the contrary, $t_{1/2}$ of an orally administered product is approx. 6-7 days in healthy human (3-4 days in patients with hyperthyroidism or 9-10 days in patients with hypothyroidism). Moreover, pharmacokinetic drug-drug interactions were described in rats (i.e. interactions occurred between levothyroxine and sodium salicylate to increase metabolism and excretion of levothyroxine, colestipol hydrochloride to decrease absorption of levothyroxine and cyclosporine A to reduce absorption of cyclosporine A due to levothyroxine induction of P-glycoprotein).

There are other drug-drug interactions identified in humans, which could be found in the labeling information of Synthroid®. Since the proposed IV levothyroxine is intended for an acute need (i.e. myxedema coma), these potential PK/PD drug-drug interactions can be managed in a hospital setting.

- Toxicology of IV levothyroxine

Clinical signs of thyroid hormone toxicity have been reported in many species (i.e. rat, guinea pig, dog, cat, rabbit, sheep and horse). These clinical signs have been seen at a wide variety of levothyroxine doses and routes of administration. The overall toxicities reported in the literature include decreased BW, increased temperature, cardiac hypertrophy, decreased bone mineral density, increased ALT and AST, and increased ALP and BUN (as well as other parameters such as leukocytosis, eosinophilia, thrombocytosis, thrombocytopenia, lymphopenia, lymphocytosis and hematocrit). Other toxicities associated with levothyroxine include disturbances in carbohydrate metabolism, abnormal pupillary reflexes, vomiting, polyuria, polydipsia, thyroid nodules or enlargement, polyphagia, reduced appetite or anorexia, altered behavior and or demeanor, changes in ejaculation, diarrhea, alopecia, scaling, altered coat color and death. These toxicities are exaggerated pharmacological activities of levothyroxine.

In addition, increased concentrations of levothyroxine during fetal and neonatal development can lead to changes in fetal lung maturation, growth stunting, neonatal mortality and suppression of the fetal thyroid. High dose of levothyroxine in neonatal rats can lead to "neo-T_4" syndrome (i.e. decreased BW, GH and TSH, increased mortality and derangements in carbohydrate metabolism).

As presented in the literature, clinical signs of thyroid hormone toxicity have been reported in rats, dogs, rabbits and etc. These clinical signs have been seen at a wide variety of levothyroxine doses and routes of administration (except no IV route). Since, there was no toxicokinetic data found in the literature, it is not possible to correlate such toxicity findings with exposure (or maximal concentration of levothyroxine). By the same token, it is also not possible to relate these toxicity findings to human exposure. One can only extrapolate that such toxicity findings are mainly exaggerated pharmacological effects of levothyroxine which observed only at very high doses, regardless routes of administration.

Moreover, no literature was found with regards to genotoxicity and/or carcinogenicity of levothyroxine. This IV levothyroxine is intended as an acute rescue therapeutic regimen (b) (4) (b) (4) The sponsor did not conduct any additional genotoxicity, carcinogenicity, reproductive or developmental studies. During pre-IND/NDA meeting in 2008, FDA did not request such studies to be conducted. Also, with current approved oral levothyroxine products, no animal studies were performed to evaluate the mutagenic or carcinogenic potential, or effect on fertility.

Lastly, impurities identified by the sponsor were all within limits. Therefore, no animal study is needed to further characterize these impurities.

- Overall nonclinical data of IV levothyroxine

Based on the available literature provided by the sponsor and the clinical use of other approved levothyroxine products, the pharmacological and toxicological profile of levothyroxine (T_4) has been well-described in animals and humans since levothyroxine has a long history of application in many therapeutic indications for reduced or absent thyroid function. The toxicology profile of levothyroxine has been associated primarily with exaggerated manifestations of excessive T_4 stimulation, resulting in physiological responses to severe hyperthyroidism. Due to the intended use of Levothyroxine Sodium for Injection (i.e. acute rescue for myxedema coma (b) (4) any adverse effects associated with levothyroxine can be easily monitored in a hospital setting.

Currently, all approved levothyroxine are in a tablet or capsule form. However, it is expected that oral and IV levothyroxine would have similar toxicity profiles. An injectable levothyroxine is more effective during acute rescue situation since it would have a higher bioavailability than oral levothyroxine. Since these patients are already in a severe hypothyroidism state, a higher dose of levothyroxine that leads to an increased risk of exaggerated pharmacological effect of T4 (i.e. hyperthyroidism) is small.

Even though there is no bridging animal study with this IV levothyroxine, one published clinical study (Maxon et al 1983) compared the PK parameters between Synthroid injection and Synthroid tablet (approx. 0.18 mg) in healthy males. The Synthroid was not an approved product back then. The study showed there were no apparent PK variations

(e.g. AUC, Cmax, tmax) between IV and oral levothyroxine in healthy volunteers. However, the NDA's proposed indication is myxedema coma. The literature showed that hypothyroidism might lead to a slight increase in thyroxine absorption. Hence, a larger dose of levothyroxine is proposed for an IV administration.

All things considered, since there is no bridging animal data with this IV levothyroxine, the pharm/tox defers to CMC to determine the similarity of this IV levothyroxine to approved oral products. According to CMC, the drug substance specification is the same as the current USP monograph for levothyroxine sodium and all the impurities and their limits are the same as in the monograph. The sponsor referenced DMF (b) (4) which has been reviewed by the CMC and used to support other approved products. As for the drug product, there is one drug metabolite (liothyronine) specified. This degradant itself is an approved drug in the US. Other unknown impurities are within ICH limits. Therefore, based on the literature, and since CMC has no further issue with the similarity of products (IV vs. other oral) and its impurity profile (IV), there is no safety nonclinical concern.

Unresolved toxicology issues (if any):
n/a

Recommendations:
The pharm/tox recommends on the approval of Levothyroxine Sodium for Injection (100, 200 and 500 mcg/vial) based on well-established published literature on levothyroxine, available clinical data with other approved levothyroxine product, and no apparent impurity and/or degradant issues associated with this product.

Pharm/tox comment with suggested labeling:
The labeling language seems reasonable. The sponsor is proposing the use of injectable levothyroxine under emergency situation (i.e. myxedema coma (b) (4) (b) (4) They defer to oral levothyroxine under non-emergency situation (i.e. maintaining a euthyroid state).

Suggested changes:

- (b) (4)

Proposed labeling:

2 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

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/s/

MIYUN M TSAI-TURTON
04/08/2011

KAREN L DAVIS BRUNO
04/08/2011

Pharmacology/Toxicology Mid-Cycle Deliverables

Mid-Cycle Deliverable	Goal Date	Status
Filing Review Meeting • **Deliverable: Regulatory history, summary of pharmacology and toxicology findings from preliminary review of existing data, adequacy of NDA submission**	**45- day of NDA submission**	Completed
Genetox Study Review (impurities and drug substance) • **Deliverable: Draft review, label recommendation/interact with genetox committee**	**2-3 months of NDA submission**	No studies to review No published literature cited
Carcinogenicity Study Review • **Deliverable: Identify statistical reviewer**	**1-month of NDA submission**	N/A
• **Deliverable: Schedule ECAC meeting**	**1-month of NDA submission**	N/A
• **Deliverable: Draft review of Carcinogenicity study with statistical input, *Interact with statistician***	**6-months of NDA submission**	N/A
• **Deliverable: ECAC review of carcinogenicity study, incorporation of eCAC comments in the review, Identification of issues from Carcinogenicity study & related post marketing commitments, labeling recommendation**	**7- month of NDA submission**	N/A
ReproTox Study Review (Seg III and I) • **Deliverable: Draft review, Identify issue/s (special studies for post marketing commitments), labeling recommendation, *Interact with Reprotox committee***	**5- month of NDA submission**	No study to review Published literature cited ▪ Sponsor is seeking Category A labeling.
Impurity/Extractable Qualification • **Deliverable: Begin review; *interact with CMC***	**3-month of NDA submission-will depend on CMC also**	CMC reviewer: Joseph Leginus
• **Deliverable: Identify whether the impurities are qualified, Identify approvability/post marketing commitment issues**	**5-6 month of NDA submission-if sponsor has final formulation**	Nine impurities identified and within limits
Toxicokinetic Study Review • **Deliverable: Draft review; *Interact with Clinical Pharmacology* for dose and ADME, *Interact with Medical Reviewer* for adverse event comparison and dose multiples**	**6- months of NDA submission**	No study to review Published literature cited
Chronic Toxicity Study Review • **Deliverable: Draft review**	**6- months of NDA submission**	No study to review Published literature cited
Pharmacology Study Review • **Deliverable: Draft review, identify PD issues**	**6- months of NDA submission**	No study to review Published literature cited
Tox findings compared in animal chronic studies and human phase 3 studies • **Deliverable: Input in ODS database**	**8- months of NDA submission**	Pending completion of comprehensive review

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/s/

MIYUN M TSAI-TURTON
02/22/2011
midcycle pt checklist

KAREN L DAVIS BRUNO
02/23/2011

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA 202231: This NDA is a 505(b)(2) application.
Submission date: August 30th, 2010
Sponsor: APP Pharmaceuticals, LLC, Schaumburg, IN
Drug: Levothyroxine Sodium for Injection

Basic Drug Product Information:

- This NDA 202231 is an electronic 505(b)(2) submission.
- It is a Levothyroxine (T_4) Sodium for Injection for treatment of myxedema coma, (b) (4) (b) (4)

  Active ingredient: levothyroxine sodium, USP (100, 200, 500 mcg/vial)
  Inactive ingredients: see table below.

APP's Levothyroxine Sodium for Injection Inactive Ingredients	Function of Inactive Ingredients
Dibasic Sodium Phosphate Heptahydrate, USP	(b) (4)
Mannitol, USP	
Sodium Hydroxide, NF	
(b) (4)	
	(b) (4)

2

- Pre-IND meeting (IND 101,385) was held with DMEP on March 2008.
- The sponsor bases the efficacy and safety of Levothyroxine Sodium for Injection on published literature reports.
- Only oral tablet formulations of levothyroxine sodium are currently approved by the FDA for treatment of hypothyroidism.
- There are two injectable thyroid hormone drug produced approved by the FDA for treatment of myxedema coma or pre-coma: Triostat (Jones Pharma Inc.) and Liothyronine Sodium (X-Gen Pharmaceuticals). Both contain Liothyronine (T_3) sodium as API. Triostat was approved on the basis of published literature for its efficacy and safety.
- The sponsor has not performed any nonclinical studies in support of Levothyroxine Sodium for Injection.

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		5 modules in eCTD submission
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		

3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		Since there is no actual animal study conducted with the drug product, the sponsor provided published literature for their nonclinical program. All journal articles cited to support the drug product were included in module 4.
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA. (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	X		PIND meeting was held in Feb 2008. The sponsor provided published literature for single-dose toxicity, repeat-dose toxicity, and repro/dev toxicity for levothyroxine. There is no information on genotoxicity, carcinogenicity, and local tolerance toxicity for levothyroxine.

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (i.e. appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?		X	There is no actual animal study conducted for this drug product. Published data was not under GLP.
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (i.e. adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		There are 9 impurities identified in the drug substance by the sponsor. There are apparently within acceptable limits. No animal studies were conducted to address any impurity and degradant issues. .

Drug substance

Table 5. Regulatory Specification for Levothyroxine Sodium, USP (effective August 1, 2008)

Test	Acceptance Criteria		Test Method[1]	Results Lot # 1001059
Related Compounds			USP	(b) (4)
A. Liothyronine	A. NMT 1.0%		10-08-01-6485	
B. B-Hydroxy-T4[3]	B. NMT 0.15%			
C. T4-Hydroxyacetic acid[4]	C. NMT 0.15%			
D. *N*-Formyl-T4[5] and T4-Acetamide[6]	D. NMT 0.15%			
E. *N*-Acetyl-T4[7]	E. NMT 0.20%			
F. T4-Acetic Acid[8]	F. NMT 0.15%			
G. T4-Aldehyde[9]	G. NMT 0.15%			
H T4-Benzoic Acid[10]	H. NMT 0.15%			
I. Individual Unspecified Impurity	I. NMT 0.10%			
J. Total Impurities	K. NMT 1.5%			
Microbial Bioburden	Total Aerobic Count	NMT 100 CFU/g	USP <61>	
	Total Yeast and Mold Count	NMT 100 CFU/g		
	Total Combined Bioburden	NMT 100 CFU/g		
Bacterial Endotoxin	NMT 22.3 EU/mg		USP <85>	

Drug product: 9 batches were tested.

Based on Q3B(R2) for drug product, Levothyroxine Sodium for Injection, if the MDD is 0.5 mg, the identification and qualification thresholds are 1.0%.

Table 26. Summary of Stability Test Results

(b) (4)

7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		The intended human exposure rote is IV (b) (4) (b) (4) Based on the published literature provided by the sponsor, there are some animal studies via IV administration.

8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	X		Section 8.1 pregnancy: the sponsor proposes the drug product as category A Section 13 nonclinical toxicity: the sponsor stated no animal studies were performed.

ITEM	YES	NO	COMMENT
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		Pharmacology and Toxicology of the drug product are entirely based on the published literature. Pharmacology/Safety Pharmacology: 30 studies were cited. Hyperthyroidism is an exaggerative pharmacologic effect. No GLP safety pharmacology study was cited but CV and CNS effect of T4 were investigated. Pharmacokinetics: 8 studies were cited. Toxicology: 25 studies were cited. These studies included single-dose, repeat-doses tox studies and repro/dev tox studies.
10) Reasons for refusal to file: None.			

Reviewing Pharmacologist: Miyun Tsai-Turton

Supervisory Pharmacologist: Karen Davis-Bruno

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/s/

MIYUN M TSAI-TURTON
10/14/2010

KAREN L DAVIS BRUNO
10/14/2010