CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022219Orig1s000

PHARMACOLOGY REVIEW(S)

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	October 15, 2013
Reviewer:	Eric Andreasen, Pharmacology/Toxicology Reviewer
NDA:	22-219 [505(b)(2)]
Applicant:	Endo Pharmaceuticals
Drug Product:	Aveed (intramuscular testosterone undecanoate)
Indication:	Replacement of testosterone in men with primary or hypogonadotrophic hypogonadism

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Background

This memo contains recommended revisions to the labeling proposed by the Sponsor in their most recent complete response (August 29, 2013). The primary nonclinical review was submitted to DARRTS on April 18, 2008. An amended nonclinical review was submitted to DARRTS on April 12, 2013 and it contains the nonclinical executive summary, a more extensive summary of the nonclinical program, and changes/corrections to the original nonclinical review.

Nonclinical Conclusion/Recommendation

The Applicant's nonclinical program, supplied references, available literature and general knowledge of testosterone provide reasonable assurance of the safety of testosterone undecanoate (TU) in hypogonadal men from a nonclinical perspective.

Recommended Labeling

Current nonclinical recommendations for labeling are provided below. Recommended labeling in the original nonclinical review of April 18, 2008 should be ignored because the Sponsor has submitted revised labeling since the original submission.

HIGHLIGHTS OF PRESCRIBING INFORMATION

AVEED™ (testosterone undecanoate) injection CIII
Initial U.S. Approval: Year 1953

----------Indications and Usage----------
AVEED (testosterone undecanoate) injection is an androgen indicated for testosterone replacement therapy in adult males for the conditions associated with a deficiency or absence of endogenous testosterone:

1 INDICATIONS AND USAGE

AVEED [redacted] is [redacted] indicated for testosterone replacement therapy in adult males for conditions associated with a deficiency or absence of endogenous testosterone.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy category X [redacted] AVEED is contraindicated [redacted] or in women who may become pregnant. Testosterone is teratogenic and may cause fetal harm. Exposure of a fetus to androgens, such as testosterone, may result in varying degrees of virilization. If AVEED is used during pregnancy, or if the patient becomes pregnant while taking AVEED, the patient should be [redacted] of the potential hazard to the fetus.

8.3 Nursing Mothers

Although it is not known how much testosterone transfers into human milk, AVEED is contraindicated in nursing women because of the potential for serious adverse reactions in nursing infants. [redacted]

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenicity, Mutagenesis, and Impairment of Fertility

Carcinogenicity

Testosterone has been tested by subcutaneous injection and implantation in mice and rats. In mice, the implant induced cervical-uterine tumors, which metastasized in some cases. There is suggestive evidence that injection of testosterone into some strains of female mice increases their susceptibility to hepatoma. Testosterone is also known to increase the number of tumors and decrease the degree of differentiation of chemically induced carcinomas of the liver in rats. [redacted]

Mutagenicity

Mutagenic effects of testosterone undecanoate were not detected in a battery of *in vitro* tests including bacterial mutation assays (Ames test) and chromosomal aberration tests in human lymphocytes. Testosterone undecanoate was also negative in an *in vivo* bone marrow micronucleus assay in mice.

Testosterone was negative in the in vitro Ames and in the in vivo mouse micronucleus assays.

Impairment of Fertility

The administration of exogenous testosterone has been reported to suppress spermatogenesis in the rat, dog and non-human primates, which was reversible on cessation of the treatment [redacted]

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/s/

ERIC A ANDREASEN
10/15/2013

LYNNDA L REID
10/15/2013
I concur.

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	April 5, 2013
Reviewer:	Eric Andreasen, Pharmacology/Toxicology Reviewer
NDA:	22-219 [505(b)(2)]
Applicant:	Endo Pharmaceuticals
Drug Product:	Aveed (intramuscular testosterone undecanoate)
Indication:	Replacement of testosterone in men with primary or hypogonadotrophic hypogonadism

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Drug History:
The original NDA was submitted August 28, 2007. From a nonclinical perspective, the nonclinical data submitted in the original submission supported approval. However, based on the need for additional clinical data, an approvable action was taken on June 27, 2008. A complete response to the approvable action was received on November 29, 2012. An Advisory Committee meeting is being held April 18, 2013, to address clinical safety. The NDA decision date for this cycle is May 29, 2013.

Recent Change in Application Type
The application type was changed from a 505(b)(1) to a 505(b)(2) due changes in regulatory interpretation (applicant's letter December 11, 2012). This does not alter the nonclinical conclusions regarding approval. The nonclinical information used to support approval of this product is complete as presented in previous reviews.

Executive Summary of Nonclinical Findings:
The toxicology of testosterone is well understood. Testosterone is a non-mutagenic rodent carcinogen (increases cervical and uterine tumors and liver tumors), and a teratogen which causes masculinization of female fetuses and adult females and acceleration of pubertal changes in juvenile males. Because of the extensive clinical and nonclinical data available in published literature on testosterone, nonclinical evaluation of TU was limited to assessing binding affinity for the human androgen receptor, ADE (absorption, distribution, and elimination) in rats, local toxicity after a single intramuscular injection in pigs, potential for toxicity after repeated intramuscular dosing in rats, and genotoxicity.

Nonclinical findings for TU include: little potential for pharmacologic activity without being metabolized, a long half-life at the injection site with expected ADE, toxicities after repeated dosing generally related to expected pharmacology or the result of large injection volumes, and negative results for *in vitro* and *in vivo* genotoxicity assays. In

summary, no significant safety concerns associated with TU administration were identified in the nonclinical program, other than toxicities related to expected pharmacology and injection site trauma.

Nonclinical Conclusion/Recommendation:
The Applicant's nonclinical program, supplied references, available literature and general knowledge of testosterone provide reasonable assurance of the safety of testosterone undecanoate (TU) in hypogonadal men from a nonclinical perspective. Changes and corrections to the original pharmacology/toxicology review are noted in the Appendix. The changes do not effect the overall conclusion.

Nonclinical Summary

Introduction
The nonclinical program addressed the in vitro affinity of testosterone undecanoate (TU) for the human androgen receptor, ADE (absorption, distribution and elimination) in rats, potential for toxicity after repeated intramuscular dosing in rats, local toxicity after a single intramuscular injection in pigs, and genotoxicity. The applicant relied upon published literature to assess the potential for reproductive toxicity and carcinogenicity.

A. Pharmacologic Activity

Testosterone undecanoate (TU) is a fatty acid ester of testosterone. TU is an inactive pro-drug which is hydrolyzed in vivo to testosterone and undecanoic acid. TU itself has little potential for pharmacological activity since its relative binding affinity for the human androgen receptor was only 1.3% of testosterone.

B. Absorption, Distribution, and Elimination

The absorption, distribution, and elimination of radiolabeled TU were characterized in rats after intramuscular administration. The distribution of radioactivity was essentially limited to the liver, kidney, and large and small intestines and their contents. Nearly half of the administered dose, based on radiolabel, remained near the dose site eight weeks after the initial injection. Most of the radioactivity was excreted in feces and to a lesser extent in urine. The fate of undecanoic acid was not directly addressed because the radioactive label was on the steroid ring. However, undecanoic acid is not predicted to be toxic since it is a fatty acid that that is readily metabolized via the fatty acid and tricarboxylic acid pathways.

C. Nonclinical Toxicology Findings

<u>Repeat-Dose Toxicity</u>
A toxicology study was conducted in male rats that were dosed intramuscularly with vehicle ((b) (4) ratio of castor oil to benzylbenzoate) or TU [50, 200, or 800 mg/kg (800 reduced to 400 after 3rd dose)] every two weeks for 14 weeks. Graded doses of TU

were achieved by varying the volume of dose solution administered. The vehicle control and high-dose group received the same dose volume. Testosterone cypionate (TC) (Depo®- Testosterone) was used as an active comparator. The vehicle for Depot-Testosterone ([b](4)% w/v cotton seed oil, [b](4)% benzylbenzoate, and [b](4)% w/v benzyl alcohol) was different from the vehicle used in the TU study. The persistence of effects was assessed 26 weeks after the last dose in the vehicle, high-dose TU, and TC groups.

Exposure to TU in the low to highest dosed rats was roughly 2, 11, and 23 times that in humans at the maximum recommend human (MRHD) dose of 750 mg TU based upon either mean AUC or mean Cmax. Maximum serum levels of testosterone increased 3, 11, and 30 times the pre-dose levels.

Exposure to TU or TC resulted in findings generally consistent with exposure to testosterone. Reduced feed intake, reduced body weight gain, slight alterations in hematology, altered organ weights, and thymic atrophy were observed at and above the lowest dose evaluated. These findings were generally mild and most could be considered affects of exaggerated pharmacology.

Consistent with the injection of an oil vehicle, local inflammation and cystic lesions were observed in all groups. The incidence of these adverse local events increased with dose volume in the TU groups and was similar between the vehicle, high-dose TU group and the TC group. Although similar pathology was observed, the extent of the local expansion of injection site reactions beyond the immediate site of application appeared to depend upon viscosity of the dosing solution with the most to least expansion being in the vehicle control, TU groups followed by the TC group, respectively.

Since there was a negative affect of both TC and TU on body weight gain but no affect on brain weight, all data discussed below was normalized to brain weight. As expected of an androgen, both TC and TU led to significant increases in the weight of the bulbocavernosus muscle and ventral prostate at exposures ≥ 2 times the MRHD and increased the weight of the kidneys and seminal vesicle at ≥ 11 times the MRHD. Adverse histopathology was not observed in these tissues. Chronic inflammation was observed in the dorsal lateral prostate in a few rats at 23 times the MRHD of TU. At TU exposures ≥ 2 times the MRHD, a low incidence of reversible renal pathology was observed including basophilic tubular cells and nephropathy, while degeneration and necrosis of the renal proximal tubule was observed only in a single rat at 23 times the MRHD. Correlating with the renal pathology was a slight increase in BUN at 23 times the MRHD and a slight reduction in phosphorous at ≥ 11 times the MRHD. In the urinary bladder, a low incidence of transitional cell hyperplasia was observed at exposures 23 times the MRHD. Diffuse thymic atrophy was observed at TU exposures ≥ 2 times the MRHD and was still observed after drug withdrawal. In the TC group and at TU exposures ≥ 2 times the MRHD, neutrophil counts were elevated (33% to 96%) while lymphocytes were reduced (18% to 43%) at ≥ 11 times the MRHD. RBC levels were not altered by TU but hemoglobin and hematocrit were slightly elevated at exposures ≥ 2 times the MRHD. The liver, testes, and thymus were reduced in weight after exposure

to TC group and at TU exposures ≥ 2 times the MRHD. Animals did not recover from the reduction in testes weight after TU withdrawal. Adverse testes pathology was not observed in rats dosed with TU likely because the reduced testes weight was a compensatory response to elevated testosterone. In the liver, a slight increase in mononuclear cell infiltration and subacute inflammation was observed at TU exposures ≥ 2 times the MRHD. Bilirubin was reduced at TU exposures ≥ 2 times the MRHD and glucose was reduced at TU exposures 23 times the MRHD.

Early in the study, high mortality/morbidity was observed in rats injected with the largest volume of vehicle alone (3.2 mL/kg) or similarly large volumes of vehicle containing TU. This large injection volume is roughly equivalent to 200 mL in humans. Because of the high mortality/morbidity, the dose volume in these groups was reduced and morbidity and death was essentially eliminated. Death and morbidity was not reported to be immediate post-injection but occurred within four days of the first or third dose. Signs of morbidity in some of the animals that were administered large dose volumes include moderate to severe tremors, languid appearance, and lack of activity; however, no signs of respiratory distress were reported. Histopathology observed in the dead and/or moribund rats receiving large dose volumes include degeneration or necrosis of the renal tubules, myocardial degeneration, adrenal congestion, and lymphoid necrosis in the thymus, spleen, lymph nodes, lung, and bone. From the available information, it could not be determined if there were any causal relationships between the mortalities and the potential formation of pulmonary microemboli related to excessive exposure to the vehicle. The cause of morbidity and death was unclear but likely due to unintended systemic exposure to large volumes of the vehicle resulting in cardiac, lymphatic, and renal toxicities.

Genotoxicity

Testosterone undecanoate was negative in a battery of *in vitro* and *in vivo* genotoxicity assays assessing mutagenicity and clastogenicity.

Carcinogenicity and Reproductive Toxicity: The risk for reproductive toxicities and cancer is considered to be similar to other approved testosterone products based upon the established effects of testosterone.

Local Tolerance

Local tolerance was assessed in pigs after a single 0.8 mL or 3 mL intramuscular dose of vehicle ((b) (4) ratio of castor oil to benzylbenzoate) or 4 mL vehicle containing TU (1000 mg). No TU related adverse affects were observed. However, as expected of post-injection trauma, injection site hemorrhaging, inflammation, presence of giant cells, and necrosis were observed four days after dosing in all groups (including vehicle). The severity increased slightly with increased dose volume. Fibrosis was observed in all groups seven days after dosing. The tissue damage essentially recovered within 42 days of dosing.

D. Nonclinical safety issues relevant to clinical use

The safety profile of testosterone is well known. Other than expected pharmacology and injection site toxicity, no significant safety concerns associated with TU at therapeutic doses were identified in the nonclinical program.

Appendix

Changes to Original Nonclinical Review for NDA 22-219 dated 4-16-08

Notations explaining the changes/corrections to the original nonclinical review are provided below. Additions are underlined and deletions are struck through. The page numbers where changes occur to the original review are provided.

Page 3 –
Labeling: Nonclinical labeling recommendations will be changed for consistency with current labeling for similar products for the same indication.

Page 20 –
The first sentence of the second paragraph mistakenly stated (b) (4) instead of 10-week.

> Since TU was not assessed in studies utilizing a loading dose and a 10-(b) (4) week dosing schedule, the more conservative use of the clinical PK data obtained from the 1000 mg TU exposure group in Part A was used to estimate the MOEs.

Page 25 –
The composition of the vehicles should have been indicated as described below.

> Route, formulation, volume, and infusion rate: The first three doses were administered via intramuscular injections into the right hind thigh, in castor oil/benzoate vehicle (<u>(b) (4) v:v castor oil to benzyl benzoate</u>). Due to injection volume-related effects, dosing was evenly divided between the right and left rear thighs starting on day 43 in the vehicle and high dose TU groups. Dose volumes varied with dose. Vehicle and high dose groups received 3.2 ml/kg for the first three doses then 1.6 ml/kg thereafter. The 50 and 200 mg/kg groups were dosed with 0.2 and 0.8 ml/kg. The 200 mg/kg TC group received 1.0 ml/kg <u>(vehicle: (b) (4) benzylbenzoate, (b) (4) w/v cotton seed oil, and (b) (4) w/v benzyl alcohol</u> (see table in results).

Page 33 –
The brain normalized ventral prostate weights were incorrectly calculated in the table. They should be 78.0% increase for the TU group and 54%, 60%, and 76% increased in the low to high-dose TU groups. The brain normalized ventral prostate weights were statistically elevated in the TC and high-dose TU group.

Affect of TC and TU on Organ Weights Normalized to Brain Weight				
	Percent Change in Organ Weight Relative to Control			
Organ	TC 200 mg/kg	TU 50 mg/kg	TU 200 mg/kg	TU 400/800 mg/kg
Bulbocavernosus	↑ **30***	↑ **27***	↑ **24***	↑ **36***
Kidney	↑ **36***	↑ 11	↑ **33***	↑ **43***
Liver	↓ **17***	↓ **12***	↓ **17***	↓ **19***
Seminal Vesicle	↑ **67***	↑ 42	↑ **71***	↑ **84***
Testes	No affect	↓ **26***	↓ **15***	↓ **8***
Thymus	↓ **50***	↓ **39***	↓ **43***	↓ **50***
Ventral Prostate	↑ (b) (4) **78***	↑ (b) (4) **54**	↑ (b) (4) **60**	↑ (b) (4) **76***
* Statistically significant from the vehicle control, $p < 0.05$. Table Adapted from Sponsor.				

Page 49 –
The injection site inflammation and cysts does not appear to be related to TU but rather to the vehicle and its viscosity. Text suggestive of a response to TU were omitted and further explained on pages 50-51.

> Paragraph 4 - A NOEL could not be determined since the adverse responses to TU and TC including reduced feed intake, decreased weight gain, exophthalmus, lacrimation, aggressive behavior, slight alterations in hematology, altered organ weights, and thymic atrophy (b) (4) -were observed at the lowest dose tested (0.4 to 2 times the exposure in men dosed with 1000 mg TU). (b) (4) -These findings were generally mild and could be considered affects of exaggerated pharmacology. Since overt toxicity was not observed below 200 mg/kg a NOAEL could be set at 50 mg/kg.

Pages 50-51-

> Nonclinical concerns regarding the Sponsor's intramuscular TU product included whether it would have similar toxicity and pharmacology as other testosterone esters and whether undecanoic acid itself could pose additional safety concerns. It was assumed that like other testosterone esters TU would not have pharmacological activity unless its ester bond is hydrolyzed releasing the active agent, testosterone, and the inactive ester, undecanoic acid. This was supported by the Sponsor's findings of minimal binding affinity of TU for the human androgen receptor. The potential toxicity of TU and undecanoic acid was assessed in a three month intramuscular TU dosing study in male rats. The results were generally consistent with exaggerated pharmacologic affects of testosterone and were similar to the comparator testosterone cypionate. Adverse toxicities unrelated to exaggerated pharmacology of testosterone were generally

not observed. Consistent with the injection of oil vehicle, inflammation and cystic lesions were observed at or near the injection site in the vehicle control and the TC and all TU groups. Although similar pathology was observed, the extent of the local expansion of injection site pathology beyond the immediate site of application appeared to depend upon viscosity of the dosing solution with the most to least expansion being in the vehicle control, TU groups followed by the TC group respectively. (b) (4)

[redacted]

Inflammation, necrosis, fibrosis, hemorrhaging and appearance of multinucleated giant cells were also observed at the injection site after intramuscular dosing in pigs (1000 mg/4 ml TU). The affects in pigs appeared to be due to injection volume and not related to TU itself. Cystic lesions referred to in the rat study were not mentioned in the porcine study, however, the pathologist in the porcine study referred to injection site fibrosis which may be similar to the cystic lesions in rats. The differences between species may be due to the difference in volume and repetition of dosing.

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/s/

ERIC A ANDREASEN
04/12/2013

LYNNDA L REID
04/12/2013
Concur

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	August 20, 2009
Reviewer:	Lynnda Reid, Ph.D. Supervisory Pharmacologist
NDA #/SS#/date:	NDA 22-219
Sponsor:	Endo Pharmaceutical Solutions Inc.
Drug Product:	Testosterone undecanoate (AVEED®)
Indication:	Male Hypogonadism

Background: Testosterone undecanoate (TU) is a new ester of testosterone. TU is relatively inactive. The ester bond is quickly hydrolyzed *in vivo* releasing testosterone and undecanoic acid. Testosterone mediates its pharmacological activity by binding to and activating the androgen receptor.

Summary of nonclinical data: A 14-week bridging study was conducted in male rats to compare physiological responses to testosterone undecanoate (TU) with another approved testosterone ester, testosterone cypionate (Depo® –Testosterone, TC). TU exposures were 2 to 20 times the exposure in men dosed with 1000 mg TU. Dose-related adverse effects observed with both TU and TC consisted of the following:

- decreases in body weight gain and food consumption
- decreased liver and thymus weights and increased kidney, ventral prostate, seminal vesicle and bulbocavernosus muscle weight
- thymic atrophy (moderate and diffuse) with recovery observed only in the TC treated animals
- increased neutrophil and decreased lymphocyte counts

Adverse affects observed in the TU animals but not in the TC group consisted of the following findings:

- non-dose responsive and non-recoverable decrease in testicular weight (8-26%)
- low incidence of reversible adverse renal and bladder histopathology (transitional cell hyperplasia, degeneration and necrosis of the renal proximal tubule and dilation of the renal pelvis at 20 times the clinical exposure

Outstanding nonclinical issues: None

Conclusion(s):

- I concur with the primary nonclinical reviewer, Dr. Eric Andreasen, that the nonclinical data support approval of testosterone undecanoate (dose) for the treatment of men with a testosterone deficiency as proposed in this NDA.

- The final label for AVEED submitted by the Sponsor on August 14, 2009 is acceptable.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22219	ORIG 1	ENDO PHARMACEUTICALS INC	NEBIDO
NDA 22219	ORIG 1	ENDO PHARMACEUTICALS INC	NEBIDO

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/s/

LYNNDA L REID
08/20/2009

REVIEW **FOOD AND DRUG ADMINISTRATION**

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

NDA: 22-219

Sponsor: Endo Pharmaceutical Solutions Inc.

Product: AVEED (testosterone undecanoate, IM)

Date: August 19, 2009

From: Eric Andreasen, Ph.D.
Pharmacologist/Toxicologist

Through: Lynnda Reid, Ph.D.
Supervisory Pharmacologist

To: File

Subject: Concurrence with final labeling submitted by the Sponsor on August 14, 2009 (DARRTS 42)

From a pharmacology/toxicology perspective the labeling for AVEED submitted by the Sponsor on August 14, 2009 (DARRTS 42) is acceptable.

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/s/

ERIC A ANDREASEN
08/20/2009

LYNNDA L REID
08/20/2009

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:	**22-219**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**03/03/09**
PRODUCT:	**Testosterone undecanoate IM**
INTENDED CLINICAL POPULATION:	**Men with testosterone deficiency**
SPONSOR:	**ENDO Pharmaceuticals Solutions, Inc.**
DOCUMENTS REVIEWED:	**CDSESUB1\NONECTD\N22219\N_000\2009-03-02**
REVIEW DIVISION:	**Division of Reproductive and Urologic Products**
PHARM/TOX REVIEWER:	**Eric Andreasen, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lynnda Reid, Ph.D.**
ACTING DIVISION DIRECTOR:	**Scott Monroe, M.D.**
PROJECT MANAGER:	**Eufrecina Deguia**

Date of review submission to Division File System (DFS): 7-09-09

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Nonclinical data support approval.

B. Recommendation for nonclinical studies: None at this time. The nonclinical program was previously reviewed on April 18, 2008. Other than new recommendations for labeling, changes to the nonclinical review are not necessary since new data was not submitted.

C. Recommendations on labeling: The major recommendations include adding sections for the use in women (5.11), affects on spermatogenesis (5.12), drug interactions with anticoagulants (7.5), use in pregnant or nursing women (8.1, 8.3), use in pediatrics (8.4,) and use in patients with impaired renal or hepatic function (8.6). The Sponsor's comments regarding hepatocellular carcinoma and prostatic hypertrophy and prostatic carcinoma in humans (b) (4) were moved to section 5.5.

LABELING REVIEW

An unadulterated copy of the Sponsor's proposed label is listed in the appendix at the end of this document. For each section where changes to the label are recommended, the Sponsors version is listed followed by a version with the recommended changes. Additions are underlined and deleted text is struck through.

(b) (4)

11 Pages of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page.

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/s/

Eric A Andreasen
7/9/2009 04:32:07 PM
PHARMACOLOGIST

Lynnda Reid
7/9/2009 04:34:06 PM
PHARMACOLOGIST

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:	**22-219**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**08/28/07**
PRODUCT:	**NEBIDO® (testosterone undecanoate) IM**
INTENDED CLINICAL POPULATION:	**Men with testosterone deficiency**
SPONSOR:	**Indevus Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**CDSESUB1\NONECTD|N22219|N_000|2007-08-24**
REVIEW DIVISION:	**Division of Reproductive and Urologic Products**
PHARM/TOX REVIEWER:	**Eric Andreasen, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lynnda Reid, Ph.D.**
ACTING DIVISION DIRECTOR:	**Scott Monroe, M.D.**
PROJECT MANAGER:	**Eufrecina Deguia**

Date of review submission to Division File System (DFS): 4-16-08

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Nonclinical data support approval.

B. Recommendation for nonclinical studies: None at this time.

C. Recommendations on labeling:
The major recommendations include adding sections on use in women (5.10), affects on spermatogenesis (5.11), drug interactions with anticoagulants (7.5), use in pregnant or nursing women (8.1, 8.3), use in pediatrics (8.4,) use in patients with impaired renal or hepatic function (8.6).

An unadulterated copy of the Sponsor's proposed label is listed in the appendix at the end of this document. For each section where changes to the label are recommended, the Sponsors version is listed followed by a version with the recommended changes. A full copy of the suggested label is listed after the Sponsor unadulterated version in the appendices at the end of this document.

1 INDICATIONS AND USAGE

Sponsor: (b) (4)

Pharm/Tox Recommendation (b) (4) (Testosterone Undecanoate, Intramuscular Injection), an ester of endogenous testosterone, is indicated for testosterone replacement therapy in males for conditions associated with a deficiency or absence of endogenous testosterone (Section 1.1-1.2).

4 CONTRAINDICATIONS

Sponsor: (b) (4)

Pharm/Tox Recommendation:
(b) (4) should not be used in the following patients:

Men with carcinoma of the breast or known or suspected carcinoma of the prostate [see Warnings and Precautions (5.3) Adverse Reactions (6.1, 6.2), and Nonclinical Toxicology (13.1)].

Women who are or may become pregnant or are breastfeeding. (b) (4) can cause fetal harm when administered to a pregnant woman. NEBIDO may cause serious adverse reactions in nursing infants. Exposure of a (b) (4) fetus or nursing infant to androgens may result in varying degrees of virilization. *[see Use in Specific Populations (8.1, 8.3 and 8.4)]*.

5 WARNINGS AND PRECAUTIONS

The Sponsor did not address precautions for use in women or the potential for affects on spermatogenesis in this section. These sections were added (5.10 and 5.11). The Sponsor's comment regarding cancer in humans was moved (b) (4) to section 5.3.

Pharm/Tox Recommendations:

5.3 (b) (4)

(b) (4)

5.10 Use in Women

Due to lack of controlled evaluations in women and potential virilizing effects, (b) (4) is not indicated for use in women [see Use in Specific Populations (8.1, 8.3, and 8.4)].

5.11 Potential for Adverse Effects on Spermatogenesis

At large doses of exogenous androgens, spermatogenesis may be suppressed through feedback inhibition of pituitary follicle-stimulating hormone (FSH) which could possibly lead to adverse effects on semen parameters including sperm count.

7 DRUG INTERACTIONS

Interactions with anticoagulants were not mentioned by the Sponsor in this section.

Pharm/Tox Recommendation:

7.5 Oral Anticoagulants

Changes in anticoagulant activity may be seen with androgens. More frequent monitoring of INR and prothrombin time are recommended in patients taking (b) (4), especially at the initiation and termination of androgen therapy.

8 USE IN SPECIFIC POPULATIONS

Sponsor:

(b) (4)

Pharm/Tox Recommendation: Add the following and renumber the geriatric section to 8.5.

8.1 Pregnancy

Pregnancy Category X (b) (4) is contraindicated (b) (4) or in women who may become pregnant. It is teratogenic and may cause fetal harm (b) (4) Exposure of a (b) (4) fetus to androgens may result in varying degrees of virilization. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be (b) (4) of the potential hazard to a fetus.

8.3 Nursing Mothers

Although it is not known how much testosterone transfers into human milk, (b) (4) is contraindicated in nursing women because of the potential for serious adverse reactions in nursing infants (b) (4)

8.4 Pediatric Use

Safety and efficacy of (b) (4) in males < 18 years old has not been established (b) (4)

8.6 (b) (4)

(b) (4)

13 NONCLINICAL TOXICOLOGY

Any reference to human carcinogenicity in this section should be omitted. The Sponsor's comments regarding human carcinogenicity were moved to the Warnings and Precautions Section (See 5.3 (b) (4)). The lack of genotoxicity of TU was also added to this section.

Sponsor:

(b) (4)

(b) (4)

Pharm/Tox Recommendation:
Testosterone has been tested by subcutaneous injection and implantation in mice and rats. The implant induced cervical-uterine tumors in mice, which metastasized in some cases. There is suggestive evidence that injection of testosterone into some strains of female mice increases their susceptibility to hepatoma. Testosterone is also known to increase the number of tumors and decrease the degree of differentiation of chemically induced carcinomas of the liver in rats.

(b) (4)

(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

A 14 week bridging study was conducted in male rats to compare physiological responses to testosterone undecanoate (TU) with another approved testosterone ester, testosterone cypionate (Depo® –Testosterone, TC). Rats were dosed intramuscularly with TU or TC every two weeks. TU exposures were 2 to 20 times the exposure in men dosed with 1000 mg TU. Exposures and results in the high dose TU group were similar to the TC group. The NOEL for this study was < 50 mg/kg (approximately 2 times the exposure in men dosed with 1000 mg TU) based upon reduced feed intake, weight loss, exophthalmus, lacrimation, aggressive behavior, slight alterations in hematology, altered organ weights, thymic atrophy and inflammation at 50 mg/kg. However with the exception of the injection site cysts and inflammatory response these findings were generally mild and could be considered affects of exaggerated pharmacology. The persistence of affects was assessed 24 weeks after the last dosing period (26 weeks after the last dose) for most endpoints in the high dose group only.

The main affects of TU and TC in animals surviving to scheduled death:

- Decreases in body weight gain (13-21%) and food consumption (4-9%). Affects tended to recover but was not complete after the recovery period.
- Altered organ weights – Dose related.
  - Decreased – liver (12-19%) and thymus (39-50%)
  - Increased – kidney (11-43%), ventral prostate (42-69%) seminal vesicle (42-84%) and bulbocavernosus muscle (24-36%).

- Histopathology -
  - Thymic atrophy – moderate and diffuse (dose related and non-recoverable). The TC dose group recovered but the TU group did not.
  - Injection site inflammation and cysts (dose related and non-recoverable).
- White cell counts- Increased neutrophils (4-96%) and decreased lymphocytes (7-43%).

Adverse affects observed in the TU animals but not in the TC group:

- Non-dose responsive and non-recoverable decrease in testicular weight (8-26%).
- Low incidence of reversible adverse renal and bladder histopathology (transitional cell hyperplasia (14% of HD group), degeneration and necrosis of the renal proximal tubule and dilation of the renal pelvis (7% HD group).

Genotoxicity

Testosterone undecanoate was negative in a battery of *in vitro* and *in vivo* genotoxicity assays assessing mutagenicity and clastogenicity.

Local Tolerance

A local tolerance study in pigs was conducted comparing intramuscular administered TU and testosterone enanthate. Drug related adverse affects were not observed however large injection volumes (3-4 ml) did cause tissue necrosis, fibrosis, inflammation and hemorrhaging which tended to recover 7-42 days after dosing.

B. Pharmacologic activity

Testosterone undecanoate is an ester of testosterone. TU is an inactive pro-drug which upon *in vivo* hydrolysis of the ester bond releases testosterone and undecanoic acid. Testosterone mediates its pharmacological activity by binding to and activating the androgen receptor. To insure that non-hydrolyzed TU itself has little potential for pharmacological activity, the ability of TU to bind to the human androgen receptor was assessed. The results suggest that TU does not have significant pharmacological activity since its relative binding affinity was only 1.3% of testosterone.

C. Nonclinical safety issues relevant to clinical use

The safety of testosterone is well known. No additional safety concerns associated with TU were identified in the nonclinical program.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

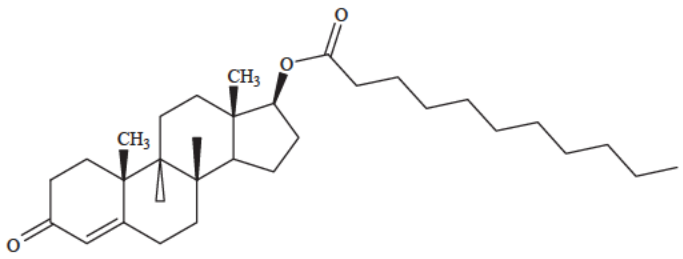
NDA number: 22-219
Review number: 1
Sequence number/date/type of submission: 000, 08/28/07, original
Sponsor and/or agent: Indevus Pharmaceuticals, Inc., Lexington, MA
Manufacturer for drug substance: There are two sources.

The clinical study supply and initial supplies for the USA market were manufactured at: [redacted] (b) (4) [redacted] (b) (4) will also begin to produce the drug substance for the USA market.

Reviewer name: Eric Andreasen
Division name: Division of Reproductive and Urologic Products
HFD #: 580
Review completion date: February 12, 2008.

Drug:

Trade name: Nebido®
Generic name: Testosterone undecanoate (TU)
Code name: ZK 5448
Chemical name: 17 β-undecanoyloxy-4-androsten-3-one
CAS registry number: 5949-44-0
Molecular formula/molecular weight: $C_{30}H_{48}O_3$ / 456.7.
Structure:



Relevant INDs/NDAs/DMFs: IND 72,297

Drug class: Androgen

Intended clinical population: Hypogonadal Men

Clinical formulation: [redacted]

Ingredients	750 mg i.m. vial	Function
Testosterone Undecanoate	750 mg	Active Ingredient
Benzyl Benzoate	1500 mg	
Castor Oil (refined for parenteral use)	885 mg	

[redacted]

Excipients: Benzyl benzoate is an FDA approved excipient used at up to 46% concentration for intramuscular injection. Castor oil is an FDA approved excipient without a listed percentage for injection in pure form. It is also a food substance generally recognized as safe.

Impurity profile: The Sponsor initially identified three impurities [redacted] which were above the limit of [redacted]% and therefore required toxicological qualification. The Sponsor's final specification for [redacted] is ≤ [redacted]%. The Sponsor noted that batches used in the genotoxicity assays were intentionally fortified with [redacted] ([redacted]% *in vitro* genotoxicity assays, and [redacted]% for mouse micronucleus assay) and [redacted] ([redacted]% in all batches used in genotoxicity) and therefore these impurities were qualified for genotoxicity. Additionally [redacted] was found at [redacted]% [redacted]

Route of administration: Intramuscular injections: 750 mg intramuscular dose of TU at baseline, then again after four weeks and every ten weeks thereafter

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

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA number 22-219 are owned by Indevus Pharmaceuticals or are data for which Indevus Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 22-219 that Indevus 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 Indevus 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 Indevus Pharmaceuticals.

Studies reviewed within this submission:

PHARMACOLOGY:

- *In Vitro* Steroid Receptor Binding of Testosterone, ZK 5448, ZK 5137 and ZK 4955 to the human androgen receptor. (GF2004.0843)

PHAMMACOKINETICS/TOXICOKINETICS:

- Pharmacokinetics and Metabolic Disposition of ^{14}C-Testosterone Undecanoate (TU) Following Intramuscular Administration to Male Rats (6530-112: CMS 81418A).

TOXICOLOGY:

Repeat-Dose Toxicity:
- Testosterone Undecanoate: Toxicity Study in Male Sprague-Dawley Rats Following Seven Bi-weekly Intramuscular Doses with Six-month Recovery Period (1630-05560).

Genotoxicity:
- Evaluation of ZK 5448 in a bacterial reverse mutation test (Ames-Test) using Salmonella typhimurium and Escherichia coli as test organisms (A07923).
- Evaluation of ZK 5448 in a bacterial reverse mutation test (Ames-Test) with preincubation using Salmonella typhimurium and Escherichia coli as test organisms (A07981).
- Chromosome aberration assay in human lymphocytes *in vitro* with ZK5448 (A08353).
- Study on the mutagenic potential of ZK 5448 in the mouse micronucleus test (A06611).

Local Tolerance:
- Local tolerance testing after intramuscular injection in pigs (JPH01496).

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Testosterone undecanoate (TU) is a pro-drug. Pharmacological activity is dependent upon *in vivo* esterase activity which releases the active free testosterone from the n-undecanoic acid side chain.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: The pharmacological activity of TU is dependent upon *in vivo* hydrolysis for release and subsequent binding of testosterone to the androgen receptor. The potential pharmacological activity TU was assessed by determining the affinity of TU for the human androgen receptor (hAR) in comparison to testosterone and two other testosterone esters, testosterone propionate (TP) and testosterone enanthate (TE) (Study GF2004.0843). Baculovirus-infected HI5 insect cells were used to produce cell fractions containing hAR for the binding assay. Binding affinity of each test compound was measured by *in vitro* competition experiments using radioactive metribolone as the standard ligand and the respective unlabelled test compounds (T, TP, TE and TU) as potential competitive ligands. Results were expressed relative to the affinity of metribolone. Results are given in the table below. This *in vitro* binding assay suggests that non-hydrolyzed TU is unlikely to have significant pharmacological activity since the relative binding affinity was only 1.3% of testosterone.

Radioligand Binding Assay with the Human Androgen Receptor		
Compound	**IC_{50} (mol/L)**	**RBA**
Metribolone	2.3 x 10-8	100
Testosterone	4.1 x 10-8	57.8
TP	5.0 x 10-8	46.7
TE	1.7 x 10-7	13.6
TU	6.6 x 10-6	0.73
IC_{50}- concentration of test compound at which competes away 50% of the radiolabeled metribolone. RBA-relative binding affinity = the IC_{50} of non-radiolabeled metribolone divided by IC_{50} of the test compound x 100. Table adapted from the Sponsor.		

2.6.2.4 Safety pharmacology

Safety pharmacology was not assessed since the safety of testosterone has been well described.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Pharmacokinetics, absorption, distribution and excretion of ^{14}C-TU were assessed in two rat strains following a single intramuscular injection (12.4 mg/kg). Radioactivity associated with

^{14}C-TU was detected in the circulation within an hour of dosing but the distribution during the subsequent eight weeks was not extensive and the release of the drug from the injection site was slow with nearly half of the dose remaining there eight weeks after dosing. Maximal tissue concentrations were generally reached 72 hrs after dosing. ^{14}C-TU equivalents were predominately detected in the excretory organs (large and small intestines and their contents and to a lesser extent in the kidneys and liver) with diminishing amounts over time. Nearly 32% of the radioactive dose was eliminated in urine (7%) or feces (25%) within eight weeks of dosing.

Metabolism was not directly investigated by the Sponsor except for analysis of TU, DHT-TU, T, DHT and E_2 following 14 weeks of intramuscular dosing in rats (see section 2.6.6.3). However the Sponsor cited an *in vitro* and *in vivo* study that suggests that hydrolysis of TU occurs primarily in target tissues and not substantially in the plasma (1). This same reference also reported that testosterone was readily metabolized into undecanoate free and undecanoate bound metabolites with testosterone being the most abundant non-esterified androgen in the plasma, bulbocavernosus muscle, and skeletal muscle accounting for 14-28% of the radioactivity.

2.6.4.2 Methods of Analysis

Absorption, distribution, elimination (ADE) of TU were assessed in male Sprague Dawley and Long Evans rats following a single intramuscular injection of ^{14}C-TU (12.4 mg/kg) (6350-112: CMS 81418A). The strains were chosen to assess the affects of melanin on distribution since Long Evans are pigmented and Sprague Dawley are non-pigmented. Quantitative whole body autoradiography was used to assess distribution in both rat strains. Routes radioactive elimination were assessed in Sprague Dawley rats by measuring radioactivity in the air, feces and urine and concentration at the injection site and whole carcass. Pharmacokinetics of the radioactivity in blood and plasma was also assessed in both strains.

Detection/quantification of radioactivity: Samples were analyzed in duplicate where possible. Blood and plasma samples were combusted and the resulting $^{14}CO_2$ was measured by liquid scintillation counting (LSC). Radioactivity in urine and cage wash was measured directly by LSC. Feces were prepared for LSC by dissolving it in 50% methanol prior to combustion. Expired radioactivity was trapped in a carbon filter and measured by LSC. The injection sites were isolated dissolved in sodium hydroxide and radioactivity was measured by LSC in duplicate. Residual carcass radioactivity was measured from homogenized whole carcass that was flash frozen, homogenized, digested with sodium hydroxide, treated with hydrogen peroxide and measured by LSC in duplicate. Whole body radiography was conducted on 40 μm frozen sagital sections. Five sections were prepared from each animal. Sections were dried and wrapped in Mylar film and exposed to phosphorimaging screens for four days along with standards for quantitative analysis. Screens were scanned using an Amersham Biosciences Storm scanner and analyzed using Specified Imaging Research Inc. AIS software.

2.6.4.3-6 Absorption, Distribution, Metabolism, Elimination

Study Title: Pharmacokinetics and Metabolic Disposition of ^{14}C-Testosterone Undecanoate (TU) Following Intramuscular Administration to Male Rats (6530-112: CMS 81418A).

Key study findings: No adverse drug events were reported. Nearly 50% of the radioactivity remained near the injection site 8 weeks after administration. C_{max} values were reached within a day of dosing. The radioactivity half life was very long ranging from 336-632 hrs in the blood. The radioactivity was primarily excreted in the feces and to a lesser extent in urine. Within one day of administration, radioactivity was primarily at the dose site but was also evident in the large and small intestines, kidneys and liver. Radioactivity was not detected in other organs. Eight weeks after dosing, radioactivity was restricted to the dose site and large and small intestines.

Study no.:	(b) (4) 6530-112: CMS 81418A
Sponsor's Submission	CDSESUB1\NONECTD\N22219|N_000|2007-08-24.
Module:	4.2.2.2 Absorption. 42-stud-rep\422-pk\4222-absorp\6530-112-legacy.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 19, 2006.
GLP compliance:	Yes
QA report:	yes (X) no ( )
Drug, lot #, and % purity:	TU- Lot N01001, unknown purity. However it was 102% in the clinical validation testing. See section 2.3 page 11 of the Sponsor's NDA submission. ^{14}C-TU – CFQ14790 Batch 1, 98.5% pure

Doses: All animals were dosed intramuscularly in the thigh with TU at 12.4 mg/kg. The dosage was a combination of 26% ^{14}C-TU (197 µCi/kg) and 74% cold TU.
Species/strain: Sprague Dawley (non-pigmented) and Long Evans (pigmented) rats.
Number/sex/group or time point (main study): All rats were male.
Group 1) Sprague Dawley (N=5): urine, feces, expired air, blood (plasma) and carcass.
Group 2) Sprague Dawley (N= 18): Blood (plasma), Carcass for whole body autoradiography.
Group 3) Long Evans (N=8): Blood (plasma), Carcass for whole body autoradiography.
Route, formulation and volume: Intramuscular injections, vehicle was castor oil/benzyl benzoate (30 day safety review reported that castor oil was (b) (4)% and benzyl benzoate was (b) (4)% of the vehicle however this was not clear in this study), 0.048 mL/kg target volume.
Age: 9 weeks old.
Weight: 263-320 g.
Sampling times:
Mortality and pain/distress observations made twice daily
Body weight was measured at randomization, prior to dosing and at sacrifice.
Group 1)
24 hr urine through 168 hrs post dosing then once weekly till week 8.

24 hr feces through 168 hrs post dosing then once weekly till week 8.
24 hr expired air through 168 hrs post dosing then once weekly till week 8.
Blood was collected after euthanization 8 weeks after exposure.
Carcass and the injection site were stored for potential radioactivity analysis.

Group 2 and 3)

Whole body autoradiography (WBA) was conducted on one animal each at 1, 24, 72 and 168 hrs post dosing and also 4 and 8 weeks after dosing.
Blood and serum were collected from the animals used for WBA and blood was also collected from an additional two animals at the same time points.
Radioanalysis of the dose site and the surrounding muscle from two rats was analyzed 1, 24, 72 and 168 hrs post-dose from. Only Sprague Dawley rats were assessed.

Summary of Sampling		
Group	No. Animals	Samples
1	5 SD	Urine, Feces, Expired Air, Blood (plasma), Carcass and Dose Site
2	18 SD	Blood (plasma), Whole Body Autoradiography and Dose Site
3	8 LE	Blood (plasma), Whole Body Autoradiography
Table modified from Sponsor. SD- Sprague Dawley. LE- Long Evans.		

2.6.4.3 Absorption

The average intramuscular dose of ^{14}C-TU in Sprague Dawley rats was 198 ± 3 µCi/kg (group 1), 197 ± 11 µCi/kg (group 2) and 197 ± 4 µCi/kg in Long Evans rats (group 3). Only a very small percentage of the dose ≤ 0.1% (assuming a blood volume of 6 mL/100g rat) was detected in blood or plasma at any time. The pharmacokinetics in the Long Evans is not reliable since only one animal was monitored per time point; however both strains of rats displayed fairly similar pharmacokinetics. A minor difference observed was that Sprague Dawley rats had slightly shorter half-lives but larger maximal concentrations of radioactivity than Long Evans rats. Radioactivity was detected in the blood and plasma in both strains of rats within an hour of delivery (see table below). The maximal blood and plasma concentrations were observed within an hour of delivery in the Sprague Dawley rats. Maximal plasma levels in the Long Evans rats were also reached in one hr while the maximal blood levels were reached at 24 hrs. The levels in the blood and plasma did not start to decline until roughly 72 to 168 hrs after administration in Sprague Dawley rats and 72 hrs in Long Evans rats. However the half-lives were very long ranging from 336 to 632 hrs in the blood and 465 to 588 hrs in plasma for Sprague Dawley and Long Evans respectively.

Pharmacokinetics of Radioactivity Associated with Intramuscular ^{14}C-TU Dosing (12.4 mg/kg)								
	Blood				Plasma			
	T_{max} (hr)	C_{max} (ng eq/g)	AUC_{0-1344} (ng eq/mL)	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng eq/g)	AUC_{0-1344} (ng eq/mL)	$T_{1/2}$ (hr)
Sprague Dawley	1	29.6	10,935	336	1	43.2	11,932	465
Long Evans*	24	27.8	12,727	632	1	38.8	13,340	588
*Blood and plasma levels in Long Evans rats were only determined in single rat per time point.								

Concentration of ^{14}C-TU (ng eq/kg) in Blood and Plasma				
Hours Post Administration	Sprague Dawley		Long Evans*	
	Blood	Plasma	Blood	Plasma
1	29.6 ± 8.2	43.2 ± 10.0	26.5	38.8
24	23.4 ± 11.4	33.4 ± 17.0	27.8	37.3
72	23.5 ± 3.7	28.1 ± 5.4	18.8	19.4
168	15.6 ± 7.0	17.9 ± 8.2	BLQ	5.8
672	4.96 ± 1.21	3.05 ± 2.68	10.6	10.6
1344	1.37 ± 2.38	3.10 ± 1.44	5.07	4.8
*Blood and plasma levels in Long Evans rats were only determined in single rat per time point. BQL- below limit of quantification.				

2.6.4.4 Distribution

In comparison to the dose, only low levels of radioactivity were detected in both strains of rats when analyzed by whole body radiography. Interpretation is limited since only one animal was assessed per time point. The tissues with detectable levels of radioactivity were similar in both strains of rats (tables below). Radioactivity was not detected in melanin containing tissues. Maximal tissue concentrations were generally reached 72 hrs after dosing. ^{14}C-TU equivalents were predominately detected in the excretory organs (large and small intestines and their contents and to a lesser extent in the kidneys) with diminishing amounts over time. Additionally low levels of radioactivity were initially detected in the liver but this declined below the level of detection by 336 hrs and 672 hrs in Long Evans and Sprague Dawley rats respectively. Radioactivity was detected in the preputial gland (no human equivalent) between 72 and 1008 hrs after dosing. The observance of radioactivity in the stomach contents but not the stomach wall in Long Evans rats was interpreted by the Sponsor to be due to grooming.

Concentrations of Radioactivity (^{14}C-TU, ng eq/g) Detected by Whole Body Radiography in Sprague Dawley Rats (n=1 per time point)						
	1 hr	24 hrs	72 hrs	168 hrs	672 hrs	1344 hrs
Cecum	ND	130	310	84.3	81.6	BQL
Cecum Contents	ND	783	2270	1190	574	407
Dose Site	985,000*	148,000*	985,000*	121,000*	198,000*	1,090,000*
Kidney	150	71	114	102	BQL	ND
Large Intestine Contents	ND	1340	3560	1740	650	374
Large Intestine	ND	132	227	BQL	BQL	BQL
Liver	198	75.2	215	188	BQL	BQL
Preputial Gland	ND	ND	209	ND	ND	ND
Renal Cortex	184	71.3	133	116	ND	ND
Renal Medulla	93	65	93	99	ND	ND
Small Intestine Contents	6,490	1,130	2,430	725	2,840	186
Small Intestine	1090	136	125	119	737	ND
Urine	957	ND	380	423	BLQ	BLQ
* Samples were above the upper limit of detection (592,000). Each value is from a single individual.						

Concentrations of Radioactivity (^{14}C-TU, ng eq/g) Detected by Whole Body Radiography in Long Evans Rats (n = 1 per time point)								
	1 hr	24 hrs	72 hrs	1 wk	2 wks	4 wks	6 wks	8 wks
Cecum	ND	67	146	110	BQL	249	BQL	ND
Cecum Contents	ND	1340	1270	2310	445	720	318	513
Dose Site*	1,030,000	1,420,000	1,100,000	1,900,000	1,220,000	1,420,000	1,420,000	1,240,000
Kidney	162	120	78	BLQ	BLQ	BLQ	BLQ	ND
Large Intestine Contents	ND	1,400	1,590	2,390	493	1,020	478	430
Large Intestine	ND	BLQ	130	167	BLQ	BLQ	BLQ	ND
Liver	231	151	169	108	BLQ	109	BLQ	BLQ
Preputial Gland	ND	BLQ	124	88	465	132	116	ND
Renal Cortex	188	108	65	BLQ	BLQ	ND	ND	ND
Renal Medulla	143	130	90	BLQ	BLQ	ND	ND	ND
Small Intestine Contents	4,920	715	754	3,390	534	371	401	947
Small Intestine	200	63	BLQ	203	BLQ	BLQ	BLQ	ND
Stomach Contents	280	183	BLQ	BLQ	BLQ	91	ND	ND
Urine	818	748	366	95	486	142	137	ND
* Samples were above the upper limit of detection (592,000). Each value is from a single individual.								

In both strains, radioactivity was below the limit of detection in the adrenals, blood, bone, bone marrow, cerebellum, cerebrum, diaphragm, epididymis, esophageal contents, esophagus, exorbital lacrimal gland, eye, fat (abdominal & brown), Harderian gland, intra-orbital lacrimal gland, lung, medulla, muscle myocardium, nasal turbinate, olfactory lobe, pancreas, pituitary gland, prostate, salivary gland, seminal vesicle, skin, spinal cord, spleen, stomach, stomach contents, testis, thymus, thyroid, urinary bladder uveal tract.

At the injection site, radioactivity remained elevated but declined after 72 hrs when it started to become elevated in the surrounding tissue in the thigh.

Average Concentration of Radioactivity (^{14}C-TU ng eq/g) Detected by LCS in Sprague Dawley Rats (N=2)						
	1 hr	24 hrs	72 hrs	1 wks	4 wks	8 wks
Injection Site	548,000	629,000	464,000	473,000	215,000	190,000
Dose Site (Thigh)	3,130	449	14,300	12,400	5,330	43
Residual Carcass	115	141	823	190	82	58

2.6.4.5 Metabolism

Metabolism was not directly investigated by the Sponsor except for analysis of TU, DHT-TU, T, DHT and E_2 following 14 weeks of intramuscular dosing in rats (see section 2.6.6.3). However a published report was provided by the Sponsor describing the metabolic profile of TU in castrated rats which were orally dosed with radiolabeled TU (1). In this study, several metabolites of TU were identified in plasma including testosterone, DHT, DHT-TU, 3β-diol, 3α-diol, epiandrosterone, androsterone, 4-androstenedione, and 5α-androstenedione (Table below). TU and DHT-TU were found in circulation demonstrating that TU can be metabolized without the release of the undecanoate side chain. This was not corroborated by the Sponsor in their three

month intramuscular bridging study since the levels of DHT-TU were too low for quantification (section 2.6.6.3). In the published report, plasma levels of undecanoate-bound androgens dominated over undecanoate free metabolites by a factor of two while in tissues (prostate, seminal vesicle, bulbocavernosus muscle, and skeletal muscle) metabolites free of the undecanoate side chain (testosterone, DHT and others) dominated over undecanoate bound androgens by a factor of 3 to 5 (see Table).

Metabolite Profile of TU in Rats Dosed Orally with ^{3}H-TU 2.5 hrs After Oral Dose (Percent of Total for Each Organ) Non-Sponsor Study

	Plasma	Prostate	Seminal Ves.	Bulbocav.	Skeletal Mus.
TU	30	12	8	10	13
5α-DHT-TU	36	9	10	11	13
Total Undecaonoates	66	21	18	21	26
T	14	9	11	28	25
5α-DHT	4	47	34	9	-
3α-diol	5	4	8	17	22
3β-diol	3	-	4	4	4
Epiandrosterone	2	-	4	5	-
Androsterone	-	4	12	-	-
4-androstenedione	2	5	-	4	4
5α-androstenedione	-	5	-	-	-
Total Metabolites free of Undecanoate	34	79	82	79	74

Table adapted from (1). Tissues- plasma, prostate, seminal vesicle, bulbocavernosus muscle, skeletal muscle.

Testosterone was the most abundant non-esterified androgen in the plasma, bulbocavernosus muscle, and skeletal muscle accounting for 14-28% of the radioactivity. Also in the published report, *in vitro* experiments with plasma, prostate and muscle homogenates suggested the plasma alone does not appreciably metabolize TU to DHT-TU or undecanoate-free androgens, while the prostate has considerable capacity to metabolize TU to testosterone. This implies that hydrolysis of TU occurs primarily in target tissues.

2.6.4.6 Excretion

In the Sponsor's ADE study, a total of 88.7% of the administered radioactivity was accounted for. Nearly 32% of the radioactive dose was eliminated in urine or feces within eight weeks of dosing. Radioactivity was primarily excreted in the feces which accounted for 24.6% of the initial dose. Excretion in feces was highest during the first week following dosing (6.7% of the administered dose) and declined thereafter. Urinary excretion accounted for 7.3% of the initial radioactive dose. Excretion in urine also was highest within 24 hrs of dosing and declined thereafter. During subsequent weeks, excretion in urine and feces declined by 33% and 23%, respectively. Expired air and cage recovery only accounted for < 0.2% of the initial dose. The dose site retained nearly 50% of the initial dose eight weeks after administration.

Percentage of Radioactive Dose					
	Urine	**Feces**	**Dose Site**	**Residual Carcass**	**Air, Cage, Cage Wash**
Week 1 total	1.87	6.73			
0-24 hrs	0.42	0.86			
24-48 hrs	0.27	0.93			
48-72 hrs	0.26	1.08			
72-96 hrs	0.25	1.03			
96-120 hrs	0.24	0.98			
120-144 hrs	0.23	0.97			
144-168 hrs	0.20	0.87			
Week 2	1.14	4.64			
Week 3	0.87	3.15			
Week 4	0.77	2.44			
Week 5	0.61	2.21			
Week 6	0.67	2.12			
Week 7	0.64	1.78			
Week 8	0.62	1.52	49.1	7.5	0.19
Total	7.31	24.6	49.1	7.5	0.19

2.6.4.7 Pharmacokinetic drug interactions: no data submitted

2.6.4.8 Other Pharmacokinetic Studies: none submitted

2.6.4.9 Discussion and Conclusions

In rats radioactivity associated with intramuscular ^{14}C-TU injection was detected in the blood at low levels within one hour of dosing. However the distribution was essentially limited to the liver, kidney and excretory tissues. Melanin had no effect on distribution or elimination patterns. The presence of radioactivity was not detected in most organs even though the amount of radioactivity injected in each rat was relatively high. Nearly half of the administered dose remained near the dose site eight weeks after the initial injection. Most of the eliminated radioactivity was excreted in feces and to a lesser extent in urine.

2.6.4.10 Tables and figures to include comparative TK summary

Toxicokinetics of TU, T, DHT-TU and E_2 were assessed in rats injected intramuscularly with TU every two weeks for 14 weeks (Section 2.6.6.3. (b) (4) Study 1630-05560). Summary tables from that study are provided below. For comparison purposes, summary pharmacokinetic data for TU (IP157-001A) and T (IP157-001C) in hypogonadal men are also presented.

Human:

Two Phase 3 clinical studies with different dosing protocols were used to compare nonclinical TU and T exposure to the exposure in man [(multiples of human exposures (MOE)] (IP157-001 Parts A and C respectively).

Human Testosterone Assessment - (157-001 Part C):
In part C, hypogonadal men were given a 750 mg intramuscular dose of TU at baseline, then again after four weeks and every ten weeks thereafter. This was referred to as the TU 750 Loading protocol and is the schedule that is being pursued for marketing. This dosing protocol was chosen for marketing because C_{max} levels for T remained below 18 ng/ml (1800 ng/dL) unlike Part A (see TU assessment below) and according to Doanh Tran (OTS Clinical Pharmacologist) the pharmacokinetics in Part C represented steady state after the third dose (days 98-168).

Pharmacokinetics of Testosterone in Human Men Following the Third Dose
TU 750 LOADING (IP157-001Part C)

TU Dose (mg)	N	Mean C_{max} (ng/ml)	Max C_{max} (ng/ml)	Mean $AUC_{(98-168)}$ (ng•day/ml)	Max $AUC_{(98-168)}$ (ng•day/ml)	Mean T_{max} (days)
T	n = 117	8.9 ± 3.5	17.6	346.5 ± 99.0	700.2	10
T† Low BMI	n = 10	12.3 ± 3.4	17.6	405.0 ± 70.6	522.6	9

†Exposure to T is shown for patients with BMI < 26 since they had the greatest exposure as a group. Mean C_{max} for testosterone in the low BMI patients was 1234 ± 340 ng/dL = 12.3 ± 3.4 ng/ml and mean AUC was 40,519 ± 7,060 ng•day/dL = 405 ± 70.6 ng•day/ml. Data derived from Table 9.2.1.8.3 (157-001 Part C).

Human TU Assessment - (157-001 Part A):
Pharmacokinetics of TU were not assessed in the TU 750 Loading protocol above so comparisons to nonclinical exposure will be derived from 157-001 Part A. Since the levels of T were fairly similar between these clinical dosing protocols, the pharmacokinetics of TU would be expected to be similar (see table above and below). In Part A of this study hypogonadal men were dosed intramuscularly with 750 or 1000 mg TU every 12 weeks for up to 48 weeks. Human C_{max} and AUC values for TU were calculated by Doanh Tran from data in the fourth dosing interval (Days 252-336). TU values were not detectable or below the limit of detection in 14 of the 101 men dosed with 750 mg TU and 14 of the 97 subjects dosed with 1000 mg TU. The time to maximal TU plasma levels was fast, being only four days in comparison to 10-11 days for testosterone in this study. Steady state for testosterone had not been reached after the fourth dose since C_{trough} concentrations of testosterone continued to rise with repeated dosing. Steady state was estimated to be 15% higher than the exposures found in the fourth interval. C_{trough} for testosterone appeared to stabilize in men dosed with 750 mg TU after the sixth dose but not in the men dosed with 1000 mg at the same time point.

Pharmacokinetics of TU and Testosterone in Human Men During the 4th Dose Interval
(IP157-001 Part A)

	TU Dose (mg)	Mean C_{max} (ng/ml)	Max C_{max} (ng/ml)	Mean $AUC_{(252-336)}$ (ng•day/ml)	Max $AUC_{(252-336)}$ (ng•day/ml)	T_{max} (days)
TU*	750 (n = 101)	1.25 ± 1.36	12.2	13.2 ± 16.4	74.1	4
	1000 (n = 97)	1.35 ± 1.13	7.4	14.8 ± 15.0	68.8	4
T	750 (n = 102)	8.0 ± 3.2	17.9	372 ±114	730	11
	1000 (n = 97)	10.0 ± 4.0	19.4	462 ± 142	879	10
T† Low BMI	750 (n = 12)					
	1000 (n = 12)	12.7 ± 3.8		523 ± 111		

*TU levels below the limit of detection (0.5 ng/ml) were set to 0. †Exposure to T is shown for patients with BMI < 26 since they had the greatest exposure as a group. C_{max} for testosterone in the low BMI patients was 1272 ± 384 ng/dL = 12.7 ± 3.8 ng/ml and AUC was 52,332 ± 11,088 ng•day/dL = 523 ± 111 ng•day/ml. Values in this table were provided by Doanh Tran, OPS Clinical Pharmacologist.

Nonclinical- Multiples of Human Exposure (MOE):
Multiples of human exposures were calculated by comparing the mean Cmax and AUC values between man and rat and more conservatively by comparing maximal Cmax or maximal AUC in humans to the mean Cmax or mean AUC in nonclinical studies.

Since TU was not assessed in studies utilizing a loading dose and a 10-month dosing schedule, the more conservative use of the clinical PK data obtained from the 1000 mg TU exposure group in Part A was used to estimate the MOEs. Doses of 1000 mg every 12 weeks had maximal and mean Testosterone Cmax and AUC levels slightly exceeding that found in men dosed with the TU 750 mg Loading protocol. Therefore the MOEs for the TU 750 Loading protocol may be under estimated. Multiples of TU exposure based upon the human mean Cmax and mean AUC were 2-20 fold in rats dosed IP with TU every two weeks at 50-400 mg/kg. However the MOEs were reduced to 0.4 to 4 when nonclinical mean AUC and Cmax values were divided by the human maximal AUC and Cmax levels.

Toxicokinetics of TU in Rats Injected Intramuscularly Every Two Weeks

Time	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (day)	AUC_{tau} (ng•day/ml)	Multiple of Human Exposure			
					Mean Rat/ Mean Human		†Mean Rat/ Max Human	
					C_{max}	AUC	C_{max}	AUC
Day 1	50	0.7	8	6.1	0.5	0.4	0.1	0.9
(After 1st Dose)	200	10.5	8	70.0	7.8	4.7	1.4	1.0
	800	32.2	8	222.0	23.9	15.0	4.4	3.2
Day 29	50	1.1	4	10.8	0.8	0.7	0.2	0.2
(After 3rd Dose)	200	7.3	8	75.0	5.4	5.1	1.0	1.1
	800	26.2	4	296.0	19.4	20.0	3.5	4.3
Day 85	50	3.0	4	29.4	2.2	2.0	0.4	0.4
(After 7th Dose)	200	14.6	4	140.0	10.8	9.5	2.0	2.0
	400	29.1	4	301.0	21.6	20.3	3.9	4.4

Multiples of human exposures (MOE) to TU were derived form mean or maximal human Cmax or AUC in men dosed with 1000 mg TU (IP157-001A). MOE = mean Cmax in rats/mean Human Cmax or mean rat AUC/mean Human AUC. †Conservative MOE = mean Cmax in rats / max Cmax in men or mean AUC in rats/ max AUC (IP157-001A). Mean Cmax for TU in men was 1.35 ± 1.1 ng/ml and the maximal was 7.4 ng/ml. Mean AUC in men was 14.8 ± 15.0 ng•days/ml while the maximal AUC was 68.8 ng/ml (IP157-001A). AUC_{tau} (AUC over the dosing interval, 14 days). Table modified from Sponsor's data ((b) (4) Study 1630-05560).

Testosterone Toxicokinetics in TU and TC Dosed Rats									
Dose (mg/kg)	Pre-dose (ng/ml)	C_{max} (ng/ml)	Fold Above Background	T_{max} (day)	AUC_{tau} (ng•day/ml)	Multiple of Human Exposure			
						Mean Rat/ Mean Human		†Mean Rat/ Max Human	
Day 1 (1st Dose)						C_{max}	AUC	C_{max}	AUC
200 TC	7.6	22.2	2.9	4	204.0	1.8	0.5	1.3	0.3
50 TU	3.0	4.4	1.5	15	46.9	0.4	0.1	0.3	0.1
200 TU	3.6	17.6	4.9	8	138.0	1.4	0.3	1.4	0.2
800 TU	2.5	44.7	17.9	12	382.0	3.6	0.9	2.5	0.5
Day 29 (3rd Dose)									
200 TC		87.3	11.5	4	822	7.1	2.0	5.0	1.2
50 TU		4.7	1.6	8	58.2	0.4	0.1	0.3	0.1
200 TU		20.6	5.7	4	258.0	1.7	0.6	1.2	0.4
800 TU		59.9	24.3	8	763.0	4.9	1.9	3.4	1.1
Day 85 (7th Dose)									
200 TC		120.0	15.9	4	1130.0	9.8	2.8	6.8	1.6
50 TU		9.3	3.1	4	119.0	0.8	0.3	0.5	0.2
200 TU		38.8	10.7	4	477.0	3.2	1.2	2.2	0.7
400 TU		74.1	30.0	8	903.0	6.0	2.2	4.2	1.3

The average pre-dose testosterone concentration was 4.2 ng/ml ± 2.7. Multiples of exposure (MOE) were derived from patients who were dosed with 750 mg TU at weeks 0, 4, 14, 24 (IP157-001 C). Mean Cmax (12.3 ng/mL) and Mean AUC (405 ng•day/ml) in low MBI men. Max C_{max} for these patients was 1759 ng/dL = 17.6 ng/ml and Max AUC was 70,017 ng•days/dL = 700 ng•days/ml. †Conservative multiple of human exposure = mean C_{max} in rats / Max C_{max} in men or mean AUC in rats/Max AUC. Table modified from Sponsor's data ((b) (4) Study 1630-05560).

Testosterone Toxicokinetics in TU and TC Dosed Rats (Corrected - Pre dose levels subtracted)				
Time	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (day)	AUC_{tau} (ng•day/ml)
Day 1 (After 1st Dose)	200 TC	14.6	4	98.8
	50 TU	1.4	15	8.3
	200 TU	14.0	8	87.5
	800 TU	42.2	12	347.0
Day 29 (After 3rd Dose)	200 TC	79.7	4	716
	50 TU	1.7	8	16.3
	200 TU	17.7	4	207
	800 TU	57.4	8	728
Day 85 (After 7th Dose)	200 TC	112	4	1025
	50 TU	6.3	4	76.9
	200 TU	35.1	4	426
	400 TU	71.6	8	868

Table modified from Sponsor's data ((b) (4) Study 1630-05560).

Dihydrotestosterone Toxicokinetics in TU and TC Dosed Rats				
Time	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (day)	AUC_{tau} (ng•day/ml)
Day 1 (After 1st Dose)	200 TC	0.8	4	8.1
	50 TU	0.1	4	0.3
	200 TU	0.7	8	4.9
	800 TU	1.8	12	15.4
Day 29 (After 3rd Dose)	200 TC	2.4	4	22.8
	50 TU	--	--	--
	200 TU	0.8	4	8.8
	800 TU	1.9	8	23.9
Day 85 (After 7th Dose)	200 TC	3.4	4	30.8
	50 TU	0.2	4	2.0
	200 TU	1.1	4	13.9
	400 TU	1.9	8	25.4
Table modified from Sponsor's data ((b) (4) Study 1630-05560).				

Estradiol Toxicokinetics in TU and TC Dosed Rats				
Time	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (day)	AUC_{tau} (ng•day/ml)
Day 1 (After 1st Dose)	200 TC	11.2	8	84.1
	50 TU	8.6	15	66.5
	200 TU	10.3	0	69.9
	800 TU	11.1	12	91.8
Day 29 (After 3rd Dose)	200 TC	9.0	8	89.8
	50 TU	3.7	12	40.3
	200 TU	5.8	12	75.4
	800 TU	8.9	4	104.0
Day 85 (After 7th Dose)	200 TC	15.1	12	109.0
	50 TU	1.6	8	12.5
	200 TU	5.7	12	57.3
	400 TU	5.8	8	66.3
Table modified from Sponsor's data ((b) (4) Study 1630-05560).				

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

None Submitted

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Repeat-dose toxicology:
A 14-week intramuscular repeat dose study was conducted in rats to evaluate the comparability of TU in terms of safety with testosterone cypionate (Depo® –Testosterone, TC), another approved testosterone replacement product. With a few possible exceptions noted below, rats responded similarly to TU and TC and the results were consistent with testosterone exposure.

Reduced testis weights, adverse renal and bladder histopathology and skin inflammation were observed in the TU groups but not in the TC group. Whether these finding are clear and unique affects of TU exposure is questionable. Brain normalized testis weights were reduced (8-26%) in the TU but not the TC dosed animals. Testicular weights were still affected after drug withdrawal in the TU groups. Although this is a known physiological response to testosterone, the lack of a dose response, adverse pathology and histopathology, and difference in testosterone pharmacokinetics reduces the confidence of the validity of this finding. Very low incidence of reversible bladder and renal transitional cell hyperplasia (14%) and degeneration and necrosis of the renal proximal tubule and dilation of the renal pelvis (7%) were observed only in the high dose TU group. Since these findings were only observed in one or two animals of the high dose TU group, it is difficult to call this a clear drug-related affect. Although injection site inflammation was observed in both the TC and TU groups, inflammation of the skin (43%) and sciatic nerve cysts (21%) near the injection site were only observed in the high-dose TU group.

Differences in response to TU and TC include differences in time to recovery from affects. Dose-related moderate thymic atrophy was observed in both the TU and TC groups, however the TC group recovered from this affect 26 weeks after the last dose but the TU group did not.

Local Tolerance: A local tolerance study in pigs was conducted comparing intramuscular injections of TU and testosterone enanthate (TE). Drug related affects were not distinguished from those in the vehicle groups. However large injection volumes (3-4 ml) caused local necrosis, fibrosis, hemorrhaging and inflammation which tended to heal by 7-42 days after dosing.

Genetic toxicology: Testosterone undecanoate was negative in a battery of *in vitro* and *in vivo* genotoxicity assays assessing mutagenicity and clastogenicity.

Carcinogenicity: Since TU is considered a pro-drug intended for delivery of testosterone, the carcinogenicity potential of TU is considered to be the same as testosterone.

Reproductive toxicology: Since TU is considered a pro-drug intended for delivery of testosterone, reproductive and developmental affects of TU exposure will be considered to be the same as testosterone.

Special toxicology: None

2.6.6.2 Single-dose toxicity

None

2.6.6.3 Repeat-dose toxicity

Study title: Testosterone Undecanoate: Toxicity Study in Male Sprague-Dawley Rats Following Seven Bi-weekly Intramuscular Doses with Six-month Recovery Period.

Key study findings: With a few exceptions noted below, rats responded similarly to TU and TC and the results were consistent with testosterone exposure. Intramuscular TU (50, 200, 400/800 mg/kg) and TC (200 mg/kg) dosing elevated T, DHT and estradiol levels. TC exposure resulted in greater Cmax spikes for testosterone and what appeared to be quicker elimination than TU. TU doses of 50, 200 and 400/800 mg/kg resulted in exposures 2, 10 and 20 times the maximal TU exposure in humans based upon mean AUC or Cmax. This decreased to 0.4 to 4 when based upon the maximal AUC or C_{max} in men. Testosterone concentrations were elevated by TU 3, 11 and 30 fold above the pre-dose levels based upon C_{max}. When assessed based upon mean C_{max}, dose multiples achieved in rats for total testosterone were 1, 3 and 6 times the mean C_{max} in men or 1, 2 and 4 time based upon the maximal C_{max} in men. TC treated rats achieved testosterone levels similar to the mid and high-dose TU group.

Main Affects of TU in Animals Surviving to Scheduled Death:
Animals generally responded to TU and testosterone cypionate (TC) in a similar manner. Adverse pathology/histology was generally not observed. Effects observed in both TU and TC treated animals included the following:

- Decreases in body weight gain (13-21%) and food consumption (4-9%). Affects tended to resolve after drug withdrawal but was not complete after the recovery period.
- Altered organ weights – Dose related but animals partially recovered after withdrawal.
  - Decreased – liver (12-19%) and thymus (39-50%)
  - Increased – kidney (11-43%), ventral prostate (42-69%) seminal vesicle (42-84%) and bulbocavernosus muscle (24-36%).
- Histopathology –
  - Thymic atrophy – moderate and diffuse (dose related).
    The TC dose group recovered from this but the TU did not.
  - Injection site inflammation and cysts (dose related and non-recoverable).
- White cell counts- Increased neutrophils (4-96%) and decreased lymphocytes (7-43%).

Adverse affects observed in the TU animals but not in the TC group:
- Non-dose responsive and non-recoverable decrease in testicular weight (8-26%).
- Very low incidence of reversible adverse renal and bladder histopathology (transitional cell hyperplasia (14% of HD group), degeneration and necrosis of the renal proximal tubule and dilation of the renal pelvis (7% HD group).
- Inflammation of the skin (43%) and sciatic nerve cysts (21%) near the injection site were only observed in the high dose TU group but not in the vehicle or TC groups.

NOEL < 50 mg/kg (approximately 2 times the maximal human dose of 1000 mg) based upon reduced feed intake, decreased weight gain, exophthalmus, lacrimation, aggressive behavior, slight alterations in hematology, altered organ weights, thymic atrophy and inflammation at 50 mg/kg. Similar findings were observed in the TC group. However with the exception of the injection site cysts and inflammatory response these findings were generally mild and could be considered affects of exaggerated pharmacology. Since overt toxicity was not observed below 200 mg/kg a **NOAEL could be set at 50 mg/kg.**

Study no.: (b) (4) study number 1630-05560
Volume #, and page #: CDSESUB1\NONECTD\N22219|N_000|2007-08-24.
Sponsor's NDA Electronic Submission Module 4.2.3.2.
42-stud-rep\423-tox\4232-repeat-dose-tox\1630-05560.pdf
Conducting laboratory and location: (b) (4)

Date of study initiation: April 5, 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Testosterone undecanoate Lot 43008, 99.95% pure.
Depo® Testosterone cypionate Lot 22 MJR, of unknown purity was supplied by (b) (4)

Methods

Doses: 0, 50, 200, 800/400 mg/kg TU biweekly (days 1, 15, 29, 43, 57, 71 and 85). The high dose was reduced from 800 to 400 mg/kg on day 43. As a positive control testosterone cypionate was dosed at 200 mg/kg biweekly.

Species/strain: Sprague-Dawley rats (Crl: CD® [SD] IGS/rats)

Number/sex/group or time point (main study): 20 males/group

Route, formulation, volume, and infusion rate: The first three doses were administered via intramuscular injections into the right hind thigh, in castor oil/benzoate vehicle. Due to injection volume-related effects, dosing was evenly divided between the right and left rear thighs starting on day 43 in the vehicle and high dose TU groups. Dose volumes varied with dose. Vehicle and high dose groups received 3.2 ml/kg for the first three doses then 1.6 ml/kg thereafter. The 50 and 200 mg/kg groups were dosed with 0.2 and 0.8 ml/kg. The 200 mg/kg TC group received 1.0 ml/kg (see table in results).

Satellite groups used for toxicokinetics or recovery: Toxicokinetics (TU, DHT-TU, T, DHT and E_2) was evaluated in all TU and TC groups with 16 rats per dose group. Blood for any one time point was collected from only 3 or 4 animals. Five animals were scheduled to study recovery in the vehicle, TC and high dose TU groups only. The recovery period was 24 weeks after the last dosing period (26 weeks after the last dose).

Age: 8 to 9 weeks at first dose.

Weight: 232.8 to 354.2 g.

Unique study design or methodology (if any): High mortality was observed in the vehicle and high dose TU groups after the first and third injections. Dose volumes (3.2 ml/kg to

1.6 ml/kg) and levels (800 mg/kg to 400 mg/kg) were reduced in the vehicle and high dose groups for the last four injections starting on day 43 (see mortality results).

Study Design and Alterations Due to High Initial Mortality

Treatment	Dose Level (mg/kg)	Dose Volume (ml/kg)	No. Animals Used		
			Main Phase	Toxicokinetics	Recovery
Vehicle	0	3.2/1.6*	8	-	4
TC	200	1.0	20	16	5
TU	50	0.2	20	16	-
TU	200	0.8	19	15	-
TU	800/400†	3.2/1.6†	14	13	4

Vehicle- castor oil and benzyl benzoate. TC- Testosterone Cypionate (Depo®- Testosterone). TU- testosterone undecanoate.
* Dose volume was reduced to 1.6 ml/kg on study day 43 (4th of 7).
† Dose was reduced to 400 mg/kg and volume to 1.6 ml/kg on study day 43.
Table adapted from the Sponsor.

Observations and times:

Mortality: More than twice daily.

Clinical signs: Prior to first dose then weekly thereafter and at termination.

Body weights: Prior to first dose then weekly thereafter and at termination.

Food consumption: Weekly

Ophthalmoscopy: Prior to necropsy.

EKG: Not examined.

Hematology: Study days 28 and 84. Complete blood count included; WBC, RBC, HGB, HCT, MCV, MCH, MCHC, MPV, PLT, red cell distribution width. Leukocyte differential count included; absolute neutrophils, lymphocytes, monocytes, eosinophils and basophils. Absolute reticulocyte count was assessed. Coagulation was assessed with prothrombin time, activated prothrombin time and fibrinogen.

Clinical chemistry: Prior to first dose then weekly thereafter and at termination. Study days 2, 28, 56 and 84. Glucose, BUN, creatinine, Na, K, Cl, Ca, Phosphorous, CO_2, cholesterol, triglycerides, total protein, albumin, globin, A/G, AST, ALT, ALKP, total bilirubin and creatine kinase.

Hormones: Study day 99. These samples were not analyzed and were discarded.

Urinalysis: Study days 15, 29 and 78 prior to dosing.

Gross pathology: All animals upon euthanization. Dead/moribund animals were evaluated but no organ weights were taken. External body, injection site (skin and underline biceps muscle), all orifices, the cranial, thoracic and abdominal cavities and their contents.

Organ weights: Whole organ weights, body weight and brain normalized weights were also provided for: adrenal gland, left bicep femoris, brain, dorsal lateral prostate, epididymides, heart, kidneys, bulbocavernosus muscle, liver, lungs, pituitary gland, seminal vesicles, spleen, testes, thymus, ventral prostate,

Histopathology: Histopathology was conducted on all animals at termination or on animals that died or were euthanized. Tissues and organs examined histologically include the following: adrenal gland, aorta, bone with bone marrow (femur and sternum), brain, bulbocavernosus muscle, cecum, colon, duodenum, epididymis, esophagus, eye, gross lesions (all groups),

Harderian gland, heart, ileum, lymph nodes (iliac, mandibular and mesenteric), injection sites, jejunum, kidney, lacrimal gland, liver, lung, mammary gland, mandibular salivary gland, optic nerve, pancreas, parathyroid gland, pituitary, prostate (dorsal, lateral and ventral), rectum, sciatic nerve (left), seminal vesicle, skeletal muscle (left and right biceps femoris), skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach, testis, thymus, thyroid gland, tongue, trachea, urinary bladder.

Adequate Battery: yes (X), no ( )—explain

Peer review: yes ( ), no (X). There was no mention of peer review but a board certified veterinary pathologist conducted the evaluation.

Toxicokinetics: Blood was collected in fasted animals after the 1st, 3rd and 7th dose. Sampling times were; prior to 1st dose, 4, 8, 12, 15 (prior to dosing), 29 (prior to dosing), 32, 36, 40, 43 (prior to dosing), 85 (prior to dosing), 88, 92, 96, 99, 113, 127 and 141. TU, DHT-TU, T, DHT and E_2 were assessed.

Results

Mortality:

High mortality/morbidity was observed in the vehicle (13/25) and high-dose TU (10/41) groups following the first and third dose (day 1 and 29). Two animals in the mid-dose TU group died, one on day 62 with chronic prostatic infection and another on day 99 of indeterminate cause.

Study Design and Alterations Due to High Initial Mortality

Treatment	Dose (mg/kg)	Dose Volume (ml/kg)	No. / Group	No. Dead	Sacrificed Moribund	No. Animals Surviving to Scheduled Termination		
						Main Phase	Toxico-kinetics	Recovery Phase
Vehicle	0*	3.2/1.6*	25	7	6	8	-	4
TC	200	1.0	41	0	0	20	16	5
TU	50	0.2	36	0	0	20	16	-
TU	200	0.8	36	2	0	19	15	-
TU	800/400†	3.2/1.6†	41	4	6	14	13	4

Vehicle- castor oil and benzyl benzoate. TC- Testosterone Cypionate (Depo®- Testosterone). TU- testosterone undecanoate.
* Dose volume was reduced to 1.6 ml/kg on study day 43 (4th of 7).
† Dose was reduced to 400 mg/kg and volume to 1.6 ml/kg on study day 43.
Table adapted from the Sponsor.

Morbidity/Mortality due to Large Injection Volume:

All of the mortality in the vehicle group and nine of the ten high dose deaths occurred within four days of the first or third dosing. This was attributed to the large dosing volumes (3.2 ml/kg) in these groups which was reduced by half (1.6 ml/kg) for the fourth and subsequent doses. Only one death of indeterminate cause occurred after this in the high dose group on day 147 (almost 7 weeks after the last dose). Due to the high mortality rate, only 8 animals in the vehicle group (4 for recovery) and 14 in the high dose TU group (4 for recovery) survived to scheduled termination. Dosing volume for TC was 1.0 ml/kg, and no deaths occurred in this group.

The Sponsor stated that, "The cause of death in these animals was unclear; however, clinical chemistry profiles of several moribund sacrificed animals suggested a possible renal dysfunction

and minimal hepatotoxicity". The Sponsor attributed the deaths to unintended systemic exposure to the vehicle and not to TU.

Prior to death the animals displayed moderate to severe tremors, languid appearance, prostration, eye lacrimation and rough hair coat. Labored breathing was not mentioned. Some of the moribund animals had elevated creatine, K, P, total protein, albumin, AST and ALT. The injection sites were inflamed (4/13 control; 8/9 high dose TU) and populated with multifocal cysts (4/13 control; 9/9 high dose TU) (Table below). Toxicity to the vehicle following unintended systemic exposure was suspected by the Sponsor to cause lymphoid necrosis in the thymus (11/13 control; 7/10 HD TU), spleen (5/13 Control; 8/10 HD TU), lymph nodes (4/13 control; 9/10 HD TU), lung (2/13 Control) and bone (2/13 Control) in addition to mild to moderate thymic atrophy (4/10 HD TU). Additionally degeneration or necrosis of the renal tubules (8/13 Control; 7/10 HD TU), myocardium (4/13 control and 7/10 HD TU), GI (2/13 Control) and liver (2/13 Control) was also observed.

Incidence of Major Adverse Histopathology in Unscheduled Dead or Moribund

Organ	Adverse Affect	Vehicle	TU	
		0 (mg/kg) N=13	200 (mg/kg) N=2	400/800 (mg/kg) N=10
Adrenal	Congestion	8	1	4
Bone Marrow	Multifocal Necrosis	2	-	-
Duodenum	Degeneration Necrosis – villi	2	-	-
Heart	Myocardial Degeneration	4	-	7
	Mononuclear cell infiltration	7	-	8
Injection Site	Inflammation	3	-	9
	Cysts – multifocal epimysial	2	-	10
Kidney	Congestion	1	-	2
	Degeneration/Necrosis – proximal tubular	8	-	7
	Nephropathy- multifocal	1	-	2
	Basophilia – multifocal tubular cell	8	-	9
Liver	Necrosis- focal coagulative	2	-	-
	Congestion Mononuclear Cell Infiltration	-	-	1
Lung	Necrosis/Inflammation	2	-	-
	Hemorrhage- acute multifocal	-	-	1
Lymph Nodes (several locations)	Necrosis- diffuse and multifocal	4	1	9
	Hyperplasia - diffuse	6	-	5
	Depletion – diffuse	0	-	1
	Inflammation	2	-	1
* Skeletal Muscle	Inflammation – chronic active	4	1	5
	Cyst – multifocal and focal	4	1	4
	Degeneration / Necrosis - multifocal	1	-	-
Skin	Inflammation and cysts - multifocal	2	-	1
Spleen	Necrosis – lymphoid diffuse	5	-	8
	Depletion – lymphoid parafollicular diffuse	7	1	4
Thymus	Necrosis- lymphoid diffuse	11	0	7
	Hemorrhage- acute	3	-	-
	Atrophy- diffuse	-	1	4

*Data was combined in some categories and may be slightly over represented.

After the first dosing, the vehicle was studied to determine if the deaths were related to microbiologic or endotoxin contamination. There were no identified quality defects in the vehicle. A new batch of vehicle was sent for subsequent dosing. Meeting minutes between the Sponsor and vehicle supplier (b) (4) noted that the same batch of vehicle was used in clinical trials without reported ill affects. Additionally the supplier reported that the same volume of vehicle from a different Lot was used in previous toxicology studies without adverse responses.

Clinical signs:
A marginal but dose responsive increase in the number and frequency of exophthalmus was observed. Essentially all animals in every group displayed rough hair coat that did not revert to normal during the recovery period. The frequency of this was slightly dose related. Other signs observed at low frequency in drug exposed groups compared to controls include, abrasions, alopecia, discoloration, hyperactivity upon touch (aggressiveness), lacrimation and thin appearance.

Incidence of Significant Clinical Signs (Incidence / Number of Animals)

Dose (mg/kg)	0	TC 200	TU (mg/kg)		
			50	200	400 / 800
HED mg/kg (mg for a 60 kg human)	0	32	8	32	66 / 129
Multiple of Human Dose (AUC) †			2.0	9.5	20.3
Number of animals: Initial (survivors)	25 (12)	41 (41)	36 (36)	36 (34)	41 (31)
Abrasions		1/1	6/2		1/1
Alopecia			9/1		
Discoloration		14/3	6/1		5/1
Eye- Exophthalmus		8/2	1/1	1/1	7/3
Hyper to Touch		11/2	4/1	21/5	
Eye- Lacrimation	1/1	7/1	4/1		19/4
Rough Hair Coat	124/13	605/41	431/36	414/36	483/35
Thin				4/2	

† Multiple of human dose = AUC in rats / AUC hypogonadal men dosed with 1000 mg TU (IP157-001, Part A). A more conservative MOE would be 0.4, 2.0 and 4.4 based upon max human AUC. The rat TU levels were obtained after the 7th biweekly dosing.

Body weights:
Mean body weight gains were reduced in all treatment groups compared to the vehicle. Body weight gains were reduced 18%, 13%, 15% and 21% in the TC and low-, mid- and high-dose TU groups respectively. Reduced weight gains were statistically significant beginning on day 22 of dosing in the high dose TU group and the TC group. Weight gains in the low- and mid-dose TU groups were significantly lower beginning on days 36 and 50 respectively. The TC and high dose TU groups were studied for recovery but the weights were still less than vehicle 24 weeks after the last dose. The percentage of body weight gain during dosing was 70, 38, 54, 48 and 34% for the vehicle, TC and low- to high-dose TU groups respectively. Upon recovery the vehicle group gained 138% of their original body weight while the TC and high dose TU group only gained 86% and 76%.

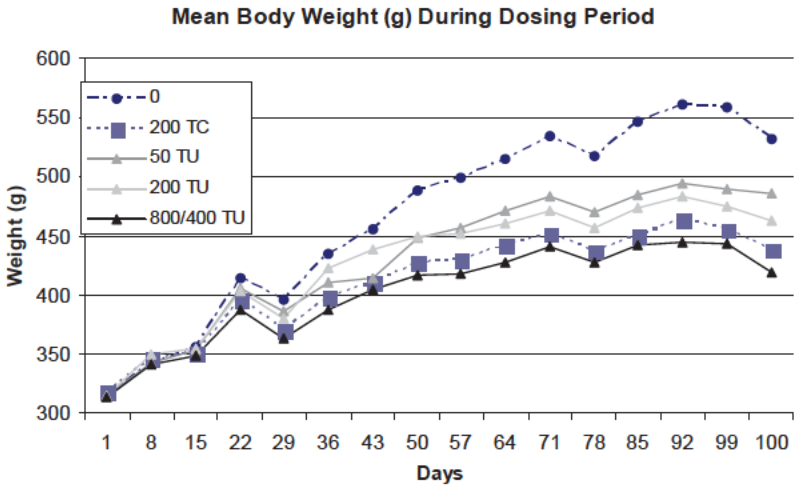


Figure Adapted From the Sponsor.

Food consumption:
Food consumption was slightly reduced relative to vehicle control in a dose responsive manner in all groups. This may account for the reduced body weight gain in the TU and TC treated rats. Food intake was reduced 5, 6, 5 and 9% relative to controls in the TC group and low-, mid- and high-dose groups TU groups respectively. The incidence of reduced food intake showed a similar response with 2, 4, 2 and 8 of the 14 feed periods below the vehicle intake in TC group and the low-, mid- and high-dose TU groups. The reduction in feed intake was not reversed during the 24-week recovery period in the high-dose TU group.

Cumulative Food Consumption Food Consumption (g)						
Dose (mg/kg)	**Dosing (Days 1-98)**			**Recovery (Days 106-266)**		
	Food Consumption (g)	**Reduction (%)**	**Incidence of Consumption Less Than Vehicle**	**Food Consumption (g)**	**Reduction (%)**	**Incidence of Consumption Below Vehicle**
0	2492	-	-	4796	-	-
TC 200	2376	4.7	**2/14***	4532	5.5	**7/23***
TU 50	2351	5.6	**4/14***	-	-	-
TU 200	2396	3.9	**2/14***	-	-	-
TU 800/400	2276	8.7	**8/14***	**4255***	11.2	**12/23***
* Statistically different from control $p < 0.05$						

Ophthalmoscopy:
Opacity of the cornea in the right eye was noted in one high-dose TU rat at test termination (day 98).

EKG: Not tested.

Hematology:

Hematological parameters were altered similarly by TU and TC. Hemoglobin was slightly elevated (4-10%) in the TC and all TU groups 28 and 84 days after dosing initiation. The hemoglobin levels were above historical controls on day 84 in both the TC and TU groups. Platelet levels were reduced on days 28 and 84 but were only statistically significant at day 28. Neutrophils were elevated in the TC group (13%) and the high-dose group (88%) on day 28. This became more apparent by day 84 since it was dose responsive in the TU groups and the levels were greater than the earlier time point. The elevation in neutrophils was slightly greater in the high-dose TU group than the TC group. Lymphocytes were slightly reduced during both time points in all TU and the TC group, increasing in severity at the later time point. Reticulocytes were reduced in all dose groups (9-30%) at both day 28 and 84. The affect on reticulocytes was less pronounced at day 84 and never exceeded the historical control levels.

Hematology Summary

Parameter	Day	TC 200 (mg/kg)	TU (mg/kg)		
			50	200	400/800
Hemoglobin	28	↑ 7.0*	↑ 6*	↑ 4*	↑ 8*
	84	↑ **10***	↑ **7***	↑ **6***	↑ **10***
Platelets	28	↓ 16%*	↓ 11%*	↓ 20%*	↓ 17%*
	84	↓ 11%	↓ 12%	↓ 16%	↓ 8%
Neutrophils	28	↑ 13%	↓ 18%*	↑ 4%	↑ **88%***
	84	↑ 61%*	↑ 33%*	↑ 64%*	↑ **96%***
Lymphocytes	28	↓ 29%*	↓ 7%	↓ 25%*	↓ 20%*
	84	↓ 41%*	↓ 18%*	↓ **43%***	↓ 32%*
Reticulocytes	28	↓ 28%*	↓19 %*	↓ 23%*	↓ 30%*
	84	↓ 10%	↓ 12%	↓ 10%	↓ 9%

* Significantly different from vehicle control $p < 0.05$. Bold text indicates that the value exceeded the historical control range. N= 9-10, however only 6 animals were used in the control group on day 84.

Clinical chemistry:
Significant changes in clinical chemistry compared to the time matched vehicle control were minimal. BUN was elevated 17% and 22 % in the high TU dose group on days 28 and 56 respectively. Glucose reduced 9% relative to the control at week 84 in the high-dose TU group. Similarly glucose was also reduced 13% in the TC group at week 84. Phosphorous was reduced 11-12% in the TC group during days 56 and 84 as well as being reduced 10% in the mid- and high-dose TU groups during days 84. Total bilirubin was reduced 30-42% in all TU groups during week 84 although only statistically significant at the high-dose. Total bilirubin was also reduced 31% at day 28 in the high-dose TU group.

Clinical Chemistry Summary

Parameter	Day	TC 200 (mg/kg)	TU (mg/kg)		
			50	200	400/800
A/G	56	↑ **11% ***			
BUN	28				↑ **17% ***
	56				↑ **22% ***
Glucose	84	↓ **13% ***			↓ **9% ***
Phosphorous	56	↓ **12% ***			
	84	↓ **11% ***		↓ **10% ***	↓ **10% ***
T. Bilirubin	28	↓ **24% ***			↓ **31% ***
	84	↓ 36%	↓ 30%	↓ 30%	↓ **42% ***

* Significantly different from vehicle control $p < 0.05$.

Urinalysis:
There was no significant affect of TC or TU on urinalysis endpoints.

Organ weights:
Since there was a negative affect of both TC and TU on body weight but no affect on brain weight, all data discussed below was normalized to brain weight.

As expected of an androgen both TC and all doses of TU lead to significant increases in the weight of the bulbocavernosus muscle, kidney, ventral prostate and seminal vesicle (Table below). Likewise the liver, testes and thymus were reduced in weight after 14 weeks of exposure to TU or TC. Adverse histopathology was observed the kidney, liver and thymus but not in the bulbocavernosus muscle, seminal vesicle, spleen, testes or ventral prostate (see table below).

Reduced testis weights were observed in a non-dose responsive manner in the TU groups however testis weights in the TC group were unaffected. This apparent testicular atrophy in the TU groups was not confirmed with gross pathology or histopathology but would not be surprising since this is a known affect of testosterone. The non-dose responsiveness of this affect suggests that it may not be TU related or the threshold for the affect to occur is below 50 mg/kg. However the lack of affect on testis weight in the TC group suggests that serum testosterone levels alone could not account for this affect since testosterone levels in the TC group were maintained above that found in the 50 mg/kg TU group which had the greatest reduction in testis weights (see pharmacokinetics below).

The organ weights affected during the dosing period in the high-dose TU and the TC groups were still affected but recovering 24 weeks after the last dosing period. Even though recovery was not complete, the organ weights were not statistically different from the control group after recovery with the exception that the testes were still statistically reduced in the high-dose TU group. Mid- and low-dose groups were not assessed after recovery.

Affect of TC and TU on Organ Weights Normalized to Brain Weight

	Percent Change in Organ Weight Relative to Control			
Organ	TC 200 mg/kg	TU 50 mg/kg	TU 200 mg/kg	TU 400/800 mg/kg
Bulbocavernosus	↑ **30***	↑ **27***	↑ **24***	↑ **36***
Kidney	↑ **36***	↑ 11	↑ **33***	↑ **43***
Liver	↓ **17***	↓ **12***	↓ **17***	↓ **19***
Seminal Vesicle	↑ **67***	↑ 42	↑ **71***	↑ **84***
Testes	No affect	↓ **26***	↓ **15***	↓ **8***
Thymus	↓ **50***	↓ **39***	↓ **43***	↓ **50***
Ventral Prostate	↑ **59***	↑ **48***	↑ **42***	↑ **69***

* Statistically significant from the vehicle control, $p < 0.05$. Table Adapted from Sponsor.

Gross pathology:

Due to the loss of moribund or dead animals there were an uneven number of animals at the termination of the study. There was a slight increase in the incidence of clear or yellow cysts at the injection site with the TU and TC groups. The investigator believed that the cysts at the injection site, biceps (right and left), and sciatic nerves were an attempt by the rats to wall off the vehicle at the injection site. Histological analysis supports this finding (see histology section). Affects on the male reproductive system were sparse including single incidences of small epididymides and testes (50 mg/kg TU), enlarged ventral prostate (200 mg/kg TC) and an irregular prostatic mass (400 mg/kg TU). The high TU group had very low incidences of what the pathologist called dilated and rough kidneys and a single occurrence of distended ureter and calculus urinary bladder.

Incidence of Significant Adverse Gross Pathology

		Vehicle		TC		TU (mg/kg)			
Organ	Adverse Affect	0 (mg/kg)		200 (mg/kg)		50	200	400/800	
		D99 N=8	R N=4	D99 N=20	R N=5	D99 N=20	D99 N=19	D99 N=14	R N=4
Epididymis	Discolored and small				4	1			
Inject. Site	Discolored yellow				2			2	
	Nodule black							1	
	Cyst clear or yellow	4	4	16	2	6	10	11	4
Sk. muscle R	Cyst, multiple	1		1			1	2	
Sk. muscle L	Cyst, multiple	1	2					2	2
Kidney	Dilation							1	
	Rough							3	
V. Prostate	Enlarged			1					
	Mass							1	
Sciatic nerve	Cyst, clear multiple							1	
Testes	Reduced size, left				4	1			
Ureter	Distention, bilateral							1	
U. Bladder	Calculus white multiple							1	

D99- Day 99 of dosing (week 14). R- Recovery group (24 weeks after last dose).

Histopathology:
Chronic inflammation and cysts were observed in a dose dependent manner at the injection site in the TU and TC groups but not in the control group. Since cysts and inflammation were observed at the injection site in the TC and TU group and not in the control group, the cause of this reaction may be testosterone itself and not just excessive injection volume. This was still apparent after the recovery period at the injection site. Subcutaneous cysts near the injection site were observed in the high-dose TU group (50%) and the TC group (5%). Additionally inflammation of the skin (43%) and sciatic nerve cysts (21%) near the injection site were only observed in the high-dose TU group but not in the vehicle or TC groups.

Moderate thymic atrophy was readily apparent and dose dependent with the incidence being 15%, 58% and 64% of the low-, mid- and high-dose TU animals and 75% of the TC animals. This was completely reversible in the TC group but not in the high-dose TU group.

In the urinary bladder, submucosal inflammation and transition cell hyperplasia were observed in 14% of the high-dose TU animals but not upon recovery. This was not observed in the TC or control groups.

Renal effects including degeneration and necrosis of the renal proximal tubule, transition cell hyperplasia, and dilation of the renal pelvis were observed in one or two high-dose TU animals. Low incidence of basophilic tubular cells and nephropathy was also observed in all TU and the TC dosed groups.

Diffuse vacuolation in the zona fasciculata of the adrenals was observed in 15/20 TC animals and 2/14 high-dose TU animals and none upon recovery.

Although affects were observed, dose dependence was not confirmed in heart, thyroid, liver, pituitary, dorsolateral prostate.

Incidence of Significant Adverse Histopathology									
Organ	Adverse Affect	Vehicle		TC		TU (mg/kg)			
		0 (mg/kg)		200 (mg/kg)		50	200	400/800	
		D99 N=8	R N=4	D99 N=20	R N=5	D99 N=20	D99 N=19	D99 N=14	R N=4
Adrenal	Diffuse vacuolation			15				2	
Epididymis	Sperm granuloma			1	5	1			
Eye	Extensive local inflammation							1	
	Phthisis bulbi			1					
Harderian	Mononuclear infiltration		1	11	4	15	10	5	3
	Basophilic acinar cell			5			1	1	
	Degeneration acinar cells			1		1	1		
Heart	Myocardial degeneration	3		1		1	2		2
	Mononuclear infiltration	5	2	9	1	5	8	4	2
Inject. Site	Chronic inflammation		2	17	5		6	14	4
	Epimysial cyst		3	17	5	4	12	14	4
Kidney	Degeneration/necrosis							1	
	Mineralization			1					
	Hyperplasia transition cell							2	
	Basophilia tubular cell			2		1	2	3	
	Nephropathy		4	5	3	3	2	5	3
	Dilation- pelvis							1	
Liver	Mononuclear infiltration	2	4	12	4	8	8	7	4
	Inflammation-subacute						1	2	1
	Necrosis- coagulative	1		2			1	1	1
	Hyperplasia		1	3					
Pituitary	Cyst						2		1
DL Prostate	Mononuclear infiltration			1	3	2	1		3
	Inflammation- chronic							3	
Sciatic nerve	Cyst- perinural				1			3	4
	Pigmentation- perinural							3	
Skin	Inflammation-chronic				1			6	
	Cyst- subcutaneous			1	2			7	
Thymus	Atrophy- diffuse		1	16		3	11	9	3
Thyroid	Cyst	2	2	5	2	3	5	5	1
U. Bladder	Inflammation-submucosal							2	
	Hyperplasia-transition cell							2	
D99- Day 99 of dosing (week 14). R- Recovery group (24 weeks after last dosing period).									

Toxicokinetics:

In addition to testosterone undecanoate (TU), toxicokinetics of several of its metabolites including dihydrotestosterone undecanoate (DHT-TU), testosterone (T), dihydrotestosterone (DHT) and estradiol were assessed after the 1st, 3rd and 7th dosing in all of the TU groups and in the TC group. TU and TC dosing elevated T, DHT and estradiol levels. TC exposure resulted in greater Cmax spikes for testosterone and what appeared to be quicker elimination than TU. The half-lives of T and DHT could not be determined because of the slow elimination in both the TU and TC groups. With repeated dosing TU accumulated and T and DHT increased roughly 2 fold in the TU dosed animals, while in the TC dose group testosterone and DHT increased 4-5 fold. Estradiol levels increased less than dose proportionally in the TU dosed animals but were

elevated 2-5 fold from the lowest to highest TU group suggesting that TU elevates estradiol levels.

Since TU was not measured in humans dosed with the proposed 750 mg loading dose regimen with repeat injections every 10 weeks, estimated MOEs for TU were based on PK data collected following clinical doses of 750 or 1000 mg administered every 12 weeks for 48 weeks (Study IP157-001-Part A). Clinical doses of 1000 mg every 12 weeks resulted in mean and maximal Cmax and AUC levels of Testosterone slightly greater than that found following the 750 mg loading dose regimen. Multiples of human exposures (MOE) were calculated by dividing the mean Cmax and AUC values in rats by that in man and more conservatively by dividing the mean Cmax or mean AUC in rats by the maximal Cmax or maximal AUC in humans.

TU:
The C_{max} and AUC values for TU increased slightly greater than the proportional increase in dose and also increased with repeated dosing. Steady state was not reached. Elimination half-lives for TU could not be determined due to the slow elimination rate. The Sponsor stated that multiples of human exposures (MOEs) of 2.5, 12 and 25-fold were achieved for TU based on average AUC. Similar results were obtained in our analysis when comparing men dosed with 1000 mg TU to rats after the 7th dose. After the seventh dose in rats, the MOEs based upon AUC for TU were 2, 10 and 20. Nearly similar multiples (2.2, 10.8 and 21.6) were achieved when comparing C_{max} values for these same groups. A more conservative estimate of MOEs would be 0.4 to 4 based upon the human max AUC or max Cmax. However confidence in the MOEs for TU and its metabolites is not very great due to the uncertainty and variability of the human TU exposures and the lack of TU exposure data following TU 750 Loading protocol.

Toxicokinetics of TU in Rats Injected Intramuscularly Every Two Weeks

Time	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (day)	AUC_{tau} (ng•day/ml)	Multiple of Human Exposure			
					Mean Rat/ Mean Human		†Mean Rat/ Max Human	
					C_{max}	AUC	C_{max}	AUC
Day 1 (After 1st Dose)	50	0.7	8	6.1	0.5	0.4	0.1	0.9
	200	10.5	8	70.0	7.8	4.7	1.4	1.0
	800	32.2	8	222.0	23.9	15.0	4.4	3.2
Day 29 (After 3rd Dose)	50	1.1	4	10.8	0.8	0.7	0.2	0.2
	200	7.3	8	75.0	5.4	5.1	1.0	1.1
	800	26.2	4	296.0	19.4	20.0	3.5	4.3
Day 85 (After 7th Dose)	50	3.0	4	29.4	2.2	2.0	0.4	0.4
	200	14.6	4	140.0	10.8	9.5	2.0	2.0
	400	29.1	4	301.0	21.6	20.3	3.9	4.4

Multiples of human exposures (MOE) to TU were derived form mean or maximal human Cmax or AUC in men dosed with 1000 mg TU (IP157-001A). MOE = mean Cmax in rats/mean Human Cmax or mean rat AUC/mean Human AUC. †Conservative MOE = mean Cmax in rats / max Cmax in men or mean AUC in rats/ max AUC (IP157-001A). Mean Cmax for TU in men was 1.35 ± 1.1 ng/ml and the maximal was 7.4 ng/ml. Mean AUC in men was 14.8 ± 15.0 ng•days/ml while the maximal AUC was 68.8 ng/ml (IP157-001A). AUC_{tau} (AUC over the dosing interval, 14 days). Table modified from Sponsor's data ((b) (4) Study 1630-05560).

DHT-TU:
Dihydrotestosterone undecanoate (DHT-TU) was in general below the limit of quantification therefore toxicokinetic analysis was not conducted.

Testosterone:
Serum testosterone was analyzed in rats either uncorrected or corrected (normalized) relative to pre-dose testosterone levels within each group. The correction to account for pre-dose testosterone levels was done by averaging the pre-dose testosterone levels for each group and subtracting that from the average of each testosterone group during the dosing period. The average pre-dose testosterone concentration for all groups was 4.2 ± 2.7 ng/ml. TU and TC dosed rats received a large increase in testosterone above pre-dose levels with the average Cmax increasing 3, 11, 30 fold in the low-, mid- and high-dose TU groups and 16 fold in the TC group after the seventh dose. When corrected for the pre-TU and TC dosing levels, testosterone increased greater than the proportional change in dose and accumulated with C_{max} and AUC levels increasing ~2 fold with repeated TU dosing and ~7-10 fold in the TC group.

Dose Proportionality for Testosterone (Corrected for Pre Dose Levels)			
Dose Interval	TU Dose Multiples	C_{max} Ratio	AUCtau Ratio
Day 1 (1st dose)	1 : 4 : 16 fold	1 : 9.8 : 30.0 fold	1 : 11.0 : 42.0 fold
Day 29 (3rd dose)	1 : 4 : 16 fold	1 : 9.8 : 33.0 fold	1 : 13.0 : 45.0 fold
Day 85 (7th dose)	1 : 4 : 8 fold	1 : 5.6 : 11.0 fold	1 : 5.5 : 11.0 fold
Table modified form the Sponsor			

When analyzed as total testosterone levels, mean C_{max} and AUC increased in proportion with TU dose (table below).

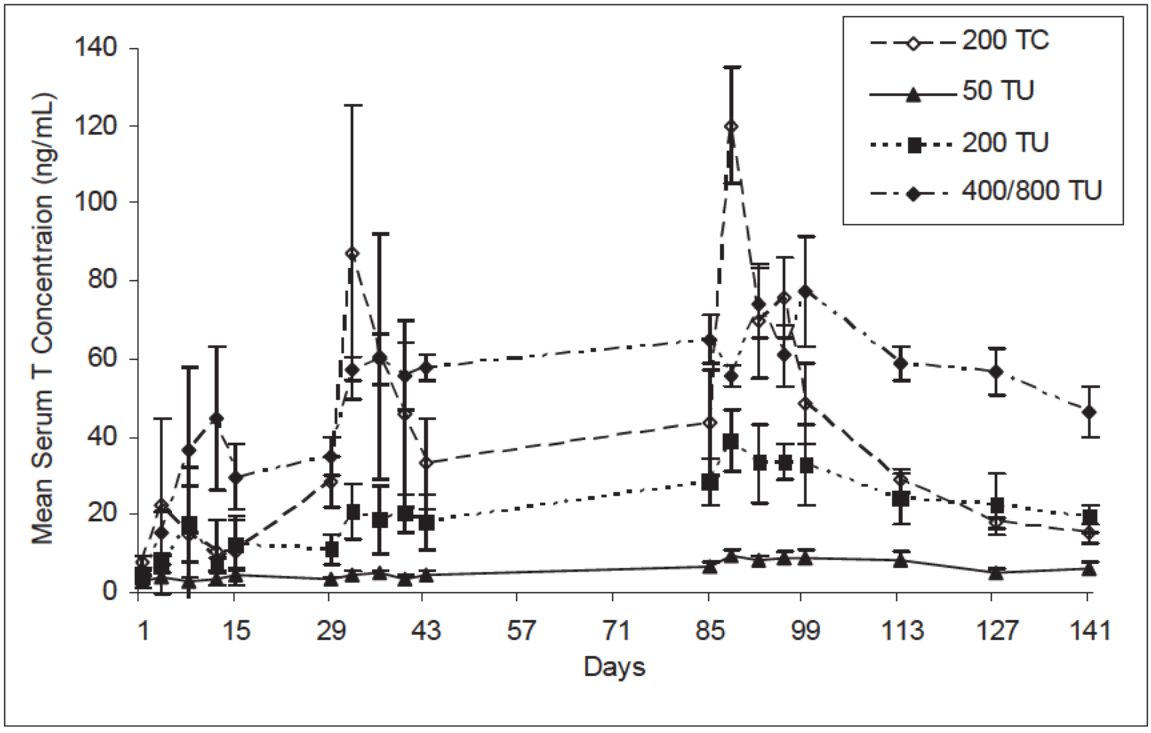
Dose Proportionality for Testosterone			
Dose Interval	TU Dose Multiples	C_{max} Ratio	AUCtau Ratio
Day 1 (1st dose)	1 : 4 : 16 fold	1 : 4.0 : 10.0 fold	1 : 2.9 : 8.1 fold
Day 29 (3rd dose)	1 : 4 : 16 fold	1 : 4.4 : 13.0 fold	1 : 4.4 : 13.0 fold
Day 85 (7th dose)	1 : 4 : 8 fold	1 : 4.2 : 8.0 fold	1 : 4.0 : 7.6 fold
Table modified form the Sponsor			

Like TU, the elimination of testosterone was too slow to estimate its half-life, however the T_{max} was generally reached within 8 hrs of dosing in the TU and TC dosed animals (Table below). MOEs for total testosterone were 0.3, 1.2 and 2.2 times the human mean AUC for low BMI men dosed with the TU 750 Loading protocol and 0.8, 3.2 and 6.0 times when based upon mean C_{max}. A more conservative estimate of human exposure based upon human maximal AUC and maximal Cmax found that these MOE were reduced to 0.2-1.3 and 0.5-4.2 respectively. The serum level of testosterone in the TC treated rats was intermittent between the high- and mid-dose TU groups. Although a proper analysis was not conducted, TC dosing appeared to result in larger Cmax spikes for testosterone after dosing and relatively quicker elimination than TU (see figure below). Additionally accumulation of testosterone was more pronounced in the TC group compared to the TU groups.

Testosterone Toxicokinetics in TU and TC Dosed Rats

Dose (mg/kg)	Pre-dose (ng/ml)	C_{max} (ng/ml)	Fold Above Background	T_{max} (day)	AUC_{tau} (ng•day/ml)	Multiple of Human Exposure			
						Mean Rat/ Mean Human		†Mean Rat/ Max Human	
Day 1 (1st Dose)						C_{max}	AUC	C_{max}	AUC
200 TC	7.6	22.2	2.9	4	204.0	1.8	0.5	1.3	0.3
50 TU	3.0	4.4	1.5	15	46.9	0.4	0.1	0.3	0.1
200 TU	3.6	17.6	4.9	8	138.0	1.4	0.3	1.4	0.2
800 TU	2.5	44.7	17.9	12	382.0	3.6	0.9	2.5	0.5
Day 29 (3rd Dose)									
200 TC		87.3	11.5	4	822	7.1	2.0	5.0	1.2
50 TU		4.7	1.6	8	58.2	0.4	0.1	0.3	0.1
200 TU		20.6	5.7	4	258.0	1.7	0.6	1.2	0.4
800 TU		59.9	24.3	8	763.0	4.9	1.9	3.4	1.1
Day 85 (7th Dose)									
200 TC		120.0	15.9	4	1130.0	9.8	2.8	6.8	1.6
50 TU		9.3	3.1	4	119.0	0.8	0.3	0.5	0.2
200 TU		38.8	10.7	4	477.0	3.2	1.2	2.2	0.7
400 TU		74.1	30.0	8	903.0	6.0	2.2	4.2	1.3

The average pre-dose testosterone concentration was 4.2 ng/ml ± 2.7. Multiples of exposure (MOE) were derived from patients who were dosed with 750 mg TU at weeks 0, 4, 14, 24 (IP157-001 C). In low MBI men Mean Cmax = 12.3 ng/mL and Mean AUC = 405 ng•day/ml. Max C_{max} for all men was 1759 ng/dL = 17.6 ng/ml and Max AUC was 70,017 ng•days/dL = 700 ng•days/ml. †Conservative multiple of human exposure = mean C_{max} in rats / Max C_{max} in men or mean AUC in rats/Max AUC. Table modified from Sponsor's data ((b) (4) Study 1630-05560).



Rats were dosed on days 1, 15, 29, 43, 57, 71 and 85. Serum samples were taken prior to dosing on days 1, 15, 29, 43 and 85. Figure generated from Sponsor's tabular data.

DHT:

Prior to dosing, DHT was only detected in the TC group and not in the TU groups. DHT increased slightly greater than the proportional increase in TU dose. DHT slightly accumulated in the TU groups especially after the seventh dose where AUC levels were elevated (~2 fold) compared to after the first dose. TC dosed animals had a much greater accumulation of DHT upon repeated dosing with Cmax and AUC levels increasing roughly four fold.

Dihydrotestosterone Toxicokinetics in TU and TC Dosed Rats

Dose (mg/kg)	C_{max} (ng/ml)	Dose Normalized C_{max} (ng/mg)/(mg/kg)	T_{max} (day)	AUC_{tau} (ng•day/ml)	Dose Normalized AUC_{tau} (ng•day /ml)/(mg/kg)
Day 1 (After 1st Dose)					
200 TC	0.8	0.004	4	8.1	0.04
50 TU	0.1	0.001	4	0.3	0.01
200 TU	0.7	0.004	8	4.9	0.04
800 TU	1.8	0.002	12	15.4	0.04
Day 29 (After 3rd Dose)					
200 TC	2.4	0.01	4	22.8	0.03
50 TU	--	--	--	--	--
200 TU	0.8	0.004	4	8.8	0.03
800 TU	1.9	0.002	8	23.9	0.03
Day 85 (after 7th Dose)					
200 TC	3.4	0.017	4	30.8	0.03
50 TU	0.2	0.004	4	2.0	0.02
200 TU	1.1	0.006	4	13.9	0.03
400 TU	1.9	0.005	8	25.4	0.03

Table modified form the Sponsor. DN- Dose normalized, toxicokinetic parameter divided by the intended dose.

Dose Proportionality for Dihydrotestosterone

Dose Interval	TU Dose Multiples	C_{max} Ratio	AUCtau Ratio
Day 1 (1st dose)	1 : 4 : 16 fold	1: 9.7 : 27 fold	1 : 18 : 57 fold
Day 29 (3rd dose)	1 : 4 : 16 fold		
Day 85 (7th dose)	1 : 4 : 8 fold	1 : 5.9 : 9.8 fold	1 : 6.9 : 13 fold

Table modified form the Sponsor

Estradiol:
Estradiol levels during the dosing period were only slightly elevated or lower than pre-dose levels. In general estradiol levels (C_{max} and AUC_{tau}) increased slightly (~2 to 5 fold) between the lowest and highest TU dose groups.

Estradiol Toxicokinetics in TU and TC Dosed Rats

Dose (mg/kg)	C_{max} (ng/ml)	Dose Normalized C_{max} (ng/mg)/(mg/kg)	T_{max} (day)	AUC_{tau} (ng•day/ml)	Dose Normalized AUC_{tau} (ng•day /ml)/(mg/kg)
Day 1 (After 1st Dose)					
200 TC	11.2	0.06	8	84.1	0.42
50 TU	8.6	0.17	15	66.5	1.33
200 TU	10.3	0.05	0	69.9	0.35
800 TU	11.1	0.01	12	91.8	0.12
Day 29 (After 3rd Dose)					
200 TC	9.0	0.05	8	89.8	0.45
50 TU	3.7	0.07	12	40.3	0.81
200 TU	5.8	0.03	12	75.4	0.38
800 TU	8.9	0.01	4	104.0	0.13
Day 85 (after 7th Dose)					
200 TC	15.1	0.08	12	109.0	0.55
50 TU	1.6	0.03	8	12.5	0.25
200 TU	5.7	0.03	12	57.3	0.29
400 TU	5.8	0.02	8	66.3	0.17

Table modified form the Sponsor. DN- Dose normalized, toxicokinetic parameter divided by the intended dose.

Dose Proportionality for Estradiol

Dose Interval	TU Dose Multiples	C_{max} Ratio	AUCtau Ratio
Day 1 (1st dose)	1 : 4 : 16 fold	1 : 1.2 : 1.3 fold	1 : 1.1 : 1.4 fold
Day 29 (3rd dose)	1 : 4 : 16 fold	1 : 1.6 : 2.4 fold	1 : 1.9 : 2.6 fold
Day 85 (7th dose)	1 : 4 : 8 fold	1 : 3.5 : 3.6 fold	1 : 4.6 : 5.3 fold

Table modified form the Sponsor

2.6.6.4 Genetic toxicology

The following genetic toxicology studies were reviewed by Dr. Leslie McKinney.

Study title: Evaluation of ZK 5448 in a bacterial reverse mutation test (Ames-Test) using Salmonella typhimurium and Escherichia coli as test organisms.

Key findings: No evidence of mutagenicity

Study no.: A07923
Original NDA Submission: CDSESUB1\NONECTD\N22219|N_000|2007-08-24. Module 4.2.3.3.1 42-stud-rep\423-tox\4233-genotox\42331-in-vitro\a07923-legacy.pdf
Conducting laboratory & location: (b) (4)
Date of study initiation: Dec 2001.

GLP compliance: Yes, see submission N002 for verification.
QA reports: yes (X) no ( ). See submission N002 for verification.
Drug, lot #, and % purity: ZK 5448, KC-0114/01, 99.88%, stability given in test report #10693AT4.doc from 14 Mar 2002.
In order to qualify impurities [redacted] (b) (4) ([redacted] (b) (4) %) and [redacted] (b) (4) ([redacted] (b) (4) %) were added to this batch.

Methods
Strains/species/cell line: *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, and T98 and *Escherichia coli* strain WP2uvrA
Doses used in definitive study: [redacted] (b) (4) mg
Basis of dose selection: not given
Range finding studies: not given
Metabolic activation system: Aroclor 1254-induced male rat liver homogenate (S9 mix)
Negative controls: DMSO, phosphate buffer, 50 µl/plate
Positive controls:

2-AA: anthracene-2-amine
2-NF: 2-nitro-9H-fluorene
CP: cyclophosphamide
EMS: ethylmethanesulfonate
BP: benzo[a]pyrene
4-NPDA: 4-nitro-o-phenylenediamine
NaN_3: sodium azide
MNNG: N-methyl-N'-nitro-N-nitrosoguanidine

S. typhimurium Strains	-S9 mix Pos. Control dose/plate	+S9 mix Pos. Control dose/plate
TA 1535	NaN_3 [redacted] (b) (4) µg	2-AA: [redacted] (b) (4) µg CP: [redacted] (b) (4) µg
TA 100	NaN_3 [redacted] (b) (4) µg	2-AA: [redacted] (b) (4) µg BP: [redacted] (b) (4) µg
TA 1537	4-NPDA: [redacted] (b) (4) µg	2-AA: [redacted] (b) (4) µg
TA 1538	2-NF: [redacted] (b) (4) µg	2-AA: [redacted] (b) (4) µg
TA 98	2-NF: [redacted] (b) (4) µg	2-AA: [redacted] (b) (4) µg BP: [redacted] (b) (4) µg
E. coli WP2uvrA	EMS [redacted] (b) (4) µg MNNG [redacted] (b) (4) µg	2-AA: [redacted] (b) (4) µg

Incubation and sampling times:
Incubation and sampling times: 72 h @ 37°C
Study design: direct plating of a suspension of bacteria, buffer, and test material ± S9 mix

Analysis:
No. of replicates: Plates prepared in triplicate
Counting method: Artek M 982B automated colony counter; arithmetic means of the number of mutant colonies of the 3 parallel plates in the negative control groups were compared with those of the compound groups.
Criteria for positive results:
A response was considered positive if the number of revertants of the compound groups compared to the number of revertants of the negative group was reproducibly higher than 2-fold. A dose-dependent increase in the number of revertants was also considered to indicate a mutagenic effect.

Results

Study validity: Methods considered valid but no documentation of GLP.

Study outcome:

- None of the six tester strains showed increased reversion, either in the absence or presence of S9 mix.
- Precipitates in the agar were found from (b) (4) mg/plate onward; most of the plates with precipitates were manually scored.
- Growth inhibition of the background lawn was not observed.
- Positive and negative controls produced the expected numbers of revertant colonies.

Conclusion: No evidence of mutagenicity

Study title: Evaluation of ZK 5448 in a bacterial reverse mutation test (Ames-Test) with preincubation using Salmonella typhimurium and Escherichia coli as test organisms.

Key findings: No evidence of mutagenicity
Study no.: A07981
Original NDA Submission: CDSESUB1\NONECTD\N22219|N_000|2007-08-24. Module 4.2.3.3.1 42-stud-rep\423-tox\4233-genotox\42331-in-vitro\a07981-legacy.pdf
Conducting laboratory & location: (b) (4)
Date of study initiation: Jan 2002
GLP compliance: Yes, see submission N002 for verification.
QA reports: yes (X) no ( ). See submission N002 for verification.
Drug, lot #, % purity, and stability: ZK 5448, KC-0114/01, 99.88%, stability given in test report #10693AT4.doc from 14 Mar 2002. In order to qualify impurities (b) (4) ((b) (4)%) and (b) (4) ((b) (4)%) were added to this batch.

Methods
Strains/species/cell line: *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, and T98 and *Escherichia coli* strain WP2uvrA
Doses used in definitive study: (b) (4) mg
Basis of dose selection: not given
Range finding studies: not given
Metabolic activation system: Aroclor 1254-induced male rat liver homogenate (S9 mix)
Negative controls: DMSO, phosphate buffer, 50µL/plate.
Positive Controls:

2-AA: anthracene-2-amine
2-NF: 2-nitro-9H-fluorene
CP: cyclophosphamide
EMS: ethylmethanesulfonate
BP: benzo[a]pyrene
4-NPDA: 4-nitro-o-phenylenediamine
NaN_3: sodium azide
MNNG: N-methyl-N'-nitro-N-nitrosoguanidine
DMNA: N-nitrosodimethylamine

S. typhimurium Strains	-S9 mix Pos. Control dose/plate	+S9 mix Pos. Control dose/plate
TA 1535	NaN_3 (b)(4) µg	2-AA (b)(4) µg CP: (b)(4) µg
TA 100	NaN_3 (b)(4) µg	2-AA (b)(4) µg DMNA (b)(4) µg
TA 1537	4-NPDA: (b)(4) µg	2-AA: (b)(4) and (b)(4) µg
TA 1538	2-NF (b)(4) µg	2-AA: (b)(4) µg
TA 98	2-NF: (b)(4) µg	2-AA (b)(4) µg BP: (b)(4) µg
E. coli WP2uvrA	EMS: (b)(4) µg MNNG: (b)(4) µg	2-AA: (b)(4) µg

Exposure conditions:
Incubation and sampling times: 72 h @ 37°C
Study design: a suspension of bacteria, buffer, and test material ± S9 mix was incubated @37°C for 60 min, then plated. Plates were kept at 37°C for 48 or 72 h.
Analysis:
No. of replicates: Plates prepared in triplicate
Counting method: Artek M 982B automated colony counter; arithmetic means of the number of mutant colonies of the 3 parallel plates in the negative control groups were compared with those of the compound groups.
Criteria for positive results:
A response was considered positive if the number of revertants of the compound groups compared to the number of revertants of the negative group was reproducibly higher than 2-fold. A dose-dependent increase in the number of revertants was also considered to indicate a mutagenic effect.

Results
Study validity: Methods considered valid but no documentation of GLP.
Study outcome:

- None of the six tester strains showed increased reversion, either in the absence or presence of S9 mix.
- Precipitates in the agar were found from (b)(4) mg/plate onward; most of the plates with precipitates were manually scored.
- Growth inhibition of the background lawn was not observed.
- Positive and negative controls produced the expected numbers of revertant colonies.

Conclusion: No evidence of mutagenicity

Study title: Chromosome aberration assay in human lymphocytes in vitro with ZK5448.

Key findings: No evidence of chromosomal aberrations in human lymphocytes *in vitro* when tested up to [redacted] µg/mL.

Study no.: 721300
Original NDA Submission: CDSESUB1\NONECTD\N22219|N_000|2007-08-24. Module 4.2.3.3.1 42-stud-rep\423-tox\4233-genotox\42331-in-vitro\721300-legacy.pdf
Conducting laboratory and location: [redacted]
Date of study initiation: November 26, 2001
GLP compliance: yes
QA reports: yes (X) no (X)
Drug, lot #, % purity, stability: ZK 5448 batch #Kc-0114/01, 99.88%, not given
In order to qualify impurities [redacted] ([redacted]%) and [redacted] ([redacted]%) were added to this batch.
Formulation/vehicle: DMSO 0.5% v/v

Methods

Strains/species/cell line: primary lymphocytes from healthy human donors.
Doses used in definitive study: [redacted] µg/mL.
Basis of dose selection: Range finding study tested [redacted] µg/mL. Cytotoxicity was observed [redacted] Dose selection was therefore based on the solubility of the test compound.
Precipitates observed at the [redacted] concentrations.
Metabolic activation system: liver S9 mix from phenobarbital/β-naphthoflavone treated male rats. S9 mix was prepared by [redacted].
Negative controls: culture medium, and also DMSO.
Positive controls: ethylmethane sulfonate (EMS) for the protocol without S9 mix; cyclophosphamide (CPA) for the protocol with S9 mix. BrdU was used to verify replication time of the lymphocytes.
Exposure conditions:

	Without S9 Mix			With S9 Mix	
	Exp 1		Exp 2	Exp 1	Exp 2
Exposure (hrs)	4	22	46	4	4
Recovery (hrs)	18	--	--	18	42
Preparation interval (hrs)	22	22	46	22	46
Sponsor's table.					

Analysis: Two independent experiments were performed. In each experimental group two parallel cultures were analyzed. Experiment 1) 4 hrs with or without S9 mix and 22 hrs without S9 mix. Experiment 2) 4 hrs with S9 mix and 46 hrs without S9 mix. Chromosomes were prepared 22 hrs (exp 1) and 46 hrs (exp 2) after initiation of test article treatment. Per culture, 100 metaphase plates were scored for structural chromosome aberrations: breaks,

fragments, deletions, exchanges, and disintegrations, but not gaps. Polyploid cells were also counted. At least 1000 cells were counted per culture for mitotic index.

Criteria for negative results: The test item was considered non-mutagenic if: the # of aberrations were in the range of historical controls (0-4%)

Criteria for positive results: The test item was considered mutagenic if: the number of aberrations were not in the historical range and either a concentrations-related or a significant increase in the number of aberrations was observed. A test item was classified as an aneugen if: the # of induced aberrations were not in the range of historical controls (0-1.5% polyploid). Statistical significance was confirmed by Fisher's exact test ($p<0.05$)

Results

Study validity: Study was considered valid on the basis of methodology and data showing that negative and positive control values were appropriate. Values for chromosomal aberrations in human lymphocytes for negative and/or solvent controls fell within the historical control data range (0-4%). The positive control substances produced a significant increase in the frequencies of aberrations.

Study outcome: No evidence of chromosomal aberrations in human lymphocytes *in vitro* when tested up to (b) (4) µg/mL.

Deviations from the protocol: Final concentration for EMS used at the 46 hr preparation interval without metabolic activation: (b) (4) mM ((b) (4) µg/mL). This is an increased concentration of the positive control only. Sponsor deems no effect on the outcome of the study.

Study title: Study on the mutagenic potential of ZK 5448 in the mouse micronucleus test.

Key findings: No clinical signs of toxicity were observed in any dose group. PCE/NCE ratio was reduced in all dose groups indicating generalized bone marrow depression. There was no increase in micronucleated polychromatic erythrocytes (PCE) or normochromatic erythrocytes (NCE) counts in any dose group. There was no evidence of mutagenicity.

Study no.: A06611
Original NDA Submission: CDSESUB1\NONECTD\N22219|N_000|2007-08-24.
Module 4.2.3.3.1
42-stud-rep\423-tox\4233-genotox\42332-in-vivo\a06611 legacy.pdf
Conducting laboratory and location: (b) (4)
Date of study initiation: July 2001
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, % purity, stability: ZK 5448, Batch # 101131, 100.4%, test article prepared for immediate use.
In order to qualify impurities (b) (4) ((b) (4)%) and (b) (4) ((b) (4)%) were added to this batch.

Formulation/vehicle: Microcrystalline suspension, Three batches were prepared, one each for the low- mid- and high-dose groups (batch #s N5131A-1, N5131B-1, N5131C-1)
Vehicle: 9 mg NaCl, 0.85 mg Myr53, 10 mg Klucel LF in 1 mL dd H_20.

Methods

Strain/species: Crl:NMRI, Br male mice
No. animals: 5 animals per time point (24 and 48 hrs post dose) per dose.
Doses used in definitive study: 0, 500, 1000, or 2000 mg/kg intraperitoneal.
Basis of dose selection: No significant clinical signs for 500, 1000, 1500, or 2000 mg/kg; therefore used highest possible dose (2000 mg/kg) was chosen.
Negative controls: Vehicle (20 mL/kg).
Positive controls: cyclophosphamide (30 mg/kg) single treatment by gavage, 5 males.
Incubation and sampling times: Treated animals were euthanized 24 and 48 hrs post-treatment.
Positive controls euthanized after 24 hrs of treatment.
Analysis: Bone marrow smears prepared and slides analyzed for incidence of micronucleated cells per 2000 PCEs or 1000 NCEs.

Results

Study validity:
Study was considered valid on the basis of methodology and data showing that negative and positive control values were appropriate.

Study outcome:
No clinical signs of toxicity were reported in any group.
The study was considered positive if a statistically significant increase (at the 5% level) in PCE and NCE counts were observed. No evidence for mutagenicity of the test article was observed.

2.6.6.5 Carcinogenicity

Since TU is a pro-drug intended for delivery of testosterone, the carcinogenic potential of TU is considered to be the same as for testosterone.

2.6.6.6 Reproductive and developmental toxicology

Since TU is a pro-drug intended for delivery of testosterone, reproductive and developmental risks associated with TU exposure will be considered to be the same as for testosterone.

2.6.6.7 Local tolerance

This study was reviewed by Dr. Leslie McKinney.

Study title: Local tolerance testing after intramuscular injection in pigs.

Key study findings: Single intramuscular injections of up to 1000 mg of testosterone undecanoate or testosterone enanthate were reasonably well tolerated. However large injection volumes (3-4 ml) did cause tissue necrosis, fibrosis, inflammation and hemorrhage which tended to recover 7-42 days after dosing.

Study no.: JPH01496
Original NDA Submission: CDSESUB1\NONECTD\N22219|N_000|2007-08-24.
Module 4.2.3.3.1
42-stud-rep\423-tox\4236-loc-tol\jph01496-legacy.pdf
Conducting laboratory and location: (b) (4)
Date of study initiation: Nov, 1996
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: TU: Batch 030795, TE: Batch 61451-207, Vehicle: Batch 030796
Methods:
Dose Solutions: TU: 250 mg/mL; TE: 250 mg/mL.

	TU mg/mL	TE mg/mL	Vehicle mg/mL
Drug Substance	250	250	--
Benzylbenzoate	500	500	662.25
Castor Oil	294.7	294.7	390.33

Species/strain: SPF pigs
Number/sex/group or time point (main study): 10/ Female / group
Route, formulation, volume, and infusion rate: separate injections in the right and left *longissimus dorsi* muscle (low and high volumes simultaneously). The TE groups received one injection of 250 mg and another of 1000 mg on the other side of the body. The TU groups received two high dose injections one on each side (1000 mg TU each).

	Low Volume (mL)	High Volume (mL)
Vehicle	0.76	3.04
TE	1.0	4.0
TU	4.0	4.0

		Right Side		Left Side	
Group	No. Pigs	ml/injection	Dose (mg/injection)	ml/injection	Dose (mg/injection)
Vehicle	5	0.76	-	3.04	-
	5	3.04	-	0.76	-
TE	5	1.0	250	4.0	1000
	5	4.0	1000	1.0	250
TU	10	4.0	1000	4.0	1000

Satellite groups used for toxicokinetics or recovery: none
Age: not given
Weight: 19-30 kg

Sampling times: Necropsy 4 days post-injection (4/group) and at 7, 21, and 42 days post-injection (2/group). Tissue from the injection site examined grossly and microscopically.

Results:
No clinical signs other than loss of body weight in two animals (one vehicle treated, one TE treated).

Injection Site Pathology/Histology:
Gross and microscopic findings at the injection site were not TU or TE dependent and appeared to be due to injection volume since the incidence and severity increased with larger volumes. These findings generally resolved 7-42 days after dosing with the exception of minimal to slight fibrosis. Healing was faster and more extensive in the low volume dose groups.

Gross findings:

- Low volume injection site: Scattered findings of pale areas, focal hemorrhage and one finding of necrosis in a vehicle treated animal. Pale areas were observed in the TE group.
- High volume injection site: Scattered findings of pale areas, focal hemorrhage in all groups and necrosis in the vehicle and TE group.

Microscopic findings observed in all groups:

- Low volume injection site: On day 4, minimal to moderate tissue necrosis with inflammation and hemorrhage. Mineralization of necrotic tissue and giant cells noted.
- High volume injection site: Similar pattern of findings present in all groups, but more severe. Fibrosis in the vehicle group changed from moderate on day 4 to minimal/slight by day 42. Minimal fibrosis was observed in the TE and TU groups on day 4 only.

Conclusion: Findings were deemed not related to test article, but to the volume of injection. It is likely that the inflammation and necrosis, etc., are due to non-specific local tissue injury. There is no direct evidence that either of the excipients are directly toxic to human tissue. Benzyl benzoate is, however, used therapeutically in the treatment of scabies because it is directly toxic to the type of house mite that causes scabies. Whether it could directly activate macrophages, which would explain the presence of giant cells at the injection site, has not been established, but has been observed for other benzoates *in vitro* (Choi et al., Arch Pharm Res 28(1):49-54 (2005).

2.6.6.8 Special toxicology studies

None

2.6.6.9 Discussion and Conclusions

Overall the nonclinical program produced results similar to what would be expected of testosterone.

Fourteen weeks of repeated intramuscular TU exposure to male rats resulted in responses consistent with exposure to testosterone. The toxicities were similar to the reference drug testosterone cypionate. Reduced testis weights, adverse renal and bladder histopathology and skin inflammation were observed in the TU groups but not in the TC group. Generally since the incidence and severity of the findings unique to TU were low, it is not possible to definitively state that there is any significant difference in adverse effects between TU and testosterone or other testosterone esters.

High mortality/morbidity was observed in rats injected with large volumes of vehicle alone or TU. The Sponsor stated that, "The cause of death in these animals was unclear; however, clinical chemistry profiles of several moribund sacrificed animals suggested a possible renal dysfunction and minimal hepatotoxicity". The Sponsor attributed the deaths to unintended systemic exposure to the vehicle and not to TU. From the data supplied it could not be concluded that there were any causal relationships between the mortalities and the potential formation of pulmonary microemboli related to excessive exposure to the vehicle. Although these animals displayed moderate to severe tremors, languid appearance, prostration, signs of respiratory distress were not mentioned in the study report.

A NOEL could not be determined since the adverse responses to TU and TC including reduced feed intake, decreased weight gain, exophthalmus, lacrimation, aggressive behavior, slight alterations in hematology, altered organ weights, thymic atrophy and injection site inflammation were observed at the lowest dose tested (0.4 to 2 times the exposure in men dosed with 1000 mg TU). However with the exception of injection site cysts and inflammation these findings were generally mild and could be considered affects of exaggerated pharmacology. Since overt toxicity was not observed below 200 mg/kg a NOAEL could be set at 50 mg/kg.

The affects on organ weights were generally still apparent after 26 weeks of drug withdrawal but were not statistically significant. Thymic atrophy was still evident in the high dose TU rats (4 to 20 times the human exposure at 1000 mg TU) but not in the TC group after the recovery period. Chronic inflammation and cysts were observed in dose dependent manner at the injection site in the TU and TC groups but not in the vehicle group. Cysts were still evident after recovery as well. Inflammation of the skin (43%) and sciatic nerve cysts (21%) near the injection site were only observed in the high-dose TU group but not in the vehicle or TC groups. These inflammatory responses may not be a major concern since a local tolerance study in pigs was conducted comparing intramuscular administered TU and testosterone enanthate without drug related affects. However injection volume related affects were observed in the pigs including injection site inflammation, necrosis, fibrosis, hemorrhaging and appearance of multinucleated giant cells after intramuscular dosing in pigs at the injection site.

In this same study, intramuscular TU or TC dosing elevated T, DHT and estradiol levels. Exposures of TU in rats ranged from 2 to 20 times the level in men dosed with 1000 mg TU

based upon the human mean AUC or Cmax or 0.4 to 4 when based upon the maximal AUC or Cmax in man. TU dosing elevated testosterone concentrations 3, 11 and 30 fold above the pre-dose levels based upon C_{max}. When assessed based upon mean C_{max}, dose multiples achieved in rats for total testosterone were 1, 3 and 6 times the mean C_{max} in men dosed with the proposed 750 mg loading dose regimen, or 1, 2 and 4 time when based upon the maximal C_{max} in men In the TU groups testosterone increased greater than the proportional increase in dose and tended to accumulate. DHT increased with increased TU doses and had slight tendency to accumulate with repeated dosing (~2 fold). DHT-TU was too low to measure. In general estradiol levels (C_{max} and AUC_{tau}) increased slightly (~2 to 5 fold) between the lowest and highest TU dose groups.

TU was negative in the standard battery of genotoxicity assays. Reproductive and developmental toxicity and carcinogenicity were not studied since the product will be labeled under Pregnancy Category X and will carry a carcinogenicity warning consistent with approved testosterone products (see section 2.6.6.5 and 2.6.6.6).

2.6.7 TOXICOLOGY TABULATED SUMMARY

None Submitted

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Several therapies involving exposure to testosterone or testosterone esters have been developed to elevate serum testosterone. Oral testosterone undecanoate (TU) has been marketed outside of the United States as Andriol Testocaps® for testosterone replacement therapy for over 20 years. The Sponsor's Nebido® or Reandron® product (injectable TU) has recently become available in 36 countries. Other esterified testosterone products have been approved for injection by the FDA including testosterone cypionate, testosterone enanthate and testosterone propionate. Exogenous exposure to testosterone or testosterone esters has resulted in common adverse responses in men including; elevated DHT and E_2 (metabolites of T), reduced fertility, gynecomastia, behavioral changes, sleep apnea, edema (retention of Na, Cl, K and inorganic phosphates), increased prostatic hyperplasia, increased platelet aggregation and thrombogenicity, altered serum lipid profile, polycythemia, liver and kidney toxicity (2;3).

Nonclinical concerns regarding the Sponsor's intramuscular TU product included whether it would have similar toxicity and pharmacology as other testosterone esters and whether undecanoic acid itself could pose additional safety concerns. It was assumed that like other testosterone esters TU would not have pharmacological activity unless its ester bond is hydrolyzed releasing the active agent, testosterone, and the inactive ester, undecanoic acid. This was supported by the Sponsor's findings of minimal binding affinity of TU for the human androgen receptor. The potential toxicity of TU and undecanoic acid was assessed in a three month intramuscular TU dosing study in male rats. The results were generally consistent with exaggerated pharmacologic affects of testosterone and were similar to the comparator testosterone cypionate. Adverse toxicities unrelated to exaggerated pharmacology of testosterone

were generally not observed. One exception is that when dosed every two weeks, the TU product (and TC) caused injection site inflammation and cystic lesions surrounding the injection material that were not observed in the vehicle group. Inflammation, necrosis, fibrosis, hemorrhaging and appearance of multinucleated giant cells were also observed at the injection site after intramuscular dosing in pigs (1000 mg/4 ml TU). The affects in pigs appeared to be due to injection volume and not related to TU itself. Cystic lesions referred to in the rat study were not mentioned in the porcine study, however, the pathologist in the porcine study referred to injection site fibrosis which may be similar to the cystic lesions in rats. The differences between species may be due to the difference in volume and repetition of dosing.

Toxicokinetics for TU, DHT-TU, T and estradiol were also assessed in the three month repeat dose study in rats. The rats received roughly 2 to 20 times the TU exposure that hypogonadal men receive after 1000 mg TU based upon either mean AUC or mean C_{max}. TU significantly elevated testosterone concentrations above the pre-dose levels 3, 11, 30 fold based upon C_{max}. TU dosing achieved testosterone levels that were 1, 3 and 6 times greater than in men dosed with 750 mg TU based upon mean C_{max} or 1, 2 and 4 times when based upon max Cmax in men. TU exposure resulted in greater than proportional increase in testosterone and testosterone tended elevate with repeated dosing. DHT increased with increased TU doses and had a slight tendency to accumulate with repeated/frequent dosing (~2 fold). In general estradiol levels (C_{max} and AUC_{tau}) increased slightly (~2 to 5 fold) between the lowest and highest TU dose groups. DHT-TU was too low to measure.

The absorption, distribution and elimination of radiolabeled TU were also characterized in rats. The distribution of radioactivity was essentially limited to the liver, kidney and excretory tissues. Nearly half of the administered dose remained near the dose site eight weeks after the initial injection. Most of the radioactivity was excreted in feces and to a lesser extent in urine. The fate of undecanoic acid was not directly addressed because the radioactive label was on the steroid ring. However it is not predicted to be toxic since it is believed to be metabolism via the fatty acid and tricarboxylic acid pathways (4). Additionally, undecanoic acid is an approved food additive in the EAFUS Food Additive Database.

A standard battery of genotoxicity assays were conducted with TU and were negative. Carcinogenicity and reproductive toxicity studies were not considered necessary since the label will contain warnings for cancer risk consistent with other approved testosterone products and the drug will be labeled as a pregnancy category X drug based upon the effects of testosterone on development.

Conclusions: The Sponsor's nonclinical program, supplied references, available literature and general knowledge of testosterone provide reasonable assurance of the safety of TU in hypogonadal men.

Unresolved toxicology issues (if any): There are no unresolved issues at this time.

Recommendations: None at this time

Suggested labeling:
See Executive Summary I (C) page 3.

A copy of the Sponsor's proposed labeling is listed in the Appendix/Attachments below. Following this a full label including the recommended changes from the pharmacology/toxicology perspective is listed.

The major recommendations include adding sections on use in women (5.10), affects on spermatogenesis (5.11), drug interactions with anticoagulants (7.5), use in pregnant or nursing women (8.1, 8.3), use in pediatrics (8.4,) use in patients with impaired renal or hepatic function (8.6).

21 Pages of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page.

APPENDIX III

References

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