

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**22-052**

**PHARMACOLOGY REVIEW**

## INTEROFFICE MEMO

TO: NDA 22052  
FROM: C. Joseph Sun, Ph. D., Supervisory Pharmacologist  
Division of Pulmonary and Allergy Products  
DATE: April 5, 2007

I concur with pharmacologist's recommendation that pharmacology and toxicology of zileuton have been adequately studied and the drug product (controlled-release tablets) is approvable from a preclinical standpoint.

Preclinical Pharmacology and toxicology assessment of zileuton is primarily based on the data submitted for the zileuton Tablets formulation (NDA 20471) which was approved in 1996.

**Pharmacology:** Zileuton is a 5-lipogenase inhibitor, inhibiting leukotriene formation. It inhibited leukotriene-dependent bronchospasm in antigen and arachidonic acid-challenged guinea pigs. It inhibited bronchoconstriction in and eosinophil migration into the lungs of antigen-challenged sheep, arachidonic acid-induced ear edema in mice and neutrophil migration in mice in response to polyacrylamide gel.

**General toxicity:** It has been examined in mice, rat, dogs and monkeys. The main target organs of toxicity were the liver (rats and mice), kidney (rats and dogs), male reproductive organs (dogs) and hematopoietic systems (dogs). No target organs of toxicity were identified in monkeys.

**Reproductive toxicity:** All phases of the reproductive process have been evaluated in rat or rabbits. No impairment of fertility was reported in rats. Increased gestation period, increased pup mortality and reduced pup weight in rats and increased incidence of cleft palate and hydrocephalus were observed in rabbits.

**Genotoxicity:** A battery of standard genotoxicity studies performed with zileuton have been negative. However, a dose-related increase in DNA adduct formation was reported in kidneys and liver of female mice treated with zileuton. Some evidence of DNA damage was observed in a UDS assay in hepatocytes isolated from the Arocolor-treated rats, no such findings was noticed in hepatocytes isolated from monkeys where the metabolic profile is more similar to that of humans.

**Carcinogenesis:** The carcinogenic potential was evaluated in mice and rats. In mice, increased incidences of liver tumors, renal tubular tumors and hemangiosarcomas in females and a trend of increases in the incidence of liver tumors in males were observed. In rats, a dose-related increase in Leydig cell tumors and increased incidence of renal tubular tumor were reported. A mechanistic explanation was provided for Leydig cell tumors. Its tumorigenesis was prevented by supplementing males rats with testosterone and no hormonal changes were observed in humans with zileuton. Therefore its relevance

No significant test article-related adverse effects were observed on the measured parameters. NOAEL was identified at high dose, 40/2 mg base/kg/day of the Zileuton/Degradant combination.

Study no.: TA97-141

Volume #, and page #: Volume 18, page 1-362

Conducting laboratory and location: \*

Date of study initiation: 5/23/1997

GLP compliance: GLP (GLP compliance was stated under the QA statement)

QA report: yes (X) no ( )

Drug, lot #, and % purity: Zileuton Lot# 02-911-AL, potency = ~~na/na~~ mg/g

Abbott- ~~na/na~~ Lot# 26881-45, potency = ~~na/na~~ mg/g

No % purity is reported.

**Methods**

Doses, Number/sex/group, and dose volume are listed in the study design table below (excerpted from Vol. 18, page 14)

Group	No. of Animals		Dosage Material	Abbott <sup>a</sup> (mg base/kg/day)	Abbott <sup>b</sup> (mg base/kg/day)	Dose Volume (mL/kg)
	Male	Female				
1	10	10	Vehicle	0	0	10
2	10	10	Zileuton	40	0	10
3	10	10	Zileuton and Degradant	40	1	10
4	10	10	Zileuton and Degradant	40	2	10

<sup>a</sup> Zileuton Potency = ~~na/na~~, lot number = 02-911-AL.

<sup>b</sup> Degradant Potency = ~~na/na~~ /g, lot number = 26881-45.

Route: Oral gavage

Formulation: The Zileuton stock solution and Degradant stock suspension were prepared separately in 0.2% hydroxypropyl methylcellulose (vehicle). Then, the Zileuton stock solution was combined with either the vehicle or the Degradant suspension at 1:1 v/v ratio. See the table listed below (excerpted from Vol. 18, page 18). The dose analysis revealed that during weeks 4-6, the concentration of ~~na/na~~ ranged from ~~na/na~~ or the 0.1 mg/mL formulation, and ~~na/na~~ for the 0.2 mg/mL formulation. From weeks 8-14, the formulations were within ~~na/na~~ of the nominal concentrations for ~~na/na~~.

Group	Initial Concentrations		Final Dosing Concentrations	
	Zileuton (Abbott- (mg/mL)	Degradant (Abbott- (mg/mL)	Zileuton (Abbott- (mg/mL)	Degradant (Abbott- (mg/mL)
1	0	0	0	0
2	8	0	4	0
3	8	0.2	4	0.1
4	8	0.4	4	0.2

to humans is considered limited. Liver tumor in the mouse were explainable based on enzyme induction and relevance of this tumor finding to humans is questioned. The renal tubular tumors were suggested to result from target organ of toxicity and increase cell proliferation. No explanation was provided for the increased incidence of hemangiosarcomas. However, it is noted that the metabolic profile for zileuton in rodents is different from that observed in humans. This difference raises the possibility that the findings observed in rodents could be secondary to metabolites of zileuton no present or present in very small quantities in humans, and therefore, not relevant to humans.

Labeling: Carcinogenesis, mutagenesis and impairment of fertility and pregnancy category C sections have been incorporated with the above-mentioned preclinical findings.

There are no outstanding preclinical issues.

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Joseph Sun  
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PHARMACOLOGIST/TOXICOLOGIST

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**PHARMACOLOGY/TOXICOLOGY REVIEW  
FOR CONSULTATION REQUEST**

**NDA number:** 22-052

**Request date:/Type of Request:** December 21, 2006/Review-General

**Requested by:** ONDQA/DPA1/Branch 2

**Information to sponsor:** Yes ( ) No (X)

**Sponsor and/or agent:** Critical Therapeutics

**Reviewer name:** Jean Q. Wu

**Division name:** Division of Pulmonary and Allergy Products

**HFD #:** 570

**Review completion date:** February 19, 2007

**Drug:**

Trade name:  (Zileuton) Extended Release Tablet

Active Drug: Zileuton

**Relevant INDs/NDAs/DMFs:** NDA 20-471

**Drug class:** Lipogenase inhibitor

**Intended clinical population:** Patients with Phrophylaxis in treatment of Chronic Asthma at age of 12 years and above

**Clinical formulation:** controlled-release (CR) tablet containing 600 mg zileuton

**Route of administration:** Oral

**Intended Dosage:** two tablets bid (2 x 600 mg bid = 2400 mg/day)

**Consultation requested:**

This chemistry consult was requested by the review chemist, Arthur Shaw, PhD to assess the acceptability of the proposed level of  identified impurities  for  for  an  for  of them  were qualified for Zyflo in the approved NDA 20-471 as the limits of  and , respectively.  a newly identified impurity.

**Review:**

**Study title:** A Three-Month Oral (Gavage) Safety Study of Abbott- Degradant) Administered With Zileuton Using Sprague Dawley Rats

**Key study findings:**

Satellite groups used for toxicokinetics or recovery: N/A  
Species/strain: Rat/Sprague Dawley  
Age: approximately seven weeks at initiation of the treatment  
Weight: males: 209-254 grams, females: 158-191 grams  
Sampling times: N/A

**Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.**

Mortality: Observed daily.

Clinical signs: Observed daily.

Body weights: recorded twice weekly for the first four weeks and weekly thereafter.

Food consumption: Recorded weekly.

Ophthalmoscopy: performed pre-treatment and near in-life termination (Day 82).

Hematology, Clinical chemistry and Urinalysis: Samples were taken at termination (Day 91 or 92)

Gross pathology: Complete gross necropsy was performed at the study termination (Day 91 or 92) or at the time of death.

Organ weights: The tissues/organs listed in the Table of Histopathology Inventory below were weighed.

Histopathology: The tissues listed in the Table of Histopathology Inventory below were preserved. Tissues were fixed in neutral buffered 10% formalin unless otherwise specified. Eyes were fixed in Davidson's solution and the testes were fixed in Bouin's solution. All preserved tissues from necropsied animals in the control and high dose groups were microscopically examined.

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

Peer review:    yes ( ), no ( x )

## Results

Mortality: No test article-related mortality was observed. One male in Group 4 was found dead on Day 6, which was attributed to mechanical trauma and not considered test article-related.

Clinical signs: There were no significant test article-related clinical observations reported in the study.

Body weights: There were no test article-related effects on body weight or body weight gain.

Food consumption: There were no test article-related effects on food consumption.

Ophthalmoscopy: Ophthalmological evaluation did not indicate any test article-related ocular abnormalities.

**Hematology, Clinical chemistry and Urinalysis:** There were no test article-related effects on measured clinical pathology parameters (including hematology, coagulation, chemistry and urinalysis).

**Gross pathology:** There were no test article-related adverse effects on gross pathology.

**Organ weights:** There were no test article-related effects on organ weights. The ratio of heart weight vs. body weight was decreased slightly in the group 4 males. However, the decrease was not observed for the absolute heart weight and was not associated to any abnormal histopathological findings.

**Histopathology:** There were no significant test article-related microscopic lesions observed in the histopathological evaluation. The observations listed in the table below and the other incidental observations were not considered toxicologically significant.

Findings	Males		Female	
	control	High dose	control	High dose
Dose group				
Number of animals	10	10	10	10
Lung/Bronchi, chronic inflammation	4 (1)	4(1)	2(1)	5(1)
Kidney (right), nephropathy	0	2 (1, 2)	1(2)	0
Kidney (left), nephropathy	0	2(1)	1(1)	0
Bone Marrow/Femur, hypoplasia	0	2(1,2)	0	0
Bone Marrow/sternum, hypoplasia	0	1(1)	0	0

**Histopathology inventory (optional)**

Study	3-month			
Species	rat			
Adrenals	X*			
Aorta	X			
Bone Marrow smear	X			
Bone (femur)	X			
Brain	X*			
Cecum	X			
Cervix				
Colon	X			
Duodenum	X			
Epididymis	X			
Esophagus	X			
Eye	X			
Fallopian tube				
Gall bladder				
Gross lesions	X			
Harderian gland				
Heart	X*			
Ileum	X			
Injection site	N/A			
Jejunum	X			
Kidneys	X*			

Lachrymal gland	X			
Larynx				
Liver	X*			
Lungs	X			
Lymph nodes, cervical				
Lymph nodes mandibular, mediastinal	X			
Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity				
Optic nerves	X			
Ovaries	X*			
Pancreas	X			
Parathyroid	X*			
Peripheral nerve				
Pharynx				
Pituitary	X*			
Prostate	X*			
Rectum	X			
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	X			
Skin	X			
Spinal cord	X			
Spleen	X*			
Sternum	X			
Stomach	X			
Testes	X*			
Thymus	X*			
Thyroid	X*			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	X			
Vagina	X			
Zymbal gland				

X, histopathology performed

\*, organ weight obtained

**Summary and Evaluation**

In the 3-month rat safety study of Abbott-~~XXXXXX~~ (degradant), the NOAEL was identified at high dose, 40/2 mg/kg/day of the Zileuton/Abbott-~~XXXXXX~~ combination.

According to the previous review of the study titled "Three-Month Oral Safety Study of Abbott-~~XXXXXX~~ and Abbott-~~XXXXXX~~ (Zileuton Impurities) in Rats" in NDA 20-471 (finalized on 8/16/1997), the NOAELs of 1.5 and 1.2 mg/kg/day were established for A-~~XXXXXX~~ and A-~~XXXXXX~~, respectively.

The safety margins of the identified impurities at the proposed specifications based on the NOAELs are listed below. A 10-fold safety margin based on the NOAELs in rat studies is required to qualify the identified impurities. Therefore, the proposed new specifications of \_\_\_\_\_ for \_\_\_\_\_ and \_\_\_\_\_ are not acceptable and the proposed new specification of \_\_\_\_\_ for \_\_\_\_\_ is acceptable.

Impurities/Degradants	NOAEL (mg/kg/day)	Proposed Specification (%)	Human intake* (mg/kg/day)	Safety Margin
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**Recommendation:**

The proposed new specifications of \_\_\_\_\_% for \_\_\_\_\_ and \_\_\_\_\_ are not acceptable and they should be lowered to NMT \_\_\_\_\_% for \_\_\_\_\_ and NMT \_\_\_\_\_ for \_\_\_\_\_. The proposed new specification of \_\_\_\_\_ for \_\_\_\_\_ is acceptable.

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

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/s/

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Jean Wu  
2/22/2007 12:12:28 PM  
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Joseph Sun  
2/22/2007 12:21:56 PM  
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I concur.

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**ADDENDUM 1**  
**PHARMACOLOGY/TOXICOLOGY REVIEW**  
**FOR CONSULTATION REQUEST**

**NDA number:** 22-052

**Request date/Type of Request:** December 21, 2006/Review-General

**Requested by:** ONDQA/DPA1/Branch 2

**Information to sponsor:** Yes ( ) No (X)

**Sponsor and/or agent:** Critical Therapeutics

**Reviewer name:** Jean Q. Wu

**Division name:** Division of Pulmonary and Allergy Products

**HFD #:** 570

**Review completion date:** March 2, 2007

**Drug:**

Trade name: \_\_\_\_\_ (Zileuton) Extended Release Tablet

Active Drug: Zileuton

**Relevant INDs/NDAs/DMFs:** NDA 20-471

**Drug class:** Lipogenase inhibitor

**Intended clinical population:** Patients with Phrophylaxis in treatment of Chronic Asthma at age of 12 years and above

**Clinical formulation:** controlled-release (CR) tablet containing 600 mg zileuton

**Route of administration:** Oral

**Intended Dosage:** two tablets bid (2 x 600 mg bid = 2400 mg/day)

**Consultation requested:**

This chemistry consult was requested by the review chemist, Arthur Shaw PhD to assess the acceptability of the proposed level of \_\_\_\_\_ identified impurities \_\_\_\_\_ for A \_\_\_\_\_, \_\_\_\_\_ for \_\_\_\_\_ and \_\_\_\_\_ for \_\_\_\_\_ of them ( \_\_\_\_\_ and \_\_\_\_\_, were qualified for Zyflo in the approved NDA 20-471 as the limits of \_\_\_\_\_% and \_\_\_\_\_, respectively. A \_\_\_\_\_ is a newly identified impurity.

In the original consultation review of February 22, 2007, a 3-month rat safety study of Abbott \_\_\_\_\_ (degradant) was reviewed and the specification of NMT \_\_\_\_\_ or \_\_\_\_\_ was recommended based on the NOAEL and 10-fold safety margin. In this addendum, two genotoxicity studies of Zileuton containing \_\_\_\_\_ impurity/degradant are reviewed.

**Review:**

**Study title:** Bacterial Reverse Mutation Assay (Ames Test) of Zileuton with Degradant Abbott-~~7~~

**Key study findings:**

Under the conditions of this study, Zileuton containing impurity/degradant Abbott-~~7~~ ( ~~7~~ w/w) did not cause a positive response either in the presence or absence of S9 activation.

**Study no.:** TX97-159

**Volume #, and page #:** Volume 18, page 363-408

**Conducting laboratory and location:** Abbott Laboratories  
Drug Safety Evaluation Division  
Abbott Park, IL 60064

**Date of study initiation:** June 3, 1997

**GLP compliance:** yes

**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:** Zileuton Lot# 02-911-AL, potency ~~7~~ mg/g (note: the potency was listed as ~~7~~ mg/g for the same lot in the 3-month toxicity study, Study No. TA 97-141). Abbott-~~7~~, Lot# 26881-45, potency= ~~7~~ mg/g  
No % purity is reported.

**Methods**

Strains/species/cell line:

*Salmonella typhimurium* bacteria TA98, TA100, TA1535 and TA1537; *Escherichia coli* WP2 *uvrA*;

Aroclor 1254-induced rat liver microsomes S9 fraction used as the metabolic activation system.

Doses used in definitive study:

One study was conducted.

Zileuton was dissolved in DMSO at a concentration of 100 mg/mL with 4.5 mg/mL of Abbott-~~7~~. For Zileuton, doses were 100, 300, 1000, 2000 and 5000 µg per plate For Abbott-~~7~~, the doses were 4.5, 13.5, 45, 90 and 225 µg per plate.

Basis of dose selection: No precipitation was observed on any of plates. Some toxicity (as would be indicated by reduced colony counts and the presence of small colonies indicative of toxicity) was observed with Zileuton at 5000 µg per plate in the nonactivation and S9 activation assays.

Negative controls: vehicle control, DMSO (dimethyl sulfoxide)

Positive controls: MNNG (N-methyl-N'-nitro-N-nitrosoguanidine), 9A (9-aminoacridine), NF (2-nitrofluorene), AA (2-aminoanthracene)

Incubation and sampling times: Vehicle control, positive controls and each dose of the test article were plated with culture of tester strains on selective agar in the presence and absence of S9. The plates were incubated for approximately 48 hours at approximately 37 °C.

## Results

Study validity : The numbers of colonies from the triplicate plates were recorded. The following criteria were used for evaluation:

1. TA 1535 and TA-1537: If the solvent control value was within the normal range, a test article that produced a positive dose response over three concentrations with the highest increase equal to three times the solvent control value, should be considered mutagenic.
2. TA-98, TA-100 and E. coli: If the solvent control value was within the normal range, a test article that produced a positive dose response over three concentrations with the highest increase equal to twice the solvent control value, should be considered mutagenic.
3. Pattern: Because TA-1535 and TA-100 are derived from the same parental strain to some extent, there was a built in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen, and such a pattern was sought.
4. Reproducibility: If a test article produced a response in a single test that could not be reproduced in additional runs, the initial positive test data lose significance.

The criteria were met and the study is valid. Positive controls in the presence or absence of S9 produced expected response in accordance with the criteria, which were similar to the historical positive control data (provided in addendum 2 of the report).

*Note: There were not evaluations at the concentrations higher than 2000 µg/plate and lower than the dose of 5000 µg/plate at which cytotoxicity was observed.*

Study outcome: Under the conditions of this study, Zileuton containing degradant Abbott- w/w relative to zileuton) did not cause a positive response in either the presence or absence of S9 activation.

Study title: In Vitro Cytogenetic Assay in Human Lymphocytes of Zileuton with Degradant Abbott

### Key findings:

- Under the assay conditions, zileuton containing the degradant, Abbott- did not show any clastogenic potential in human peripheral blood lymphocytes.

**Study no.:** TX97-160

**Volume #, and page #:** Volume 18, page 409-444

**Conducting laboratory and location:** Abbott Laboratories  
Drug Safety Evaluation Division



metaphases were read from each of the media or 1% DMSO control and the positive cultures. Criteria for an acceptable assay are negative control data with approximately 5% or less metaphases with aberrations, positive control data significantly higher than the negative control, and evidence of test article toxicity to approximately 50% reduction in mitotic index (number of mitotic cells x 100/1000 total cells). The mitotic index decreased 28%, 55.1% and 89.7% in non-activation assay, and 18.6%, 46.1% and 83.3% in activation assay at concentration of 93, 185 and 370 µg/mL, respectively. The criteria were met in the test. Numbers of aberration from the vehicle controls were within the vehicle control range and numbers of aberrations from the positive controls were within the historical positive control range (Addendum 2 of the report). Osmolarity was checked in the assay and stayed below the range of 600 mOsm which has been shown to increase chromosome aberrations in CHO cells.

Study outcome: The cultures treated with test article in the absence or presence of S9 activation system did not show statistically significant increase in aberrations over the vehicle control. Under the assay conditions, zileuton containing the degradant Abbott- did not show any clastogenic potential in human peripheral blood lymphocytes.

**Summary**

In the original consultation review on February 22, 2007, the NOAEL was identified at high dose, 40/2 mg/kg/day of the Zileuton/Abbott- combination, in the 3-month rat safety study of Abbott- (degradant). Based on 10-fold safety margin, it was recommended that the specification of should be lowered to NMT . In addition to the original consultation review, the genotoxicity studies of Zileuton containing impurity/degradant were reviewed in this addendum. is not an identified structure alert compound. The submitted genotoxicity studies showed that zileuton with ( w/w relative to zileuton) were negative in both bacterial reverse mutation assay and in vitro cytogenetics assay in human lymphocytes under the test conditions. The results have no impact on the recommended specification of NMT in the original consultation.

**Recommendation:**

As recommended in the original consultation review on February 22, 2007, the proposed limit of for / not acceptable and it should be lowered to NMT

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

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I concur.

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PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-052
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	07/31/06
PRODUCT:	ZYFLO XR™ (zileuton extended-release tablets)
INTENDED CLINICAL POPULATION:	Prophylaxis and treatment of chronic asthma in adults and children 12 years of age and older
SPONSOR:	Critical Therapeutics, Inc.
DOCUMENTS REVIEWED:	Vol. 11-23
REVIEW DIVISION:	Division of Pulmonary and Allergy Products
PHARM/TOX REVIEWER:	Jean Q. Wu
PHARM/TOX SUPERVISOR:	C. Joseph Sun
DIVISION DIRECTOR:	Badrul Chowdhury
PROJECT MANAGER:	Anthony Zeccola

Date of review submission to Division File System (DFS): March 29, 2007

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## ***EXECUTIVE SUMMARY***

### **I. Recommendations**

- A. Recommendation on approvability: Approvable
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling: See the suggested label changes in page 20.

### **II. Summary of nonclinical findings**

#### **A. Brief overview of nonclinical findings**

The toxicology of zileuton has been extensively evaluated in mice, rats, dogs and monkeys. In sub-chronic and chronic studies, the main target organs of toxicity were the liver, kidney, reproductive organs and hematopoietic systems. Findings included hepatocytomegaly associated with microsomal enzyme induction (rats and mice), prolongation of the estrus cycle and increased chronic nephritis /nephropathy (rats), and renal tubular atrophy and inflammation, renal tubular epithelial karyomegaly, reduced red blood cells parameters, neutropenia, thrombocytopenia and prostatic and testicular atrophy (dogs). There were no target organs of toxicity identified in monkey studies.

In 2-year carcinogenicity studies, increases in the incidence of liver, kidney, and vascular tumors in female mice and a trend toward an increase in the incidence of liver tumors in male mice were observed. An increase in the incidence of kidney tumors was observed in both male and female rats. The dose-related increase in benign Leydig cell tumors was observed in the testes of male rats, presumably due to a disruption of the hypothalamic-pituitary-gonadal axis as demonstrated by lower testosterone response following HCG challenge in Zileuton-treated rats. This mechanism of explanation was supported by a study report that Leydig cell tumorigenesis was prevented by replacement therapy with testosterone in male rats. An absence of any hormonal change in humans supports the absence of relevant risk for Leydig cell tumors in humans. Thus, relevancy of this tumor finding to humans is considered limited.

Zileuton was negative in battery of genotoxicity studies. However, increase in DNA adduct formation was reported in kidneys and livers of female mice treated with Zileuton. Some evidence of DNA damage was observed in unscheduled DNA synthesis (UDS) assay in hepatocytes isolated from Aroclor-1254 treated rats. But no such findings were observed in hepatocytes isolated from monkeys. The negative results of UDS assay in hepatocytes isolated from monkeys where the metabolic profile of zileuton is more similar to that of humans outweighed the concern for human exposure to Zileuton with regard to its rodent genotoxic and carcinogenic activities (liver and kidney).

Zileuton produces no effects on fertility in rats but reduced fetal implantation, increased gestation periods, prolongation of estrous cycle, decreased litter size, and increased stillbirths. An increase in skeletal variations and ossification delays were also observed in rats. Cleft palates or domed head and hydrocephalus were observed in rabbit fetuses and decreased pup weight and pup survival rate were noted in a rat perinatal/postnatal study.

**B. Pharmacologic activity**

Zileuton is an orally active, direct inhibitor of 5-lipoxygenase, a pivotal enzyme in the arachidonic acid cascade leading to the generation of leukotrienes. The efficacy of zileuton is due to binding to the lipoxygenase enzyme and the subsequent interaction of the enzyme's active site iron with N-hydroxyurea contained within zileuton. It has shown efficacy in numerous pharmacodynamic studies in various animal models, which include the arachidonic acid-induced ear edema model in mice, the pleural Arthus reaction in the rat, the acrylamide granuloma model in mice, inhibition of smooth muscle contraction in guinea pig, inhibition of arachidonic acid and antigen-induced bronchospasm in guinea pig, relieving bronchoconstriction and airway hyperreactivity in antigen-challenged sheep, and inhibition of allergic inflammation in a mouse model. Receptor for 5-oxo-E<sub>2</sub>E was highly expressed in human lung biopsy samples from severe asthmatics but not from normal controls.

**C. Nonclinical safety issues relevant to clinical use: None.**

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## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** NDA 22-052

**Review number:** 001

**Sequence number/date/type of submission:** 000/July 31, 2006/Original

**Information to sponsor:** Yes (X) No ( )

**Sponsor and/or agent:**

Critical Therapeutics, Inc.  
60 Westview Street  
Lexington, MA 02421

**Manufacturer for drug substance:**

**Finished Product Release:**

Critical Therapeutics, Inc.  
60 Westview Street  
Lexington, MA 02421

**Reviewer name:** Jean Q. Wu

**Division name:** Division of Pulmonary and Allergy Drug Products

**HFD #:** 570

**Review completion date:** March 29, 2007

**Drug:**

Trade name: ZYFLO XR™ (zileuton extended-release tablets) (changed in the submission of February 8, 2007 from the name of: ™ Controlled-Release Tablets in the original submission)

Generic name: Zileuton Extended-Release Tablets

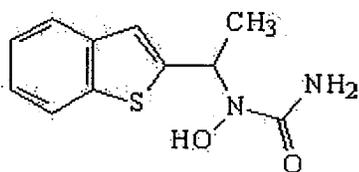
Code name: N/A

Chemical name: (+)-1-(1-Benzo[b]thien-2-ylethyl)-1-hydroxyurea

CAS registry number: 111406-87-2

Molecular formula/molecular weight: C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S/236.29

Structure:



**Relevant INDs/NDAs/DMFs:** NDA 20-471 (ZYFLO™, zileuton tablets), IND 30,661(zileuton tablets), IND 47,561 (zileuton CR tablets),

**Drug class:** 5-lipoxygenase inhibitor for inhibiting leukotriene synthesis

**Intended clinical population:** Prophylaxis and chronic treatment of asthma in adults and children 12 years of age and older.

**Clinical formulation:** ZYFLO XR extended-release (ER) tablet containing 600 mg zileuton (Excerpted from Vol. 2, Item 3.4 Page 078-079).

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Ingredient	Amount per Tablet (mg)			
	Formulation C	Formulation 4E	Formulation 56	Formulation 24
<b>Slow-Release Layer:</b>				
<b>Barrier Layer:</b>				



Study Title (Study No. CTI-02-P05-001R): The Effect of Zileuton and Montelukast in Allergen-Induced Airway Infiltration of Eosinophils and Neutrophils in a Mouse Model

Study Title (Study No. CTI-02-P06-004R): Expression of Receptor for 5-oxo-ETE in Human Asthmatic Airways

Study Title (Study No. CTI-03-P06-001R): Effect of Zileuton on hERG Tail Current Recorded from Stably Transfected HEK293 Cells

Study Title (Study No. CTI-03-P06-002R): Effect of A66193 on hERG Tail Current Recorded from Stably Transfected HEK293 Cells

Study Title (Study No. CTI-03-P06-003R): A Study of the Pharmacokinetic Behavior of Zileuton and Its Metabolite (A-66193) Following Single Oral Administration to Female New Zealand White Rabbits

Study (No. CTI-03-T06-001R): In Vitro Chromosomal Aberrations Test with A-66193

Study (No. CTI-03-T06-002R) Bacterial Mutation Test (Ames Test) with A-66193

**Studies not reviewed within this submission:**

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

Refer to NDA 20-471 for the numerous primary, secondary and safety pharmacology studies.

In addition, three new pharmacology studies were submitted in this application. Zileuton inhibited neutrophil and eosinophil influx, reduced the levels of multiple cytokines in BALF, and reduced serum IgE levels in a mouse model of allergic inflammation. The 5-oxo-EETE receptor was highly expressed in lung biopsy samples from severe asthmatics but not from normal controls. The inhibition of hERG tail current in HEK293 cells was not observed at 100  $\mu$ M of Zileuton but was significant at 200 and 400  $\mu$ M with  $IC_{25}$  of 278.6  $\mu$ M. The major metabolite, A-66193, did not result in a significant inhibition of hERG channel at concentration up to 100  $\mu$ M.

### 2.6.2.2 Primary pharmacodynamics

Mechanism of action: refer to NDA 20-471

#### **Study Title (Study No. CTI-02-P06-004R): Expression of Receptor for 5-oxo-EETE in Human Asthmatic Airways**

It has been suggested that the arachidonic acid metabolite 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-EETE) may be an important mediator in asthma. A cell membrane receptor for 5-oxo-EETE, the oxoeicosanoid (OXE) receptor has been described

and characterized. To investigate the expression of OXE receptors in asthmatic and normal lungs, sections from 5 severe asthmatics and 5 control subjects were stained with polyclonal antibodies using a peroxidase-antiperoxidase (PAP) technique. The data showed that the 5-oxo-ETE receptor (OXE receptor) was highly expressed in lung biopsy samples from severe asthmatics but not from normal controls. Staining was detected mostly in eosinophils, epithelial cells, mostly non-mucous-secreting cells and to a lesser extent in smooth muscle cells.

Drug activity related to proposed indication: refer to NDA 20-471

**Study Title (Study No. CTI-02-P05-001R): The Effect of Zileuton and Montelukast in Allergen-Induced Airway Infiltration of Eosinophils and Neutrophils in a Mouse Model**

Male BALB/c mice received i.p. injection of 100 µg ovalbumin (OVA) on Days 1 and 14. Intranasal (IN) allergen challenge of 100 µg OVA was given on Days 29, 30 and 31. Treatment with montelukast (0.17 mg/kg p.o., qd), zileuton (10 mg/kg p.o. qid), or a positive control, dexamethasone (4 mg/kg p.o. qd) was performed 30 minutes prior to each IN challenge. Bronchoalveolar lavage (BAL) was performed on Day 32, 24 hours post the last IN challenge. Both montelukast and zileuton treatments reduced total cell index (51% and 61%, respectively), inhibited eosinophils (>99%) in the BAL fluid, and improved airway hyperresponsiveness. Zileuton inhibited neutrophil influx (88%) much greater than montelukast (24%). Unlike montelukast, zileuton also reduced serum IgE levels (57.6%). Dexamethasone treatment also inhibited cell influx and cytokines in the BAL.

**2.6.2.3 Secondary pharmacodynamics**

Refer to NDA 20-471.

**2.6.2.4 Safety pharmacology**

Neurological effects: Refer to NDA 20-471. — (see Section 2.6.2.4 in Appendix 1).

Cardiovascular effects: Refer to NDA 20-471. — (see Section 2.6.2.4 in Appendix 1) and the following two study reviews.

**Study Title (Study No. CTI-03-P06-001R): Effect of Zileuton on hERG Tail Current Recorded from Stably Transfected HEK293 Cells**

The test article, zileuton, was formulated in DMSO and tested at final target concentrations of 100, 200 and 400 µM with 0.2% DMSO. The effects of the test article on a human ether-a-go-go-related gene (HERG)-encoded channel tail current recorded from human embryonic kidney 293 (HEK293) cells stably transfected with HERG cDNA was assessed with the whole-cell patch-clamp technique. The reference standard was E-4031 at 100 nM in bath solution. Exposure of zileuton at 100, 200 and 400 µM resulted in

11.4%, 24.6% and 37.2% inhibition on HERG tail current while vehicle control showed 7.3% inhibition and the reference compound, E-4301, showed 86.6% inhibition. A statistically significant inhibition was observed at 200 & 400  $\mu\text{M}$  of zileuton. There was no inhibition of hERG tail current at 100  $\mu\text{M}$ . The  $\text{IC}_{25}$  of was identified at 278.6  $\mu\text{M}$ .

**Study Title (Study No. CTL-03-P06-002R): Effect of A66193 on hERG Tail Current Recorded from Stably Transfected HEK293 Cells**

The test article, A-66193, was formulated in DMSO and tested at the final target concentration of 100  $\mu\text{M}$  with 0.2% DMSO. The effects of the test article on a human ether-a-go-go-related gene (HERG)-encoded channel tail current recorded from human embryonic kidney 293 (HEK293) cells stably transfected with HERG cDNA was assessed with the whole-cell patch-clamp technique. The reference standard was E-4031 at 100 nM in bath solution. Exposure of A-66193 at 100  $\mu\text{M}$  for approximately 15 minutes resulted in 17.5% inhibition on HERG tail current while vehicle control showed 12.9% inhibition and the reference compound, E-4301, showed 91.7% inhibition. Therefore, treatment with A-66193 at 100  $\mu\text{M}$ , the highest soluble concentration tested, did not result in significant inhibition of hERG tail current in HEK293 cells.

Pulmonary effects: — (see Section 2.6.2.4 in Appendix 1).

Gastrointestinal effects: Not available.

Others: N/A

**2.6.2.5 Pharmacodynamic drug interactions**

N/A

**2.6.3 PHARMACOLOGY TABULATED SUMMARY**

N/A

**2.6.4 PHARMACOKINETICS/TOXICOKINETICS**

**2.6.4.1 Brief summary**

Refer to NDA 20-471 for most pharmacokinetics/toxicokinetics studies.

In recent rabbit PK study, zileuton was absorbed rapidly following a single oral administration ( $T_{1/2}=1.57$  hours). The metabolite, A-66193, was present following the single dose of zileuton and its plasma exposure comprised 7.5% of the zileuton  $\text{AUC}_{0-\infty}$  in rabbits.

**2.6.4.2 Methods of Analysis:**

Refer to NDA 20-471.

**2.6.4.3 Absorption**

Refer to NDA 20-471.

**Study Title (Study No. CTI-03-P06-003R): A Study of the Pharmacokinetic Behavior of Zileuton and Its Metabolite (A-66193) Following Single Oral Administration to Female New Zealand White Rabbits (vol. 23, page 208)**

Four female New Zealand White rabbits were given a single oral dose of Zileuton (Lot No. 3048372) at 50 mg/kg with a dose volume of 4 mL/kg. Blood samples were collected at pre-dose and the selected time points up to 48 hours post dose. Plasma samples were analyzed for zileuton and its metabolite using LC-MS/MS method. The PK parameters were summarized in the table below.

	AUC <sub>0-t</sub> (ng.hr/mL)	AUC <sub>0-∞</sub> (ng. hr/mL)	C <sub>max</sub> (ng/mL)	T <sub>1/2</sub> (hr)	T <sub>max</sub> (hr)	CL (mL/hr.kg)
zileuton	44502.5	45581.3	8050.85	1.57	3.13	1156.3
A-66193	2704.2	3441.2	483.38	3.91	4.75	NC

**2.6.4.4 Distribution**

Refer to NDA 20-471.

**2.6.4.5 Metabolism**

Refer to NDA 20-471.

**2.6.4.6 Excretion**

Refer to NDA 20-471.

**2.6.4.7 Pharmacokinetic drug interactions**

Refer to NDA 20-471.

**2.6.4.8 Other Pharmacokinetic Studies**

Refer to NDA 20-471.

**2.6.4.9 Discussion and Conclusions**

N/A

**2.6.4.10 Tables and figures to include comparative TK summary**

N/A

**2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

N/A

**2.6.6 TOXICOLOGY**

**2.6.6.1 Overall toxicology summary**

Refer to NDA 20-471 for overall toxicology evaluation.

Additional genotoxicity studies of the major metabolite, A-66193, showed that A-66193 was negative in a bacterial mutation test and in an *in vitro* mammalian chromosome aberration assay.

**2.6.6.2 Single-dose toxicity**

Refer to NDA 20-471.

**2.6.6.3 Repeat-dose toxicity**

Refer to NDA 20-471

**2.6.6.4 Genetic toxicology**

Refer to NDA 20-471 for genotoxicity studies of Zileuton and Ames Test studies of the metabolites.

**Study title:** Bacterial Mutation Test (Ames Test) with A-66193**Key study findings:**

No substantial increases in the revertant colony counts were obtained with any test strains following exposure to A-66193 in the presence and absence of S9 mix. Therefore, under the conditions of this study, A-66193 did not show any evidence of mutagenic activity in the presence and absence of S9 activation.

**Study no.:** CTI-03-T06-002 ( ) No. 961211)**Volume #, and page #:** Volume 17, page 80-129**Conducting laboratory and location:****Date of study initiation:** April 26, 2006**GLP compliance:** yes**QA report:** yes (X) no ( )**Drug, lot #, and % purity:** A-66193, Reference # DUD/RCX2130/23/3; Purity = 100 %.**Methods****Strains/species/cell line:**

*Salmonella typhimurium* bacteria TA1535 *hisG46 rfa ΔuvrB*, TA1537 *hisC3076 rfa ΔuvrB*, TA98 *hisD3052 rfa ΔuvrB* pKM101, TA100 *hisG46 rfa ΔuvrB* pKM101, *Escherichia coli* WP2 *trp uvrA*;

Phenobarbital/5,6-benzoflavone induced SD rat liver S9 fraction used as the metabolic activation system.

**Doses used in definitive study:**

A-66193 was dissolved in DMSO in a stock solution at concentration of 50 mg/mL. The doses for confirmatory test (definitive study) were 5.0, 15.8, 50, 158, 500 and 1581, 5000 µg per plate.

Basis of dose selection: The precipitation was observed at highest dose level in both initial and confirmatory tests. In the initial test, toxicity as indicated by reduced colony counts following exposure to A-66193 at the highest level tested (5000 µg per plate).

Negative controls: vehicle control, DMSO (dimethyl sulfoxide)

Positive controls:

In the absence of S9: NaAz (sodium azide), 9AC (9-aminoacridine), 2NF (2-nitrofluorene);

In the presence of S9: 2AA (2-aminoanthracene), BaP (benzo[a]pyrene)

Incubation and sampling times: In the initial test, vehicle control, positive controls and each dose of the test article were plated with culture of tester strains on selective agar in the presence and absence of S9. In the confirmatory test, the

## Results

Study validity: The triplicate plates per dose were examined visually and, if necessary, with the aid of an inverted microscope. Revertant colony counts were routinely collected using an automated colony counter (version 3.6, Symbiosis) unless there were precipitation or other artifacts interfered with the colony counter. The plates from at least five non-toxic dose levels of the test article were assessed in each experiment.

The following criteria were used for evaluation:

*Positive:* If treatment with the test article produced a dose-related increase in revertant colony numbers to at least 2 X the concurrent vehicle control with any strain (1.5 X for TA100) in the presence and absence of S9.

*Negative:* If treatment with the test article did not produce a dose-related increase of at least 1.5 (for TA100) or 2 (for any other strains) times the concurrent vehicle controls, it was considered to show no evidence of mutagenicity activity in the test system.

*Equivocal:* If the results obtained failed to satisfy the criteria for a clear "positive" or "negative" response, the results were considered equivocal.

The revertant colony counts of the vehicle controls were closed to or within the laboratory historical control range. The positive controls produced increase in revertant colony numbers at least 1.5 (for TA100) or 2 (for any other strains) times the concurrent vehicle controls. Therefore, the study is considered valid.

Study outcome: No substantial increases in the revertant colony counts were obtained with any test strains following exposure to A-66193 in the presence and absence of S9 mix. Therefore, under the conditions of this study, A-66193 did not show any evidence of mutagenic activity in the presence and absence of S9 activation.

**Study title:** In Vitro Mammalian Chromosome Aberration Test with A-66193

**Key findings:**

Under the assay conditions, A-66193 did not show any evidence of clastogenic potential in human peripheral blood lymphocytes.

**Study no.:** CTI-03-T06-001R ( ~~CTI-03-T06-001R~~ No. 961192)

**Volume #, and page #:** Volume 17, page 039-079

**Conducting laboratory and location:**

**Date of study initiation:**

April 26, 2006

**GLP compliance:** yes

**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:** A-66193, Reference # DUD/RCX2130/23/3; Purity:

**Methods**

Strains/species/cell line:

Human peripheral lymphocytes ± rat liver S9 fraction

Doses used in definitive study:

A-66193 was dissolved in DMSO in a stock solution at concentration of 50 mg/mL. The doses selected were 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 50, 100 and 200 µg/mL.

Basis of dose selection: The toxicity of the test article was assessed by evaluating changes in the mitotic indices relative to the solvent control. The precipitation was visible at concentration of 200 µg/mL in the culture media. The concentrations chosen for metaphase evaluation were 50, 100 and 200 µg/mL. Mitotic indices were decreased slightly at highest concentration tested. RMI was 95% and 84% of control after 4 hours in the presence of S9 and 21 hours treatment in the absence of S9, respectively.

Negative controls: DMSO

Positive controls:

MMC, Mitomycin C (0.1 µg/mL) and CP, Cyclophosphamide (8.0 µg/mL) were used as positive controls for the non-activated and activated assays, respectively.

Incubation and sampling times: The duplicate cultures were prepared for each test concentrations.

## Results

Study validity: Duplicate cultures were used. At least 2 slides per culture were stained. Metaphases were selected on the basis of good morphology. Mitotic index was determined by examination at least 500 cells per culture for the selected treatment groups. The relative mitotic index (RMI) was calculated as a percentage ratio compared with the concurrent vehicle control group. For each regime and phase, the highest dose level selected for examination of aberration is the highest dose level tested in the case of test article showing relatively low toxicity or the lowest concentration which causes a reduction in the RMI to below 50%. In this case, the highest dose level tested which precipitated in culture medium and the next two lower dose levels were selected for examination. Slides selected for examination of aberration were examined by microscopy and a total of 200 readable metaphases per experimental point were examined using oil-immersion optics.

A positive response is indicated by a statistically significant increase in incidence of aberrant cells for the treatment group compared with the concurrent control group. A negative result is indicated where the incidences of aberrant cells for the treatment group are not significantly greater than the incidences for the concurrent control group, and where these values fall within or close to the historical control range. An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response. Numbers of aberration from the vehicle controls were within the vehicle control range. The positive control produced a significant increase in the incidence of aberrant cells compared with the concurrent control. The study is considered valid.

Study outcome: A-66193 did not cause any statistically significant increases in the proportion of aberrant metaphases at any experimental point. Under the assay conditions, A-66193 did not show any evidence of clastogenic potential in human peripheral blood lymphocytes.

### 2.6.6.5 Carcinogenicity

Refer to NDA 20-471.

### 2.6.6.6 Reproductive and developmental toxicology

Refer to NDA 20-471.

### 2.6.6.7 Local tolerance

Not applicable for the intended route of administration.

### 2.6.6.8 Special toxicology studies

Refer to NDA 20-471

**2.6.6.9 Discussion and Conclusions**

N/A

**2.6.6.10 Tables and Figures**

N/A

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

N/A

**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

## Conclusions:

ZYFLO™ (zileuton tablets) in the immediate release (IR) dosage form was originally developed by Abbott Laboratories and was approved on December 9, 1996 (NDA 20-471). The products, including zileuton, of Abbott Laboratories were later acquired by Critical Therapeutics, Inc.

Zileuton is a direct inhibitor of 5-lipoxygenase, a pivotal enzyme in the arachidonic acid cascade leading to the generation of leukotrienes. The drug substance is identical for both zileuton IR and zileuton ER (extended-release) tablets. The approved clinical dose of zileuton (IR) for the indication of prophylaxis and chronic treatment of asthma is 600 mg q.i.d. in adults and children 12 years of age and older. The zileuton ER is intended to use both as monotherapy and as adjunctive therapy in patients with moderate, chronic asthma. The proposed clinical dose for ZYFLO XR™ is two tablets (600 mg/tablet) b.i.d. in adults and children 12 years of age and older. The human systemic exposure of Zileuton in ER formulation (ZYFLO XR) is 64 µg.hr/mL, and the exposure in IR formulation (ZYFLO) is 77.4 µg.hr/mL under fed condition (bioavailability study CTI-03-C-05-103).

Zileuton is an orally active, specific inhibitor of 5-lipoxygenase. It has shown efficacy in numerous pharmacodynamic studies in various animal models, which include the arachidonic acid-induced ear edema model in mice, the pleural Arthus reaction in the rat, the acrylamide granuloma model in mice, inhibition of smooth muscle contraction in guinea pig, inhibition of arachidonic acid and antigen-induced bronchospasm in guinea pig, relieving bronchoconstriction and airway hyperreactivity in antigen-challenged sheep, and inhibition of allergic inflammation in a mouse model.

The toxicology of zileuton has been extensively evaluated in mice, rats, dogs and monkeys. In sub-chronic and chronic studies, the main target organs of toxicity were the liver, kidney, reproductive organs and hematopoietic systems. Findings included hepatocytomegaly associated with microsomal enzyme induction (rats and mice), prolongation of the estrus cycle and increased chronic nephritis/nephropathy (rats), and renal tubular atrophy and inflammation, renal tubular epithelial karyomegaly, reduced red blood cells parameters, neutropenia, thrombocytopenia and prostatic and testicular

atrophy (dogs). There were no target organs of toxicity identified in monkey studies. The No-Observed-Adverse-Effect-Level s (NOAELs) of the major toxicity studies and the corresponding safety margin were listed in the table below (based on previous review of NDA 20-471 and the summary table 1 on Page 141 of Vol. 2 in the current submission).

Species	Study	Sex	NOAEL (mg/kg)	AUC (µg.hr/mL)	Safety Margin	Sampling Period
Human	<sup>1</sup> Bio (IR)	M/F	N/A	77.4	N/A	Day 6
	<sup>1</sup> Bio (ER)	M/F	N/A	64.0	N/A	Day 6
Dog	6-mon	M/F	20	288.5	4.5	Week 22
	1-year	M/F	10	115	1.8	Week 48
Monkey	1-year	M/F	500	32.8 <sup>2</sup>	0.5 <sup>2</sup>	Day 153
Rat	1-year	M	50	263.4	4.1	Day 345
		F		596.8	9.3	
	<sup>3</sup> 2-year (diet)	M	170	498	7.8	Week 75-76
		F		1043	16.3	
Mouse	<sup>3</sup> 2-year	M	450	529	8.3	Week 93-94
		F		340	5.3	

1. Bioavailability study CTI-03-C05-103. IR- zileuton immediate release formulation, ER- zileuton extended release formulation.

2. There are differences in Zileuton metabolism and pharmacokinetics among species. In monkey, high plasma level of the major metabolite, A-66193 (267.2 µg.hr/mL) was observed, which is approximately 3 times the clinical exposure of A-66193 following ZYFLO XR administration. No target organ of toxicity was identified in the monkey studies.

3. 2-year carcinogenicity study

In 2-year carcinogenicity studies, increases in the incidence of liver, kidney, and vascular tumors in female mice and a trend toward an increase in the incidence of liver tumors in male mice were observed at 450 mg/kg/day. In rats, an increase in the incidence of kidney tumors was observed at 170 mg/kg/day. The dose-related increase in benign Leydig cell tumors was observed in the testes of male rats at dose levels of 40, 80 and 170 mg/kg/day, presumably due to a disruption of the hypothalamic-pituitary-gonadal axis as demonstrated by lower testosterone response following HCG challenge in Zileuton-treated rats. This mechanism of explanation was supported by a study report that Leydig cell tumorigenesis was prevented by replacement therapy with testosterone in male rats. An absence of any hormonal change in humans supports the absence of relevant risk for Leydig cell tumors in humans. Thus, relevancy of this tumor finding to humans is considered limited.

Zileuton was negative in battery of genotoxicity studies. However, increase in DNA adduct formation was reported in kidneys and livers of female mice treated with Zileuton. Some evidence of DNA damage was observed in unscheduled DNA synthesis (UDS) assay in hepatocytes isolated from Aroclor-1254 treated rats. But no such findings were observed in hepatocytes isolated from monkeys. The negative results of UDS assay in hepatocytes isolated from monkeys where the metabolic profile of zileuton is more similar to that of humans outweighed the concern for human exposure to Zileuton with regard to its rodent genotoxic and carcinogenic activities (liver and kidney).

Zileuton produces no effects on fertility in rats at oral doses up to 300 mg/kg/day but reduced fetal implantation and decreased pup weight at oral doses of 150 mg/kg/day and higher. Increase in gestation periods, prolongation of estrous cycle, decreased litter size, and increase in stillbirths were observed at 70 mg/kg/day and/or higher. An increase in skeletal variations and ossification delays were observed in rats at 300 mg/kg/day in rat. Three of 118 rabbit fetuses had cleft palates and two rabbit fetuses had domed head and hydrocephalus at dose of 150 mg/kg/day (the highest dose tested). In a perinatal/postnatal study in rats, decreased pup weight and pup survival rate were noted at 300 mg/kg/day.

The metabolite A-66193 was 5-fold higher in human exposure for CR formulation than IR formulation. The toxicology studies submitted in NDA 20-471 indicated the exposure of A-66193 in monkey and rat are approximately 2-3 folds of the human exposure of A-66193 under fed condition [93 µg.hr/mL] at proposed maximum human dose of CR formulation. The bacterial mutation test and in vitro mammalian chromosome aberration test with A-66193 were negative. Treatment with 100 µM of A-66193 did not result in a significant inhibition of hERG channel in HEK293 cells.

Based on the information referred to approved IR formulation of Zileuton, ZYFLO (NDA 20-471), and a few additional pharmacology and toxicology data provided in this submission, the proposed use of ZYFLO XR™ Controlled-Release Tablets is considered approvable from a preclinical perspective.

Unresolved toxicology issues (if any): None

Recommendations: The proposed use of ZYFLO XR™ (zileuton extended-release tablets) is approvable from a preclinical perspective.

Suggested labeling:

1. Section 10, **OVERDOSAGE**. In the 2<sup>nd</sup> paragraph, since no additional information submitted to support the changes made in the proposed ZYFLO XR™ label from the current ZYFLO™ label, suggest add multiples of the human exposure at the determined lethal doses as reflected in the current ZYFLO™ label.

The oral minimum lethal doses in mice and rats were 500-4000 and 300-1000 mg/kg, respectively (providing greater than 3 and 9 times the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose, respectively). In dogs, at an oral dose of 1000 mg/kg (providing in excess of 12 times the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose), no deaths occurred but nephritis was reported.

2. Section 12.1 **Mechanism of Action**. The statements in the 1<sup>st</sup> paragraph recommended to be removed are additional to the current ZYFLO™ label. Modify the section as suggested below or provide the supporting information/reference for the proposed additional statements and update the current ZYFLO™ label.

1<sup>st</sup> paragraph

3. Section 12.2 Pharmacodynamics. Zileuton as an active inhibitor of LTB<sub>4</sub> formation in the species other than human has been discussed in the previous section (12.1).

Zileuton is an orally active inhibitor of *ex vivo* LTB<sub>4</sub> formation in human.

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

**APPENDIX 1**

Appears This Way  
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