CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-006

PHARMACOLOGY REVIEW(S)

Comments on N22288 bepotastine besilate Bepreve
From A. Jacobs
8/27/09

I concur that there are no outstanding pharm/tox issues and the pregnancy category should be C, since the adverse effects might be applicable to the use of the API for other indications for which systemic exposure might be considerably higher.

My previous comments to the reviewer on the pharm/tox portion of labeling and on the pharm/tox review have been addressed.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22288	ORIG 1	ISTA PHARMACEUTICALS	BEPOTASTINE BESILATE OPHTHALMIC SOLUTION

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ABIGAIL ABBY C C JACOBS
08/27/2009

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:	**22-288**
SERIAL NUMBER:	**0000, 0008, 0014, 0016 and 0017**
DATE RECEIVED BY CENTER:	**11/14/08**
PRODUCT:	**Bepreve™**
INTENDED CLINICAL POPULATION:	**allergic conjunctivitis in patients aged 3 years and older**
SPONSOR:	**ISTA, Pharmaceuticals®, Inc., Irvine, CA**
DOCUMENTS REVIEWED:	**Module 4 Documents submitted 11/14/2008 and 3/11/2009, 5/29/2009, 6/5/2009 and 6/12/2009**
REVIEW DIVISION:	**Division of Anti-Infective and Ophthalmology Products (HFD-520)**
PHARM/TOX REVIEWER:	**Theresa Allio, Ph.D.**
PHARM/TOX SUPERVISOR:	**Wendelyn Schmidt, Ph.D.**
DIVISION DIRECTOR:	**Wiley Chambers, M.D.**
PROJECT MANAGER:	**Rafael Rodriguez**

Date of review submission to Division File System (DFS): July 21, 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

There are no objections to the approval of this NDA from a pharm/tox perspective

B. Recommendation for nonclinical studies

No additional nonclinical studies are recommended.

C. Recommendations on labeling

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogensis, Mutagenesis and Impairment of Fertility

Long term dietary studies in mice and rats were conducted to evaluate the carcinogenic potential of bepotastine besilate. Bepotastine besilate did not significantly induce neoplasms in mice receiving a nominal dose of up to 200 mg/kg/day for 21 months or rats receiving a nominal dose of up to 97 mg/kg/day for 24 months. These dose levels represent systemic exposures approximating 350 and 200 times that achieved with human topical ocular use. The no observable adverse effect level for bepotastine besilate based on nominal dose levels in these carcinogenicity tests were 18.7 to 19.9 mg/kg/day in mice and 9.58 to 9.8 mg/kg/day in rats (representing exposure margins of 60 and 17.7 times the systemic exposure anticipated for human topical ocular use).

There was no evidence of mutagenicity in the Ames test, in CHO cells (chromosome aberrations), in mouse hepatocytes (unscheduled DNA synthesis) or in the mouse micronucleus test.

When oral bepotastine was administered to male and female rats at doses up to 1,000 mg/kg/day, there was a slight reduction in fertility index and surviving fetuses. Infertility was not seen in rats given 200 mg/kg/day oral bepotastine besilate (approximately 3330 times the maximum systemic concentration anticipated for topical ocular use in humans).

8.1 Pregnancy

Pregnancy Category C: Teratogenicity studies have been performed in animals. Bepotastine besilate was not found to be teratogenic in rats at oral doses up to 200 mg/kg/day (representing a maximal systemic concentration approximately 3300 times that anticipated for topical ocular use in humans), but did show some potential for causing skeletal abnormalities at 1000 mg/kg/day. There were no teratogenic effects seen in rabbits at oral doses up to 500 mg/kg/day (>13,000 times the dose in humans on a mg/kg basis).

Evidence of infertility and conceptus loss was seen in rats given oral bepotastine besilate 1000 mg/kg/day; however, no evidence of infertility was observed in rats given 200 mg/kg/day (representing a maximal systemic concentration approximately 3330 times that anticipated for topical ocular use in humans). The concentration of radiolabeled bepotastine besilate was similar in fetal liver and maternal blood plasma following a single 3 mg/kg oral dose. The concentration in other fetal tissues was one-third to one-tenth the concentration in maternal blood plasma. There are no adequate and well-controlled studies of bepotastine besilate in pregnant women. Because animal

reproduction studies are not always predictive of human response, Brepreve™ (bepotastine besilate ophthalmic solution) 1.5% should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

8.2 Labor and Delivery In rats given oral doses of 100 mg/kg/day, an increased incidence of stillborns were observed. At the 1000 mg/kg/day dose level in this same study, an increase in stillborns, decreased survival and decreased rate of development were observed in pups. There were no effects observed in rats treated with 10 mg/kg/day (representing a maximal systemic concentration less than 93 times that anticipated for topical ocular use in humans). There are no adequate and well-controlled studies in pregnant women. Brepreve™ (bepotastine besilate ophthalmic solution) 1.5% should be used during labor and delivery only if the potential benefit justifies the potential risk do the fetus.

8.3 Nursing Mothers
Reduced nursing was observed in lactating rats given an oral dose of 1,000 mg/kg/day. A reduction in nursing was not observed in rats given 100 mg/kg/day (representing a maximal systemic concentration slightly higher than 957X times that anticipated for topical ocular use in humans). Following a single 3 mg/kg oral dose of radio-labeled bepotastine besilate to nursing rats 11 days after delivery, the maximum concentration of radioactivity in milk was 0.40 µg eq/mL 1 hour after administration; at 48 hours after administration the concentration was below detection limits. The milk concentration was higher than the maternal blood plasma concentration at each time of measurement. It is not known if bepotastine besilate is excreted in human milk. Caution should be exercised when **Bepreve™** (bepotastine besilate ophthalmic solution) 1.5% is administered to a nursing woman.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings
Bepotastine besilate in the Bepreve™ formulation did not cause ocular inflammation or histopathologic changes in rabbits or dogs. There are some data that suggest that Bepreve™ may have an affinity for melanin binding based on the observation of presence in the iris in an ocular toxicity study and to pigmented tissues in a radiolabeled study. This association with melanin appears to be reversible, reaching levels below limit of detection when given enough time for clearance after dosing (e.g. 30 days after single dose of radiolabeled compound, bepotastine besilate was no longer detected in pigmented tissues).

The pivotal study for the proposed indication was a 26 week study in dogs using 4 and 8X per day dosing with the 1.5% TAU-284 solution. The 4X/day dosing paradigm was determined to be the NOAEL based on decreases in A and B wave amplitude in electroretinograms (ERG) in the 8X/day dose group. When considering systemic exposures seen in this study, the identified NOAEL for ERG endpoints provides a 15X safety factor over that of anticipated systemic exposures seen with topical ocular use in humans. Several short term ocular toxicity studies demonstrated that bepotastine besilate solutions up to 2% in concentration were well tolerated in various animal species.

Bepreve™ also did not cause strong hypersensitivity reactions with repeated use.

Although bepotastine besilate appears to be a substrate for Cyp450 metabolism in rodents, it does not appear to be a target/inhibitor of human CYP450 enzymes. In both rats and dogs, test article is primarily excreted in feces and urine. Additional information may be found in the clinical pharmacology review.

The exec-CAC concluded that bepotastine besilate did not significantly induce neoplasms in 2 year dietary carcinogenicity studies in mice (at margin of exposure relative to human after ophthalmic use of 353) or in rats (at a margin of exposure relative to human of 200) .

As an antihistamine, bepotastine besilate, may eventually be used for other indications besides ocular. Pregnancy category C is recommended for this product due to the observation of a rare skeletal malformation seen in the fertility/early embryo development study in rats at the 1000 mg/kg dose. The approximate margin of exposure for the 200 mg/kg/day NOAEL identified in this study was 3,300X that of anticipated human systemic exposure with topical ocular use. In rats given oral doses of 100 mg/kg/day, an increased incidence of stillborns were observed (~200X human systemic exposure for ocular use). At the 1000 mg/kg/day dose level in this same study, an increase in stillborns, decreased survival and decreased rate of development were observed in pups. There were no effects observed in rats treated with 10 mg/kg/day (representing a maximal systemic concentration approximately 18 times that anticipated for topical ocular use in humans).

From a radiolabeled study in pregnant rats, it is recognized that bepotastine besilate can rapidly distribute to the yolk sac/placenta and to the fetus. Bepotastine besilate was transferred to the yolk sac/placenta at levels nearly equivalent to maternal maximal plasma concentration, ~33-55% of bepotastine besilate was transferred to the developing fetus. At 24 hours following a single oral administration of 3 mg/kg, ~ 5.9% and 3.1% of maximal plasma TAU-284 concentrations were detected in the brain and liver of the fetus at 24 hours postdose. Bepotastine besilate was also noted to be transferred to milk in lactating rats, with milk concentrations being 1.5 to 2 times maximal plasma concentrations by 1 hour postdose and reaching levels below the limit of detection by 48 hours postdose.

B. Pharmacologic activity

Bepotastine besilate demonstrated ability in preventing histamine induced inflammation and edema in Guinea pig and rabbit models. The sponsor believes that this activity is due to antagonism of the histamine H1 receptor, although direct enzyme kinetic measurements were not evaluated.

C. Nonclinical safety issues relevant to clinical use

None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

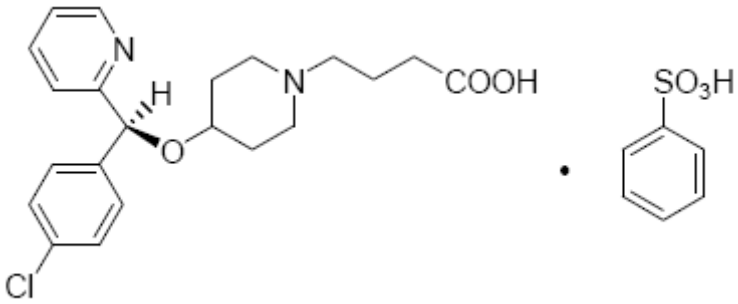
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-288
Review number: 0000
Sequence number/date/type of submission: 0000/14 November 2008/original NDA
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: ISTA, Pharmaceuticals®, Inc., Irvine, CA
Manufacturer for drug substance: Ube Industries, Inc., Yamaguchi Prefecture, Japan

Reviewer name: Theresa Allio, Ph.D.
Division name: DAIOP
HFD #: 520
Review completion date: Draft 3, June 17, 2009

Drug:

Trade name: Bepreve™
Generic name: bepotastine besilate
Code name: TAU-284
Chemical name: (S)-4[4-[(4-chlorophenyl) (2-pyridyl) methoxyl] piperidino] butyric acid monobenzenesulfonate
CAS registry number: 190786-44-8
Molecular formula/molecular weight: $C_{21}H_{25}ClN_2O_3 \cdot C_6H_6O_3S$/ 547.06
Structure:



Relevant INDs/NDAs/DMFs: 66,864 (IND), 19,966 (DMF)

Drug class: Histamine H_1 receptor antagonist

Intended clinical population: allergic conjunctivitis in patients ages 3 years and older

Clinical formulation: The table below was taken from the Sponsor's NDA.

Table 1. Drug Product Quantitative Composition of Bepreve™ (Bepotastine Besilate Ophthalmic Solution)

Components	Function	Amount/mL	(b) (4) Batch Composition	(b) (4) Batch Composition	% w/v
Bepotastine besilate	Active pharmaceutical ingredient	15 mg[1] or 10 mg[2]	(b) (4)		1.5%[1] or 1.0%[2]
Sodium chloride					(b) (4)
Monobasic Sodium Phosphate, Dihydrate					
Benzalkonium chloride	Preservative	0.05 mg			(b) (4)
Sodium hydroxide	pH adjuster	qs to pH 6.8			
Water for Injection	Solvent	qs			

[1] For Bepreve™ 1.5% drug product

[2] For Bepreve™ 1.0% drug product

Route of administration: ocular instillation

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

Studies reviewed within this submission:

Primary pharmacodynamics

SNJ-HO-1 Inhibitory Effect of TAU-284 on Histamine-Provoked Acute Conjunctival Edema on Guinea Pigs

SNJ-HO-2 Suppressive Effects of TAU-284 on Allergic Conjunctivitis in Guinea Pigs

SNJ-HO-3 Inhibitory Effect of TAU-284 Ophthalmic Solution for Passively Sensitized Allergic Conjunctivitis in Guinea Pigs – Study of Minimal Effective Concentration and Effective Saturation Concentration

SNJ-HO-4 Suppression Effects of TAU-284 Ophthalmic Solutions on Passively Sensitized Allergy Conjunctivitis in Guinea Pigs: Efficacy Comparison with Similar Drugs

SNJ-HO-5 Inhibition Effect of Bepotastine Besilate (TAU-284) Ophthalmic Solution on Passively Sensitized Allergic Conjunctivitis in Guinea Pigs – Examination of Effect Duration

SNJ-HO-6 Effect of TAU-284 on Histamine-Induced Conjunctivitis in Guinea Pigs

SNJ-HO-12 Comparative Investigation of the Inhibitory Actions of TAU-284 and Ketotifen on Protein Secretion from Rabbit Lacrimal Glands

SNJ-HO-10 Inhibitory Action of TAU-284 on Histamine Release from Rat Peritoneal Mast Cells (Preliminary Investigation)

SNJ-HO-11 Effect of TAU-284 on Chemotaxis of Peritoneal Eosinophils in Guinea Pigs

SNJ-HO-9 Inhibitory Effect of the TAU-284 Ophthalmic Solution on Passive Sensitization Allergic Conjunctivitis in Rats

SNJ-HO-8 Drug Efficacy Comparative Study of TAU-284 and Other Drugs in a Guinea Pig Active Sensitization Model

Safety Pharmacology

TNB-HO-12 Effects of a Novel Anti-Allergy Drug Bepotastine Besilate (TAU-284) on Canine Electrocardiograms

TNB-HO-15 Binding Inhibition Activity of Bepotastine Besilate (TAU-284), a New Anti-Allergy Drug, in Relation to Various Types of Receptors – a Comparison with Other Drugs

Methods of Analysis

SNJ-NI-7 Validation Study of a Measurement Method for the Concentration of Bepotastine Besilate

Absorption

SNJ-HE-1 The Dynamic Examination of Applying Eye Drop Medication TAU-284 – The Distribution Test Within An Eye Tissue When Carrying Out Single Time Medication of the 1.5% ^{14}C-TAU-284 Eye Drop Liquid in a Colored Rabbit

TNB-HO-14 Blood Plasma Concentration Transition in Guinea Pigs During Inhibitory Activity Testing of a New Anti-allergy Drug Bepotastine Besilate (TAU-284) in Relation to Histamine-induced Shock

TNB-HE-04 Internal Dynamics of Bepotastine Besilate in Canines

Distribution

TNB-HE-05 Nerve Transfer of Bepotastine Besilate (TAU-284)

Metabolism

TNB-HE-07 Metabolism of Bepotastine Besilate (TAU-284) in Human Liver Microsomes and the Main Effects on P450 Activity

TNB-NI-28 Effects of Bepotastine Besilate on Mouse Liver Cytochrome P-450—Using Testosterone Metabolic Activity and Alkoxyresorufin O-Dealkylation Activity as Indices

TNB-NI-27 Influence of Bepotastine Besilate on Hepatic Cytochrome P-450 in Rats – Using Testosterone Metabolism Activity as the Index

Excretion

TNB-HE-06 Internal Dynamics of Bepotastine Besilate (TAU-284) in Canines- Effects of Concomitant Use of Probenecid

Single-dose Toxicity

TNB-NI-01 Acute Toxicity Study on Bepotastine Besilate – Single Dose Oral Toxicity Study in Rats

TNB-NI-02 Acute Toxicity Study on Bepotastine Besilate – Single Dose Intravenous Toxicity Study in Rats

TNB-NI-02 Acute Toxicity Study on Bepotastine Besilate – Single Dose Oral Toxicity Study in Dogs

Repeat-dose Toxicity

SNJ-NI-1 The Ocular Irritancy Test for Anterior Eye with TAU-284 Ophthalmic Solution Being Administered to Rabbit 8 Times a Day

SNJ-NI-9 Investigation of Ocular Lesions after 8 Instillations in 1 Day of TAU-284 and Ocular Lesions After Repeated Instillation 8 Times per Day for 7 Days

SNJ-NI-8 Study of Ocular Lesions after 8 Instillations of TAU-284 in 1 Day

SNJ-NI-10 Study of Anterior Ocular Segment Irritation after 8 Instillations of TAU-284 to Rabbits

SNJ-NI-2 Study on Anterior Ocular Segment Irriration Due to Seven-Day Repeated Administration of the TAU-284 Ophthalmic Solution to Rabbits

SNJ-NI-3 Toxicity Test by 4-Week Repeated Ophthalmic Administration of TAU-284 Ophthalmic Solution to Rabbits

SNJ-NI-4 13-Week Repeated Ophthalmic Dose Toxicity Study on the TAU-284 Ophthalmic Solution in Dogs

SNJ-NI-5 Toxicity Test by 26-Week Repeated Ophthalmic Administration of Butyric Acid Monobenzenesulfonate (TAU-284) Ophthalmic Solution in Dogs

TNB-NI-05 Subacute Toxicity Test of Bepotastine Besilate Oral Administration Four-Week Repeated Dose Toxicity Study and Four-Week Recovery Study on Dogs

TNB-NI-7 Chronic Toxicity Study on Bepotastine Besilate – 26-Week Repeated Oral Dose Toxicity Study and Four-Week Recovery Study in Dogs

TNB-NI-04 Subacute Toxicity Test of Bepotastine Besilate Oral Administration Four-Week Repeated Dose Toxicity Study and Four Week Recovery Study on Rats

TNB-NI-06 Long-Term Toxicity Test of Bepotastine Besilate – A 26-Week Oral Toxicity Study in Rats Followed by a 4 Week Recovery Test

Genetic Toxicology
TNB-NI-17 Reverse Mutation Tests Using Bepotastine Besilate Bacteria

TNB-NI-18 Chromosomal Aberration Tests Using Bepotastine Besilate Cell Cultures

TNB-NI-26 Unscheduled DNA Synthesis (UDS) Tests Using Bepotastine Besilate in Primary Mouse Hepatocyte Cultures

TNB-NI-19 Micronucleus Tests with Bepotastine Besilate in Mice

Carcinogenicity
TNB-NI-22R Study on 14-Day Oral Administration to Rats with Food

TNB-NI-24R Other Toxicity Testing of Bepotastine Besilate – 14-Day Mixed Feed Oral Administration Testing in Mice

TNB-NI-25 90 Day Carcinogenicity Pre-Examination in Mice

TNB-NI-23 Preliminary 90-Day Carcinogenicity Study in Rats

TNB-NI-21 Carcinogenicity Study on Bepotastine Besilate – 24 – Month Carcinogenicity Study in Mice

TNB-NI-20 Carcinogenicity Study on Bepotastine Besilate – 24-Month Carcinogenicity Study in Rats

Fertility and Early Embryonic Development
TNB-NI-10 Reproductive and Developmental Toxicity Study on Bepotastine Besilate—Study on Oral Administration to Rats Prior to and During Early Stage of Pregnancy

Embryofetal Development
TNB-NI-11 Bepotastine Besilate Reproductive/Developmental Toxicity Testing – Fetal Organ Formation Stage Administration Testing Through Oral Administration to Rats

TNB-NI-12 Bepotastine Besilate Reproductive/Developmental Toxicity Testing – Fetal Organogenesis Stage Administration Testing Through Oral Administration to Rabbits

Prenatal and Postnatal Development
TNB-NI-13 Reproductive and Developmental Toxicity Study on Bepotastine Besilate—Study on Oral Administration to Rats During the Perinatal and Lactation Periods

Local Tolerance
SNJ-NI-6 Skin Sensitization Test with TAU-284 Ophthalmic Solution in Guinea Pigs (Maximization Test Method)

TNB-NI-15 Antigenicity Study on Bepotastine Besilate in Guinea Pigs – Active Systemic Anaphylaxis (ASA) Reactions, Homologous Passive Cutaneous Anaphylaxis (PCA) Reactions, and Enzyme-linked Immunosorbent Assay (ELISA) Method in Guinea Pigs

TNB-NI-16 A Study on Antigenicity in Mice Using Bepotastine Besilate – Rat Passive Cutaneous Anaphylactic (PCA) Reaction

Studies not reviewed within this submission:
All studies submitted were reviewed in this submission.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

Mechanism of action: The sponsor claims bepotastine besilate works as a histamine h1 receptor antagonist. The pharmacology studies conducted in Guinea pigs and rabbits support this proposed mechanism although effects on H1 receptor kinetics were not directly measured.

Drug activity related to proposed indication: The sponsor conducted a series of preclinical proof of concept studies to demonstrate that bepotastine besilate was effective in preventing histamine-induced inflammation and edema in Guinea pigs and rabbits. These data support use for the proposed indication of allergic conjunctivitis.

Study title: Inhibitory Effect of TAU-284 on Histamine-Provoked Acute Conjunctival Edema on Guinea pigs

Key study findings: The 1.0 and 1.5% TAU-284 (bepotastine besilate) concentrations appear to have equivalent activities against histamine activation. Both solutions were determined not to be significantly different from 0.05% ketotifen and statistically superior to 0.025% levocabastine, based on the amount of dye leakage in the eye.

Study no.: SNJ-HO-1
eCTD location: 4.2.1.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 18 March 2002
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity:
Water based ophthalmic solution with 0.01%, 0.1%, 1% and 1.5% bepotastine besilate (lot 02H071 for all solutions), % purity not indicated

Comparator drugs: 0.025% levocabastine, lot 067AAJ and 0.05% ketotifen fumaric acid, lot ML998

Control: physiological saline solution, lot # 1K74N from (b) (4)

Methods

Doses: 0 (with and without histamine exacerbation), 0.01, 0.1, 1.0, 1.5% bepotastine besilate ophthalmic drops, 0.05% ketotifen ophthalmic drop group, and 0.025% levocabastine ophthalmic drop group, 10 µl/eye: bepotastine besilate solutions were within 99.5 to 101.9% of target dose and were considered stable for the duration of the study.

Doses were selected based on a preliminary study in which the ability of 0.05% ketotifen to inhibit edema induced by 0.1, 0.2, and 0.5% histamine concentrations was determined to be 90.1, 76.4 and 97.6%, respectively. Based on these results, the sponsor used 0.2% histamine in the main study.

Species/strain: Guinea pigs of Std:Hartley group, from (b) (4)
Number/sex/group: males only, 10/group, only 8/group included in statistical analysis
Route, formulation, volume: ocular instillation, ophthalmic solutions, 10 µl/eye

Age: 5-6 weeks at first dose
Weight: 248-292 grams at receipt

Unique study design or methodology (if any):

- Treatment: Test article (bepotastine besilate, ketotifen or levocabastine) was applied to left eyes 30 minutes before edema was provoked by histamine injection into the upper palpebral conjunctiva of the left eye.
- Detection method: 2% Evans' blue stain was injected into the caudal vein (1 ml/kg) just prior to histamine application.
- Challenge: histamine solution was injected into the upper palpebral conjunctiva of the right eye. Animals were sacrificed 30 minutes after histamine challenge for edema evaluation.
- Conjunctival tissue was isolated, dye was extracted and amount of dye leakage was determined spectrophotometrically.

1 animal in the 0.01% bepotastine besilate group was excluded from the study due to an error in histamine injection.

Results:

Effects of TAU-284 (bepotastine besilate), Ketotifen and Levocabastine on conjunctival edema induced by an injection of 0.2% histamine in Guinea pigs

Group	# of animals	Body weight (g)	Amount of dye (μg/eye)	vs controls	vs ketotifen	vs levocabastine	% inhibition
Normal[a]	8	376 ± 14	6.1 ± 0.7	--	--	--	--
Control[b]	8	385 ± 13	84.0 ± 5.0	$p < 0.01$ vs normal	--	--	--
0.01% TAU-284	8	381 ± 16	63.5 ± 3.0	$p < 0.01$	$p < 0.01$	$p < 0.01$	26.3
0.1% TAU-284	8	380 ± 13	45.8 ± 3.1	$p < 0.01$	$p < 0.01$	NS[C]	49.0
1.0% TAU-284	8	375 ± 14	14.6 ± 1.5	$p < 0.01$	NS	$p < 0.01$	89.1
1.5% TAU-284	8	384 ± 14	17.2 ± 2.2	$p < 0.01$	NS	$p < 0.01$	85.8
0.05% Ketotifen	8	376 ± 17	13.5 ± 1.4	--	--	--	90.5
0.025% levocabastine	8	383 ± 15	47.1 ± 2.0	--	--	--	47.4

[a] Normal = saline treatment, no histamine challenge
[b] Control = saline treatment with histamine challenge
[c] NS = not significant
[d] Statistics based on Dunnett multiple comparison test

Study title: Suppressive Effects of TAU-284 on Allergic Conjunctivitis in Guinea Pigs

Key study findings: 1.0 and 1.5% bepotastine besilate ophthalmic solutions were significantly different from saline controls and similar to 0.05% ketotifen in effectiveness of suppressing allergic conjunctivitis in Guinea pigs. No difference in effectiveness between the 1.0 and 1.5% bepotastine besilate solutions and 0.025% levocabastine were also seen.

Study no.: SNJ-HO-2
eCTD location: 4.2.1.1.1
Conducting laboratory and location: (b) (4)

Date of study initiation: 25 March 2002
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Water based ophthalmic solution with 0.01%, 0.1%, 1% and 1.5% bepotastine besilate (lot 02H071 for all solutions), % purity not provided

Comparator drugs: 0.025% levocabastine, lot 067AAJ and 0.05% ketotifen fumaric acid, lot ML998

Control: physiological saline solution, lot # K1F84 from (b) (4).

Methods

Doses: 0 (with and without histamine exacerbation), 0.01, 0.1, 1.0, 1.5% bepotastine besilate ophthalmic drops, 0.05% ketotifen ophthalmic drop group, and 0.025% levocabastine ophthalmic drop group, 10 µl/eye: bepotastine besilate solutions were within 99.5 to 101.9% of target dose and were considered stable for the duration of the study.

Species/strain: Slc:Hartley guinea pigs (SPF) from (b) (4)
Number/sex/group: males only, 8/group
Route, formulation, volume: ocular instillation, ophthalmic solutions, 10 µl/eye

Age: 5 weeks
Weight: 276 – 355 grams

Unique study design or methodology (if any):

- Sensitization: 50 µl of physiological saline or anti-ovalbumin (OVA) serum was administered to the conjunctiva of the upper right eyelids.
- Treatment: 48 hours after sensitization, 10 µl of test article or saline was instilled in the right eye of animals
- Challenge: 30 minutes after test article application, 3 mg/kg OVA/20 mg/kg Evans Blue dye in a 2 ml/kg volume was injected into the side back veins of the hind legs
- Sacrifice: Animals were sacrificed 30 minutes after challenge
- Sample collection and analysis: eyelids and conjunctiva were isolated, pigments were extracted and values were read spectrophotometrically to determine the amount of dye leakage.

5 animals were excluded from the study due to errors with the palpebral conjunctival injections of antiserum. 3 animals were excluded from evaluation due to either presumed error in the injection of antiserum (1) or error in induction (2).

Results:

Effects of TAU-284 on passive sensitized allergic conjunctivitis in Guinea pigs

Treatment	Suppression vs saline controls (%)	Vs saline	Vs ketotifen	Vs levocabastine
Normal[a]	--	--		
Saline control[b]	--	--		
0.01% TAU-284	-29.3	ns	$p < 0.01$	ns
0.1% TAU-284	11.0	ns	$p < 0.01$	ns
1.0% TAU-284	63.5	$p < 0.05$	ns	ns
1.5% TAU-284	67.1	$p < 0.05$	ns	ns
0.05% Ketotifen	82.9	--		
0.025% Levocabastine	24.7	--		

[a] Normal = saline sensitization, saline treatment
[b] Control = anti-OVA sensitization, saline treatment
[c] ns = not significant
[d] statistical comparison to ketotifen and levocabastine = Dunnett multiple comparison (2-sided), for saline treated group: Jonckheere, Shirley-Williams (1-sided)

Study title: Inhibitory effect of TAU-284 ophthalmic solution for passively sensitized allergic conjunctivitis in Guinea pigs – study of minimal effective concentration and effective saturation concentration

Key study findings: 0.1%, 1% and 1.5% TAU-284 (bepotastine besilate) solutions all performed better than saline treated controls in inhibiting sensitized allergic conjunctivitis in guinea pigs. The effectiveness of the 1% and 1.5% TAU-284 (bepotastine besilate) solutions were similar.

Study no.: SNJ-HO-3
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Research Laboratory, Senju Pharmaceutical Co., Ltd., Kobe-city, Japan
Date of study initiation: 27 November 2002
GLP compliance: No
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Water based ophthalmic solution with 0.1%, 1% and 1.5% bepotastine besilate (lot 02H071), and 0.3% bepotastine besilate (lot 02X181), % purity not provided, stated no easily detectable insoluble impurities were found.

Control: bepotastine besilate ophthalmic solution base, lot # 02H071

Methods
Doses: 0 (with and without anti-chicken ovalbumin antiserum sensitization), 0.1, 0.3, 1.0, and 1.5% bepotastine besilate ophthalmic drops, 10 µl/eye: bepotastine besilate solutions were said to be stable for the duration of the study, % of target dose not indicated, inferred that the 0.1, 1 and 1.5% bepotastine besilate solutions were within 96.7 to 101.9% based on previous studies with same solutions.

Species/strain: Slc:Hartley guinea pigs (SPF) from (b) (4)
Number/sex/group: males only, 5 for the normal, unaffected control group, 9-10 animals for all remaining groups
Route, formulation, volume: ocular instillation, ophthalmic solutions, 10 µl/eye

Age: 6 weeks
Weight: mean 395.5 grams

Unique study design or methodology (if any): 4 animals, one from each of the following groups: bepotasine besilate base and 0.1, 0.3 and 1.5% bepotastine besilate groups were excluded due to errors during inflammation induction.

- Sensitization: 50 µl of physiological saline or anti-chicken ovalbumin (OVA) serum was administered to the conjunctiva of the upper right eyelids.
- Treatment: 47.5 hours after sensitization, 10 µl of bepotastine besilate test article or base was instilled in the right eye of animals
- Challenge: 30 minutes after test article application, 1.5 mg/ml OVA/1 mg/ml Evans Blue dye in a 2 ml/kg volume was injected into the side back veins of the hind legs

- Sacrifice: Animals were sacrificed 30 minutes after challenge
- Sample collection and analysis: eyelids and conjunctiva were isolated, pigments were extracted and values were read spectrophotometrically to determine the amount of dye leakage.

Results:

Effects of TAU-284 on passive conjunctival anaphylaxis in Guinea pig conjunctiva

Treatment	Number of animals	Suppression vs vehicle controls (%)	Vs vehicle control[c]
Normal[a]	5	--	--
Vehicle control[b]	9	--	--
0.1% TAU-284	9	40.9	$p < 0.01$
0.3% TAU-284	9	49.4	$p < 0.01$
1.0% TAU-284	10	60.7	$p < 0.01$
1.5% TAU-284	9	72.8	$p < 0.01$

[a] Normal = saline sensitization, saline treatment
[b] Control = anti-OVA sensitization, bepotatine besilate (TAU-284) vehicle treatment, significantly different from normal control in 1 sided student t test, $p < 0.01$.
[c] statistical significance based on 1-sided Williams test following linear regression analysis
[d] statistical comparison to ketotifen and levocabastine = Dunnett multiple comparison (2-sided), for saline treated group: Jonckheere, Shirley-Williams (1-sided)

Study title: Suppression Effects of TAU-284 Ophthalmic Solutions on Passively Sensitized Allergy Conjunctivitis in Guinea Pigs: Efficacy Comparison with Similar Drugs

Key study findings: 1.0% bepotastine besilate solution was similar in efficacy to 0.069% ketotifen fumarate and 0.111% olopatadine hydrochloric acid solutions and better than 0.027% levocabastine hydrochloric acid solution in suppressing effects of sensitized allergic conjunctivitis in guinea pigs.

Study no.: SNJ-HO-4
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Research Laboratory, Senju Pharmaceutical Co., Ltd., Kobe-city, Japan
Date of study initiation: 23 December 2002
GLP compliance: No
QA report: yes (x) no ( )
Drug, lot #, and % purity: Note, % purity not provided for any of the solutions.

Test article: Water based ophthalmic solution with 1% bepotastine besilate (lot 02H071), % purity not indicated but stated that no easily detectable insoluble impurities were found

Controls: bepotastine besilate ophthalmic solution base, lot # 02H071
Physiologic saline (Otsuka normal saline, lot # 2G74N)

Comparators: 0.069% ketotifen fumarate ophthalmic solution (0.05% Zaditen® ophthalmic solution, lot NH047)

0.111% olopatadine hydrochloric acid ophthalmic solution (0.1% Patanol®, lot # 36466F)

0.027% levocabastine hydrochloric acid suspension (0.025% Livostin®, lot # 073AAK)

Methods

Doses: 0 (both bepotastine besilate base and physiologic saline), 1.0% bepotastine besilate ophthalmic drops, 0.05% ketotifen fumarate, 0.111% olopatadine hydrochloric acid, 0.027% levocabastine hydrochloric acid suspension, 10 µl/eye: bepotastine besilate solutions were said to be stable for the duration of the study. Concentrations of the 0.1, 1 and 1.5% bepotastine besilate solutions were within 99.5 to 101.6% of target concentration.

Species/strain: Hartley guinea pigs (SPF) from (b) (4)
Number/sex/group: males only, 2 for the normal, unaffected control group; 5-6 animals for all remaining groups
Route, formulation, volume: ocular instillation, ophthalmic solutions, 10 µl/eye

Age: 6 weeks
Weight: average 384.0 grams

Unique study design or methodology (if any): 15 animals were excluded from study due to failed injections of antiserum dilution. A total of 70 animals were properly sensitized.

- Sensitization: 50 µl of physiological saline or anti-chicken ovalbumin (OVA) serum was administered to the conjunctiva of the upper right eyelids.
- Treatment: 47.5 hours after sensitization, 10 µl of bepotastine besilate test article or base was instilled in the right eye of animals.
- Challenge: 30 minutes after test article application, 1.5 mg/ml OVA/10 mg/ml Evans Blue dye in a 2 ml/kg volume was injected into the side back veins of the hind legs.
- Sacrifice: Animals were sacrificed 30 minutes after challenge.
- Sample collection and analysis: right eyelids and conjunctiva were isolated, pigments were extracted and values were read spectrophotometrically to determine the amount of dye leakage.

Results:

Inhibitory effect of 1.0% TAU-284 ophthalmic solution and other drugs on increase of vascular permeability induced by passive conjunctival anaphylaxis in Guinea pig conjunctiva

Group	Number of animals	Inhibition (%)	Vs control	Vs 1.0% TAU-284
Normal[a]	4	--	$p < 0.01$[c]	--
Control[b]	11	--	--	$p < 0.01$[d]
1.0% TAU-284	11	66.6	$p < 0.01$[d]	--
0.05% ketotifen	10	78.8	$p < 0.01$[d]	NS[e]
0.1% olopatadine	11	52.6	$p < 0.01$[d]	NS[e]
0.025% levocabastine	11	27.9	$p < 0.05$[d]	$p < 0.01$[e]

[a] Normal = nonsensitized, saline treated control
[b] Control = sensitized, saline treated control, statistical significance based on 1-sided student's t-test
[c] statistical significance based on 1-sided student's t-test
[d] statistical significance based on 1-sided Dunnett's multiple comparison test
[e] NS, no significance, statistical significance based on 2-sided Dunnett's multiple comparison test

Study title: Inhibition effect of bepotastine besilate (TAU-284) ophthalmic solution on passively sensitized allergic conjunctivitis in Guinea pigs – Examination of effect duration

Key study findings: 1.0 % bepotastine besilate is similarly effective against passively sensitized allergic conjunctivitis in guinea pigs as compared to 0.1% olopatadine and 0.05% ketotifen. All solutions retained activity 1 and 4 hours post-instillation, effectiveness was lost by 8 hours post-instillation.

Study no.: SNJ-HO-5
eCTD location: 4.2.1.1.1
Conducting laboratory and location: (b) (4)

Date of study initiation: 07 August 2003
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Test article: Water based ophthalmic solution with 0.5%, 1.0% bepotastine besilate (lot 03H63), percent purity not indicated but certificate of analysis declares the solutions acceptable

Control: Physiologic saline (Otsuka normal saline, lot # K3C93)

Comparators:

0.069% ketotifen fumarate ophthalmic solution (0.05% Zaditen® ophthalmic solution, lot PE096)

0.111% olopatadine hydrochloric acid ophthalmic solution (0.1% Patanol®, lot # 36466F)

Methods

Doses: 0 (both bepotastine besilate base and physiologic saline), 1.0% bepotastine besilate ophthalmic drops, 0.05% ketotifen fumarate, 0.111% olopatadine hydrochloric acid, 0.027% levocabastine hydrochloric acid suspension, 10 µl/eye. bepotastine besilate solutions were said to be stable for the duration of the study, dosing solutions were 100.5 to 101.8% of target concentration.

Species/strain: Hartley guinea pigs (SPF) from (b) (4)
Number/sex/group: males only, 5 for the normal, unaffected control group, 12-16 animals for all remaining groups
Route, formulation, volume: ocular instillation, ophthalmic solutions, 10 µl/eye

Age: 6 weeks
Weight: 326 to 478 grams

Unique study design or methodology (if any):

- Sensitization: 50 µl of physiological saline or anti-chicken ovalbumin (OVA) serum was administered to the right eye top palpebra.
- Treatment: 10 µl of bepotastine besilate test article or base was instilled in the right eye of animals at one of the following timepoints following sensitization: 40 ± 1 hours, 44 ± 1 hours, 47 ± 1 hours to evaluate protection from induction 1, 4 and 8 hours after test article instillation.
- Challenge: 1.5 mg/ml OVA/4 mg/ml Evans Blue dye in a 2 ml/kg volume was injected into the side back veins of the hind legs at 1, 4 or 8 hours after test instillation, staggered such that final collection was ~ 48.5 hours after the initial sensitization.
- Sacrifice: Animals were sacrificed 30 minutes after challenge.
- Sample collection and analysis: right palpebra and conjunctiva were isolated, pigments were extracted and values were read spectrophotometrically to determine the amount of dye leakage.

Results:

Effects of bepotastine besilate, olopatadine and ketotifen on passively sensitized allergic conjunctivitis in Guinea pigs

Treatment	Time of induction post-instillation (hours)	# of animals	Suppression of dye leakage (%)	Vs 1.0% Bepotastine besilate[a]	Vs induced, saline treated controls[b]
0.5% bepotastine besilate (TAU-284)	1	14	32.2	--	ns[c]
1.0% bepotastine besilate	1	15	52.4	--	$p < 0.05$
0.1% olopatadine	1	14	46.8	ns	ns
0.05% ketotifen	1	14	64.1	ns	$p < 0.01$
0.5% bepotastine besilate	4	14	34.3	--	ns
1.0% bepotastine besilate	4	12	38.3	--	ns
0.1% olopatadine	4	14	53.8	ns	ns
0.05% ketotifen	4	13	55.2	ns	ns

0.5% bepotastine besilate	8	15	-8.8	--	ns
1.0% bepotastine besilate	8	15	6.1	--	ns
0.1% olopatadine	8	15	-15.3	ns	ns
0.05% ketotifen	8	16	-0.2	ns	ns

[a]Statistical significance based on Dunnett multiple comparison (two-sided)
[b] statistical significance based on Dunnett multiple comparison (one-sided)
[c] ns = non-significant

Study title: Effect of TAU-284 on histamine-induced conjunctivitis in Guinea pigs

Key study findings: Bepotastine besilate concentrations of 0.001% and higher showed protection against inflammation. 1.0% concentrations of bepotastine besilate appeared to have similar efficacy as 0.069% ketotifen fumarate and 0.111% olopatadine hydrochloric acid ophthalmic solutions.

Study no.: SNJ-HO-6
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Senju Pharmaceutical Co., Ltd., Kobe City, Japan
Date of study initiation: 06 November 2000
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: % purity not indicated for any of the solutions
Test article: Water based ophthalmic solution with 0.5%, 1.0% bepotastine besilate (lot 03H63)
Control: Physiologic saline (Otsuka normal saline, lot # K3C93)

Comparators: 0.069% ketotifen fumarate ophthalmic solution (0.05% Zaditen® ophthalmic solution, lot PE096)

0.111% olopatadine hydrochloric acid ophthalmic solution (0.1% Patanol®, lot # 36466F)

Methods
Doses:

Test 1: 0.0001, 0.001, 0.01, 0.1 and 1.0% of bepotastine besilate, and 0.05% ketotifen (Zaditen® eye drops), physiological saline for control; 10 µl/eye

Test 2: 1.0% TAU-284, 0.1% olopatadine (Patanol® eye drops), physiological saline for control; 10 µl/eye

Bepotastine besilate dosing solutions were inferred to be 100.5 to 100.9% of target concentration.

Species/strain: Hartley guinea pigs from (b) (4)
Number/sex/group: males only, 6 animals per dose group
Route, formulation, volume: ocular instillation, ophthalmic solutions, 10 µl/eye

Age: 5 weeks

Unique study design or methodology:

- Treatment: 10 µl of bepotastine besilate, ketotifen, olopatadine or saline solution was instilled in both eyes of animals
- Inflammation induction: 20 mg/ml, 2.5 ml/kg of 2.0% Evans' Blue solution was injected into a hindlimb vein, immediately after 50 µl of 0.1% histamine dihydrochloride was injected into the upper eyelid conjunctiva to induce conjunctivitis
- Sacrifice: Animals were sacrificed 30 minutes after inflammation induction
- Sample collection and analysis: eyelid conjunctivae showing dye leakage were isolated, pigments were extracted and values were read spectrophotometrically to determine the amount of dye leakage.

Results:
Bepotastine besilate concentrations of 0.001% and higher showed protection against inflammation (10-85% protection). 1.0% concentrations of bepotastine besilate appeared to have similar efficacy as the 0.069% ketotifen fumarate and 0.111% olopatadine hydrochloric acid ophthalmic solutions (85% vs 90% and 75% vs 56%, respectively).

Study title: Comparative Investigation of the Inhibitory Actions of TAU-284 and Ketotifen on Protein Secretion from Rabbit Lacrimal Glands

Key study findings: Results from this study suggest that bepotastine besilate will not affect lacrimal fluid secretion given the proposed clinical 1.5% formulation. Although ketotifen showed slight inhibitory activity at the 100 µM concentration, this concentration is much higher than would be achieved by the 0.05% clinical formulation of ketotifen.

Study no.: SNJ-HO-12
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Senju Pharmaceutical Co., Ltd., Kobe City, Japan
Date of study initiation: 06 July 2005
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:

Test article: TAU-284 (lot 104002), % purity ~ 100%

Comparator: ketotifen fumarate (b) (4) Lot No. 38H0374)

Positive control: 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP, (b) (4) Lot No. 084K4618), an M3 receptor inhibitor

Challenging agent (promotes protein secretion): Carbamylcholine chloride (b) (4) Lot No. M3M3123)

Methods
Doses: See unique study design section. % targeted dose not indicated

Species/strain: Japanese white rabbits from (b) (4)
Number/sex/group: males only, 5-6 animals per dose

Weight: 1.5 to 1.99 kg

Unique study design or methodology: The main lacrimal gland was extracted from animals and cut in ½ and cultured alone, with 10 μM carbachol, 10 μM carbachol + 100 μM 4-DAMP, 10 μM carbachol + 1 - 100 μM ketotifen or 10 μM carbachol + 1 – 100 μM TAU-284.

Results:
Bepotastine besilate did not demonstrate inhibitory action towards protein secretion at any dose. Significant inhibitory action was seen with 100 μM ketotifen. Near complete inhibition was seen with 100 μM 4-DAMP.

Study title: Inhibitory Action of TAU-284 on Histamine Release from Rat Peritoneal Mast Cells (Preliminary Investigation)

Key study findings: The clinical effects of bepotastine besilate do not appear to involve inhibition of histamine release.

Study no.: SNJ-HO-10
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Senju Pharmaceutical Co., Ltd., Kobe City, Japan
Date of study initiation: 20 February 2004
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:

Test article: bepotastine besilate (lot 104002), % purity estimated to be 99% and was within acceptable limits

Comparator: ketotifen fumarate ((b) (4), Lot No. 38H0374)

olopatadine hydrochloride (b) (4) Lot No. YS-002491)
Levocabastine hydrochloride ((b) (4), Lot No. P5700q)

Positive control: Sodium cromoglycate ((b) (4), Lot No. 2318E)

Methods
Doses:

For Study of A23187-Induced Histamine Release: 10, 100 and 1000 µM for TAU-284, ketotifen, olopatadine and cromoglycic acid and 10 and 100 µM for levocabastine; 0.01 µM A23187.

For Study of Compound 48/80 Induced Histamine Release: 10, 100 and 1000 µM for bepotastine besilate; 0.1, 1, 10, 100 and 1000 µM for ketotifen; 0.1 µg/ml Compound 48/80. % targeted dose not indicated.

Species/strain:

Wistar rats (b) (4) for A23187 induced histamine release study

Sprague Dawley rats from (b) (4) for compound 48/80 induced histamine release

Number/sex/group: Males only, number of animals from which peritoneal cells were isolated was not indicated.

Weight: 220 - 320 g

Unique study design or methodology: Mast cells were isolated from rat peritoneal cavities. Cells were preincubated with test articles for 2 hours prior to the addition of challenging agent (A23187 or Compound 48/80).

Results: The clinical effects of bepotastine besilate do not appear to involve inhibition of histamine release.

Study title: Effect of TAU-284 on Chemotaxis of Peritoneal Eosinophils in Guinea Pigs

Key study findings: Bepotastine besilate may potentially inhibit eosinophil chemotaxis action, and do so at a greater level of efficacy than olopatadine hydrochloride.

Study no.: SNJ-HO-11
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Senju Pharmaceutical Co., Ltd., Kobe City, Japan
Date of study initiation: 04 February 2005
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:

Test article: bepotastine besilate (lot 104002), % purity not indicated but ~ 100% from certificate of analysis

Comparator: ketotifen fumarate ((b) (4), Lot No. 38H0374)

olopatadine hydrochloride (b) (4) Lot No. YS-002491)

Positive control: Rolipram ((b) (4), Lot No. 112K4604)

Methods
Doses:
10^{-5} M, 10^{-4} M and 10^{-3} M for bepotastine besilate, ketotifen and olopatadine, 10^{-5}M and 10^{-4}M for levocabastine and 2 x 10^{-5} M for Rolipram. % targeted dose not indicated.

Species/strain: Hartley guinea pigs from (b) (4)

Number/sex/group: Males only, number of animals used for eosinophil isolation not indicated.

Weight: 300-400 g

Unique study design or methodology: Eosinophils were isolated from peritoneal cavity. Cells were preincuated with test articles for 2 hours prior to the addition of challenging agent (leukotriene B4).

Results: Chemotaxis rates were ketotifen < bepotastine besilate < olopatadine at 10^{-3} M, suggesting that TAU-284 may potentially inhibit eosinophil chemotaxis action and be more effective than olopatadine.

Study title: Inhibitory Effect of the TAU-284 Ophthalmic Solution on Passive Sensitization Allergic Conjunctivitis in Rats

Key study findings: 1.5% bepotastine besilate showed similar ability to 0.5% ketotifen fumarate (Zaditen®) in inhibiting passive conjunctival anaphylaxis induction in rat conjunctivae.

Study no.: SNJ-HO-9
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Senju Pharmaceutical Co., Ltd., Kobe City, Japan
Date of study initiation: 01 February 2002
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:
Test article: 0.001, 0.01, 0.1, 1.0 and 1.5% bepotastine besilate ophthalmic solutions (all from lot 02H071), % purity not indicated, considered ~ 100% as certificate of analysis states no easily detectable insoluble impurities were found

Comparator: Zaditen, 0.05% ketotifen fumarate ophthalmic solution (b) (4) Lot No. ML998)

Negative control: physiological saline

Methods
Doses: 0.001, 0.01, 0.1, 1.0 and 1.5% bepotastine besilate, 0.05% ketotifen, physiological saline for the negative control group. % of target dose not indicated, inferred that the 0.1, 1 and 1.5% bepotastine besilate solutions were within 99.5 to 101.9% based on previous studies with same solutions.

Species/strain: Wistar rats (Jcl: Wistar) from Senju Pharmaceutical Co., Ltd, Kobe, Japan

Number/sex/group: 7-8 animals/group; males only
Age: 7 weeks
Weight: 300 g

Unique study design or methodology: 10 µl/eye test article administered 60, 40 and 20 minutes before inflammation induced. Conjunctivas were removed 6 hours after inflammation was induced.

Results: 1.5% bepotastine besilate was similar to 0.05% ketotifen in inhibiting vascular permeability induced by passive conjunctival anaphylaxis in rats. All concentrations lower than 1.5% bepotastine besilate were inferior to 0.05% ketotifen. The table below was taken from the Sponsor's NDA.

Group	Inhibition (%)
0.001% TAU-284	14.8
0.01% TAU-284	-3.1
0.1% TAU-284	22.4
1.0% TAU-284	40.8
1.5% TAU-284	62.6
0.05% Ketotifen	67.4

Inhibition (%) = {1−(X−N)/(C N)} × 100. X,N and C; these initials indicate the mean value of each treated, Normal and Control group, respectively (n=7-8).

Study title: Drug Efficacy Comparative Study of TAU-284 and Other Drugs in a Guinea Pig Active Sensitization Model

Key study findings: 1.0% bepotastine besilate was effective in inhibiting conjunctival edema and eosinophil infiltration (considered to be immediate and delayed symptoms, respectively). 1.0% bepotastine besilate was similar to the 0.05% ketotifen solutions for both the edema and eosinophil infiltration observations. 1.0% bepotastine besilate was more potent against edema but less effective against eosinophil infiltration as compared to the 0.1% betamethasone solution.

Study no.: SNJ-HO-8
eCTD location: 4.2.1.1.1
Conducting laboratory and location: Kobe Creative Center, Senju Pharmaceutical Co., Ltd., Kobe City, Japan
Date of study initiation: 26 October 2001
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: bepotastin besilate, Lot 19, 100.14% pure

Methods

Doses: 1.0% bepotastine besilate, 0.05% ketotifen (Zaditen), and 0.1% betamethasone (Rinderon) ophthalmic solutions. Physiological saline for the negative control group. bepotastine besilate solutions were not tested for stability, but were said to be known to be stable for the duration of the experiment

Species/strain: Male Guinea pigs from (b) (4)

Number/sex/group: males only
Weight: 400 g

Unique study design or methodology: 10 µl/eye test article or physiological saline was administered 6, 4, 2, 1, 0.5 and 0.25 hours before each of the first and second antigen stimulations.

Results: 1.0% bepotastine besilate significantly inhibited conjunctival edema and decreased eosinophil infiltration (which was not significant from controls, but is considered a delayed reaction). 1.0% bepotastine besilate effects were similar to that of the 0.05% ketotifen solutions for both edema and eosinophil infiltration observations. 1.0% bepotastine besilate was more potent against edema but less effective against eosinophil infiltration as compared to the 0.1% betamethasone solution.

2.6.2.3 Secondary pharmacodynamics
No data submitted.

2.6.2.4 Safety pharmacology

Neurological effects:
No data were submitted beyond what was included in the chronic toxicology studies. No neurological effects were observed beyond mydriasis seen at the highest dose in many of the oral toxicology studies in both rats (300-1000 mg/kg, safety factors of at least 3,300 X that of maximal systemic concentration based on topical ocular administration in humans). This is likely due to local effects on the parasympathetic nervous system, as bepotastine besilate was not found to significantly cross the blood brain barrier. The sponsor did conduct an additional study which determined that bepotastine besilate did not substantially cross the blood brain barrier displaying an AUC_{brain}/AUC_{plasma} ratio of 0.069 vs 16.862 for ketotifen measured 8 hours after a 3 mg/kg i.v. dose given to male Wistar rats.

Cardiovascular effects:
The sponsor did however conduct a non-GLP safety pharmacology study in anesthetized dogs. The results of this study showed that bepotastine caused only slight depression in vertebral blood flow, heart rate and blood pressure in a dose dependent manner, while it did not affect QTc, QT, QRS or PR intervals at doses up to 10 mg/kg (a safety factor of ~4890 X that of the anticipated human systemic concentrations for ophthalmic use. Due to the ocular instillation route of application for the proposed indication, this study was considered to provide sufficient confidence that bepotastine besilate would not pose a significant risk to cardiac effects in humans. A thorough QTc studying humans was not conducted for bepotastine besilate but would be expected if the sponsor pursued an indication that required a higher systemic concentration.

Study title: Effects of a novel anti-allergy drug bepotastine besilate (TAU-284) on Canine electrocardiograms

Key study findings: This was a non-GLP report. Bepotastine besilate caused a slight depression in vertebral blood flow amount, heart rate and blood pressure in a dose response manner, and no effects on QTc, QT, QRS and PR intervals in the anesthetized dog. The effects of the positive control articles (terfenidine and ketotifen) caused effects as expected.

Study no.: TNB-HO-12
eCTD location: 4.2.1.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: 06 November 2000
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: Bepotastine besilate, terfenidine and ketotifen, lots and % purity not provided

Methods
Doses: 0.1, 0.3, 1.0, 3.0 and 10 mg/kg bepotastine besilate, terfenidine and ketotifen plus vehicle treated controls. No information on bepotastine besilate solution stability or % of targeted concentration was provided.

Species/strain: mixed breed dogs
Number/sex/group: males and females only, 6 animals/dose group, #/sex not specified
Route, formulation, volume: i.v., 0.1% nicolic acid in physiologic saline, 0.4 ml/kg over 20 minute period

Age: 9 months or older
Weight: 11.8 to 18.0 kg
Unique study design or methodology (if any):

- Sodium pentobarbital anesthetized dogs.
- Animals were assigned to a specific test article group, doses were administered in a dose escalation format, separated by 20 minute intervals.

Results:
Terfenadine caused significant ($p < 0.05$) QTc prolongation (20 to 30 msec) at 3 mg/kg and higher, while bepotastine besilate and ketotifen had no effects.

Terfenadine and ketotifen showed a significant increase ($p \leq 0.05$) in QT interval at 10 mg/kg dose (55 to 57 msec) compared to controls. The differences were also statistically significant for terfenadine at 1 and 3 mg/kg dose levels (20 to 25 msec increase, respectively). A trend for an increased QT interval was also seen for the 3 mg/kg ketotifen dose group, but values were not significantly different from controls (23.3 ± 9.8 vs 7.7 ± 6.8 msec). Values for bepotastine besilate ranged from 3.6 ± 1.6 to 31.0 ± 7.4 msec vs -0.3 ± 2.2 to 14.0 ± 7.4 msec for control.

There were no test article effects on QRS interval or on R wave height. Ketotifen demonstrated an increase in PR interval only at the 10 mg/kg dose as compared to vehicle treated controls. (14.0 ± 4.7 vs -1.3 ± 3.4 msec).

Hemodynamic effects were noted for the terfenadine and ketotifen treated animals. A decrease in blood pressure, heart beats/minute, and vertebral artery blood vessel resistance were all seen at the 10 mg/kg dose level, vertebral artery flow was increased at the 10 mg/kg dose level. The effects on blood vessel resistance were also seen in the 1 and 3 mg/kg terfenadine dose group and 3.0 mg/kg ketotifen dose group. Terfenidine's effects on vertebral artery blood flow were also seen at the 1 and 3 mg/kg dose levels. Bepotastine besilate caused only a slight depression in vertebral blood flow amount, heart rate and blood pressure in a dose response manner, but the effects were not considered significant when compared to vehicle treated contols. The tables below were copied from the Applicant's application.

Heart rate (Δbeats/min)

Group	Dose (mg/kg, i.v.)						
	Baseline	0.0	0.1	0.3	1	3	10
Vehicle	128.3±10.4	1.3±1.8	4.5±3.3	5.0±5.1	5.2±5.6	4.0±7.3	0.0±8.6
Betotastine	130.0±5.3	-2.0±0.7	-1.8±2.5	-2.7±2.7	-4.8±3.1	-7.2±3.4	-11.3±4.3
Terfenadine	143.8±4.5	-1.3±0.9	-4.0±1.9	-7.2±3.1	-9.5±3.7*	-9.2±4.6	-30.8±7.0**
Ketotifen	141.2±10.6	-2.3±1.4	-3.3±2.9	-3.3±3.0	1.3±2.7	-5.3±7.2	-31.7±5.7**

Vertebral artery blood flow amount (Δml/min)

Group	Dose (mg/kg, i.v.)						
	Baseline	0.0	0.1	0.3	1	3	10
Vehicle	22.7±3.8	1.3±0.3	4.5±2.1	4.3±2.2	1.2±1.3	1.5±1.2	-0.2±1.6
Betotastine	20.7±3.2	-0.2±0.3	-0.3±0.3	0.0±0.3	0.8±0.7	-0.8±0.2	-1.7±0.4
Terfenadine	32.3±7.9	0.5±0.3	4.0±2.1	4.7±1.1	18.3±3.1**	46.0±4.1**	46.5±10.0**
Ketotifen	22.8±5.0	-0.7±0.8	-1.2±1.1	-1.3±1.5	1.3±2.5	8.3±2.6	28.8±7.1**

Vertebral artery blood vessel resistance (ΔmmHg/ml/min)

Group	Dose (mg/kg, i.v.)						
	Baseline	0.0	0.1	0.3	1.0	3.0	10.0
Vehicle	6.0±0.9	-0.30±0.11	-0.64±0.25	-0.51±0.21	0.03±0.42	-0.22±0.27	0.26±0.39
Betotastine	6.0±0.7	-0.01±0.09	0.07±0.10	-0.18±0.11	-0.27±0.14	0.07±0.13	0.04±0.32
Terfenadine	4.4±0.8	-0.04±0.10	-0.35±0.16	-0.71±0.25	-1.78±0.38*	-3.04±0.68**	-3.38±0.83**
Ketotifen	7.5±2.1	0.60±0.58	1.66±1.59	2.37±2.12	0.08±0.63	-3.34±0.91**	-5.00±1.14**

Toxicokinetics: Values are from n = 6 animals, 3 minutes post-dose

Group		Plasma bepotastine besilate concentration (μg/ml)				
Dose (mg/kg, i.v.)		0.1	0.3	1	3	10
Bepotastine	Unchanged	0.29 ± 0.04	0.96 ± 0.13	3.38 ± 0.52	10.95 ± 1.74	35.87 ± 5.53
Terfenadine	Unchanged	0.04 ± 0.01	0.09 ± 0.04	0.27 ± 0.10	0.89 ± 0.21	3.08 ± 0.69
	Acid metabolite	ND*	ND	ND	0.03 ± 0.01	0.13 ± 0.05
	N-dealkylated metabolite	ND	ND	ND	ND	ND
Ketotifen	Unchanged	0.02 ± 0.01	0.09 ± 0.01	0.29 ± 0.08	0.73 ± 0.19	2.37 ± 0.40

*ND = not detectable, limit of detection = 0.02 μg/ml

Pulmonary effects: No data provided beyond what is included in chronic toxicity studies. No pulmonary effects were observed nor expected due to low systemic exposure seen with topical ocular use.

Renal effects: No data provided beyond what is included in the chronic toxicity studies. No renal effects were observed nor expected due to low systemic exposure seen with topical ocular use. Bepotastine besilate is noted to undergo renal excretion, primarily due to glomerular filtration.

Gastrointestinal effects: No data provided beyond what is included in the chronic toxicity studies. No gastrointestinal effects were observed nor expected due to low systemic exposure seen with topical ocular use.

Abuse liability: No data provided.

Other: Due to the low systemic exposures expected with topical ocular use, cross reactivity of bepotastine besilate binding different receptors is believed to represent a low level of risk. However, the sponsor did submit data indicating that 3 μg/ml concentration of bepotastine,besilate caused 50% inhibition of sigma receptor binding activity and 24, 28 and 36% inhibition of quisqualate, NMDA and Muscarinic M1 receptors, respectively. The results for the comparator agents terfenadine and ketotifen confirmed the validity of the study.

Study title: Binding Inhibition Activity of Bepotastine Besilate (TAU-284), a New Anti-allergy Drug, in Relation to Various Types of Receptors – a Comparison with Other Drugs

Key study findings: 3 μg/ml concentration of bepotastine besilate, caused 50% inhibition of sigma receptor binding activity and 24, 28 and 36% inhibition of quisqualate, NMDA and Muscarinic M1 receptors, respectively. The results for the comparator agents terfenadine and ketotifen confirmed the validity of the study.

Study no.: TNB-HO-15
eCTD location: 4.2.1.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: February 18, 1992

GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: Bepotastine besilate, terfenidine, ketotifen, and cetirizine, lots and % purity not provided

Methods
Doses: 3 µg/ml concentration was used for all test drugs evaluated in this study. Screen against 30 receptors/ ion channels.

Results:
3 µg/ml concentration of bepotastine besilate, caused 50% inhibition of sigma receptor binding activity and 24, 28 and 36% inhibition of quisqualate, NMDA and Muscarinic M1 receptors, respectively. Terfenadine showed binding inhibition activity of ≥50% for the Na channel. Ketotifen showed 50% inhibition activity toward the muscarinic M1, M2 and M3 receptors, the serotonin $5HT_{1A}$ and $5HT_3$ receptors, and the sigma receptor. Cetirizine showed 50% binding inhibition activity towards the NMDA receptor. The effects of terfenadine on the Na channel and ketotifen on the muscarinic M1 receptor was 97% and 99% respectively, which was in alignment with the pharmacologically relevant concentrations of these drugs.

2.6.2.5 Pharmacodynamic drug interactions
No studies were conducted to evaluate drug interactions.

2.6.3 PHARMACOLOGY TABULATED SUMMARY
Copied from the NDA:

2.6.3.2 Primary Pharmacology **Test Article: bepotastine besilate**

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose[1]	Drug Product Lot #	Gender and n per Group	Noteworthy Findings	Study Numb
Histamine-induced conjunctival edema	Std:Hartley guinea pigs	Topical in the conjunctival cul-de-sac	10 µL of 0.01, 0.1, 1.0 and 1.5% topical solutions	02H071-0.01% 02H071-0.1% 02H071-1.0% 02H071-1.5%	10 males	Dose-related reduction in edema formation as measured by Evan's blue dye leakage. Reduction in dye leakage was similar to 0.05% ketotifen and significantly ($p<0.01$) less than 0.025% levocabastine.	SR 0113((SNJ-HO-
Ovalbumin-induced allergic conjunctivitis	Std:Hartley guinea pigs	Topical in the conjunctival cul-de-sac	10 µL of 0.01, 0.1, 1.0 and 1.5% topical solutions	02H071-0.01% 02H071-0.1% 02H071-1.0% 02H071-1.5%	8 males	Dose-related reduction in conjunctivitis as measured by Evan's blue dye leakage. Reduction in dye leakage was similar to 0.05% ketotifen and significantly ($p<0.05$) less than 0.025% levocabastine.	JBS-02-GXAG (SNJ-HO-
Ovalbumin-induced allergic conjunctivitis	Std:Hartley guinea pigs	Topical in the conjunctival cul-de-sac	10 µL of 0.01, 0.1, 0.3, 1.0 and 1.5% topical solutions	02H071-0.01% 02H071-0.1% 02H071-1.0% 02H071-1.5% 02H071-vehicle 02X181-0.3%	10 males	Dose-independent reduction in conjunctivitis as measured by Evan's blue dye leakage. All doses of 0.1% and above were significantly ($p<0.01$) better than vehicle.	B2003A04 (SNJ-HO-
Ovalbumin-induced allergic conjunctivitis	Hartley guinea pigs	Topical in the conjunctival cul-de-sac	10 µL of 1.0% topical solution	02H071-1.0% 02H071-vehicle	12 males	Significant reduction in conjunctivitis as measured by Evan's blue dye leakage. Reduction in dye leakage was similar to 0.05% ketotifen and 0.1% olopatadine and significantly ($p<0.01$) less than 0.025% levocabastine.	B2003A04 (SNJ-HO-
Ovalbumin-induced allergic conjunctivitis	Std:Hartley guinea pigs	Topical in the conjunctival cul-de-sac	10 µL of 0.5 and 1.0% topical solutions	03H63-0.5% 03H63-1.0%	12-15 males	Reduction in conjunctivitis as measured by Evan's blue dye leakage. Reduction in dye leakage was similar to 0.05% ketotifen and 0.1% olopatadine. This effect was evident at 1 and 4 hours after instillation, but not at 8 hours.	JBS-03-GXAG (SNJ-HO-

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose[1]	Drug Product Lot #	Gender and n per Group	Noteworthy Findings	Study Num
Histamine-induced conjunctival edema	Hartley guinea pigs	Topical in the conjunctival cul-de-sac	10 µL of 0.0001, 0.001, 0.01, 0.1, and 1.0 topical solutions	not available	males (n not specified)	Dose-related reduction in edema formation as measured by Evan's blue dye leakage. Reduction in dye leakage with 1.0% was similar to 0.05% ketotifen and less than 0.1% olopatadine.	B2001B0 (SNJ-HO-
Cardiovascular	Mixed breed dogs	Intravenous	0.1 - 10 mg/kg	not available	6 (gender not specified)	Positive control drugs: terfenadine, ketotifen. Bepotastine showed no significant effect on QTc, slight hypotensive activity and heart rate reduction at 10 mg/kg. Terfenidine showed significant extension of QTc at doses of 3 mg/kg and up. Terfenidine also showed hypotension, heart rate reduction and increase in vertebral artery blood flow from 1 mg/kg and up. Ketotifen showed no significant effect on QTc, but dose-dependent reduction in blood pressure, heart rate, and increased vertebral artery pressure from 1 mg/kg.	13028 (TNB-HO-
Ligand binding inhibition	mice, rats, guinea pigs, rabbits, cultured human cancer cells	n/a	n/a	not available	n/a	See Table 1 and Table 2.	13030 (TNB-HO-

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

A radiolabeled ocular instillation study in pigmented rabbits revealed that bepotastine besilate distributed to multiple subocular compartments with minimal systemic exposure. The ocular compartments with the highest concentrations included the conjunctiva, cornea, retina, iris, ciliary body and choroid. The association with the iris suggests that there may be some affinity for bepotastine besilate binding to melanin, although a separate melanin binding study was not conducted. Tmax was achieved in 0.25 hours. The half life of bepotastine besilate was between 0.5 and 2 hours. This was the only study in which subocular distribution of test article was measured, toxicokinetic assessments from the pivotal toxicology studies provided further support of the observation of low systemic exposure following ocular instillation ($AUC_{0\text{-}INF}$ Blood = 945.68 ng eq.*h/ml vs 325.62 to 186279.18 ng eq*h/ml or gram for ocular subcompartments).

Systemic toxicokinetics were also evaluated in the 26 week ocular instillation dog study (Study SNJ-NI-5), with the maximum $AUC_{0\text{-}24}$ being 3310 ± 500 ng*h/ml. There was a trend for slight accumulation (approximately 50%) of bepotastine besilate over the 26 week dosing period, and may be due to some potential for melanin binding that was identified in the radiolabeled ocular instillation study. Affinity for melanin binding was further confirmed in the radiolabeled study to determine whole body distribution of bepotastine besilate, and transfer of radiolabeled compound to fetus and milk in pregnant and lactating dams (Study TNB-NI-15). This binding was transient, showing complete dispersion by 30 days after a

single oral dose of radiolabeled bepotastine besilate. There were no differences between males and females in terms of exposure levels. In contrast, systemic exposures following oral dosing were generally 1000X higher than that seen with ocular instillation, with AUC ranging from 169.25 to 1707.12 µg*h/ml in week 26 of the 26 week dog oral toxicity study. These results provided additional support that systemic exposure following ocular dosing was low and did not accumulate with multiple dosing, although in the case of the dogs, the Tmax was generally achieved between 6-8 hours post-dose.

In metabolism studies, bepotastine besilate did not appear to be a substrate for human liver microsome metabolism or inhibit CYP 3A4, 2C9 or 2C19 metabolism of classic substrates at concentrations up to 200 µM. In female mice, 2 weeks oral administration of bepotastine besilate increased Cyp450 protein content 2.1 X that of controls, with specific induction of Cyp2b, Cyp2c, Cyp2d, Cyp2a and Cyp1a1/2, with the effects on Cyp2b being particularly strong achieving 3.5 to 6.5 fold that of controls. Two week oral dosing in rats demonstrated induction of Cyp2b1/2, Cyp3a1/2, Cyp2a1 and likely Cyp2c11. Increased Cyp450 content was also found in the liver of rats in multiple dose toxicology studies (TNB-NI-04 and TNB-NI-06), providing further confirmation of this induction. Cyp450 enzymes were not induced in a multiple dose toxicology study in dogs (TNB-NI-05), suggesting that Cyp450 induction is rodent specific.

The sponsor submitted several study reports that evaluated pharmacokinetics/toxicokinetics of bepotastine besilate following oral administration in guinea pig, rat and dog. These data indicate that bepotastine besilate does not significantly accumulate in the body, with Tmax being achieved by 0.5 hours and an average half life of ~4 hours. Bepotastine besilate appears to be renally excreted in dogs, via glomerular filtration. In a radiolabeled study in rats (TNB-NI-15), bepotastine besilate was excreted in an approximate 60:40 fashion in feces and urine, respectively. There is a small tendency for bepotastine besilate to accumulate with multiple dosing, as seen in rats dosed with 3 mg/kg/day for 21 days. Plasma concentrations were ~9% higher on Day 21 than on Day 1.

Bepotastine besilate was transferred to the yolk sac/placenta at levels nearly equivalent to maximal plasma concentration, ~33-55% of bepotastine besilate was transferred to the developing fetus. At 24 hours following a single oral administration of 3 mg/kg, ~ 5.9% and 3.1% of maximal plasma bepotastine besilate concentrations were detected in the brain and liver of the fetus at 24 hours postdose. Bepotastine besilate was also noted to be transferred to milk in lactating rats, with milk concentrations being 1.5 to 2 times maximal plasma concentrations by 1 hour postdose and reaching levels below the limit of detection by 48 hours postdose.

Toxicokinetic data collected during the 2 year carcinogenicity studies in rats and mice showed that systemic exposures exhibited a dose dependent relationship. In the case of the rodent studies, Bepotastine besilate concentrations were often reduced with chronic treatment, further supporting the suggestion of hepatic enzyme induction.

2.6.4.2 Methods of Analysis

A validated HPLC method of analysis was used to measure the concentration of bepotastine besilate in plasma from dog. Upper and lower limits of detection were 10 and 300 ng/ml. This covers the methods of analysis used for the pivotal 26 week toxicology study in dogs (Study SNJ-NI-5), which was the only toxicology report in which toxicokinetics were evaluated.

Study title: Validation Study of a Measurement Method for the Concentration of Bepotastine Besilate (TAU-284) in Dog Plasma

Key study findings: HPLC method of analysis. Calibration curve was linear between plasma concentrations of 10 and 300 ng/mL.

Study no.: SNJ-NI-7
eCTD location: 4.2.3.7.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 08 October 2003
GLP compliance: no
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, Lot #8, 99.8% pure

2.6.4.3 Absorption

Study title: The Dynamic Examination of Applying Eye Drop Medication TAU-284. – The Distribution Test Within An Eye Tissue When Carrying Out Single Time Medication of the 1.5% ^{14}C-TAU-284 Eye Drop Liquid in a Colored Rabbit

Key study findings: Tmax was generally reached within 0.25 hours of dosing in ocular tissues with that in some substructures extending out to 4 hours with test article still being detected at the 72 hour timepoint. Tissues with the highest bepotastine besilate exposure included conjunctiva, cornea, iris and ciliary body, retina and choroid. The association with the iris suggests there may be some affinity for bepotastine besilate binding to melanin. Plasma exposure was 668.56 ng eq * hr/ml, blood exposure was 945.68 ng eq*hr/ml (values given as $AUC_{0\text{-}inf}$). Tmax for plasma and blood was 0.5 hours, $t_{1/2}$, ranged from 0.63 to 0.99 hours.

Study no.: SNJ-HE-1
eCTD location: 4.2.2.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 18 February 2002
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity:
1.5% ^{14}C-bepotastine besilate, lot CFQ12978, 98.6 % radiochemical purity
Nonlabeled bepotastine besilate, lot 104002, % purity not indicated

Methods
Doses: 0.75 mg/0.5 MBq/50µl/eye of 1.5% ^{14}C-TAU-284, both eyes were treated, single administration

Species/strain: Kbl:Dutch Rabbit from (b) (4)
Number/sex/time point: 2 males

Route, formulation, volume: ocular instillation, bepotastine besilate eye drop 1.5% basis agent ingredient, 50µl/eye

Age: 13-14 weeks at start of acclimation, acclimation was said to be > 1 week
Weight: 1.23 to 1.69 kg at end of acclimation
Sampling times: 0.25, 0.5, 1, 2, 4, 8, 24 and 72 hours post-dose

Results:
Radioactivity concentrations in tissues after single instillation of 1.5% ^{14}C-bepotastine besilate ophthalmic solution at a dose of 0.75 mg/50 µl/eye to male pigmented rabbits are presented in the table below.

Pharmacokinetic parameters						
Tissue	Tmax (h)	Cmax (ng eq/ml or g)	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	$AUC_{0\text{-}24h}$ (ng eq*h/ml or g)	$AUC_{0\text{-}INF}$ (ng eq.*h/ml or g)
Blood	0.5	198.1	0.63 (0.5-2h)	22.40 (2-24h)	528.86	945.68
Plasma	0.5	214.8	0.99 (0.5-4h)	22.43 (4-24h)	438.80	668.56
Aqueous humor	1.0	1989.8	1.38 (1-8h)	5.87 (8-24h)	6137.54	6214.60
Cornea	0.25	27080.3	1.42 (0.25-8h)	33.53 (8-72h)	45592.16	50222.02
Conjunctiva	0.25	13075.9	1.89 (2-8h)	13.32 (24-72h)	141894.76	145844.76
Extraocular muscle	1.0	826.1	2.50 (0.25-8h)	37.46 (8-72h)	5754.31	7094.48
Iris + ciliary body	2.0	5294.5	5.22 (2-8h)	47.35 (8-72h)	124024.39	186279.18
Lens	4.0	75.2	62.47 (0.5-72h)	--	3290.86	6012.81
Vitreous body	0.25	31.0	14.54 (0.25-72h)	--	313.04	325.62
Retina + choroid	0.25	2808.8	20.13 (0.25-72h)	--	44035.19	48380.48
Sclera	0.25	3231.8	2.63 (0.25-8h)	31.20 (8-72h)	21237.24	24757.28

Note, eye variables averaged over 4 eyes/timepoint, blood variables averaged over 2 animals/timepoint.

Study title: Blood Plasma Concentration Transition in Guinea Pigs During Inhibitory Activity Testing of a New Anti-allergy Drug Bepotastine Besilate (TAU-284) in Relation to Histamine-induced Shock

Key study findings: Single oral doses of 0.3 and 1.0 mg/kg bepotastine besilate were evaluated. Tmax was achieved at 0.5 hours post-dose. Cmax and $AUC_{0\text{-}24}$ for the low and high doses were 66.8 and 273.4 ng/ml and 187.7 and 621.0 ng*hr/ml.

Study no.: TNB-HO-14
eCTD location: 4.2.2.2.1
Conducting laboratory and location: Ube Laboratory Medical Research Department at Ube Industries, Ltd., Japan

Date of study initiation: June 1991
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, lot number and percent purity not indicated

Methods
Doses: 0, 0.3 and 1.0 mg/kg, % of targeted dose not indicated
Species/strain: Hartley-type guinea pigs from (b) (4)
Number/sex/group: males only, 4 in CMC vehicle control, 7 in remaining dose groups

Route, formulation, volume: oral gavage; 0.5% CMC solution; 5 ml/kg
Age: 5 weeks
Weight: 270-360 g
Sampling times: Blood taken at 0.5, 1, 2, 4, 8, 12 and 24 hours after dosing

Results:
Bepotastine besilate pharmacokinetic parameters

Dosage (mg/kg)	Tmax (hr)	Cmax (ng/ml)	AUC_{0-24} (ng*hr/ml)
0.3	**0.5**	**66.8**	**187.7**
1	**0.5**	**273.4**	**621.0**

Study title: Internal Dynamics of Bepotastine Besilate in Canines

Key study findings: Bepotastine besilate systemic exposures increased in a dose dependent fashion following single oral doses of 5, 10 and 20 mg/animal. There was little interindividual variation seen, Cmax was typically achieved within the first hour after dosing, $t_{1/2}$ was ~ 4 hours for all doses.

Study no.: TNB-HE-04
eCTD location: 4.2.2.2.1
Conducting laboratory and location: (b) (4) inferred from report cover page
Date of study initiation: November 7, 1990
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, lot number and % purity not indicated

Methods
Doses: 5, 10 or 20 mg bepotastine besilate, single dose, test article concentration and stability confirmation not provided

Species/strain: beagle dogs from animal stocks at (b) (4)
Number/sex/group: 4 males used in this study, each animal received all doses of bepotastine besilate, each dosing occasion was separated by 7 days

Route, formulation, volume: oral gavage, formulation not indicated, 30 ml volume

Age: Not indicated
Weight: 11.0 to 14.5 kg
Sampling times: 0.5, 1, 2, 3, 4, 5, 6, and 7 hours post-dose

Results:
The table below provides a summary of the results. Mean (SE)

Dosage (mg)	Cmax (ng/ml)	Tmax (hr)	$t_{1/2}$ (hr)	AUC_{0-7} (ng*hr/ml)	AUC_{0-inf} (ng*hr/ml)
5	357.8 (24.8)	1.1 (0.3)	3.9 (0.4)	1690.2 (146.5)	2467.7 (263.8)
10	701.1 (20.9)	0.9 (0.1)	3.9 (0.2)	3095.4 (94.1)	4482.9 (239.4)
20	1453.7 (85.6)	0.8 (0.1)	4.0 (0.4)	6483.5 (432.8)	9523.9 (1042.3)

2.6.4.4 Distribution

Study title: **Nerve transfer of Bepotastine Besilate (TAU-284)**

Key study findings: Bepotastine besilate did not show a high propensity to cross the blood brain border.

Study no.: TNB-HE-05
eCTD location: 4.2.2.3.1
Conducting laboratory and location: (b) (4) inferred from report cover page
Date of study initiation: October 26, 1995
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:
TAU-284, lot #13, 99.93% pure
Ketotifen fumarate, lot #3, % purity not indicated
Terfenadine, lot #4, % purity not indicated
Epinastine hydrochloride, lot #1, % purity not indicated
Cetirizine dihydrochloride, lot #4, % purity not indicated
Terfenadine acid, lot #3, % purity not indicated

Methods
Doses: 3 mg/kg of each ketotifen, terfenadine, epinastine, cetirizine and bepotastine besilate, % of targeted dose was not indicated.
Species/strain: Wistar type rats from (b) (4)
Number/sex/group: 3 males/group

Route, formulation, volume: i.v., terfenadine was dissolved in 2.5% DMA-PEG and administered as 3 mg/kg/0.5ml; all other test articles were prepared in saline solution and administered 3 mg/kg/ml

Age: 7 weeks

Sampling times: 2, 6, 15 and 30 minutes and 1, 2, 4, 6, and 8 hours post-dose

Unique study design or methodology (if any): Brain concentrations of test article were evaluated by perfusing brain with saline solution after each blood collection.

Results: Bepotastine besilate did not show a propensity to cross the blood-brain barrier, suggesting bepotastine besilate would have only limited sedative effects. Key data are summarized in the table below.

Table of AUC values for plasma, brain and brain/plasma ratios. AUC values in µg*hr/ml or g

	TAU-284	Ketotifen	Terfenadine	Ter-acid*	Epinastine	Cetirizine
AUC_{0-8}, plasma	1.587	0.457	0.154	0.875	0.779	12.497
AUC_{0-8}, brain	0.109	7.706	0.082	0.288	0.030	0.052
AUC_{brain}/AUC_{plasma} ratio	0.069	16.862	0.532	0.329	0.039	0.004

*Ter-acid = acid metabolite form of terfenadine

2.6.4.5 Metabolism

Study title: Metabolism of Bepotastine Besilate (TAU-284) in Human Liver Microsomes and the Main Effects on P450 Activity

Key study findings: Bepotastine besilate did not appear to be a substrate for human liver microsome metabolism or inhibit CYP 3A4, 2C9 or 2C19 metabolism of classic substrates at concentrations up to 200 µM.

Study no.: TNB-HE-07
eCTD location: 4.2.2.4.1
Conducting laboratory and location: (b) (4) inferred from report cover page
Date of study initiation: November 1996
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:
bepotastine besilate, lot #13, 99.4% pure
Terfenadine, lot #3, % purity not indicated

Methods
Unique study design or methodology (if any): Potential for metabolism by human liver microsomes of bepotastine besilate and terfenadine were evaluated at 10 µM final concentration. CYP450 isozyme inhibition potential was evaluated over a range of 2 to 200 µM of TAU-284 or positive control (ketoconazole, sulfaphenazole, or omeprazole).

Results: Bepotastine besilate showed virtually no metabolism during a 2 hour incubation with human liver microsomes, with 99% of parent compound remaining at the end of incubation compared to only 18% of parent compound terfenadine remaining after the same incubation period. Further studies evaluating the inhibition potential of bepotastine besilate on CYP450 3A4, 2C9 and 2C19 showed little potential for bepotastine besilate causing drug-drug interactions with compounds metabolized by these isozymes.

Study title: Effects of Bepotastine Besilate on Mouse Liver Cytochrome P-450 – Using Testosterone Metabolic Activity and Alkoxyresorufin O-Dealkylation Activity as Indices

Key study findings: 2 week oral administration of bepotastine besilate (20 mg/kg) to female CD-1 mice did not increase liver weights as compared to controls but did increase Cyp450 protein content by 2.1 fold higher than controls. Further investigation revealed that bepotastine besilate induced Cyp2b, Cyp2c, Cyp2d, Cyp2a and Cyp1a1/2. The effect on Cyp2b was particularly strong (3.5-6.5 fold that of controls based on PROD and EROD activity, respectively).

Study no.: TNB-NI-28
eCTD location: 4.2.2.4.1
Conducting laboratory and location: (b) (4)
Date of study initiation: March 1997
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:
TAU-284, lot #13, 99.4% pure

Methods
Doses: test articles and respective vehicle controls
20 mg/kg bepotastine besilate, 1X/day for 2 weeks
8 mg/kg Phenobarbitol, 1X/day for 4 days
8 mg/kg β –naphthoflavone, 1X/day for 3 days

Species/strain: Crj:CD-1 mice from (b) (4)
Number/sex/group: 5/group, females only
Route, formulation, volume:
TAU-284: oral gavage, 0.5% carboxymethylcellulose sodium to 20 mg/ml concentration, 10 ml/kg volume
β –naphthoflavone: i.p., in olive oil to 8 mg/ml concentration, 10 ml/kg
phenobarbitol: i.p., in 0.9% NaCl to 8 mg/ml concentration, 10 ml/kg

Age: 8-9 weeks
Weight: 25-33 g

Unique study design or methodology: Similar findings were not seen in the rat 2 year bioassay. Livers were collected 24 hours after final dose and microsomes were prepared. In vitro metabolism of testosterone, 7-ethoxyresorufin and 7-pentoxyresorufin were evaluated.

Results: 200 mg/kg x 14 days p.o. bepotastine besilate did not induce significant increases in liver weights as did 80 mg/kg x 3 days i.p. β –naphthoflavone or 80 mg/kg x 4 days i.p. phenobarbitol. However, bepotastine besilate did induce testosterone hydroxylation and alkoxyresorufin o-dealkylation activities. All three compounds caused a ~2 fold increase in Cyp450 content as compared to controls.

The specific Cyp450 isozymes induced were determined by the hydroxylation patterns of testosterone and PROD and EROD activity. Oral administration of bepotastine besilate caused induction of Cyp2b, Cyp2c, Cyp2d, Cyp2a and Cyp1a1/2. The effects on Cyp2b was particularly strong (3.5-6.5 fold that of controls based on PROD and EROD activity, respectively).

β –naphthoflavone caused the induction of Cyp1a1/2 and Cyp2b, with a particularly strong effect on Cyp1a1/2 (5 fold that of controls based on PROD and EROD activity). Phenobarbitol caused induction of Cyp2b, Cyp2c, Cyp2d, Cyp3a and Cyp1a1/2, with a particularly strong effect on Cyp2b (7-8 fold that of controls based on PROD and EROD activity).

Study title: Influence of Bepotastine Besilate on Hepatic Cytochrome P-450 in Rats – Using Testosterone Metabolism Activity as the Index

Key study findings: 2 weeks of oral dosing with bepotastine besilate was able to induce Cyp2B1/2, Cyp3A1/2 , Cyp2A1 and likely Cyp2C11. The extent of enzyme induction was dose dependent, with levels reaching statistical significant with 100 and 300 mg/kg doses.

Study no.: TNB-NI-27
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: August 1996
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, Lot #13, 99.4% pure

Methods
Doses: 30, 100, and 300 mg bepotastine besilate/day oral gavage for 2 weeks.
80 mg/kg Phenobarbital ip for 4 days
Control: 0.5% carboxymethylcellulose sodium 1x/day for 2 weeks

Note, % target dose and stability of test articles was not indicated.

Species/strain: Crj: Sprague-Dawley male rats from (b) (4)
Number/sex/group (main study): 4, males only

Route, formulation, volume:
bepotastine besilate: oral gavage, 0.5% carboxymethylcellulose sodium, 10 ml/kg
Phenobarbital: IP, 0.9% saline, 2 ml/kg
Control: oral gavage, 0.5% carboxymethylcellulose sodium, 10 ml/kg

Age: 7-8 weeks
Weight: 250 to 370 g

Unique study design or methodology: Hepatic microsomes were prepared 24 hours after final dose. Total amount of protein and P450 content were measured and microsomes were studied for their ability to metabolize testosterone in vitro.

Results: Both bepotastine besilate and phenobarbitol treatment caused a dose-dependent increase in Cyp450 enzyme content in the liver, with levels of induction being statistically significant at the 100 and 300 mg bepotastine besilate doses and the phenobarbital group. Rates of testosterone metabolism were significantly elevated in the 100 and 300 mg/kg group similar to that of phenobarbital. Bepotastine besilate distinguished itself from the phenobarbital pattern of testosterone metabolism in generating androstendione, 2α-hydroxylation, and 16α-hydroxylation. Both compounds caused elevation in testosterone 16β-hydroxylation, 6β-hydroxylation, 2β-hydroxylation, and 7α-hydroxylation.

Phenobarbitol was inferred to induce Cyp3A1/2, Cyp2A1 and Cyp2B1/2.
TAU-284 was inferred to induce Cyp2B1/2, Cyp3A1/2 and Cyp2A1. Cyp2C11 induction is also likely.

2.6.4.6 Excretion
Study title: **Internal Dynamics of Bepotastine Besilate (TAU-284) in Canines – Effects of Concomitant Use of Probenecid**

Key study findings: Probenecid did not significantly affect pharmacokinetic parameters of bepotastine besilate following a single oral administration, suggesting that renal clearance of bepotastine besilate is primarily by glomerular filtration.

Study no.: TNB-HE-06
eCTD location: 4.2.2.5.1
Conducting laboratory and location: (b) (4)
Date of study initiation: March 25, 1996
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:
Bepotastine besilate, lot #13, 99.4% pure
Ketotifen fumarate, lot #3, % purity not indicated
Terfenadine, lot #4, % purity not indicated
Epinastine hydrochloride, lot #1, % purity not indicated
Cetirizine dihydrochloride, lot #4, % purity not indicated
Terfenadine acid, lot #3, % purity not indicated
Methods
Doses: Single dose, latin square crossover design, with dosing periods separated by at a minimum 1 week drug holiday.

Test 1: 5 or 10 mg bepotastine besilate + 200 mg probenecid (administered 30 minutes prior to bepotastine besilate)
Test 2: 10 mg bepotastine besilate + 900 mg probenecid (20 ml administered 30 minutes prior to bepotastine besilate and 16 ml administered 2 hours after administration)

Species/strain: Male beagle dogs from stock maintained at [redacted] (b) (4)

Number/sex/group: 4 males/group Test 1, 3 males/group Test 2

Route, formulation:
bepotastine besilate: oral, 5 and 10 mg tablets
Probenecid: oral gavage, 10 mg/ml in a 50% macrogol suspension
Age: 7 weeks
Weight: 8.5 to 13.5 kg
Sampling times: pre-bepotastine besilate dose, and 0.25, 0.5, 1, 2, 3, 5, 7, and 24 hours post-bepotastine besilate dose.

Results: The study was done because 70-90% of unchanged bepotastine besilate is excreted in urine in humans within 24 hours of administration, and to determine the contribution of renal tubular secretion activities to this mechanism.

Probenecid did not significantly affect pharmacokinetic parameters of bepotastine besilate following a single oral administration, suggesting that renal clearance of bepotastine besilate is primarily by glomerular filtration. This result may or may not be relevant to the human condition, it is noted that renal clearance in humans is greater than that in dogs, and may be the result of a combination of glomerular filtration and active tubular secretion. The table below summarizes the data from this study.

Summary of PK parameters following oral dosing of TAU-284 ± Probenecid (average ± SE)

TAU-284 dose (mg/animal)	Probenecid administration	Tmax (hr)	Cmax (ng/ml)	AUC_{24} (ng*hr/ml)	AUC_{inf} (ng*hr/ml)	$t_{1/2}$ (hr)
Test 1*						
5	--	**1.0 ± 0.4**	**363.3 ± 44.2**	**2588.8 ± 185.1**	**2627.6 ± 200.2**	**4.2 ± 0.3**
	++	**0.7 ± 0.2**	**392.4 ± 35.2**	**2771.6 ± 209.7**	**2824.8 ± 211.4**	**4.8 ± 0.4**
10	--	**1.0 ± 0.4**	**738.8 ± 89.3**	**5195.2 ± 321.4**	**5277.4 ± 319.9**	**4.1 ± 0.1**
	++	**0.9 ± 0.1**	**703.4 ± 40.5**	**5367.3 ± 325.6**	**5500.8 ± 336.5**	**4.6 ± 0.2**
Test 2**						
10	--	**1.2 ± 0.4**	**711.8 ± 105.6**	**5637.5 ± 584.3**	**5771.3 ± 634.5**	**4.4 ± 0.4**
	++	**0.7 ± 0.2**	**800.1 ± 43.5**	**4935.8 ± 261.4**	**5044.1 ± 269.1**	**4.5 ± 0.1**

***n = 4 animals, ** n = 3 animals**

2.6.4.7 Pharmacokinetic drug interactions

No studies submitted.

2.6.4.8 Other Pharmacokinetic Studies

No studies submitted.

2.6.4.9 Discussion and Conclusions

Bepotastine besilate is distributed to multiple subocular compartments with minimal systemic exposure being seen. Tissues with highest exposure included conjunctiva, cornea, iris and ciliary body, retina and choroid. The association with the iris suggests there may be some affinity for bepotastine besilate binding to melanin. Plasma exposure was 668.56 ng eq * hr/ml, blood exposure was 945.68 ng eq*hr/ml (values given as $AUC_{0\text{-}inf}$). Tmax for plasma and blood was 0.5 hours, $t_{1/2}$, ranged from 0.63 to 0.99

hours. From in vitro studies, it does not appear that bepotastine besilate is a substrate for human CYP450 metabolism or induction, while in rodents bepotastine besilate has demonstrated the ability to induce Cyp450 isozymes. From evaluatation of Cyp450 enzyme content in livers in the chronic toxicology study, bepotastine besilate also does not appear to be a Cyp450 target in dogs either. In dogs, bepotastine besilate is primarily excreted by the kidney by glomerular filtration.

2.6.4.10 Tables and figures to include comparative TK summary

Not provided.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

The applicant did not provide an appropriate summary table in the application.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Ocular, oral and intravenous toxicology studies were conducted with bepotastine besilate. Ocular studies established the NOAEL as 1.5% bepotastine besilate when given 4X/day for up to 26 weeks in dogs (SNJ-NI-5).

Transient ocular irritation was observed with test article administration in the first few days of administration, but incidence was reduced with chronic administration. Some decrease in a- and b-wave amplitudes were observed in electroretinogram exams in males and females following 8X/day treatment for 26 weeks. These observations were not seen in the 4X/day treatment group. The applicant also studied ocular administration of bepotastine besilate in rabbits (SNJ-NI-3) which demonstrated a NOAEL of 1.5% TAU-284 solution given 8X/day for up to 4 weeks. A 2.0% bepotastine besilate solution was also evaluated in rabbits (8X/day for 1-7 days, Study SNJ-NI-1 and SNJ-NI-9). The results revealed that the 2.0% solution caused slightly more irritation than the 1.5% solution.

Mydriatis was not observed with topical ophthalmic application of bepotastine besilate, but was often seen with high doses of bepotastine besilate administered orally. The data collected from the single dose intravenous study in rats and multiple dose oral toxicity studies up to 26 weeks in rats and dogs provided additional assurance regarding the safety margins associated with bepotastine besilate ocular application and the proposed clinical use.

NOAELs in single dose oral toxicity studies in rats and dogs were 500 mg/kg and <500 mg/kg, respectively. No test article related deaths were observed for either species at doses up to 2000 mg/kg. A single dose intravenous study in rats established a NOAEL of 75 mg/kg for both males and females and an LD_{50} of 130 mg/kg for males and 126 mg/kg for females.

Oral toxicity studies:

The NOAEL was 30 mg/kg/day in a 26 week oral toxicity study in dogs and was 20 mg/kg/day in a 26 week oral toxicity study in rats, resulting in safety factors of 2876X and 93X, respectively, when comparing systemic exposures to anticipated maximal concentrations seen with human topical ophthalmic use. The primary clinical signs of toxicity seen in these oral toxicity studies were vomiting

and salivation in dogs and liver pathologies associated with metabolic induction in the rats. The sponsor also submitted a single dose intravenous study in rats in which the LD_{50} was determined to be 130 mg/kg for males and 126 mg/kg for females. Systemic exposure following the oral and intravenous dosing routes were far above what occurs after ocular administration of bepotastine besilate.

Ocular toxicity studies:
The pivital study for the proposed indication was a 26 week study in dogs using 4 and 8X per day dosing with the 1.5% bepotastine besilate solution. The 4X/day dosing paradigm was determined to be the NOAEL, due to the observation of decreases in A and B wave amplitude in electroretinogram exams in the 8X/day dose group. When considering systemic exposures seen in this study, the identified NOAEL provides a 15X safety factor over that of anticipated systemic exposures seen with topical ocular use in humans. Several short term ocular toxicity studies were also conducted in several species and demonstrated bepotastine besilate solutions of up to 2% were well tolerated.

Genetic toxicology: Bepotastine besilate did not demonstrate mutagenic/genotoxic potential in any of the tests conducted. This supports the suggestion that liver tumors seen in mice were due to enzyme induction rather than due to a genotoxic mechanism. The details of the genetic toxicology studies are bulleted below:

- negative; Ames assay, concentrations up to 5000 µg/plate for both with and without metabolic activation
- negative; chromosome aberration assay, concentrations up to 2100 µg/ml for 24 hour direct mutagenicity, up to 1000 µg/ml for the 48 hour direct mutagenicity and up to 5500 µg/ml in the metabolic activation study
- negative; unscheduled DNA synthesis in primary mouse hepatocyte cultures concentrations up to 1000 µg/ml
- negative; in vivo micronucleus test in mice, doses up to 1000 mg/kg x 2 days (lethal dose was 2000 mg/kg)

Carcinogenicity: Bepotastine besilate was mixed in feed and administered ad libitum to mice and rats to evaluate carcinogenic potential. Oral administration of bepotastine besilate was found to induce hepatocellular carcinomas/tumors in female mice at the highest dose tested (nominal dose 190 mg/kg/day) for 21 months which represents a margin of exposure of approximately 353 times that anticipated that of systemic concentration seen with topical ocular application in humans. The Applicant concluded that the liver neoplasms in the female mice were drug related, but the incidence of these neoplasms were not statistically significant according to criteria applied by CDER. There were no similar tumor findings in the rat study which assessed doses up to 97.9 mg/kg/day for 24 months (provide a relative safety factor approximately 200 times that anticipated for systemic concentration seen with topical ocular application in humans. Given the ocular dose of the use of this drug, bepotastine besilate is not believed to pose a significant risk for cancer induction in humans.

Reproductive toxicology:
The pregnancy category C was applied to this drug due to the observation of a slight potential for teratogenicity in rats (Study TNB-NI-10). Although this risk is not considered significant for ocular use, it could be considered more relevant for other indications where much higher levels of systemic exposure would be expected.

Reproductive toxicology:

Overall view:

Pregnancy Category C is recommended for the label, based on the observation of a rare skeletal abnormality (an increase in pre-sacral vertebra and lumbar rib seen in 11.1% of litters in the 1000 mg/kg dose group vs none in controls) seen in the rat study evaluating fertility and development through day 7 of gestation (TNB-NI-10). The NOAEL for this observation was 200 mg/kg/day. The sponsor did not measure toxicokinetics values for the reproductive toxicology studies. A safety factor of 3330X was estimated by referring to systemic concentrations seen in the 200 mg/kg/day group in the 26 week oral toxicology study in rats on day 1, to the anticipated human systemic concentration seen with topical ocular use.

NOAEL for pregnant female toxicities was 10 mg/kg for rats and rabbits due to the observation of mydriasis at the next higher dose in the different studies. Additional toxicities were seen with the higher doses. Mortality was seen at 1000 mg/kg dose levels. There were no effects on organ development observed in rats or rabbits. There was an increase incidence of still borns and survival at day 4 postbirth in 100 and 1000 mg/kg dosed rats. Developmental delays were also seen in the 1000 mg/kg dose group. Due to the anticipated topical ocular use of this product, the potental risk for reproductive effects in humans is considered low.

Additional details for each study are provided in the text below:

Fertility and early pregnancy (through Gestation Day 7): Sprague-Dawley Rats, TAU-284 doses of 0, 8, 40, 200 and 1000 mg/kg/day examined.

Parent body toxicities: Oral administration of bepotastine besilate displayed a NOAEL of 40 mg/kg/day due to the observation of mydriasis in 100% of animals on at least one occasion in the 200 and 1000 mg/kg/day dose groups. Two males in the 1000 mg/kg/day group died on days 49 and 57 of dosing.

Fertility: NOAEL for reproductive capacity was 200 mg/kg/day for both males and dams, based on observation of 20% infertility (both males and dams) in the 1000 mg/kg/day dose group. There were no pathologies seen at necropsy to explain this, and there were no assessments done on sperm motility or morphology.

NOAEL for embryotoxicity and teratogenic potential through gestation day 7 was 200 mg/kg/day due to increased preimplantation loss (approximately 2-fold higher) in the 1000 mg/kg/day group as compared to controls. There may have also been a slight ability to serve as a teratogen with 3/132 fetuses (2.27% or 11.1% on a per litter basis) in the 1000 mg/kg/day group displaying an increase in pre-sacral vertebra and lumbar rib, with none seen in any of the control fetuses. This was also seen in 1/238 fetuses (0.004%) in the 40 mg/kg/day group, but this was considered to be an incidental finding. According to a 1993 publication from Charles River Laboratories, this is considered a very rare finding.

Developmental toxicity: Fetal Organ formation (Gestation Days 7 to 17) in Sprague-Dawley Rats; bepotastine besilate doses 0, 10, 100 and 1000 mg/kg/day

Parent body toxicity: NOAEL 10 mg/kg/day for dams due to mydriasis seen at the 100 and 1000 mg/kg/day dose levels.

Developmental toxicity (organ development stage):
NOAEL was 1000 mg/kg/day, the highest dose examined in this study. There were no apparent test article effects on either the F1 or F2 generations.

Developmental toxicity: Fetal Organ formation (Gestation days 6 to 18) in NZW Rabbits; bepotastine besilate doses 0, 20, 100 and 500 mg/kg/day

Parent body toxicity: A NOAEL was not established but is considered to be < 20 mg/kg/day. Miscarriage was noted in one 20 and one 100 mg/kg/day dam on gestation days 19 and 29, respectively, although these deaths were not clearly test article related. Bleeding was noted in 2 additional dams in the 20 mg/kg group on Gestation days 28 and 29. Red urine was seen in 5/14 animals in the 100 mg/kg group after the completion of dosing, starting on Gestation day 22 and in 7/14 females in the 500 mg/kg group starting on gestation day 14. This was not considered indicative of miscarriage but may be due to a species specific metabolite.

NOAEL for the organogenesis stage of development was considered to be 500 mg/kg/day. There were no apparent test article effects on the F1 generation.

Perinatal and Lactation period toxicity (dams treated Gestation day 17 through Day 21 post-delivery): Developmental and reproductive toxicity Sprague-Dawley rats; bepotastine besilate, 0, 10, 100 and 1000 mg/kg/day

Parent body toxicity: NOAEL = 10 mg/kg/day due to the retention of a dead fetus in the uterus of one dam in the 100 mg/kg/day group. Other observations were made in the 1000 mg/kg/day dose group included deaths of several dams, and a higher incidence of still births. Mydriasis and soiling of lower abdomen was also seen in this high dose group.

F1 generation effects: NOAEL = 10 mg/kg/day due to increased incidence of stillborns in the 100 and 1000 mg/kg dose groups as compared to controls (6.01% and 10.18% vs 3.79%). There was also a decrease in the number of animal surviving from birth to post-delivery day 4 in the 1000 mg/kg dose group as compared to controls (46.09% vs 94.10%). There was no difference in distributions of effects between male and female offspring.

Also seen in the 1000 mg/kg dose group were a tendency toward the delay of the differentiation of pinna detachment (50% of animals), incisor eruption (~10% of animals), and eyelid separation (~10% of males needed 1 additional day). A 20-30% decrease in righting reflex rate, suppression of weight gain (-11 to -15%), and transient decreased food consumption between days 28 to 56 after birth were seen in the 1000 mg/kg pups as compared to controls. No effects of test article on motor coordination, learning ability, emotionality, or reproductive capacity, and no abnormalities were observed with regard to the development of F1 fetuses.

F2 effects: NOAEL = 100 mg/kg/day, due to the observations of slightly higher total number of early resorptions seen in the 1000 mg/kg dose group as compared to controls.

The sex ratio of embryos (males/females) was ~50% lower in the 1000 mg/kg dose group as compared to controls.

Special toxicology:
Bepotastine besilate is not expected to cause skin sensitization or antigenicity based on studies conducted in Guinea pigs and mice.

2.6.6.2 Single-dose toxicity

Study title: Acute Toxicity Study on Bepotastine Besilate – Single Dose Oral Toxicity Study in Rats

Key study findings: LD_{50} of bepotastine besilate was > 2000 mg/kg and the NOAEL of bepotastine besilate was < 500 mg/kg by oral dosing in Crj:CD(SD) male rats. Transient mydriasis, decreased body weight gain and food intake were observed in all males in the 1000 and 2000 mg/kg groups. Mydriatis was also seen in 2/6 males in the 500 mg/kg dose group. All observations resolved by 2 days after treatment. There were no test-article related pathologies identified at necropsy or in any of the female rats.

Study no.: TNB-NI-01
eCTD location: 4.2.3.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: November 2, 1990
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot #4, 98.54% pure

Methods
Doses: Single dose, 0, 500, 1000 and 2000 mg/kg bepotastine besilate administered to fasted animals. Doses were not corrected for purity. Test article stability was confirmed for the dosing period and was estimated to be 97.8 to 101.89% of targeted dose.

Note, doses were based on results from a preliminary study in which single doses of 500, 1000 and 2000 mg/kg were administered to 3 male and 3 female Crj:CD(SD) rats. Mydriatis was observed in the 1000 and 2000 mg/kg groups on the day of dosing, while decreases in body weight and food consumption were seen in these same groups the day after dosing. There were no fatalities.

Species/strain: CrJ:CD (SD) rats) from (b) (4)
Number/sex/group: 6
Route, formulation, volume: oral gavage, 0.5% methylcellulose 1500, 10 ml/kg

Age: 6 weeks
Weight: 177.8 to 192.8 g for males and 128.9 to 144.8 g for females

Sampling times:
Unique study design: Animals observed for 14 days after dosing.

Results
Mortality (first 6 hours after dosing, twice daily thereafter): No mortalities were observed for the duration of the study.

Clinical signs (first 6 hours after dosing and twice daily thereafter): Mydriasis was seen ~15 minutes after dosing in 2/6 males in 500 mg/kg group and 6/6 males in each the 1000 and 2000 mg/kg groups. Resolution was seen in all remaining animals by the second day after dosing. No effects were seen in females.

Body weights (day 0, 1, 3, 5, 7, 10 and 14): Transient decrease in body weight gain associated with decreased food intake was seen the first 2 days after dosing in the 2000 mg/kg males compared to controls (14.2 g vs 26.2 g change in weight gain between day 0 to day 1), but this recovered to normal levels by day 3. No effects were seen in females.

Food consumption (day -1, 1, 3, 5, 7, 10 and 14): Decreased food consumption was noted the first 2 days after treatment in the high dose males compared to controls (-3.2 g/day vs +4.4 g/day change in food intake between day -1 and day 1), but returned to normal levels by day 3 after dosing. No effects were seen in females.

Gross pathology (scheduled necropsy Day 14): No test article related changes observed.

Study title: Acute Toxicity Study on Bepotastine Besilate – Single Dose Intravenous Toxicity Study in Rats

Key study findings: NOAEL = 75 mg/kg for both males and females. LD_{50} was calculated to be 130 mg/kg for males and 126 mg/kg for females.

Study no.: TNB-NI-02
eCTD location: 4.2.3.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: November 2, 1990
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )-
Drug, lot #, and % purity: bepotastine besilate, lot #4, 98.54% pure

Methods
Doses: Single dose 0, 75, 100, 120, 150 and 200 mg/kg bepotastine besilate. Doses were not corrected for purity. Solutions were confirmed to be stable for the duration of dosing and were within 97.5 to 101.4% of targeted dose.

Note, doses were chosen from a preliminary study in which 75, 150 and 300 mg/kg doses were administered to 3 male and 3 female CrJ:CD(SD) rats. 2/3 males and 2/3 females in the 150 mg/kg group and 100% of animals in the 300 mg/kg group died after dosing. From these results, 200 mg/kg was chosen as the highest dose for the confirmatory study.

Species/strain: CrJ:CD(SD) rats

Number/sex/group: 6

Route, formulation, volume: i.v., Japanese pharmacopoeia saline solution, 10 ml/kg

Age: 6 weeks
Weight: 182.2 to 217.4 g for males and 135.9 to 172.1 g for females

Unique study design or methodology: Animals were evaluated for 14 days post-treatment.

Results
Mortality (first 6 hours after dosing, twice daily thereafter): Deaths were observed within 4 minutes of dosing in 2/6 males and 3/6 females in the 120 mg/kg; 5/6 males and 5/6 females in the 150 mg/kg group and 100% of animals in the 200 mg/kg groups. The LD_{50} was calculated to be 130 mg/kg for males and 126 mg/kg for females.

Prior to death, the rats presented hypoactivity and ataxic gait or prone position followed by convulsions, dyspnea and loss of righting reflex.

Clinical signs (first 6 hours after dosing, twice daily thereafter): Hypoactivity, prone position, tachypnea and mydriasis were observed in the surviving rats in the 100 (females only), 120 and 150 mg/kg dose groups, but these symptoms resolved by 6 hours after dosing. No effects were seen in controls or in the 75 mg/kg dose groups.

Body weights (Day 0, 1, 3, 5, 7, 10 and 14): No test article related effects observed.

Food consumption (Days -1, 1, 3, 5, 7, 10 and 14): No test article related effects observed.

Gross pathology (day 14 for scheduled necropsy): No test article related effects observed.

Study title: Acute Toxicity Study on Bepotastine Besilate – Single Dose Oral Toxicity Study in Dogs

Key study findings: Bepotastine besilate LD_{50} was determined to be > 2000 mg/kg following a single oral administration in beagle dogs. NOAEL was < 500 mg/kg. Transient vomiting was observed following dosing in all bepotastine besilate groups, resolving by 6 hours after dosing. Transient salivation was also seen in the 1000 and 2000 mg/kg groups, resolving by 2 hours after dosing. Systemic Cmax was achieved within 1.5 to 3 hours post-dose, with nearly complete elimination occurring by 24 hours post-dose.

Study no.: TNB-NI-03
eCTD location: 4.2.3.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: November 2, 1990
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot #4, 98.54% pure

Methods
Doses: Single dose, 0, 500, 1000, and 2000 mg/kg administered to fasted animals. Doses were not corrected for purity. Dosing solutions were confirmed to be stable for the duration of dosing.

Note, doses were based on a preliminary study in which up to 300 mg/kg oral doses were evaluated in beagle dogs. No clinical observations other than vomiting were observed. Based on these results, 2000 mg/kg (maximum permissible dose for suspension) was chosen as the high dose for this study.

Species/strain: beagle dogs from (b) (4)
Number/sex/group: 2

Route, formulation, volume: oral gavage, 0.5% methylcellulose 1500 solution, 10 ml/kg
Age: 7 months
Weight: 8.7 to 9.8 kg for males and 8.1 to 9.3 kg for females

Unique study design or methodology (if any): Animals were observed for 14 days post-dose.

Results
Mortality: No premature mortalities were observed.

Clinical signs (first 6 hours post-dose, twice daily thereafter): Vomiting following dosing was observed in all dose groups, and was accompanied by increased salivation in some animals in the 1000 and 2000 mg/kg dose groups. All observations were resolved by 1 (salivation) and 6 (vomiting) hours after treatment. Vomiting in the 1000 mg/kg dose group occurred within 2 hours after feeding, while vomiting in the 2000 mg/kg dose group occurred before feeding in ~ half of the cases and after feeding in the remaining half of cases.

No other clinical signs were observed.

Body weights (first 6 hours post-dose, twice daily thereafter): No test article effects observed.

Food consumption (first 6 hours post-dose, twice daily thereafter): No test article effects observed.

Hematology (1 week predose and 1 and 2 weeks post-dose): No test article effects observed.

Clinical chemistry (1 week predose and 1 and 2 weeks post-dose): No test article effects observed.

Gross pathology (Day 14): No test article effects observed.

Toxicokinetics (day -1 and at 1.5, 3, 6 and 24 hours after dosing): Test article measured by LC method, lower limit of detection = 0.01 µg/ml. Test article concentration in blood reached Cmax within 1.5 to 3 hours, and was nearly completely eliminated by 24 hours after dosing.

Plasma concentrations of TAU-284; Mean ± SE

Dose mg/kg	Plasma concentration (µg/ml) Time after treatment			
	1.5 hr	3 hr	6 hr	24 hr
500				
Males	58.45 ± 0.65	56.38 ± 0.16	9.13 ± 1.47	0.03
Females	39.31 ± 0.33	31.54 ± 15.41	7.73 ± 0.36	--
1000				
Males	39.14 ± 0.24	35.17 ± 0.49	10.68 ± 1.01	--
Females	24.16 ± 0.25	46.98 ± 2.04	5.32 ± 0.77	0.12 ± 0.01
2000				
Males	54.94 ± 0.25	49.23 ± 0.00	9.95 ± 0.37	--
Females	49.23 ± 0.82	79.15 ± 3.67	13.08 ± 1.53	0.40 ± 0.08

2.6.6.3 Repeat-dose toxicity

Study title: The Ocular Irritancy Test for Anterior Eye with TAU-284 Ophthalmic Solution Being Administered to Rabbit 8 Times a Day

Key study findings: Transient ocular irritation was seen with the 1.5% and 2.0% bepotastine besilate solutions, the degree of severity was considered slight in the 1.5% treated eyes and slight to moderate in the 2.0% treated eyes.

Study no.: SNJ-NI-1
eCTD location: 4.2.3.2.1
Conducting laboratory and location: Kobe Creative Center at Senju Pharmaceutical Co., Ltd., Hyogo, Japan
Date of study initiation: 05 March 2003
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, 1.5% solution Lot # 03K241; 2.0% solution Lot # 03K241; % purity not given

Methods
Doses: 1.5 and 2.0% bepotastine besilate, in right eye, physiological saline solution in left eye; administered 8X, 1X/hour for 1 day, Dose solutions were 101% of target dose and were stable over the duration of treatment.

The 2.0% solution was chosen as the high dose based on a preliminary experiment in which transient ocular irritation was seen with this dose.

Species/strain: Japanese white rabbits from (b) (4) labs.
Number/sex/group: 5, males only
Route, formulation, volume: ocular instillation, 1.5 and 2.0% ophthalmic solution, 100 µl

Age: 10 weeks
Weight: 1.93 to 2.09 kg

Results
Mortality: No premature mortality observed.

Clinical signs (day -1, 1 and 2): No test article effects observed.

Body weights (Day of treatment and day after): No test article effects observed.

Ophthalmoscopy: Slit lamp examination on day -1 and 30 minutes after the 8th administration. The conjunctiva was observed by microscope 30 minutes after the 2nd, 4th, and 6th administration and the day after treatment.

Conjunctival conjestion was seen following the 2nd instillation to a slight degree in 3/5 treated eyes in the 1.5% treatment group and in 5/5 treated eyes in the 2% treatment group, with progression to a moderate degree in one of these animals after the 6th instillation.

Also observed in the 2.0% treatment group was a slight degree of secretion in 2/5 eyes and a very slight degree of corneal staining stigma in 3/5 cases.

These findings resolved by the day after treatment. There were no events observed in the saline treated eyes.

Study title: Investigation of Ocular Lesions after 8 Instillations in 1 Day of TAU-284 and Ocular Lesions after Repeated Instillation 8 Times per Day for 7 Days

Key study findings: The NOAEL for the 7 day study was 1.5% bepotastine besilate. In the 1 day study, 2.0% bepotastine besilate caused ocular irritation, this dose was not used in the 7 day study. Test article did not cause mydriasis at any dose level.

Study no.: SNJ-NI-9
eCTD location: 4.2.3.2.1
Conducting laboratory and location: Kobe Creative Center at Senju Pharmaceutical Co., Ltd., Hyogo, Japan
Date of study initiation: 14 February 2001
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, Lot # 19, 100.14% pure

Methods
Doses:

1.0, 1.5 and 2.0% bepotastine besilate given hourly, 8X/day for 1 day.
1.0 and 1.5% bepotastine besilate solutions given hourly, 8X/day for 7 days .
Doses placed in right eye only. Left eye was treated with vehicle.

Species/strain: Japanese white rabbits from (b) (4)
Number/sex/group: 3 males/dose
Route, formulation, volume: ocular instillation, 1.0, 1.5 and 2.0% bepotastine besilate ophthalmic solution, 100 µl/eye

Weight: 2 kg

Results
8X/day for 1 day
Macroscopic ocular observations (predose, 30 minutes after the 2nd, 4th, 6th and 8th doses, slit lamp exam before dosing and after the 8th dose): Mild bulbar conjunctival redness and palpebral conjunctival redness were observed beginning with the 2nd instillation and continuing for the duration of dosing for the 2.0% formulation. Mild bulbar conjunctival redness was seen after the 2nd instillation with 1.5% bepotastine besilate, but did not persist across the other doses. There were no effects seen with the 1.0% dosing solution.

8X/day for 7 days
Ocular examinations (predose and 30 minutes after last dose on days 1, 4, and 7): No effects seen for the 1.0 and 1.5% solutions.

Pupil size (predose and 30 minutes after the final dose on days 1, 4, and 7): Light reflex was measured at the end of examination on day 7. No mydriatic activity was seen with the 1.0 or 1.5% doses.

Study title: Study of Ocular Lesions after 8 Instillations of TAU-284 in 1 Day

Key study findings: NOAEL = 0.3% bepotastine besilate, the highest dose examined in this study.

Study no.: SNJ-NI-8
eCTD location: 4.2.3.2.1
Conducting laboratory and location: Kobe Creative Center at Senju Pharmaceutical Co., Ltd., Hyogo, Japan
Date of study initiation: 24 April 2000
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, Lot # 802002, purity was ~ 99.8 to 100.1%
Methods
Doses:

0.05, 0.1 and 0.3% bepotastine besilate given hourly, 8X/day for 1 day
Doses placed in right eye only. Left eye was treated with vehicle.

Species/strain: Japanese white rabbits from (b) (4).
Number/sex/group: 3 males/dose
Route, formulation, volume: ocular instillation, 0.05, 0.1 and 0.3% bepotastine besilate ophthalmic solution, 100 µl/eye

Weight: 2 kg

Results
8X/day for 1 day
Macroscopic ocular observations (predose, 30 minutes after the 2nd, 4th, 6th and 8th doses, slit lamp exam before dosing and after the 8th dose): No ocular abnormalities observed.

Study title: Study of Anterior Ocular Segment Irritation after 8 Instillations of TAU-284 to Rabbits

Key study findings: NOAEL = 1.5% bepotastine besilate given 8X/day for 1 day due to mild ocular irritation seen with the 2.0% bepotastine besilate solution. No induction of mydriatis was seen with either the 1.5 or 2.0% bepotastine besilate solutions.

Study no.: SNJ-NI-10
eCTD location: 4.2.3.2.1
Conducting laboratory and location: Kobe Creative Center at Senju Pharmaceutical Co., Ltd., Hyogo, Japan
Date of study initiation: 08 November 2001
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, Lot # 104002, 100.0% pure

Methods
Doses:
1.0, 1.5 and 2.0% given hourly, 8X/day for 1 day.
Doses placed in right eye only. Left eye was treated with vehicle.

Species/strain: Japanese white rabbits from (b) (4)
Number/sex/group: 3 males/dose
Route, formulation, volume: ocular instillation, 1.0, 1.5 and 2.0% ophthalmic solution, 100 µl/eye

Age: ~9 weeks
Weight: 2.02 to 2.31 kg

Results
Macroscopic ocular observations (predose, 30 minutes after the 2nd, 4th, 6th and 8th doses, slit lamp exam before dosing and after the 8th dose): Conjunctiva examined after 2nd, 4th, and 6th administrations. Corneal assessment was performed at the end of dosing and the following day.

NOAEL = 1.5% bepotastine besilate solution due to low levels of conjunctival redness and secretion and fluorescein corneal staining seen in the 2.0% bepotastine besilate treated group.

Pupil size (predose and 30 minutes after the 4th and 8th doses): No effects observed for either the 2.0 or 1.5% bepotastine besilate solutions.

Study title: Study on Anterior Ocular Segment Irritation Due to Seven-Day Repeated Administration of the TAU-284 Ophthalmic Solution to Rabbits

Key study findings: NOAEL = 1.5% bepotastine besilate solution following 8X/day X 7 day dosing administration. This was the highest dose examinined in this study.

Study no.: SNJ-NI-2
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 10 December 2001
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity:
1.0% bepotastine besilate, lot # 1Y28, % purity not given
1.5% bepotastine besilate, lot # 1Y28, % purity not given
bepotastine besilate vehicle, lot # 1Y28

Methods
Doses: 8X/day administered hourly for 7 days , 1.0 and 1.5% bepotastine besilate + vehicle and saline controls. Bepotastine besilate/vehicle treatment was applied to the right eye, saline was applied to the left eye.

All test articles were within 100.0% of target dose and were stable for the duration of treatment.

Species/strain: Japanese white rabbits Kbl:JW from (b) (4)
Number/sex/group: 5, males only

Route, formulation, volume: ocular instillation, ophthalmic solution, 100 µl

Age: 12 weeks
Weight: 2.41 to 2.65 kg

Results
Mortality: No premature mortalities observed.

Clinical signs (once daily during treatment and day after treatment completion): No test article effects observed.

Body weights (first day of dosing and day after treatment completion): No test article effects observed.

Ophthalmoscopy (predose, day 1 and 30 minutes after final dose on days 4 and 7): No abnormalities were seen in the corneas, anterior chambers, irises, conjunctiva or pupil diameter measurements.

Study title: Toxicity Test by 4-Week Repeated Ophthalmic Administration of TAU-284 Ophthalmic Solution to Rabbits

Key study findings: NOAEL = 1.5% bepotastine besilate given 8X/day for 4 weeks. This was the highest dose tested in this study.

Study no.: SNJ-NI-3
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 04 December 2001
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate (both 1.0 and 1.5% solutions), lot 1Y28, purity = 100.0%

Methods
Doses: 8X/day administered hourly for 4 weeks (28 days); 1.0 and 1.5% bepotastine besilate and physiologic saline for negative controls. Doses were applied to both eyes. Test articles were within 100% of target dose and were stable for the dosing duration.

Species/strain: Dutch KBL pigmented rabbits from (b) (4)
Number/sex/group: 5, males only

Route, formulation, volume: ocular instillation, ophthalmic solution, 100 µl

Age: 9 weeks
Weight: 1.24 to 1.47 kg

Results
Mortality: No premature mortalities observed.

Clinical signs (daily): No test article effects observed.

Body weights (weekly): No test article effects observed.

Food consumption (daily): No toxicologically relevant effects observed.

Ophthalmoscopy (sample timing provided below): No toxicologically relevant effects were observed.

Anterior observation of both eyes: Day 1, Week 2 and Week 4, 30 minutes after 8th instillation

Cornea, iris and conjunctiva photographs were taken predose, and at week 4.

Optic media and fundus examination of the left eye was done predose and at weeks 2 and 4 between the 1st and 2nd instillation on day after anterior eye examination.

Pupil diameter of right eye was assessed predose and at weeks 2 and 4, 20 minutes after the 8th instillation and prior to anterior eye examination.

Electroretinogram examination of right eye was done predose and in Week 4 between 1st and 2nd instillations.

Hematology (predose and week 4): Coagulation testing done the day after the last dose. No toxicologically relevant observations.

Clinical chemistry (predose and week 4): No test article effects observed.

Urinalysis (predose and week 4): Analysis done on 24 hour collections. Urine volume was significantly increased and specific gravity was significantly lowered in the 1.5% bepotastine besilate group compared to negative controls. This finding is not considered toxicologically relevant.

	Saline Control	**1.0% TAU-284**	**1.5% TAU-284**
Number of animals	5	5	5
Volume (ml/d)	38 ± 9	34 ± 14	84 ± 34*
Specific Gravity	1.064 ± 0.007	1.063 ± 0.010	1.046 ± 0.016

Organ weights : No test article effects observed.

Brain, pituitary, thyroids, salivary glands, thymus, lungs, heart, liver, spleen, adrenal gland, kidney and testis were weighed.

Histopathology: Adequate Battery: yes ( ), no (x)—explain Only the liver, eye ball, optic nerve and palpebral conjunctiva were assessed.
Peer review: yes ( ), no ( ) – not specified

No toxicologically relevant observations.

Study title: 13-Week Repeated Ophthalmic Dose Toxicity Study on the TAU-284 Ophthalmic Solution in Dogs

Key study findings: NOAEL = 1.5% bepotastine besilate administered 8X/day for 13 weeks, this was the highest dose examined in this study.

Study no.: SNJ-NI-4
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 08 July 2002
GLP compliance: Yes, Japan
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate 1.0 and 1.5% solutions, lot # 2R18, % purity not indicated

Methods
Doses: 8X/day administered hourly for 13 weeks: 1.0 and 1.5% bepotastine besilate, physiological saline used for negative control, doses applied to both eyes. The 1.0% solution was within 98.0% of target dose, while the 1.5% solution was within 98.7% of the target dose. The test articles were considered stable for the duration of the study.

Species/strain: beagle dogs (TOYO) from [redacted] (b) (4)

Number/sex/group (main study): 4, males only

Route, formulation, volume: ocular instillation, ophthalmic solution, 100 µl/eye

Age: 8-9 months
Weight: 10.20 to 11.40 kg

Results
Mortality: No premature mortalities observed.

Clinical signs (daily): No test article effects observed.

Body weights (once weekly): No test article effects observed.

Food consumption (once weekly): No test article effects observed.

Ophthalmoscopy (frequency specified below): No toxicologically relevant effects observed.

> Pupil diameter measurements and electroretinograms were done on right eyes. Measurements were done pre-dose and in Weeks 4, 8 and 13 at ≥30 minutes after the 8th instillation of the day.
>
> Intermediate optic media observations and fundus examinations were done on left eyes. Evaluations were done predose and in Weeks 4, 8 and 13 between the 1st and 2nd instillation.
>
> Anterior ocular segment observations on both eyes. Assessments were done pre-dose and once weekly at ≥30 minutes after 8th instillation of the day. The cornea, iris and conjunctiva were photographed pre-dose and in Week 13.
>
> Electroretinograms were done predose and in Weeks 4, 8, and 13 between the 1st and 2nd instillations of the day.

Hematology (pre-dose and in Weeks 4 and 13): No test article effects observed.

Clinical chemistry (pre-dose and in Weeks 4 and 13): No test article effects seen.

Urinalysis (pre-dose and Weeks 4 and 13): Urine collected over a 24 hour period. No toxicologically relavant effects observed.

Gross pathology: No test article effects observed. Swelling in both thyroid glands was observed in one animal in the 1.0% bepotastine besilate group.

Organ weights (see histopath table): The absolute and body weight ratios of the left adrenal gland were significantly decreased in the 1.5% bepotastine besilate group. This is not considered to be toxicologically relevant.

Adrenals, brain, heart, kidneys, liver, lungs, pituitary, salivary gland, spleen, testes, thymus, and thyroid were all weighed.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )—not indicated

Although a complete battery of tissues was collected, histopathological assessment was only done on the left eye ball, left palpebral conjunctiva, left ocular nerve and the organs/tissues having abnormal findings at necropsy (i.e. thyroid gland).

Study title: Toxicity Test by 26-Week Repeated Ophthalmic Administration of Butyric Acid Monobenzenesulfonate (TAU-284) Ophthalmic Solution in Dogs

Key study findings: NOAEL = 1.5% bepotastine besilate, 4x/day for 26 weeks. This was the highest dose tested in the study. This was based on the observation of decreased amplitude of A and B waves in electroretinogram measures in both males and females treated with 1.5% bepotastine besilate 8X/day.

Study no.: SNJ-NI-5
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 11 September 2003
GLP compliance: Yes, Japan
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lots 03X061 and 03Z171, % purity not indicated

Methods
Doses: 4-8X/day administered for 26 weeks, 1.5% bepotastine besilate and physiological saline control (12 or 24 mg bepotastine besilate/body/day). Dosing was hourly for the 8X/day group and every 2.5 hours for the 4X/day group. The saline group received 8X/day hourly doses as well. Test article was within 100-106.7% of target dose and was considered to be stable over the duration of the trial.

Species/strain: beagle dogs from (b) (4)

Number/sex/group: 4
Route, formulation, volume: ocular instillation, ophthalmic solution, 100 µl

Age: 8-9 months
Weight: 7.70 to 12.75 kg males and 6.35 to 9.75 kg females

Results

Mortality: No premature mortality was observed.

Clinical signs (daily): No test article effects were observed.

Body weights (weekly): No test article effects observed.

Food and water consumption (weekly): No test article effects observed.

Ophthalmoscopy (predose, week 13 and 26): Included examination of pupil diameters, anterior eye examinations, intermediate optic media observation, fundus examination, intraocular pressure and electroretinogram examinations.

A significant decrease in intraocular pressure (IOP, 21.2 ± 2.5 vs 26.1 ± 1.7, $p < 0.05$) was seen in the male 1.5% bepotastine besilate 8X/day group at week 13 of treatment as compared to saline treated control. However, this difference was not significant when comparing pretest IOP values within the same dose group (21.2 ± 2.5 vs 22.4 ± 0.8). Because this change in IOP was also not seen at week 26 or in similarly dosed females it is not considered toxicologically significant.

When comparing mean group values to control values, the A and B wave amplitudes in electroretinogram exams were significantly lower than pretreatment controls in the 1.5% bepotastine besilate 8X/day females at Week 13 (21-23% reduction from pretreatment controls). This same group showed a significant decrease in A wave amplitude after 26 weeks of 1.5% bepotastine besilate (average 33% less than pretreatment controls), while the effects on B wave amplitude , also showed a trend toward reduction (average 15% less than pretreatment controls). These trends were only seen in 1.5% bepotastine besilate males 8X/day after 26 weeks of treatment (average of 24% and 20% reduction from pretreatment controls). These values reached statistical significance over saline treated control eyes.

Although there were some variations in A and B wave amplitude for males and females in the 1.5% bepotastine besilate group treated 4X/day, these effects were considered to be within the limits of standard variation and were not considered toxicologically relevant.

Hematology (pre-dose and Weeks 13 and 26): No toxicologically relevant effects observed.

Clinical chemistry (pre-dose and Weeks 13 and 26): No test article related effects observed.

Urinalysis (pre-dose and Weeks 13 and 26): 24 hour collections assessed. No test article effects were observed.

Gross pathology: No test article effects observed.

Organ weights (see histopath table):

Histopathology: Adequate Battery: yes ( ), no (x)—explain only eyes and organs where gross pathology observed were examined, despite a full battery being listed in the materials and methods.

Peer review: yes ( ), no ( ) –not indicated

No test article effects observed.

Toxicokinetics (Day 0, Week 13 and 26):
Timepoints for 4X/day group: Blood taken predose and at 0.5 hours after 1st and 2nd administration, and at 0.5, 1, 2, 4, and 8 hours after 4th administration on each collection day.

Timepoints for control and 8X/day group: Blood taken at predose 0.5 hr after the first, second and fourth administrations, 0.5, 1, 2, 4, 8 and 17 hours after 8th dose on each collection day.
The table below summarizes the key results from this study, note that there was some increase in exposure with multiple dosing. Females showed a slight, but not significant, increase in systemic exposure as compared to males.

Summary of toxicokinetic data from blood.

Group	Cmax (ng/mL)	Tmax (h)	$AUC_{(0\text{-}24h)}$ (ng·h/ml)
1.5% TAU-284, 4X/day			
Males			
Day 0	81.5 ± 49.4	8.5 ± 0.7	944 ± 458
Week 13	89.9 ± 21.3	3.0 ± 0.0	1390 ± 180
Week 26	93.2 ± 23.5	8.5 ± 0.0	1400 ± 180
Females			
Day 0	63.2 ± 22.0	8.3 ± 0.3	847 ± 276
Week 13	110 ± 15	1.8 ± 1.4	1740 ± 170
Week 26	101 ± 4	6.4 ± 3.9	1750 ± 130
1.5% TAU-284, 8X/day			
Males			
Day 0	137 ± 12	7.6 ± 0.3	1770 ± 180
Week 13	162 ± 27	8.0 ± 0.0	2600 ± 330
Week 26	181 ± 27	7.8 ± 0.3	2810 ± 450
Females			
Day 0	190 ± 43	7.5 ± 0.0	2210 ± 480
Week 13	227 ± 21	7.5 ± 0.0	3340 ± 430
Week 26	229 ± 39	7.6 ± 0.3	3310 ± 550

Study title: Subacute Toxicity Test of Bepotastine Besilate Oral Administration Four-Week Repeated Dose Toxicity Study and Four-Week Recovery Study on Dogs

Key study findings: NOAEL = 60 mg/kg/day, based on vomiting and salivation seen at the 200 and 600 mg/kg dose levels. $T_{1/2}$ was approximately 5.5 hours on days 1 and at the end of week 3. It was unable to be determined whether test article accumulated with multiple dosing in this study due to vomiting seen on day 1. There may have been a slightly lower exposure in females than in males. Maximum systemic exposure was 4003.06 ± 1891.25 µg*hr/ml in the 600 mg/kg males and 2918.89 ± 337.36 µg*hr/ml in the 600 mg/kg females at the end of week 3. Bepotastine besilate had no effects on drug metabolizing enzymes in the liver.

Study no.: TNB-NI-05
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: October 19, 1990
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot 4, 98.54% purity

Methods
Doses: 4 weeks, daily dosing with 0, 60, 200 and 600 mg/kg It was not clear if dose was adjusted for purity. Test article solutions were stable for the duration of the trial. The controls were treated with 0.5% methylcellulose vehicle.

Species/strain: beagle dogs from (b) (4)
Number/sex/group (main study): 4
Route, formulation, volume: oral gavage, prepared in 0.5 W/V% methylcellulose 1500, 10 ml/kg

Satellite groups used for recovery: 2 each sex for 4 week recovery period
Age: 7 months
Weight: 6.6 to 9.8 kg for males, 7.0 to 9.1 for females

Results

Mortality (twice daily): No premature deaths were observed.

Clinical signs (twice daily): Vomiting was seen in all animals receiving ≥ 200 mg/kg/day within both 1 and several hours after dosing. Increased salivation was seen in nearly all animals in the 200 and 600 mg/kg/day groups. The sponsor suggests that the increased salivation was due to the bitterness of the test article.

Occasional vomiting was observed in the 60 mg/kg dose females, but the events were not considered to be test article related.

Body weights (twice weekly): A slight suppression in body weight gain was seen in the 600 mg/kg males (gain of 0.126 to 0.867 kg vs 0.755 to 1.085 kg), primarily affecting 3 males, but this was not enough to be statistically significant. These effects were not seen following the 4 week recovery period. Similar effects were not seen in the 600 mg/kg/day females.

Food consumption (twice weekly): No test article effects observed.

Ophthalmoscopy (predose, at start of 4th week and at end of recovery period): No test article effects observed.

Respiration rates (1 and 2 weeks predose, at the beginning of the 4th week of dosing and in last week of recovery period): Rates were monitored visually for 1 minute. No test article effects observed.

Body temperature (1 and 2 weeks predose, at beginning of week 4 and during last week of recovery period): Rectal temperatures were measured. No test article effects observed.

EKG (1 and 2 weeks predose, before the start of week 4 dosing and during week 4 of recovery period): No test article effects observed on heart rate, PR and QT intervals, QRS width and mean QRS electrical axis, blood pressure.

Hematology (1 and 2 weeks predose, during weeks 1, 2 and 4 of dosing and during week 4 of recovery): Blood was taken from animals that had been fasted 16 to 18 hours. No test article related changes observed.

Clinical chemistry (1 and 2 weeks predose and during weeks 1, 2, and 4 of dosing and week 4 of recovery): No test article effects observed.

Urinalysis (1 and 2 weeks predose, during week 4 of dosing and during week 4 of recovery): Analysis done on 4 hour collections taken between 8 AM and 12 PM on sampling day. Increased urine protein was seen in 6/6 males and 3/6 females in the 600 mg/kg group during week 4 of dosing.

Gross pathology (end of treatment and recovery periods): No toxicologically relevant effects seen at the end of treatment or at the end of recovery.

Organ weights (see histopath table, taken at end of treatment and recovery periods): No test article effect observed.

Histopathology (see histopath table for list of organs):

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )—not indicated

A slight increase in the mucosal epithelium of the bladder was seen in one 600 mg/kg male at the end of treatment. There were no histopathological findings at the end of the recovery period. Although the incidence of this finding is low, it does correlate with test article related pathology seen in the 4 week rat study.

Toxicokinetics (predose, and 0.5, 1, 2, 4, 6 and 24 hours post dose on first day and just before 4th week): Note, values relating to the 200 and 600 mg/kg group were not considered reliable for the first day of dosing due to vomiting observed in these groups.

The week 4 timepoint indicated that blood levels increased in a dose dependent manner, with t1/2 not significantly changing from ~5.5 hour median value across the dose range. Exposures in the females were lower than that in males at the end of week 3/beginning of week 4 timepoint.

Summary of toxicokinetic data for TAU-284:

Dose (mg/kg)	Day 0				Week 4			
	AUC (µg*hr/ml)	Cmax (µg/ml)	Tmax (hr)	T1/2 (hr)	AUC (µg*hr/ml)	Cmax (µg/ml)	Tmax (hr)	T1/2 (hr)
Males								
60	331.95 ± 66.63	35.87 ± 10.31	1.02 ± 0.28	5.88 ± 1.17	391.03 ± 82.12	42.73 ± 9.14	0.95 ± 0.28	5.68 ± 0.88
200	840.55 ± 268.65	105.80 ± 25.94	0.74 ± 0.33	4.92 ± 0.65	1365.77 ± 356.98	159.07 ± 31.38	0.95 ± 0.41	5.20 ± 0.65
600	1256.58 ± 532.66	149.60 ± 39.92	1.01 ± 0.36	4.92 ± 1.19	4003.06 ± 1891.25	364.90 ± 160.87	1.67 ± 0.76	6.11 ± 1.09
Females								
Dose (mg/kg)	Day 0				Week 4			
60	358.63 ± 60.60	39.39 ± 6.43	1.02 ± 0.32	5.61 ± 0.86	433.01 ± 46.53	48.19 ± 6.74	0.97 ± 0.39	5.55 ± 0.54
200	882.89 ± 203.45	121.66 ± 31.23	1.11 ± 0.25	4.38 ± 1.54	934.88 ± 388.96	127.11 ± 48.20	0.77 ± 0.29	4.56 ± 1.49
600	851.48 ± 311.61	110.97 ± 18.06	0.45 ± 0.31	5.03 ± 1.57	2918.89 ± 337.36	282.18 ± 42.28	1.37 ± 0.14	6.22 ± 0.85

Other: Measurement of drug-metabolizing enzyme activity in the liver evaluated at the end of treatment and recovery periods. No test article effects were observed.

Study title: Chronic Toxicity Study on Bepotastine Besilate – 26-Week Repeated Oral Dose Toxicity Study and Four-Week Recovery Study in Dogs

Key study findings: NOAEL = 30 mg/kg, with toxic dose due to vomiting being 100 mg/kg. Bepotastine besilate did not accumulate with multiple dosing, exposures in males and females were similar. TAU-284 did not have an effect on drug metabolizing enzymes in the liver.

Study no.: TNB-NI-7
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: 13 May 1991
Drug, lot #, and % purity: bepotastine besilate, lot #11 99.45% pure and lot #12 purity 99.43% were used for this study.

GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Methods
Doses: 0, 30, 100 and 300 mg/kg daily for 26 weeks. Dosing formulations were confirmed to be stable and uniform for the dosing duration. It is not apparent that dose solutions were adjusted for purity. Dose

solutions were within 98.3 and 104.1% of target dose and were considered homogenous and stable as used in the study.

Species/strain: beagle dogs from (b) (4)
Number/sex/group (main study): 4
Route, formulation, volume: oral gavage, 0.5% methylcellulose 1500 solution, 10 ml/kg
Satellite groups used for recovery: 2 males and females for 4 week recovery
Age: 7 months

Weight: 6.7 to 9.3 kg for males and 6.3 to 8.8 kg for females

Results
Mortality (twice daily): No premature deaths caused by test article.

One male in the 300 mg/kg group died on Day 38 due to administration error. Vomiting, hypoactivity, abdominal breathing and recumbency were all observed before the death. Dark red spots in the lungs, an accumulation of pleural effusion and light red bubbles in the trachea were identified upon necropsy. Histopathological assessment revealed pneumocyte necrosis involving pulmonary hemorrhage and inflammatory cellular infiltration and desquamation of the bronchiolar epithelium.

Clinical signs (twice daily): Increased vomiting and salivation were seen in the 100 mg/kg and higher dose groups. Vomiting generally occurred within 1 hour of dosing but was also seen several hours later. Salivation was seen immediately after dosing. These symptoms persisted for the duration of test article administration and resolved with cessation of dosing.

Mild hypoactivity and abnormal breathing were seen in 1 male in the 100 mg/kg group before dosing on day 4, but these symptoms resolved by the following day.

Body weights (twice weekly through week 13, once weekly thereafter): No toxicologically relevant effects observed.

Food consumption (twice weekly through week 13, once weekly thereafter): No toxicologically relevant findings were observed.

Ophthalmoscopy (1 week predose, before dosing in Weeks 4, 13 and 26 and Week 4 of recovery): No test article effects observed.

ERGS (week 23 for males, week 22 for females): No test article changes observed.

Respiration (2 and 1 week predose and before dosing in weeks 4, 13 and 26 and in week 4 of recovery): Respiration was monitored by a 1 minute visualization period. There were no test article effects observed beyond the 100 mg/kg male presenting clinical manifestations on Day 4 of dosing, but recovered by the following day. Due to lack of dose response, this observation was not considered test article related.

Body temperatures (once weekly, beginning 2 weeks before dosing): Rectal temperatures were taken. There were no test article effects on body temperature observed.

EKG (1 and 2 weeks predose and predose in Weeks 4, 13 and 26 and in week 4 of recovery): There were no test article effects on EKG measurements.

Blood pressure (1 and 2 weeks predosing and before dosing in weeks 4, 13 and 26 and in week 4 of recovery): Systolic and diastolic pressure were measured. There were no test article effects observed.

Hematology (1 and 2 weeks predose and before dosing in weeks 1, 4, 13, and 26, and in week 4 of recovery): Animals were fasted 16-18 hours prior to blood collection. No test article effects observed.

Clinical chemistry (same collection times as hematology samples): No toxicologically relevant findings. The following table summarizes the incidence of clinical chemistry perturbations.

Summary of clinical chemistry changes.

Dose	Week	Observation
100 mg/kg male	Week 1	↑ GPT
100 mg/kg male	Week 26	Slightly ↑ GOT and GPT
300 mg/kg female	Week 1	Mild ↑ GPT

Urinalysis (1 and 2 weeks predose and during weeks 4, 13, and 26 of dosing and week 4 of recovery): 4 hour collections between 8 AM and 12 PM, collections were taken before test article administration on dosing days. No test article effects observed.

Gross pathology: No test article related findings seen at the end of treatment or recovery.

Organ weights (see histopath table): There were no definitive test article related findings at the end of treatment and end of recovery periods.

Histopathology (see histopath table):

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )—not indicated

No test article related findings were observed at the end of treatment or end of recovery periods.

Toxicokinetics (predose and 0.5, 1, 2, 4, 6, and 24 hours post dose on Day 1, and in Weeks 4, 13 and 26): Majority of drug is excreted within 24 hours of dosing, so accumulation with multiple dosing is not expected. Exposure in males and females was similar. The table below summarized the measured toxicokinetic parameters on Day 0 and in Week 26.

Males								
Dose mg/kg	Day 0				Week 26			
	Cmax (µg/ml)	AUC (µg*h/ml)	Tmax (hr)	T1/2 (hr)	Cmax (µg/ml)	AUC (µg*h/ml)	Tmax (hr)	T1/2 (hr)
30	16.80 ± 3.54	112.31 ± 33.42	1.41 ± 0.73	3.36 ± 1.90	19.55 ± 2.20	169.25 ± 52.36	1.01 ± 0.21	5.47 ± 2.78
100	57.77 ± 16.04	461.90 ± 79.15	0.72 ± 0.22	5.38 ± 1.92	71.32 ± 11.19	502.21 ± 117.92	1.34 ± 0.37	3.82 ± 1.36
300	114.76 ± 38.79	1080.61 ± 684.27	1.10 ± 0.39	6.18 ± 5.11	211.62 ± 56.66	1707.12 ± 717.79	1.16 ± 0.34	4.74 ± 2.29
Females								

Dose mg/kg	Day 0				Week 26			
	Cmax (µg/ml)	AUC (µg*h/ml)	Tmax (hr)	T1/2 (hr)	Cmax (µg/ml)	AUC (µg*h/ml)	Tmax (hr)	T1/2 (hr)
30	18.27 ± 4.43	101.81 ± 17.90	1.03 ± 0.21	3.40 ± 2.28	21.10 ± 4.79	446.40 ± 706.06	1.29 ± 1.01	20.26 ± 41.38
100	54.71 ± 14.59	336.04 ± 86.84	0.90 ± 0.57	3.64 ± 1.70	85.68 ± 15.73	476.25 ± 55.75	1.31 ± 0.38	2.76 ± 1.01
300	80.95 ± 41.76	578.61 ± 238.18	0.70 ± 0.35	4.76 ± 1.96	248.74 ± 36.26	1485.32 ± 316.71	1.66 ± 0.52	2.66 ± 1.20

Other: Measurement of hepatic drug metabolizing enzyme activity performed at the end of treatment and end of recovery. Test article had no effect on hepatic drug metabolizing enzymes.

Study title: Subacute Toxicity Test of Bepotastine Besilate Oral Administration Four-Week Repeated Dose Toxicity Study and Four Week Recovery Study on Rats

Key study findings: NOAEL was set at 100 mg/kg. The sponsor claims MTD dose should be 1000 mg/kg based on decreased weight gain, but could be 300 mg/kg due to hepatocellular changes. Lethalities observed in the 1000 mg/kg group were not considered test article related. There were no residual pathologies observed following the recovery period.

Study no.: TNB-NI-04
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: November 2, 1990
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot # 4, 98.1 to 98.54% pure

Methods
Doses: 0, 30, 100, 300 and 1000 mg/kg daily for 4 weeks. The control group was dosed with methylcellulose vehicle. Dosing solutions were within 95.4 to 106.2% of targeted concentration and were considered homogenous throughout the dosing solution.

Species/strain: Crj: CD(SD) rats
Number/sex/group (main study): 12
Route, formulation, volume: oral gavage, 0.5% methylcellulose 1500, 10 ml/kg
Satellite groups used for recovery: 8 animals/sex/dose

Age: 6 weeks
Weight: 195.5 to 238.8 g for males and 142.0 to 178.5 g for females

Results
Mortality (twice/day): 2 males and 1 female in the 1000 mg/kg dose group died in the first two weeks of the study, due to dosing errors. A handling error resulted in the death of an additional female from the 1000 mg/kg group during the final week of the recovery period.

Clinical signs (twice/day): Pupil dilation was noted in the 300 and 1000 mg/kg dose groups with onset 30 – 60 minutes after dosing and symptoms remaining to the following day for the 1000 mg/kg males. Excessive salivation was seen immediately after dosing in the 300 and 1000 mg/kg males and in many of the 1000 mg/kg females. Urine contamination of the lower abdomen was observed at weeks 3 and 4 in 5 males and 6 females in the 1,000 mg/kg dose group. All of these observations resolved when dosing was completed.

Body weights (twice/week): Suppression of weight gain (3-10% less than controls) was seen in the 1000 mg/kg males by 1 week after treatment, but resolved by week 3 of the recovery period. No test article effects on female body weights were observed.

Food consumption (twice/week): Decreased food intake (4-19% less than controls) was seen in the 1000 mg/kg males compared to controls during much of the treatment period and resolved during the recovery period. This corresponded to decreased weight gain in this group. No effects on female food consumption were observed.

Ophthalmoscopy (predose, in week 4, and at end of recovery): Slit lamp examination only. No test article effects observed.

Hematology (at end of dosing and recovery): Prothrombin time and activated partial thromboplastine (APPT) times were shortened at the end of dosing in 1000 mg/kg males (17.1 ± 1.5 vs 27.6 ± 5.9 sec, and 28.5 ± 5.5 vs 51.2 ± 11.7 sec, respectively) but recovered by the end of the recovery period. No effects on prothrombin or APPT were observed in females.

Clinical chemistry:
End of treatment:
For 1000 mg/kg males, total protein and albumin were elevated as compared to controls (6.1 ± 0.3 vs 5.4 ± 0.4 g/dl and 4.4 ± 0.2 vs 3.0 ± 0.2 g/dl, respectively). GPT and triglycerides were also increased in the 1000 mg/kg males as compared to controls (16 ± 2 vs 21 ± 2 IU/l and 15 ± 3 vs 26 ± 10 mg/dl, respectively). β-globulin (both % and mg/dl values) levels were also increased but only in the 1000 mg/kg males as compared to controls (14.7 ± 0.9 vs 13.3 ± 0.8%, respectively). % albumin was significantly decreased in both males and females at 1000 mg/kg dose. A1-globulin levels were significantly higher in 1000 mg/kg males than controls (1268.9 ± 118.6 vs 1069.1 ± 140.4 mg/dl).

A2-globulin was increased in a dose dependent fashion in females, just as in males, starting as early as the 30 mg/kg dose and reaching significance with the 100 mg/kg dose as determined by both percent and mg/ml measures. A2-globulin was slightly increased in a dose-dependent manner in males as early as 30 mg/kg, reaching statistical significance by the 100 mg/kg dose (both % and mg/ml measurements).

ALP levels were noted to be significantly decreased in 1000 mg/kg females compared to controls (160 ± 22 vs 194 ± 20 IU/l, respectively).

Calcium levels were increased slightly in both males and females at the 1000 mg/kg dose and at the 300 mg/kg dose for females (~9.5 vs 9.1 mEq/l for females and 10.1 vs 9.2 mEq/l for males). Calcium levels were slightly decreased in the 300 and 1000 mg/kg males at the end of the recovery period (~8.6-8.7 ± 0.2-3 vs 9.2 ± 0.2 mg/dl).

These findings were not considered toxicologically relevant.

Urinalysis (predose, at end of dosing and end of recovery): Urine was collected from 8AM to 12PM from fasted animals on collection days. Urine pH of the 1000 mg/kg group was decreased ~ 1 point in both males and females at the end of dosing. No abnormalities were observed at the end of the recovery period.

Gross pathology: An accentuated lobular pattern was seen in the liver of one male and two females in the 1000 mg/kg group. A brownish yellow spot was observed on the serous surface of the bladder of one female subject in the 1000 mg/kg group.

At end of recovery: There were no test article related changes observed.

Organ weights (see histopath table):
End of treatment: Absolute liver weight was significantly increased ~11-20% in the 1000 mg/kg dose group compared to controls, while relative liver weights were significantly increased in the 300 mg/kg males (14% higher than controls) and 1000 mg/kg males and females (44% and 15% higher than controls, respectively). Absolute and relative weights of the spleen were reduced 10-25% in 1000 mg/kg males as compared to controls. Relative brain weight was significantly increased in 1000 mg/kg males (0.69 ± 0.05 g vs 0.60 ± 0.04 g , $p < 0.01$), but this was not considered toxicologically significant.

End of recovery: No test article effects on organ weights were observed.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )—not indicated

Lesions on bladder and liver were considered test article related. Lesions were bladder epithelial hyperplasia and liver changes associated with enzyme induction, rather than phospholipidosis.

Centrolobular hypertrophy of hepatocytes seen in 8/12 males from the 300 mg/kg group and 11/11 males and 8/12 females from the 1000 mg/kg group.

An increase in the mucosal epithelium of the bladder was seen in 1/12 300 mg/kg females and in 2/11 males and 6/12 females from the 1000 mg/kg group. Ulcers associated with the formation of granulation tissue were confirmed in one female subject from the 1000 mg/kg group.

Sections of the livers and kidneys from 2 males and 2 females from each dose group at the end of treatment and recovery were examined by electron microscopy. Electron microscopy further defined the liver lesions to be smooth endoplasmic reticula of the hepatocytes observed growing into small cysts. This pathology was observed in one 300 mg/kg male and 2 males and 2 females from the 1000 mg/kg group. Myelin like structures were seen in centrilobular hepatocytes in one male subject in the 1000 mg/kg group, and are also believed to be related to induction of drug metabolizing enzymes rather than phospholipidosis.

There were no liver pathologies observed at the end of recovery.

Toxicokinetics: No toxicokinetic data was collected.

Other: Liver enzyme induction was assessed in livers from 6 males and 6 females at the end of dosing and recovery periods (sections were reserved for histopathology prior to preparation of microsomes). The table below summarizes the findings from the end of treatment period.

Nearly all enzyme levels returned to baseline values at the end of the recovery period. The two exceptions were an increase in cytochrome P-450 (0.281 ± 0.25 nmol/mg protein vs 0.225 ± 0.031 nmol/mg protein) and a decrease in aniline hydroxylase activity (0.246 ± 0.042 nmol/min/mg protein vs 0.346 ± 0.059 nmol/min/mg protein) in the 100 mg/kg males as compared to controls seen at the end of the recovery period. These were not considered toxicologically relevant due to the absence of a dose response.

Summary of enzyme induction seen at the end of treatment; mean ± SD

Sex	Dose (mg/kg)	Number of animals	Protein concentration (mg/g liver)	Cytochrome P-450 (nmol/mg protein)	Aminopyrine-N-demethylase (nmol/min/mg protein)	Aniline hydroxylase (nmol/min/mg protein)
Male	0	6	145.222 ± 8.849	0.187 ± 0.021	0.935 ± 0.138	0.308 ± 0.062
	30	6	137.752 ± 11.362	0.234 ± 0.044	1.257 ± 0.203*	0.385 ± 0.061
	100	6	149.677 ± 6.062	0.328 ± 0.039**	1.376 ± 0.242**	0.388 ± 0.041
	300	6	159.293 ± 13.297	0.454 ± 0.048**	1.509 ± 0.244**	0.516 ± 0.078**
	1000	6	145.867 ± 20.380	0.639 ± 0.071**	1.874 ± 0.194**	0.722 ± 0.102**
Female	0	6	115.865 ± 16.911	0.075 ± 0.018	0.449 ± 0.072	0.099 ± 0.010
	30	6	108.062 ± 15.395	0.106 ± 0.015	0.622 ± 0.062	0.118 ± 0.024
	100	6	122.288 ± 11.773	0.146 ± 0.029**	0.879 ± 0.156*	0.114 ± 0.044
	300	6	129.228 ± 10.035	0.185 ± 0.037**	0.832 ± 0.215	0.148 ± 0.033*
	1000	6	123.862 ± 7.926	0.279 ± 0.037**	1.248 ± 0.231**	0.247 ± 0.040**

*P < 0.05, ** p<0.01 compared to control.

Study title: Long-term toxicity test of bepotastine besilate – A 26-week oral toxicity study in rats followed by a 4 week recovery test

Key study findings: NOAEL = 20 mg/kg/day, due to liver pathologies seen at 60 mg/kg and higher. All observations were reversible in the 4 week recovery period.

Study no.: TNB-NI-06
eCTD location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: March 6, 1991
GLP compliance: Yes, Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot 8, 100.08 to 100.16% pure

Methods
Doses: 0, 20, 60, 200 or 600 mg/kg given daily for 26 weeks, dosing solutions were within 10% of target dose and were considered stable for the dosing duration. Controls were dosed with 0.5% methyl cellulose 1500 vehicle. Doses were within 99.22 and 101.89% of target dose and were homogeneous and stable throughout the mixture.

Doses were said to be chosen from a toxicity test of TAU-2840 A 4-week oral toxicity study in rats followed by a 4 week recovery test (doses of 30, 100, 300 and 1,000 mg/kg evaluated, test number 29024). Due to increased liver weight and hepatic drug metabolizing enzymes seen at 300 mg/kg and greater, the top dose chosen was 600 mg/kg.

Species/strain: Crj:CD(SD) rats from (b) (4)
Number/sex/group (main study): 12
Route, formulation, volume: oral gavage, 0.5 W/V% methyl cellulose 1500, 10 ml/kg

Satellite groups used for toxicokinetics and recovery: 8 animals/sex/dose for 4 week recovery period, 5 rats/sex/dose/timepoint for TK assessment on day 1 and at week 26.

Age: 6 weeks
Weight: 205.3 to 235.5 g for males and 143.7 to 192.4 g for females in main study and recovery groups, 195.4 to 242.8 g for males and 156.9 to 192.5 g for females in the TK group

Results
Mortality (twice daily): The sponsor stated that there were no deaths associated with the dosing of test substance throughout the study. However, there were several deaths noted in the study as follows. One female in the 20 mg/kg dose group (#800) on day 7. Three animals in the 200 mg/kg dose group 1 male (691) on day 18, one female (860) on day 7 and one female (843) sometime between days 92 and 98. There were three females that died in the 600 mg/kg dose group: animal 737 died on day 154, animal 721 died on day 26, and animal 887 died on day 161.

Clinical signs: Pupillary dilatation, salivation and urinary soiling of the hypogastric region were seen in the 600 mg/kg animals and attributed to test article.

Transient pupillary dilatation was seen in 25 to 100% of males treated with $\geq$ 200 mg/kg and in 100% of females at 600 mg/kg 1 hour after dosing but resolved by the following day. The incidence of pupillary dilation was reduced as study duration proceeded. No dilation was seen in 200 mg/kg males after week 21 of dosing.

Salivation after dosing was seen in both males and females at 600 mg/kg/day at week 4 and beyond.

Urinary infection of the hypogastric region was seen in both males and females in the 600 mg/kg dose group as dosing progressed, with 1-4 cases in males each week after week 18, and 1-7 cases in females after week 5.

Body weights: Weight gain was reduced at 600 mg/kg in males starting at week 11, generally running 10-15% less than control levels. Body weights recovered during the recovery period.

Food consumption (twice weekly until week 13, once weekly thereafter): No significant changes in food intake were observed.

Ophthalmoscopy (day 0, week 5, 13, 26 of treatment and week 4 of recovery): Slit lamp examiniation of cornea, iris, conjunctiva, sclera, crystalline lens, vitreous body, fundus oculi examination by fundus camera.

No changes were observed at the end of treatment or recovery.

Hematology (end of treatment and recovery periods): Decrease in prothrombin time was noted in 600 mg/kg males at the end of treatment. There were no effects seen at the end of the recovery period.

Clinical chemistry (end of treatment and recovery periods): At the end of dosing, a 25% decrease in phospholipid blood levels was seen in males dosed with ≥ 200 mg/kg. Reduced triglyceride (-80%) and glucose (-16%) were seen in 600 mg/kg males. There were no effects in females.

There were no effects observed at the end of recovery.

Urinalysis (week 0, 5, 13 and 26 of dosing and week 4 of recovery): Urinalysis of 4 hour collections taken between 8 AM and 12 PM after an overnight fast.

No toxicologically relevant effects were seen either during treatment or at the end of recovery.

Gross pathology: Liver hypertrophy was seen in two 200 mg/kg males and five 600 mg/kg males.

No test article related changes were observed at the end of the recovery period.

Organ weights (see histopath table):
Absolute liver weights were increased ~26% in 200 and 600 mg/kg males and trended 16-20% higher in 200 and 600 mg/kg females, although values did not reach statistical significance for the latter sex. Relative liver weights were increased 23-47% in 200 and 600 mg/kg males and 20% in 600 mg/kg females.

Additional changes in organ weights that were observed, but did not correlate with any histologic pathology included the following. Absolute and relative left kidney weight was increased ~34% in 600 mg/kg females, while relative weight of both kidneys were elevated 27-35% in 600 mg/kg males. Slight increases in relative thyroid weights were seen in 600 mg/kg males and females. Lung weights were slightly decreased in 600 mg/kg males.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )- not indicated

Histopathology was only reported for the 600 mg/kg and control animals. Additional doses were studied if pathologies were noted at the high dose. Lesions attributed to test article were identified in the liver, parotid gland and bladder.

Hypertrophy of centrilobular hepatocytes was observed in 11/11 and 7/11 males in the 600 and 200 mg/kg dose groups, respectively. 6/12 females in the 600 mg/kg dose group were also affected. In general, the males presented more extensive liver pathologies than the females. Electron microscopic evaluation revealed that the majority of these liver pathologies were due to induction of metabolic enzymes, including vesicular growth of the smooth endoplasmic reticulum of the hepatocytes.

Additional observations included:
Concentric membrane or myelin-like structures were also observed in hepatocytes from the centrolobular to the intermediate regions in both male subjects from the 600 mg/kg group. Minute lipid droplets wrapped in a limiting membrane within centrolobular hepatocytes were observed in one male subject from the 600 and 200 mg/kg groups. Large lipid droplets within centrolobular or intermediate lobular hepatocytes were observed in both 200 mg/kg males and one 60 mg/kg male. This was in a location where hepatocyte hypertrophy was small. Bizarre mitochondria in centrolobular hepatocytes and giant mitochondria within the green regions surrounding the lobules were observed in one male from the 600 and 200 mg/kg groups.

An increase in the fat content of the centrolobular or interlobular hepatocytes was observed in 4/12 males and 2/12 females from the 600 mg/kg group and in 7/11 males and 1/11 females from the 200 mg/kg group. 5/12 males from the 60 mg/kg group were also affected.

Acinar cell hypertrophy of the parotid gland was seen in 7/11 males and 1/12 females treated with 600 mg/kg.

Mild epithelium mucous membrane growth was seen in bladders of 4/12 600 mg/kg females. Protein casts were observed in 2/12 600 mg/kg females.

Focal hyperplasia in the adrenal cortex was noted in 1 male and 1 female in the 600 mg/kg dose group.

Mammary gland hyperplasia was seen in 3/7 females in the 600 mg/kg group vs only 1/8 females in the control group in the recovery animals.

There were no test article related pathological changes observed following the recovery period.

Toxicokinetics (one hour post-dose on day 1 and week 26): Dose related blood concentrations were observed on day 1, with lower blood levels of test article seen in week 26 reflecting induction of drug metabolizing enzymes. There were no differences between males and females. The table below presents the TK data included in the NDA.

Summary of toxicokinetic data for TNB-NI-06

Sex	Group and dose	Number of animals	Plasma concentration (μg/ml)	
			Day 1	26 weeks
Male	Control	5	N.D.[a]	N.D.
	20 mg/kg	5	0.68 ± 0.55	0.18 ± 0.17
	60 mg/kg	5	5.95 ± 1.70	4.17 ± 1.15
	200 mg/kg	5	24.43 ± 8.29	13.58 ± 3.58

	600 mg/kg	5	50.71 ± 7.09	19.53 ± 3.21
Female	Control	5	N.D.	N.D.
	20 mg/kg	5	1.67 ± 0.51	1.44 ± 0.36
	60 mg/kg	5	7.02 ± 0.40	5.14 ± 0.83[b]
	200 mg/kg	5	25.55 ± 7.25	20.98 ± 2.68
	600 mg/kg	5	62.70 ± 14.51	39.13 ± 7.01 [b]

a) N.D.: not detectable (< 10 ng/ml); b) N = 4

Other:
Liver enzyme analysis (end of treatment and end of recovery): Right lobe used for enzyme analysis.

At the end of treatment, increased Cyp450 and aminopyrine N-demethylase activities were seen in males treated with 200 mg/kg and higher and in 600 mg/kg females (200-600X controls). Anyline hydroxylase activities were increased in both males and females at doses ≥ 200 mg/kg (200 to 400X controls).

No significant differences in drug metabolizing enzyme activity was seen in treated animals as compared to controls at the end of the recovery period.

2.6.6.4 Genetic toxicology

Study title: **Reverse Mutation Tests using Bepotastine Besilate Bacteria**

Key findings: Bepotastine besilate did not demonstrate mutagenic potential in the Ames assay at concentrations up to 5000 µg/plate.

Study no.: TNB-NI-17

eCTD location: 4.2.3.3.1
Conducting laboratory and location: (b) (4)

Date of study initiation: January 24, 1991
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot #4, 98.54% pure

Methods
Strains/species/cell line: E coli WP2 uvrA, S. typhimurium TA98, TA100, TA1535 and TA1537

Doses used in definitive study: 78.1, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate ± S9 (Sprague-Dawley S9 activated with phenobarbital and 5,6 benzoflavone). DMSO was used as the solvent. Solutions were determined to be stable for the duration of the study and were between 86.5 and 107.3% of the target concentration.

Basis of dose selection: Dose range finding using 4.9, 19.5, 78.1, 312.5, 1250 and 5000 µg/plate. Solutions were determined to be stable for the duration of the study.

Negative controls: Solvent (DMSO)

Positive controls: 9-aminoacridine HCl (9 AA, 80 µg/plate), 2-aminoanthracine (2 AA, 20 µg/plate), 2-(2-guryl)-3-(5-nitro-2-furyl) acrylamide (AF 2, 0.01-0.1 µg/plate), Sodium azide (SAZ, 0.5 µg/plate).

Incubation: 48 hour plate incubation

Results

Study validity : Dose range finding study was done with duplicate plates, second and third study were done with triplicate plates/dose. Outcomes for positive and negative controls were as expected, supporting validity of results.

Study outcome: No increase in revertant colonies was seen with the test article or inhibition of bacterial lawn growth were seen in either the preliminary or confirmatory studies.

Study title: Chromosomal Aberration Tests using Bepotastine Besilate Cell Cultures

Key findings: Bepotastine besilate did not cause structural or numerical aberrations in any of the assays with or without S9 metabolic activation. Concentrations evaluated were up to 2100 µg/ml in the 24 hour direct mutagenicity assay, 1000 µg/ml for the 48 hour direct mutagenicity assay, and up to 5500 µg/ml in the metabolic activation study.

Study no.: TNB-NI-18

eCTD location: 4.2.3.3.1.1
Conducting laboratory and location: (b) (4)

Date of study initiation: March 15, 1991
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot #4, % 98.54% pure

Methods
Strains/species/cell line: CHU/IU cells from Chinese hamsters

Doses used in definitive study: Metabolic activation of rat liver S9 used a combination of phenobarbital and 5,6-benzoflavone. For the confirmatory 24 hour direct mutagenicity assay, doses were set at 262.5, 525, 1050 and 2100 µg/ml. The doses used in the confirmatory 48 hour direct mutagenicity assay were 125, 250, 500 and 1000 µg/ml.

For the confirmatory metabolic activation study, doses of 687.5, 1375, 2750 and 5500 µg/ml were used.

Basis of dose selection: Preliminary studies were conducted to establish the top dose based on cytostatic effects. For the 24 hour direct mutagenicity study the 50% cytostatic effect was 2015.75 µg/ml. For the 48 hour direct mutagenicity assay, the 50% cytostatic dose was determined to be 947.47 µg/ml.

For the metabolic activation method, cytotoxicity was observed at 5500 but not 2750 µg/ml.

Negative controls: DMSO solvent control.

Positive controls: mitomycin C (MMC, 0.03-0.05 µg/ml) and cyclophosphamide (CP, 8 µg/ml)

Incubation and sampling times: 24 and 48 hour incubations were used for the direct mutagenicity study, for the metabolic activation incubations cells were incubated with test article for 6 hours and then cultured for an additional 18 hours prior to preparation of cell spreads.

Results
Study validity (comment on replicates, counting method, criteria for positive results, etc.): Each dose was studied in duplicate. 200 metaphase cells were assayed per dose (100/plate). The results for the positive and negative control articles were as expected, confirming the validity of the studies.

Study outcome: Bepotastine besilate did not cause structural or numerical aberrations in any of the assays with or without S9 metabolic activation.

Study title: Unscheduled DNA Synthesis (UDS) Tests using Bepotastine Besilate in Primary Mouse Hepatocyte Cultures

Key findings: This study was conducted due to the increase in numbers of hepatocyte tumors (hepatomas and cancers) seen in female mice. Bepotastine besilate did not cause unscheduled DNA synthesis in mouse hepatocyte cells at concentrations up to 1000 µg/ml. Metabolic activation of bepotastine besilate was not evaluated in this study, while metabolic activation of the positive control compound cyclophosphamide was assessed.

Study no.: TNB-NI-26

eCTD location: 4.2.3.3.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: October 1, 1996
GLP compliance: OECD, but not GLP
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, lot 13, 99.74% pure, test article was stable for the course of the study

Methods
Strains/species/cell line: female mouse primary hepatocyte cultures taken from 8 week old mice

Doses used in definitive study: 250, 500 and 1000 µg/ml were used. No metabolic activation of bepotastine besilate was evaluated in this study. Solutions were within 86.5 and 91.5% of targeted concentration.

Basis of dose selection: A preliminary study evaluated 250, 500, 1000, 2000 and 4000 µg/ml, with the top dose being declared the solubility limit of the compound (unlike the previously done genetox assays). Nontreated and solvent controls were included in this preliminary study. Less than 50% survival was seen at the 1000 µg/ml concentration, 100% lethality was seen at 2000 µg/ml.

Negative controls: DMSO vehicle and nontreated controls.

Positive controls: Mitomycin C (1 µg/ml) and cyclophosphamide (300 µg/ml + metabolic activation).

Incubation and sampling times: Incubations for both the preliminary and confirmatory studies were 4 hours followed by a 20 hour cultivation period.

Results

Study validity (comment on replicates): 2 slides/dose were evaluated. The positive and negative controls confirmed the validity of the study.

Study outcome: Bepotastine besilate did not induce unscheduled DNA synthesis at any dose.

Study title: Micronucleus Tests with Bepotastine Besilate in Mice

Key findings: Bepotastine besilate did not cause an increase in micronucleated cells at doses up to 1000 mg/kg given daily for 2 days. The lethal dose of bepotastine besilate was 2000 mg/kg.

Study no.: TNB-NI-19

eCTD location: 4.2.3.3.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: March 22, 1991
GLP compliance: Yes, Japanese
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: bepotastine besilate, lot # 4, 98.54% pure

Methods
Strains/species/cell line: SLC:BDF male and female mice (C57BL/6 x DBA/2) from (b) (4)

Age: 8 weeks
Weight: 26.5 to 31.8 g for males and 20.9 to 24.5 g for females

Doses used in definitive study: 250, 500 and 1000 mg/kg bepotastine besilate, 2 doses administered 24 hours apart. Vehicle was 0.5% methyl cellulose 1500. 6 mice per sex/dose were evaluated in the definitive study. Test article was confirmed to be both stable and homogenous and between 98.6 and 101.89% of targeted dose.

Basis of dose selection: Preliminary study assessing 500, 1000 and 2000 mg/kg and vehicle control given as 2 doses separated by 24 hours using 5 mice per sex/dose. All mice in the 2000 mg/kg dose group died by 48 hours after the second dose, there were no deaths or toxic effects seen at any of the other doses.

Negative controls: Vehicle was 0.5% methyl cellulose 1500.

Positive controls: 1 mg/kg mitomycin C

Incubation and sampling times: Bone marrow smears were prepared at 24 and 48 hours following the second dose. In the preliminary study, mice were followed for up to 48 hours after the second dose.

Results

Study validity : The study was considered valid. Frequency of micronuclei were determined by examining 1000 polychromatic erythrocytes and determining the ratio of PCE's to NCE's (normochromatic erythrocytes). Study validity was confirmed by positive and negative controls.

Study outcome: Bepotastine besilate did not cause an increase in micronucleated cells at doses up to 1000 mg/kg given daily for 2 days. The lethal dose of bepotastine besilate was 2000 mg/kg.

2.6.6.5 Carcinogenicity
Study title: Study on 14-Day Oral Administration to Rats with Food

Key study findings: Results show that the liver is the target organ of toxicity and led to the choice of 100, 200, 400 and 800 mg/kg/day doses for the 90 day carcinogenicity study in rats.

Study no.: TNB-NI-22R
eCTD location: 4.2.3.4.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: 14 March 1992
GLP compliance: Yes, Japanese and US GLP
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate provided by (b) (4) lot # 8, 100.08% pure

Methods
Doses: 0, 200, 400, 800, 1,600 and 3,200 mg/kg/day for 14 days, mixed in diet. Test article mixed in diet was determined to be homogenous and within 101 to 105% of target dose. Actual doses were determined on a mean mg/kg/day cage consumption and are summarized in the table below taken from the Sponsor's NDA.

Main Text Table 1: Group mean test substance intake during the administration period (mg/kg/day)

Sex	Dose group (mg/kg/day)				
	200	400	800	1,600	3,200
Male	143 (72)	293 (73)	573 (72)	1,109 (69)	1,626 (51)
Female	161 (81)	323 (81)	651 (81)	1,277 (80)	2,021 (63)

( ): Percentage of the prescribed dose

Species/strain: Fischer SPF (F344/DuCrj) rats from (b) (4)
Number/sex/group (main study): 10
Route, formulation: oral, mixed in feed

Age: 6 weeks
Unique study design: 5 animals of the same sex were housed in the same cage.

Results
Mortality: No premature deaths were observed.

Clinical signs (daily): Signs of poor grooming and perinasal or perioral red substance were observed in 60% and 100 % of animals in the 1,600 and 3,200 mg/kg/day dose groups, respectively. Two females were severely emaciated in the 3,200 mg/kg/day dose group.

Body weights (weekly): Decreases in body weight gain was seen in the 800 mg/kg/day dose group while overt body weight loss was seen in the 1,600 and 3,200 mg/kg/day dose groups.

Food consumption (weekly): Refusal to eat was seen in the 800 mg/kg/day group and higher, as early as after 1 week of treatment.

Hematology (end of treatment): Statistically significant hematology measures are presented in the table below taken from the Sponsor's NDA.

Main Text Table 2: Hematological examination items with statistically significant differences

Examination item	Sex	Dose group (mg/kg/day)				
		200	400	800	1,600	3,200
Mean corpuscular volume (MCV)	Male			↓98	↓97	↓98
	Female		↓99		↓97	↓97
Mean corpuscular hemoglobin (MCH)	Female				↓97	↓98
Mean corpuscular hemoglobin concentration (MCHC)	Male				↑102	
	Female					↑101
Platelet count	Male				↓73	↓75
White blood cell count	Male					↓84
	Female					(81)
Lymphocyte count	Male					↓83
	Female					↓79

Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.
Numbers in the table are expressed as percentages of the control group values.
( ): Does not statistically significantly differ but tends to fluctuate.
-: Not examined.

Clinical chemistry (end of treatment): Statistically significant changes are presented in the table below, taken from the Sponsor's NDA. Changes in GOT, GGTP, blood urea nitrogen, CPK activity, alkaline phosphatase and triglycerides. Decreases in blood cholesterol levels track with the same observation in the 90 day mouse study and had been thought to be related to liver changes. Increases in blood calcium levels were also observed in the 90 day mouse study, but were not correlated with any other pathologic change. Note, total cholesterol was decreased to 76% of controls in the 1,600 mg/kg/day females NOT males (error in table below).

Main Text Table 3: Blood biochemistry examination items with statistically significant differences

Examination item	Sex	Dose group (mg/kg/day)				
		200	400	800	1,600	3,200
Alkaline phosphatase (AlP)	Male		↓88	↓73	↓45	↓37
	Female		↓88	↓84	↓63	↓46
Glutamate oxaloacetate transaminase (GOT)	Male					↑831
	Female					(496)
γ-glutamyl transptidase (GGTP)	Male					(*)
	Female					↑200
Creatine phosphokinase (CPK)	Male		↓75	↓74	↓80	(274)
	Female				(211)	↑241
Creatinine	Male				↓89	
Urea nitrogen (BUN)	Male	↑114	↑111	↑111	↑118	↑134
	Female				↑131	↑182
Total protein (TP)	Male			↑105		
	Female					↑106
Globulin (Glob)	Male			↑109		↑106
	Female					↑111
Albumin/globulin ratio (A/G ratio)	Male			↓93		↓95
Blood glucose	Male			(89)	↓74	↓77
	Female				↓86	↓84
Total cholesterol (T. Chol)	Male			↓73	↓76	
	Female		↑114			↓83
Triglyceride (TG)	Male	(81)	(45)	↓25	↓28	↓33
	Female				(80)	↓63
Total bilirubin (T. Bil)	Male		↓73		↓73	↓73
Calcium	Male		↑105	↑108	↑106	↑106
	Female			↑104	↑106	↑106
Potassium	Male			↑115	↑111	
Chlorine	Male					↓98
	Female					↓97

Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.
Numbers in the table are expressed as percentages of the control group values.
( ): Does not statistically significantly differ but tends to fluctuate.
*: The activity levels of the males of the 3,200 mg/kg/day group (level 1: 5 animals, level 2: 4 animals) tended to be higher than those of the control group (level 0: 3 animals, level 1: 7 animals).

Urinalysis (week 2): Positive occult blood tests were seen in 1-2 males in the 1,600 and 3,200 mg/kg/day group and ½ of females in the 3,200 mg/kg/day group (severity being noted as slight). The pH of the urine decreased 1.5 to 1 point in all test article treated females. Protein in the urine increased in females dosed with ≥ 400 mg/kg/day, with severity increasing from slight to moderate in a dose dependent fashion. Decreases in urine specific gravity were also recorded in the 3,200 mg/kg/day group.

Gross pathology (week 2): Discoloration (dark color), swelling and an accentuated lobular pattern of the liver, paleness of the spleen, and perinasal and perioral red substance were the major gross observations at autopsy. The following table summarizes the incidences of these findings.

Site & Lesion	Dose (mg/kg/day)	0	200	400	800	1,600	3,200
	# animals examined	10	10	10	10	10	10
Spleen: Pale color							
Males		0	0	0	0	7**	8**
Females		0	0	0	0	6**	7**
Liver							
Accentuated lobular pattern							
Males		0	0	0	9**	8**	7**
Females		0	0	0	4	6**	1
Enlargement							
Males		0	0	2	10**	10**	3
Females		0	0	0	2	3	0
Dark color							
Males		0	1	5	9**	9**	10**
Females		0	0	0	0	1	4
Skin							
Red adhesive substance in perinasal and/or perioral regions(s)							
Males		0	0	0	0	5*	7**
Females		0	0	0	0	2	7**
Soiled fur in external genital region							
Males		0	0	0	0	0	1
Females		0	0	0	0	4*	6**
No abnormalities detected							
Males		10	9	4	0	0	0
Females		9	10	10	5	2	0

Organ weights (see histopath table): Liver weights were increased in all test article treated animals. Note error in the table: absolute weight of the lungs was not increased in the 800 mg/kg/day dose group, although relative lung weight was increased 110%. Adrenal weights were not elevated for the 1,600 and 3,200 mg/kg/day dose groups.

Main Text Table 4-1: Organ weights with statistically significant differences (males)

Organ name		Dose group (mg/kg/day)				
		200	400	800	1,600	3,200
Brain	Absolute weight				↓95	↓93
	Relative weight			↑117	↑143	↑159
Pituitary	Absolute weight				↓73	↓77
	Relative weight					↑129
Thyroid	Absolute weight				↓75	↓74
Heart	Absolute weight			↓81	↓65	↓59
Lungs	Absolute weight			↑110	↓85	↓86
	Relative weight				↑126	↑144
Thymus	Absolute weight			(80)	↓34	↓16
	Relative weight				↓51	↓27
Liver	Absolute weight	↑118	↑129	↑124	(106)	(93)
	Relative weight	(118)	↑134	↑148	↑159	↑159
Kidneys	Absolute weight					↓84
	Relative weight		↑111	↑119	↑135	↑143
Spleen	Absolute weight			↓84	↓62	↓53
	Relative weight				↓95	↓91
Adrenals	Relative weight				↑141	↑164
Testes	Absolute weight				↓58	↓45
Male accessory genitalia	Absolute weight			(57)	↓26	↓24
	Relative weight			(67)	↓39	↓39
Salivary glands	Absolute weight				↓80	↓87
	Relative weight				↑123	↑150

Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.
Numbers in the table are expressed as percentages of the control group values.
( ): Does not statistically significantly differ but tends to fluctuate.

Main Text Table 4-2: Organ weights with statistically significant differences (females)

Organ name		Dose group (mg/kg/day)				
		200	400	800	1,600	3,200
Brain	Absolute weight				↓97	↓94
	Relative weight				↑118	↑145
Pituitary	Absolute weight				↓74	↓63
Thyroid	Absolute weight					↓74
Heart	Absolute weight			↓91	↓81	↓66
Lungs	Absolute weight			↓92	↓88	↓83
	Relative weight					↑125
Thymus	Absolute weight			(90)	↓61	↓18
	Relative weight				↓75	↓26
Liver	Absolute weight	(105)	↑112	↑111	↑121	(101)
	Relative weight	(104)	(112)	↑121	↑149	↑153
Kidneys	Absolute weight		↑107			↓89
	Relative weight			↑108	↑115	↑135
Spleen	Absolute weight			↓85	↓67	↓50
	Relative weight				↓81	↓73
Adrenals	Absolute weight				↓87	↓85
	Relative weight					↑129
Ovaries	Absolute weight				↓70	↓65
Uterus	Absolute weight				↓26	↓25
	Relative weight				↓35	↓39
Salivary glands	Relative weight				↑117	↑146

Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.
Numbers in the table are expressed as percentages of the control group values.
( ): Does not statistically significantly differ but tends to fluctuate.

Study title: Other Toxicity Testing of Bepotastine Besilate – 14-day Mixed Feed Oral Administration Testing in Mice

Key study findings: This study was done to determine dosing for 90 day preliminary carcinogenicity study in mice. Based on inhibition of body weight gain and effects on liver function seen at ≥ 3200 mg/kg/day doses, 1600 mg/kg/day was taken as the high dose for the 90 day study.

Study no.: TNB-NI-24R
eCTD location: 4.2.3.4.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: March 14, 1991
GLP compliance: Yes, Japanese and US FDA
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot # 8, 100.08% pure

Methods
Doses: 0, 400, 800, 1600, 3200 and 6400 mg/kg/day test article was considered to be stable over the course of the study, with doses being 100 to 105% of targeted dose.

Main Text Table 1 Group average test substance uptake amounts during the administration period (mg/kg/day)

Sex	Dosage group (mg/kg/day)				
	400	800	1600	3200	6400
Males	306 (77)	625 (78)	1131 (71)	1722 (54)	1761 (28)
Females	386 (97)	700 (88)	1251 (78)	1692 (53)	2500 (42)

a: Results at Week 1 of administration
( ): The percentage ratio in relation to the established dosage.

Species/strain: ICR-type SPC Crj:CD-1 mice from [redacted] (b) (4)

Number/sex/group (main study): 10
Route, formulation: oral, mixed in feed

Age: 6 weeks

Results:
Mortality: 1 male in the 6400 mg/kg/day group died on day 10, the remaining 9 males were sacrificed the same day due to worsening clinical condition. There were no other cases of premature fatalities seen during the course of this study.

Clinical signs (daily, detailed exams weekly): Emaciation, eye discoloration and soiling of the fur on the external genitals was seen in the 6400 mg/kg males. No other clinical abnormalities were seen in the study.

Body weights (weekly): Reduction in body weight (9-37% lower than controls) was seen in 1600 mg/kg/day males and in 3200 mg/kg/day females by the end of week 1.

Food consumption (weekly): Food avoidance (21 to 65% less than controls) was seen in 1600 mg/kg/day dose groups and higher (both males and females). Food efficiency was also decreased in these groups from 3.9 to 111.9% less than controls.

Hematology (end of dosing): Performed on surviving animals.

Main Text Table 2 Hematological examination items that showed a statistically significant difference

Examination item	Sex	Dosage group (mg/kg/day)				
		400	800	1600	3200	6400
Mean corpuscular hemoglobin concentration (MCHC)	Males	⇧ 105	↑ 104			-
White blood cell count	Males				⇩ 61	-
	Females					(70)
Lymphocyte count	Males	-	-	-	⇩ 51	-
	Females	-	-	-	-	⇩ 58

Statistically significant difference: ⇧ ⇩: P<0.01; ↑ ↓: P<0.05
The numbers in the table represent the percentage ratio in relation to the control group values.
( ): Indicates that there is a trend for variation, although there is no statistically significant difference.
-: No examination performed

Clinical chemistry (end of dosing): An increase in GPT activity was seen in ≥ 800 mg/kg/day males and in ≥ 1600 mg/kg/day females.

Main Text Table 3 Blood biochemistry examination items that showed a statistically significant difference

Examination item	Sex	Dosage group (mg/kg/day)				
		400	800	1600	3200	6400
Glutamate oxaloacetate transaminase (GOT)	Males			⇧ 178		-
Glutamate pyruvate transaminase (GPT)	Males		↑ 267	⇧ 622	↑ 278	-
	Females			(290)	↑ 330	⇧ 410
Total protein (TP)	Males			⇧ 114	⇧ 115	-
	Females					⇧ 116
Blood sugar	Females			⇧ 136	⇧ 139	
Total cholesterol (T. Chol)	Males		⇩ 67	⇩ 57		-
	Females		(68)	⇩ 59	(70)	
Calcium	Males			⇧ 111	⇧ 114	-
	Females					(115)

Statistically significant difference: ⇧ ⇩: P<0.01; ↑ ↓: P<0.05
The numbers within the table represent the percentage ratio in relation to the control group values.
( ): Indicates that there is a trend for variation, despite the fact that there is no statistically significant difference.
-: No examination performed

Urinalysis (days 9 and 10): Studied in control and 6400 mg/kg/day groups. Urine pH dropped approximately 1 point in bepotastine besilate treated males and females. No other test article related effects observed.

Gross pathology: Liver enlargement and accentuated lobular pattern clear lobules were seen in all bepotastine besilate male treatment groups and in females receiving 1600 mg/kg/day and higher. Darkening/discoloration of the liver were seen in the 1,600 and 3,200 mg/kg/day males and in the 3,200 and 6,400 mg/kg/day females. Pale spleens were also noted in both males and females in the 1,600 mg/kg/day and above groups. Incidence levels are included in the table below.

Site and Lesion	Dose (mg/kg/day)	0	400	800	1,600	3,200	6,400
# animals examined		10	10	10	10	10	10
Spleen							
Pale color	males	0	0	0	2	3	0
	females	0	0	8**	9**	8**	1
Liver							
Accentuated lobular pattern	males	0	6**	10**	9**	10**	0
	females	0	0	0	4*	8**	9**
Enlargement	Males	0	5*	10**	6**	10**	0
	females	0	0	0	1	5*	5*
Dark color	Males	0	0	0	2	3	0
	females	0	0	0	0	1	2

* and ** : significantly different from control at 5 and 1% levels of probability, respectively

Organ weights (see histopath table): An increase in liver weight was noted in ≥ 400 mg/kg/day males and in ≥ 800 mg/kg/day females. Organ weights were not measured for the 6400 mg/kg/day males.

Main Text Table 4-1 Organ weights that showed a statistically significant difference (males)

Organ name	Dosage group (mg/kg/day)			
	400	800	1600	3200
Brain, relative weight			⇧ 116	⇧ 116
Pituitary gland, absolute weight				⇩ 71
Thyroid gland, absolute weight	(128)	↑ 150	↑ 153	(137)
relative weight	(133)	↑ 156	⇧ 189	↑ 167
Heart, absolute weight			↓ 91	↓ 87
Lungs, absolute weight				↓ 88
relative weight			⇧ 122	
Thymus gland, absolute weight			↓ 66	⇩ 53
relative weight			(77)	⇩ 62
Liver, absolute weight	↑ 129	⇧ 143	⇧ 140	⇧ 148
relative weight	(129)	⇧ 150	⇧ 167	⇧ 178
Kidneys, absolute weight			⇩ 78	⇩ 71
relative weight				⇩ 85
Spleen, absolute weight				⇩ 70
Adrenal gland, absolute weight		↑ 144	(127)	(127)
relative weight		↑ 146	↑ 146	↑ 146
Male accessory reproductive glands, absolute weight		↓ 82	⇩ 54	⇩ 50
relative weight			⇩ 63	⇩ 60

Statistically significant difference: ⇧ ⇩: P<0.01; ↑ ↓: P<0.05
The numbers in the table represent the percentage ratio in relation to the control group values.
( ): Indicates that there is a trend for variation, although there is no statistically significant difference.

Main Text Table 4-2 Organ weights that showed a statistically significant difference (females)

Organ name	Dosage group (mg/kg/day)				
	400	800	1600	3200	6400
Brain, absolute weight				↓ 94	⇩ 91
relative weight					↑ 111
Pituitary gland, absolute weight				⇩ 68	⇩ 64
Heart, absolute weight				↓ 90	⇩ 78
Lungs, relative weight					↑ 117
Thymus gland, absolute weight			↓ 68	↓ 72	⇩ 37
relative weight			↓ 74		⇩ 43
Liver, absolute weight		⇧ 134	⇧ 157	⇧ 167	⇧ 155
relative weight		⇧ 138	⇧ 165	⇧ 181	⇧ 191
Kidneys, absolute weight			↓ 89		⇩ 82
Spleen, absolute weight			↓ 66	⇩ 61	⇩ 49
relative weight			↓ 71	⇩ 68	⇩ 61
Adrenal gland, absolute weight			↓ 79		↓ 81
Ovaries, absolute weight				⇩ 68	⇩ 54
relative weight				↓ 73	⇩ 66
Uterus, absolute weight					⇩ 25
relative weight					⇩ 31

Statistically significant difference: ⇧ ⇩: P<0.01; ↑ ↓: P<0.05
The numbers in the table represent the percentage ratio in relation to the control group values.
( ): Indicates that there is a trend for variation, although there is no statistically significant difference.

Study title: **90 Day Carcinogenicity Pre-Examination in Mice**

Key study findings: The results from this study led to the choice of 200 mg/kg/day to be the high dose for the 24 month mouse carcinogenicity study. A denominator of 3 was applied to reach the lower doses of 60 and 20 mg/kg/day. Liver toxicity was the main finding, seen in males from all dose groups (200, 400, 800 and 1,600 mg/kg/day) and in females in the 400 mg/kg/day and greater groups.

Study no.: TNB-NI-25
eCTD location: 4.2.3.4.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: October 25, 1991
GLP compliance: Yes, Japanese and US FDA
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate from (b) (4) lot # 8, 100.08% pure

Methods
Doses: 0, 200, 400, 800 and 1,600 mg/kg/day mixed in feed for 90 days (given as mean intake); test article concentration was 92 to 102% of target value, homogeneity of test article throughout feed was considered acceptable. Actual mean test article intake is summarized in the table below.

Main Text Table 1 Mean Test Substance Intake Amount per Group (mg/kg/day)

Dosage Group (mg/kg/day)	Male	Female
200	188 (94)	188 (94)
400	376 (94)	375 (94)
800	759 (95)	751 (94)
1,600	1,536 (96)	1,535 (96)

( ): Percentage of the established dosage amount

Basis of dose selection: 14 day oral administration in feed (study IET 91-0007), which evaluated 0, 400, 800, 1,600, 3,200 and 6,400 mg/kg/day.

Species/strain: ICR-type SPF mice (Crj: CD-1), (b) (4).

Number/sex/group (main study): 12
Route, formulation: oral, mixed in feed
Frequency of dosing: daily

Satellite groups used for toxicokinetics: 5/sex/dose each for day 1 and day 90

Age: 6 weeks
Animal housing: 3 same sex animals/cage main study; 5 same sex animals/cage tk study

Restriction paradigm for dietary restriction studies: None
Drug stability/homogeneity: Drug was considered stable and diet mix was homogeneous.

Results
Mortality: No premature deaths were observed.

Clinical signs (daily): 2 males in the high dose group were said to have soiled themselves. This was believed to be representative of generalized worsened clinical condition. Several observations related to poor grooming or hair loss were seen to collectively increase in a dose dependent manner in males, while only one female in the high dose group had hair loss in the nasorosiral region. No other abnormalities were observed in the other groups.

Body weights (once weekly): Significant decreases in body weight (9-19% loss from baseline) were observed in the 800 and 1,600 mg/kg/day dose groups. These changes were seen as early as week 1 of treatment. This correlates to a decrease in food consumption in males in these dose groups, which was also seen starting at week 1.

Food consumption (cage values measured once weekly): Food consumption was reduced (average 6-23%) in males in the 800 and 1,600 mg/kg/day doses as compared to controls. Food consumption was not significantly affected by test article in females.

Feeding Efficiency (weekly): Feeding efficiency showed a dose dependent decrease (average 10 to 55%) compared to controls.

Eye examinations (pre-dose and week 13): Light reflection decay accompanying slight mydriasis was observed in 5/12 males and 3/12 females in the 1,600 mg/kg/day group as well as 1/12 females in the 800 mg/kg/day group. The incidences were significantly different for the 1,600 mg/kg/day males as compared to controls.

Urine tests (week 13): Urine pH decreased in the 800 and 1,600 mg/kg/day dose groups, suggesting that test article is excreted unchanged in the urine.

Hematological tests (end of dosing): Mean corpuscular volume (MCV) was significantly decreased in males and females. Platelet counts (Plt) were significantly decreased in males 400 mg/kg/day and greater. Table below was taken from the Sponsor's NDA.

Main Text Table 2 Hematological Test Items that Showed Statistically Significant Differences

Test Items	Sex	Dosage Groups (mg/kg/day)			
		200	400	800	1,600
Mean corpuscular volume (MCV)	Male				95 ↓
	Female			96 ↓	95 ↓
Mean corpuscular hemoglobin concentration (MCHC)	Male		103 ↑	104 ⇧	
Platelet count (Plt)	Male		84 ↓	83 ↓	85 ↓

Statistically Significant Difference: ⇧ ⇩, $P \leq 0.01$; ↑ ↓, $P \leq 0.05$
Numbers in the table are percentages compared to the control group values

Clinical Chemistry (end of dosing): The table below summarizes changes in clinical chemistry parameters and was taken from the NDA. All noted changes were significant as compared to controls.

Main Text Table 3 Blood Biochemistry Test Items that Show Statistically Significant Differences

Test Items	Sex	Dosage Groups (mg/kg/day)			
		200	400	800	1,600
Glutamate Pyruvate Transaminase (GPT)	Male			269 ⇧	246 ⇧
	Female				267 ⇧
Total Protein (TP)	Female			93 ↓	
Total Cholesterol (T. Chol)	Male		77 ↓	57 ⇩	58 ⇩
	Female			59 ⇩	58 ⇩
Calcium (ca)	Male				110 ⇧

Statistically Significant Difference: ⇧ ⇩, $P \leq 0.01$; ↑ ↓, $P \leq 0.05$
Numbers in the table are percentages compared to the control group values.

Gross pathology: The table below was taken from the sponsor's NDA.

Main Text Table 4 Occurrence Frequency of Main Autopsy Findings

Findings	Sex	Dosage Group (mg/kg/day)				
		0	200	400	800	1,600
Liver enlargement	Male	0/12	1/12	3/12	7/12 ⇧	9/12 ⇧
	Female	0/12	0/12	0/12	0/12	6/12 ⇧
Liver darkening	Male	0/12	0/12	0/12	1/12	6/12 ⇧

Number of animals with pathological changes/number of animals tested
Statistically Significant Difference: ⇧ ⇩, $P \leq 0.01$

Organ weights: Increases in brain, and liver weights and decreases in spleen weights were noted in both males and females. Kidney weights were decreased in males, but increased in females. Thyroid weights were increased in males. The tables below were taken from the Sponsor's NDA and summarize the statistically significant findings.

Organ weight data for males:

Organ Name		Dosage Group (mg/kg/day)			
		200	400	800	1,600
Brain	Relative Weight			111 ⇧	117 ⇧
Liver	Absolute Weight		121 ↑	131 ⇧	127 ⇧
	Relative Weight		127 ⇧	147 ⇧	157 ⇧
Kidneys	Absolute Weight		85 ⇩	82 ⇩	73 ⇩
	Relative Weight		89 ↓		89 ↓
Spleen	Absolute Weight				79 ↓
Thyroid	Relative Weight			136 ↑	155 ⇧

Statistically Significant Difference: ⇧ ⇩, $P \leq 0.01$; ↑ ↓, $P \leq 0.05$
Numbers in the table are percentages compared to the control group values.

Main Text Table 5-2 Organ Weights that Show Statistically Significant Differences (Female)

Organ Name		Dosage Group (mg/kg/day)			
		200	400	800	1,600
Brain	Relative Weight			113 ↑	120 ⇧
Liver	Absolute Weight				131 ⇧
	Relative Weight			134 ⇧	156 ⇧
Kidneys	Relative Weight				116 ⇧
Spleen	Absolute Weight			73 ⇩	74 ⇩

Statistically Significant Difference: ⇧ ⇩, $P \leq 0.01$; ↑ ↓, $P \leq 0.05$
Numbers in the table are percentages compared to the control group values.

Histopathology: The following table summarizes the histopathological findings observed in this study. The fatty changes in the liver coupled with decreased blood cholesterol levels suggests changes in fat metabolism.

Main Text Table 6 Occurrence Frequency of Main Histopathological Findings

Findings	Sex	Dosage Group (mg/kg/day)				
		0	200	400	800	1,600
Liver: Centrilobular hypertrophy of hepatocytes	Male	0/12	11/12 ⇧	12/12 ⇧	12/12 ⇧	12/12 ⇧
	Female	0/12	0/12	0/12	9/12 ⇧	12/12 ⇧
Liver: centrilobular fatty change of hepatocytes	Male	0/12	1/12	0/12	1/12	6/12 ⇧
	Female	0/12	0/12	0/12	0/12	0/12 ⇧
Liver: single-cell hepatocyte necrosis	Male	0/12	0/12	3/12	7/12 ⇧	7/12 ⇧
	Female	0/12	0/12	0/12	2/12	1/12
Liver: eosinophilic corpuscles within hepatocyte cytoplasm	Male	0/12	0/12	0/12	6/12 ⇧	7/12 ⇧
	Female	0/12	0/12	5/12 ↑	10/12 ⇧	9/12 ⇧

Number of animals with pathological changes/Number of animals tested
Statistically Significant Difference: ⇧ ⇩, $P \leq 0.01$; ↑ ↓, $P \leq 0.05$

Toxicokinetics (Day 1 and Day 90):

Dosage Group (mg/kg/day)	Plasma Concentration of the Test Substance (μg/ml, mean value ± standard deviation)			
	Male		Female	
	Day 1	Day 90	Day 1	Day 90
0	< 0.08	< 0.08	< 0.08	< 0.08
200	0.61 ± 0.154	0.48 ± 0.084	0.51 ± 0.145	0.33 ± 0.111
400	1.21 ± 0.237	1.14 ± 0.385	0.97 ± 0.297	0.72 ± 0.295
800	2.06 ± 0.144	1.72 ± 0.677	1.42 ± 0.227	1.41 ± 0.399
1,600	2.73 ± 0.622	4.27 ± 0.922	3.74 ± 1.305	2.63 ± 0.518

The observations and tests in the following section were not carried out on the animals in the satellite group, and the animals were disposed of after blood collection.

Study title: Preliminary 90-Day Carcinogenicity Study in Rats

Key study findings: A NOAEL was not observed in this study, increased liver weights and hepatocellular swelling were observed at the 100 mg/kg/day dose level (actual doses estimated to be 86.5 and 89.8 mg/kg/day for males and females, respectively).

Study no.: TNB-NI-23
eCTD location: 4.2.3.4.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: June 21, 1991
GLP compliance: Yes, Japanese and US FDA
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot # 8, 100.08% pure

Methods
Doses: 0, 100, 200, 400 and 800 mg/kg/day provided in feed. Feed mixes were considered homogeneous. Actual intake is presented in the table below:

Main Text Table 1: Group mean test substance intake (mg/kg/day)

Dose (mg/kg/day)	Male	Female
100	86.5	89.8
200	173	180
400	349	359
800	717	730

Basis of dose selection (MTD, MFD, AUC etc.): High dose based on suppression of body weight gain and increases in absolute liver weight seen in 800 mg/kg/day group in 14 day study.

Species/strain: Fischer SPF rats (F344/DuCrj) from (b) (4)

Number/sex/group (main study): 12
Route, formulation: oral, mixed in diet
Frequency of dosing: continuous

Satellite groups used for toxicokinetics: 5 animals/sex for 1 and 90 day TK

Age: 6 weeks
Animal housing: 3 animals same sex/cage main study, 5 animals/same sex for TK

Results
Mortality (daily): There were no premature deaths in the study.

Clinical signs (daily): Soiled fur was observed in the 400 and 800 mg/kg/day males and in the 200, 400, and 800 mg/kg/day females.

Body weights (weekly): Body weight loss (5-25%) was seen in 800 mg/kg/day males and 400 and 800 mg/kg/day females, as early as week 2 of dosing.

Food consumption (weekly): Food consumption decreased (76.6 to 87.3%) in the 800 mg/kg/day group in comparison to the control group in both males and females, with changes seen as early as week 1.

Food efficiency (weekly): Food efficiency decreased (24-30%) in the 800 mg/kg/day males and in females 400 mg/kg/day and higher.

Ophthalmologic examinations (pre-dose and end of treatment): No abnormalities observed in any of the test groups.

Urinalysis (Week 13): The pH decreased 0.5 to 1.5 points in males and females of all dose groups. In the 800 mg/kg/day group, increases in epithelial cells in urinary sediment were observed in males and increases in urinary output and protein in urine were observed in females.

Hematology (Week 13):

Main Text Table 2: Hematological examination items with statistically significant differences

Examination item	Sex	Dose group (mg/kg/day)			
		100	200	400	800
Hematocrit (Ht)	Male			↓97	↓97
	Female				↓95
Hemoglobin (Hb)	Male		↓98	↓95	↓94
	Female				↓95
Mean corpuscular volume (MCV)	Male			↓96	↓95
	Female				↓96
Mean corpuscular hemoglobin (MCH)	Male		↓97	↓93	↓93
	Female				↓96
Mean corpuscular hemoglobin concentration (MCHC)	Male			↓97	↓98
Platelet count	Male	89↓			

Numbers represent percentages of the control group values.
Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.

Clinical chemistry (Week 13):

Main Text Table 3: Blood biochemistry examination items with statistically significant differences

Examination item	Sex	Dose group (mg/kg/day)			
		100	200	400	800
Alkaline phosphatase (AlP)	Male		↓93	↓83	↓78
	Female			↓85	↓81
Glutamate oxaloacetate transaminase (GOT)	Male			↓98	↓86
	Female			↓87	↓82
Glutamate oxaloacetate transaminase (GPT)	Male			↑121	↓74
	Female			↓86	↓79
γ-glutamyl transptidase (GGTP)	Male			↓a	
Creatine phosphokinase (CPK)	Male		↓88		
Urea nitrogen (BUN)	Male				↑117
	Female				↑115
Total protein (TP)	Male	↑102	↑103	↑106	↑108
	Female	↑105	↑106	↑107	↑109
Albumin (Alb)	Male		↑102	↑104	↑107
	Female	↑103	↑104	↑105	↑106

Numbers represent percentages of the control group values.
Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.
a: Under the detection limit

Main Text Table 3

Examination item	Sex	Dose group (mg/kg/day)			
		100	200	400	800
Globulin (Glob)	Male	↑104	↑104	↑107	↑109
	Female	↑107	↑109	↑109	↑113
Albumin/globulin ratio (A/G ratio)	Female		↓96		↓94
Total cholesterol (T. Chol)	Male		↓84	↓79	
	Female	↑112	↑114		↑112
Triglyceride (TG)	Male		↓43	↓26	↓22
	Female				↓81
Total bilirubin (T. Bil)	Male		↓79	↓79	
Calcium (Ca)	Male		↑102	↑104	↑109
	Female				↑105
Potassium (Ka)	Male				↑106
Chlorine (Cl)	Male			↓98	↓96
	Female				↓98

Numbers represent percentages of the control group values.
Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.

Gross pathology : Swelling and dark coloration of the liver were observed in males in the 400 mg/kg/day group and higher and in the 800 mg/kg/day females. Soiled fur in the external genital region was observed in the 400 mg/kg/day and higher males and in the 200 mg/kg/day females. No particularly noteworthy changes were observed in the other groups.

Organ weights:

Main Text Table 4: Organ weights with statistically significant differences (males)

Organ name		Dose group (mg/kg/day)			
		100	200	400	800
Brain	Absolute weight				↓97
	Relative weight				↑134
Liver	Absolute weight	↑116	↑129	↑142	↑136
	Relative weight		↑124	↑147	↑188
Kidneys	Absolute weight	↑107	↑112	↑116	↑109
	Relative weight		↑109	↑119	↑150
Spleen	Absolute weight			↓91	↓70
	Relative weight	↓94	↓94	↓94	
Adrenals	Relative weight				↑140
Testes	Absolute weight				↓94
	Relative weight				↑130

Numbers represent percentages of the control group values.
Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.

Main Text Table 5: Organ weights with statistically significant differences (females)

Organ name		Dose group (mg/kg/day)			
		100	200	400	800
Brain	Relative weight				↑122
Liver	Absolute weight		↑107	↑108	↑124
	Relative weight		↑108	↑117	↑153
Kidneys	Relative weight			↑115	↑129
Spleen	Absolute weight			↓88	↓75
	Relative weight	↓91		↓95	↓91
Adrenals	Relative weight			↑115	↑126
Ovaries	Relative weight				↑122

Numbers represent percentages of the control group values.
Statistically significant difference: ↑↓, P≤0.01; ↑↓, P≤0.05.

Histopathology : Centrilobular hepatocellular swelling was observed in all males in the ≥100 mg/kg/day group and in females receiving 200 ≥ mg/kg/day and higher (100% of animals being affected by 400 mg/kg/day dose level). Hepatocellular fatty changes were observed in males receiving ≥100 mg/kg/day (80 to 100% of animals affected). In examinations of the bladder, mild mucosal epithelial hyperplasia was observed in the 800 mg/kg/day group 5/12 females and 7/12 males. Other changes were assessed as incidental based on the conditions under which they developed.

Toxicokinetics (Day 1 and 90): The table below was taken from the Sponsor's NDA.

Dose group (mg/kg/day)	Test substance concentration in plasma (μg/ml, mean ± standard deviation)			
	Male		Female	
	Day 1	Day 90	Day 1	Day 90
0	<0.05	<0.05	<0.05	<0.05
100	0.65±0.063	1.27±0.136	0.54±0.208	1.13±0.140
200	1.41±0.286	2.17±0.044	0.72±0.086	2.55±0.227
400	2.95±.135	3.97±0.652	1.70±0.538	4.62±0.552
800	5.35±0.394	7.41±0.946	3.09±0.498	8.11±1.697

Study title: Carcinogenicity Study on Bepotastine Besilate – 24 – Month Carcinogenicity Study in Mice

Key study findings: An increase in hepatocellular tumors seen in the high dose females was not considered statistically significant based on criteria applied by CDER. Given the intended clinical route of administration of ocular instillation, this finding do not represent a concern for human risk given the proposed indication and dosing paradigm. Additional details regarding study design and data interpretation provided below.

Adequacy of the carcinogenicity study and appropriateness of the test model: Study protocol and doses were not approved by CAC prior to conduct of the study.

Evaluation of tumor findings: No increase in animals with tumors, increases in incidence of specific tumors or time of tumor onset was seen in any of the treatment groups. However, the overall incidence of hepatocellular tumors (total number of animals among all of the animals examined with hepatocellular adenoma or carcinoma) increased in high dose females (nominal dose 190 mg/kg/day) as compared to controls. A similar positive trend in hepatocellular tumor induction was observed in high dose males males (nominal dose 200 mg/kg/day), although the values did not reach statistical significance.

The Applicant concluded that the liver neoplasms in female mice were drug related, the incidence of neoplasms was not considered statistically significant according to the criteria used by CDER. Given the intended clinical route of administration of ocular instillation, this finding do not represent a concern for human risk given the proposed indication and dosing paradigm.

Study no.: TNB-NI-21
eCTD location: 4.2.3.4.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: February 22, 1993
GLP compliance: Yes, Japanese and US FDA
QA report: yes (x) no ( )
Drug, lot #, and % purity: TAU-284, lot number 13, 99.42 to 99.66 % pure
CAC concurrence: Yes

Methods

Doses: 0, 20, 60 and 200 mg/kg/day for 21 months, test article was mixed in food. Nominal doses are provided in the table below. Concentration in food was 87 to 95% of targeted concentration. Test article in feed was determined to be 92 to 98% stable.

Group mean test substance intake (mg/kg/day*)

Dose (mg/kg/day)	Male	Female
20	19.9 (100)	18.7 (94)
60	60.1 (100)	57.0 (95)
200	200 (100)	190 (95)

*Note, test substance intake based on the assumption of 100% purity in the test article.

Basis of dose selection (MTD, MFD, AUC etc.): Preliminary 90 day carcinogenicity study in TAU-284 mice studying 0, 200, 400, 800 and 1,600 mg/kg/day doses of TAU-284. Suppression of weight gain was seen at the 800 and 1,600 mg/kg doses, while hepatocellular swelling and fatty changes were observed in all TAU-284 treated males and females treated with ≥ 400 mg/kg/day. Based on these findings, 200 mg/kg/day was chosen as the highest dose for the 24 month bioassay.

Species/strain: IRC SPF mice (Crj:CD-1) from (b) (4)

Number/sex/group (main study): 52

Route, formulation: oral, mixed in food
Frequency of dosing: daily

Satellite groups used for toxicokinetics: 16 males and 16 females/dose
Age: 6 weeks

Animal housing: 4 animals of same sex housed per cage

Restriction paradigm for dietary restriction studies: None
Drug stability/homogeneity: Test article concentration was determined to be 91 to 103% of target concentration, stability over a 4 to 6 week period ranged from 98 to 92% of freshly prepared values.

Dual controls employed: No

Interim sacrifices: None.

Deviations from original study protocol: Protocol originally called for 24 months of treatment, but was shortened to 21 months based on survival rates in all groups, including controls. At the time of analysis, males were 91 weeks old, females were 90 weeks old.

Results
Mortality: Lowest survival rates of all of the groups after Week 78 of administration were 67.3% for females and 59.6% for males. Mortality rates were similar between the TAU-284 treated groups and controls over the course of the study.

Clinical signs (daily observations, detailed examinations weekly): No toxicologically relevant effects observed.
Body weights (weekly through Week 16, then monthly thereafter): No toxicologically relevant effects observed.

Food consumption (weekly through Week 16, monthly thereafter): No toxicologically relevant effects observed.

Hematological examinations (Weeks 52, 78 and last week of administration): 10 random animals/sex/timepoint evaluated. There were no test article effects on differential leukocytes at weeks 52, 78 and the end of treatment.

Gross Pathology: No toxicologically relevant findings observed.

Organ Weights (at necropsy): Weights of the following organs were measured in the 10 animals/sex/timepoint evaluated for hematological examinations: brain, liver, kidneys, spleen, adrenals, and testes. Liver and kidney weights increased in males of all TAU-284 treated groups and findings were supported by histopathological findings of centrilobular hepatocellular swelling. Absolute and relative spleen weights were also increased in TAU-284 groups (150 to 190% of controls). There were no changes in organ weights in any of the females.

Main Text Table 4: Organ weights with statistically significant differences

Organ name		Dose (mg/kg/day)		
		20	60	200
(Males)				
Liver:	Absolute weight	160⇧	143↑	138⇧
	Relative weight	166⇧	149⇧	142⇧
Kidneys:	Absolute weight	150	205	184↑
	Relative weight	155↑	205↑	190⇧

Statistically significant difference: ↑↓, $P \leq 0.05$; ⇧⇩, $P \leq 0.01$. (Dunnett's test)
Numbers in the table are expressed as percentages of the control group values.

Histopathology (see histopathology table):
Full battery: yes (x), no ( )
Peer review: yes ( ), no ( )—not indicated

Non-neoplastic: An increased incidence of centrilobular hepatocellular swelling, single-cell necrosis of hepatocytes, hepatocellular intracytoplasmic eosinophilic bodies, and acidophilic foci of cellular alterations were observed in mid and high-dose males. The incidence of centrilobular and diffuse hepatocellular fatty changes and intracytoplasmic eosinophilic bodies significantly increased in high dose females. The table below summarizes the incidences of these observations.

Summary of liver pathology findings with test article related findings:

Examination period	Terminally sacrificed animals				Deaths/imminently sacrificed animals				All animals examined			
Dose (mg/kg/day)	0	20	60	200	0	20	60	200	0	20	60	20
Males												
No. of tissues examined	20	18	18	18	32	34	34	34	52	52	52	52
Single-cell necrosis of hepatocytes	1	3	10*	14*	1	0	3	6	2	3	13*	20
Centrilobular hepatocellular swelling	0	2	12*	14*	0	0	1	10*	0	2	13*	24
Foci of cellular alterations acidophilic cells	3	1	4	6	2	5	6	9*	4	6	10	15
Intracytoplasmic eosinophilic bodies	0	0	3	10*	0	1	7*	13*	0	1	10*	23
Females												
No of animals examined	32	28	27	26	20	24	25	26	52	52	52	52
Centrilobular hepatocellular fatty change	0	0	0	8*	0	1	0	6**	0	1	0	14
Diffuse hepatocellular fatty change	3	0	5	8**	0	2	1	5**	3	2	6	13
Intracytoplasmic eosinophilic bodies	0	0	0	5**	0	0	0	3	0	0	0	8*

* statistically significant P ≤ 0.01, ** statistically significant P ≤ 0.05 (Fisher's exact probability test)

Neoplastic: There were no increases in the number of animals with tumors, the incidence of specific tumors or time of tumor onset. However, an increase in hepatocellular tumors (adenomas + carcinomas) was observed for high dose females as compared to controls. There were no test article effects in time to tumor onset or number of deaths due to hepatocellular adenomas. There were no significant test article effects on tumor related data in males, although hepatocellular adenoma incidence did show a dose related trend to increase in the mid and high dose males as compared to controls. The sponsor considered this increase in neoplasms to be test article related, but the incidence was not considered to be statistically significant according to the criteria applied by CDER. The table below was copied from the Applicant's NDA.

{Females}

Testing time	Planned sacrificed				Death/Euthanization				Total number of animals tested			
Dose (mg/kg/day)	0	20	60	200	0	20	60	200	0	20	60	200
{Male} Number of the animals tested	20	18	18	18	32	34	34	34	52	52	52	52
Liver: Number of tissues tested	20	18	18	18	32	34	34	34	52	52	52	52
Hepatocellular adenoma	7	5	12	9	7	10	11	10	14	15	23	19
Hepatocellular carcinoma	6	7	8	6	7	2	13	13	13	9	21	19
Hepatocellular tumor	10	9	16	10	12	12	17	17	22	21	33 ↑	27
{Female} Number of the animals tested	32	28	27	26	20	24	25	26	52	52	52	52
Liver: Number of tissues tested	32	28	27	26	20	24	25	26	52	52	52	52
Hepatocellular adenoma	0	1	2	3	1	1	0	1	1	2	2	4
Hepatocellular carcinoma	1	0	0	2	0	0	0	3	1	0	0	5
Hepatocellular tumor	1	1	2	5	1	1	0	3	2	2	2	8 ↑
Harderian gland adenoma: Number of tissues tested	32	28	27	26	20	24	25	26	52	52	52	52
	3	1	0	1	3	3	0	1	6	4	0 ↓	2

Statistical significance: ↑↓, P≤0.05 (Fisher's exact test)

Toxicokinetics (day 1, Week 52 and final week of study): 5 mice/dose/sex/timepoint were sacrificed for TK assessment. Bepotastine besilate concentrations in blood increased in a dose dependent manner, with some lowering with extended treatment duration, indicative of hepatic enzyme induction. The TK data is summarized in the table below taken from the Sponsor's NDA.

Dose group (mg/kg/day)	Test substance concentration in plasma (μg/ml, mean ± standard deviation)					
	Male			Female		
	Day 1	Week 52	Week 91	Day 1	Week 52	Week 90
0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
20	0.36 ±0.046	0.28 ±0.058	0.19 ±0.025	0.44 ±0.074	0.41 ±0.036	0.35 ±0.128
60	0.95 ±0.143	1.03 ±0.236	0.51 ±0.093	1.02 ±0.151	0.78 ±0.175	0.78 ±0.195
200	2.75 ±0.815	3.84 ±0.767	1.64 ±0.634	2.36 ±0.866	2.59 ±0.443	1.88 ±0.510

Study title: Carcinogenicity Study on Bepotastine Besilate – 24-Month Carcinogenicity Study in Rats

Key study findings: There were no increases in tumors for any of the test article groups as compared to controls.

Adequacy of the carcinogenicity study and appropriateness of the test model:
Study protocol was not approved by CAC prior to study conduct. The final CAC meeting minutes for the final study review are included in the appendix.

The majority of animals survived the duration of the trial, there were no signs of toxicities seen for any of the dose groups. Although the doses used in this study did not reach a level of toxicity that would normally cause this study to be considered adequate for carcinogenic assessment, the doses are considered to provide an adequate safety margin for predicting carcinogenesis risk for the proposed ophthalmic use. This deficiency in dose levels should be addressed if the drug is considered for a use that leads to higher systemic exposure.

Evaluation of tumor findings: Data was evaluated at risk rates of 5 and 1% using the following methods. Sponsor assessment was considered adequate, a separate statistical consult for the rat tumor data was not requested.

Dunnett's or Scheffe's multiple comparison test for body weight, food consumption, organ weight, and differential leukocytes.

Fisher's exact probability test for clinical symptoms, death rate, necropsy findings and histopathological exam results.

Study no.: TNB-NI-20
eCTD location: 4.2.3.4.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: December 24 1992
GLP compliance: Yes, Japanese and US GLP
QA report: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot # 13, 99.67% pure
CAC concurrence: yes

Methods
Doses: 0, 10, 30 and 100 mg/kg/day in food (i.e. animals were not food restricted). Concentration in food was 91-103% of targeted dose. Test article was determined to be 92-98% stable. Actual doses based on mean food intake (assuming 100% of target dose) are provided in the table below, which was copied from the Applicant's NDA.

Main Text Table 2: Group mean test substance intake (mg/kg/day)

Dose (mg/kg/day)	Male	Female
10	9.58 (96)	9.81 (98)
30	29.2 (97)	29.5 (98)
100	97.0 (97)	97.9 (98)

( ): Percentage of the prescribed dose

Basis of dose selection (MTD, MFD, AUC etc.): Preliminary 90 day carcinogenicity study in rats evaluating 0, 100, 200, 400 and 800 mg/kg/day doses. Suppression of weight gain in 800 mg/kg males and 400 & 800 mg/kg females and increases in liver weight and hypertrophy seen in all dose groups for males and at doses ≥ 200 mg/kg/day for females were the observations that led to the selection of 100 mg/kg/day as the high dose used in this study.
Species/strain: Fischer SPF rats (F344/DuCrj) from (b) (4)

Number/sex/group (main study): 50

Route, formulation: oral, mixed in food
Frequency of dosing: daily for 24 months
Satellite groups used for toxicokinetics: 12 animals/sex/group, 5 animals were sacrificed at each timepoint on day 1, and week 52. 5 animals from the main study group were evaluated at week 104

Age: Males were 6 weeks and females were 7 weeks of age at start of dosing.
Animal housing: 5 animals of same sex/cage

Restriction paradigm for dietary restriction studies: No dietary restriction was employed.

Drug stability/homogeneity: Drug concentrations were confirmed to be stable for up to 14 days of storage, ranging from 87 to 95% of targeted dose. Test article concentration assessment for target dose indicated doses were within 91-103% of the targeted dose.

Dual controls employed: None
Interim sacrifices: None
Deviations from original study protocol: None

Results
Mortality: Test article did not impact death rates over the duration of the trial.

Clinical signs (observations daily, detailed exams weekly): No toxicologically significant effects were observed.

Body weights (weekly through week 16, monthly from week 20 to 104): No toxicologically relevant effects observed.

Food consumption (once per week to week 16, monthly from week 20 to 104): Food efficiency was evaluated through week 16. There were no test article effects on food consumption or food efficiency.

Hematological Examinations (weeks 52, 78 and 104): 10 animals/sex/dose selected at random for blood smear analysis. No test article effects on differential leukocytes were observed.

Gross pathology: Enlargement of the liver (17/50 vs 3/50), coarsening of the kidney surface (16/50 vs 1/50), and increased testes mass (48/50 vs 39/50) were observed in the high dose males. Values given were for complete dataset comprising animals from 24 month scheduled necropsy and unscheduled deaths. However, the majority of these observations were in animals that were terminally sacrificed.

Organ weights: Organ weights of animals used in the hematological examinations in Week 104 were measured (10 animals/sex/dose). Brain, liver, kidneys, spleen, adrenals and testes were all weighed. The weights of the liver and kidneys increased in both the males and females of bepotastine besilate treated animals. Adrenal weights were increased in the high dose males.

Main Text Table 4: Organ weights with statistically significant differences

Organ name	Dose (mg/kg/day)		
	10	30	100
(Males)			
Liver: Absolute weight	96	100	108
Relative weight	96	103	127 ⇧
Kidneys: Absolute weight	100	100	105
Relative weight	100	102	124 ⇧
Adrenals: Absolute weight	86	78	238
Relative weight	86	79	379 ↑
(Females)			
Liver: Absolute weight	112	112	112
Relative weight	111 ↑	108	111 ↑
Kidneys: Absolute weight	106	107	113 ⇧
Relative weight	105	103	114

Statistically significant difference: ↑↓, P ≤ 0.05; ⇧⇩, P ≤ 0.01. (Dunnett's test)
Numbers in the table are expressed as percentages of the control group values.

Histopathology (see histopathology table for organ list):

Non-neoplastic: In the high dose group, the frequencies of centrilobular hepatocellular swelling and clear cell alterations were increased in males, while centrilobular hepatocellular fatty changes, foci of acidophilic cell alterations and chronic nephropathy were increased in both males and females.

In the mid dose males, the frequencies of slight clear cell hepatocyte growth and chronic nephropathy were increased.

Summary of pathological findings

Examination period	Terminally sacrificed animals				Deaths/imminently sacrificed animals				All animals examined			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100	0	10	30	100
Males												
No. of tissues examined	30	40	41	44	20	10	9	6	50	50	50	50
Centrilobular hepatocellular fatty change	0	1	1	13*	0	0	0	0	0	1	1	13*
Centrilobular hepatocellular swelling	0	0	0	14*	0	0	0	0	0	0	0	14*
Foci of cellular alterations (acidophilic cells)	8	9	11	28*	1	1	1	0	9	10	12	28*
Foci of cellular alterations (clear cells)	10	17	26**	28*	0	0	0	0	10	17	26*	28*
Hepatocellular sponge-like cysts	2	4	3	5	0	1	1	2**	2	5	4	7
Kidneys: No of animals	30	40	41	44	20	10	9	6	50	50	50	50
Chronic nephropathy	29	38	40	44	6	3	5	4	35	41	45**	48*
Female												
Liver # animals examined	38	37	40	43	12	13	10	7	50	50	50	50
Centrilobular hepatocellular fatty change	0	0	0	8*	1	0	1	0	1	0	1	8**
Foci of cellular alterations	3	8	5	15*	1	1	1	1	4	9	6	16*

(acidophilic cells)												
Kidney: No of tissues examined	38	37	40	43	12	13	10	7	50	50	50	50
Chronic nephropathy	24	30	31	40*	6	6	6	6	30	36	37	46*

* statistically significant $P \leq 0.01$, ** statistically significant $P \leq 0.05$ (Fisher's exact probability test)

Neoplastic: There were no increases in the number of animals with tumors, increases in specific tumors or increases in tumor incidence rates or earlier tumor development in any of the dose groups throughout the study period.

Toxicokinetics (day 1, week 52 and week 104): Test article concentrations in blood exhibited dose dependent toxicokinetics at all timepoints.

Dose group (mg/kg/day)	Test substance concentration in plasma (μg/ml, mean ± standard deviation)					
	Male			Female		
	Day 1	Week 52	Week 104	Day 1	Week 52	Week 104
0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10	0.05 ±0.009	0.13 ±0.042	0.14 ±0.024	0.08 ±0.007	0.10 ±0.012	0.13 ±0.013
30	0.15 ±0.023	0.27 ±0.043	0.61 ±0.119	0.19 ±0.016	0.54 ±0.043	0.44 ±0.110
100	0.38 ±0.074	0.85 ±0.212	2.34 ±0.619	0.63 ±0.172	1.46 ±0.305	1.23 ±0.302

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Reproductive and Developmental Toxicity Study on Bepotastine Besilate – Study on Oral Administration to Rats Prior to and During Early Stage of Pregnancy

Key study findings: NOAEL for general toxicity for both males and dams was 40 mg/kg/day, due to the observation of mydriasis in 100% of animals on at least one occasion in the 200 and 1000 mg/kg/day dose groups. Two males in the 1000 mg/kg dose group died on days 49 and 57 of dosing.

NOAEL for reproductive capacity was 200 mg/kg/day for both males and dams, based on the observation that 20% of males and females in the 1000 mg/kg dose group were infertile, without any accompanying pathologies seen at necropsy. Sperm motility and morphology assessments were apparently not conducted.

NOAEL for embryotoxicity and teratogenic potential through gestation day 7 was 200 mg/kg/day. Decreased numbers of corporea lutea, implants and live fetuses (approximately 40% of control values) and a ~1.5-fold increase in pre-implantation losses were seen in the 1000 mg/kg/day group as compared to controls. An increase in pre-sacral vertebra and lumbar rib was noted in the 1000 mg/kg dose group as compared to controls (2.27% vs 0% on a per embryo basis, 11.1% vs 0% incidence on a per litter scale). This was also seen in 1 fetus in the 40 mg/kg dose group, but this latter finding was considered an incidental finding. According to a 1993 publication from Charles River, this is considered a very rare finding. It is this finding that lead to the application of the Pregnancy Category C to the labeling.

Study no.: TNB-NI-10
eCTD location: 4.2.3.5.1.1
Conducting laboratory and location: (b) (4)

Date of study initiation: October 12, 1990
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lots 4, 5, and 6, 98.54 to 99.60% pure

Methods
Doses: 0, 8, 40, 200 and 1000 mg/kg once daily, test article preparation was stable and actual dose was within 5 to 10% of target dose.

Doses were chosen from a 2 week dose finding study which evaluated 30 to 1000 mg/kg/day doses.

Species/strain: Sprague-Dawley rats (Crj:CD) from (b) (4)
Number/sex/group: 25
Age of animals: 6 week old males, 10 week old females
Body weights: 183.5 to 222.1 g for males and 208.4 to 256.0 g for females

Route, formulation, volume: oral gavage, 0.5% W/V% methylcellulose 1500 vehicle, 10 ml/kg

Study design: Males were treated for 63 days before starting of the mating period and during the mating period. Females treated for 14 days before mating, during mating and through Day 7 of pregnancy.

Results
Mortality (daily): One male died on day 57 of treatment, and one male was found moribund on day 49 of administration. Both animals were in the 1000 mg/kg/day group. One control male died on day 23 due to a test article administration error.

Clinical signs (daily): At least one occurrence of mydriasis was seen in nearly 100% of animals in the 200 and 1000 mg/kg/day groups at some point during the study. The incidence of affected animals generally increased with increasing duration. Almost all animals in the 1000 mg/kg group had lower abdominal soiling due to urine at some point during the dosing period.

Pale eyes, pale skin, perirhinal soiling, abdominal breathing or bradypnea, dyspnea, cyanosis, salivation, hypoactivity, reduced body temperature, prone position or recumbence and tonic convulsions were seen from Day 47 to 48 of administration in 3 males of the 1000 mg/kg group, including the male that was found moribund on day 49 and the male that died on day 57. The surviving male experienced symptoms only on Day 47.

Body weight: In the 1000 mg/kg dose group, body weights were reduced by 3 and 10% of controls, in males and females, respectively.

Food consumption: No toxicologically relevant observations.

Organ weights: Weights of the testes, epididymis, prostate and seminal vesicles were measured. Relative testis weight was increased approximately 10% in the 1000 mg/kg group, but because the standard deviations of the weights overlapped with that of control values this finding was not considered toxicologically relevant. No other test article related findings were observed.

Necropsy: Kidney and liver enlargement were seen in 25% and 50% of animals, respectively. The degree of enlargement was not indicated.

Fertility parameters (estrus cycles, mating/fertility index, corpora lutea, preimplantation loss, etc.): There were no test article effects on mean estrous counts or estrous cycles measured two weeks before mating. Mating rates were 100% in the first mating, with the number of days to first copulation not affected by treatment.

5 females and 5 males in the 1000 mg/kg dose group were infertile, but there were no reproductive organ pathologies seen at necropsy. No analysis of sperm motility or morphology was conducted. There was no test article effect on estrous cycling in females.

The number of females impregnated in the first mating period was reduced ~22% in the 1000 mg/kg dose group, with 78.26% of females becoming pregnant vs 100% of controls.

The numbers of corporea lutea, numbers of implants and number of live fetuses were decreased by approximately 40% and the number of pre-implantation losses increased by approximately 150% in the 1000 mg/kg group compared to controls (although these were not apparent on a per litter basis). There were no test article effects on total number of fetal deaths, the number of early resorptions, the number of late resorptions, the number of dead fetuses, the sex ratio of surviving fetuses, or the body weights and placental weights of surviving fetuses.

External examination of F1 fetuses: No test article effects observed.

Visceral examination of F1 fetuses: Unilateral dilatation of the ureter was observed at 7.7% higher incidence on a per litter basis in the 1000 mg/kg group vs controls. This was not considered toxicologically significant.

Skeletal examination of F1 fetuses: An increase in the pre-sacral vertebrae, and lumbar rib was observed at a higher incidence in the 1000 mg/kg group as compared to control (2.27% vs 0% on a per embryo scale, 11.1% vs 0% incidence on a per litter scale). This was also seen in one pup in the 40 mg/kg group (4% incidence on a per litter scale). The increase in presacral vertebrae and lumbar rib suggest there is some potential for teratogenicity at the high dose, although data are not available on a per litter basis to accurately evaluate. Inference is made by comparing to 1993 MARTA historical controls for the Crl:CD BR Rat publication produced by Charles River. It is this observation that led to the recommendation that Pregnancy category C be applied to the label.

There were no other toxicologically significant effects observed.

Embryofetal development

Study title: Bepotastine Besilate Reproductive/Developmental Toxicity Testing – Fetal Organ Formation Stage Administration Testing Through Oral Administration to Rats

Key study findings: NOAEL for dams was 10 mg/kg/day due to observation of mydriasis at doses of 100 and 1000 mg/kg/day. There was one maternal death seen at the 1000 mg/kg dose on Gestation Day 12, cause of death not determined.

NOAEL for developmental effects was 1000 mg/kg/day. There was a slightly higher incidence (on a per fetus basis) of runts in the 1000 mg/kg dose group. This was attributed to 5 runts appearing in a single litter, the status of the dam was not known. There was one incidence of complete inversion of the internal cavity in one F1 male from the 1000 mg/kg group, but this may be considered to be within normal historical controls. There were no toxicologically relevant effects on the fertility, learning, emotions or motor coordination of the F1 generation. There were no apparent effects on F2 offspring.

It should be noted that data was not provided on an individual fetus or per litter basis an assessment beyond that of normal background rate cannot be made. Since the overall incidence of abnormalities is similar across dose groups, it does not appear that test article acts as a teratogen in this study.

Study no.: TNB-NI-11
eCTD location: 4.2.3.5.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: March 13, 1991
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot # 8, 99.5 to 100.8% pure

Methods
Doses: 0, 10, 100 and 1000 mg/kg/day 1X/day from gestation day 7 to 17. Dosing solutions were confirmed to be stable and were within 5-10% of targeted dose.

Doses were selected based on a 2 week dose range finding study in rats.

Species/strain: Sprague-Dawley rats (Crj:CD), (b) (4)

Number/sex/group: 36 initially planned; 35, 34, 36 and 36 pregnant dams were actually obtained for dosing purposes
Age: ≥ 12 weeks for both males and females
Weight: 256.6 to 312.9 g for females, 392.0 to 467.9 g for males
Route, formulation, volume: oral gavage in 0.5% methyl cellulose 1500, 10 ml/kg

Study design: Dams were treated with test article from day 7 to 17 of gestation.
Parameters and endpoints evaluated: Caesarean section on GD 20 for 2/3 of animals, natural birth for the remaining 1/3 of animals.

Results

Mortality (dams): 1 fatality was seen in the 1000 mg/kg dose group on Gestation Day 12. Autopsy revealed a partial whitening and blackening of the bladder, fatty agglutination of the serosa of the urinary bladder, red-colored urinary accumulation within the bladder, atrophy of the thymus gland, blackened spots on the glandular stomach mucosal membrane and blackened spots on the lungs were seen. In the uterus, 15 corporea lutea and 15 implantation sites were seen.

Clinical signs (dams): Mydriasis seen in 1 and 3 dams in the 100 and 1000 mg/kg/day groups, respectively. A slight incidence of soiled lower abdomen was seen in the 1000 mg/kg/day dams. No test article effects were seen in the 10 mg/kg dose group.

Body weight (dams): No toxicologically significant test article effects observed. It is noted that weights of 3 dams, 1 each in control, 10 mg/kg and 1000 mg/kg dose groups exhibited signs of dosing errors. A reduction in body weight and in food uptake were seen in these three animals, but recovered after several days. Body weights and food uptake amounts from these 3 animals were not included in the evaluation, but all other reproductive parameters were evaluated.

Food consumption (dams): No toxicologically significant test article effects observed. It is noted that weights of 3 dams, 1 each in control, 10 mg/kg and 1000 mg/kg dose groups exhibited clinical signs related to dosing errors. A reduction in body weight and in food uptake were seen in these three animals, but recovered after several days. Body weights and food uptake amounts from these 3 animals were not included in the evaluation, but all other reproductive parameters were evaluated.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): A red-colored or ash-white colored nodular protuberance on the urinary bladder mucosa surface was seen in 6/23 1000 mg/kg dams but was not seen in any other dose group. No test article effects were seen for the number of corpus luteum, number of implantations, early stage absorbed embryo count, late stage absorbed embryo count, fetal mortality count, surviving fetus count or gender ratio, fetal body weight or placental weight for surviving fetuses.

Offspring (malformations, variations, etc.):

F1

No test article effects on weight, food consumption, body weight change. 355, 341, 334 and 340 fetuses were examined in the control, 10, 100 and 1000 mg/kg dose groups, respectively.

External examination: The number of fetuses with external abnormalities, complex abnormalities and runts were 1.77%, 0.29% and 0.91% higher in the 1000 mg/kg/day dose group as compared to controls. There were 2 litters in the control group, each having 1 runt. There was one litter in the 1000 mg/kg group with 5 runts. These instances were not considered to be related to decreased food consumption or decreased body weight of the dams. Single instances of body trunk shortening, pygmyism, edema, astomia, agnathia, aproctia, complications of vestigial tail and club food and complications of aproctia and vestigial tail were also noted in the 1000 mg/kg dose group. These findings were within the normal background of variation.

Visceral examination: The number of total animals with visceral abnormalities was not affected by bepotastine besilate treatment. One to three animals in the 100 and 1000 mg/kg dose groups displayed either unilateral or bilateral dilatation of the renal pelvis and ureter while no cases were observed in the control or 10 mg/kg dose groups. One case of ventricular septal defect was seen in the 10 mg/kg group. Abnormal lobulation of the lungs was seen in one fetus in the control group. 5-8 cases of remnant thymic glands were seen in each treatment group. These data were considered to be due to background variation.

Skeletal examination: No toxicologically significant effects were observed. The number of fetuses with skeletal variations was increased in the 1000 mg/kg dose group as compared to controls (7.41% vs 4.38% in 216 vs 228 fetuses, respectively). This increase was primarily due to a 0.95% increase in unilateral cervical rib variations and a 0.46% decrease in presacral vertebra and shortened 13th rib. A 5.03 to 5.41% decrease in phalange ossification of the forelimb was also noted. These incidences were considered to be within the limits of background incidence.

Postnatal course of F1 generation: No apparent test article effects at birth, survival or weaning. 12 week necropsy: No apparent test article effects, although complete visceral inversion of the abdominal cavity was observed in one male in the 1000 mg/kg dose group.

Motor coordination, learning capacity and emotion examinations conducted by the rota rod, water maze and open field testing. There was no test article effect on any of the study endpoints.

F1 fertility assessment: 24 mating pairs were examined. Although there was a slight decrease in the copulation rates between the control and 1000 mg/kg groups (95.83% vs 87.5%), the conception rates among the dose groups was unaffected.

External examination of F2 fetuses: No significant test article findings observed.

Overall conclusion: NOAEL for developmental effects was 1000 mg/kg/day

Study title: Bepotastine Besilate Reproductive/Developmental toxicity testing – Fetal Organogenesis Stage Administration Testing Through Oral Administration to Rabbits

Key study findings: A NOAEL was not established for dams but is considered to be < 20 mg/kg/day. Miscarriage was noted in one 20 and one 100 mg/kg/day dam on gestation days 19 and 29, although this was not clearly related to test article administration. Bleeding was also noted in 2 additional cases in the 20 mg/kg group, on GD 28 and 29. Red urine was seen in 5/14 animals in the 100 mg/kg group after the completion of dosing starting from GD 22 and in 7/14 females in the 500 mg/kg group starting at GD 14. This was not considered indicative of miscarriage. Red urine is a common phenomena in rabbit, and in the absence of correlating pathologies, this finding is not considered toxicologically significant.

NOAEL for the organogenesis stage of development was considered to be 500 mg/kg/day.

Study no.: TNB-NI-12
eCTD location: 4.2.3.5.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: August 26, 1991
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, Lot #8, 99.53 to 99.84% pure

Methods
Doses: 0, 20, 100 and 500 mg/kg/day from gestation day 6 to 18; doses were within 5 to 10% of target concentration.

Basis of dosing choice: Preliminary testing of 250, 500, 1000 and 2000 mg/kg in which 5/6 and 6/6 dams died in the 1000 and 2000 mg/kg dose levels, respectively. Miscarriage was noted in the remaining female in the 1000 mg/kg dose group. Red urine was seen in females in the 500 mg/kg/day and greater groups starting on Gestation day 8-16. Mydriasis, reduction in weight and food uptake and elimination of food uptake were seen in the 1000 and 2000 mg/kg/day dose groups. Early stage absorption and fetal fatality was said to be observed at increased rates in the 250 and 500 mg/kg dose groups, but incidence was not provided. The 500 mg/kg dose was chosen as the high dose as this was the highest dose from which a viable fetus was obtained. This dose range finding study was not submitted to the NDA (test number 49114).

Species/strain: Kbs:NZW rabbits from (b) (4)

Number/sex/group: 4 males/dose each mated to 15 untreated females
Age: 6 months males, 5 months females
Weight: 3.202 to 3.741 kg for females, 3.402 to 4.030 kg for males
Route, formulation, volume: oral gavage in 0.5% methyl cellulose 1500, 10 ml/kg

Parameters and endpoints evaluated: Caesarean section on GD 29.

Results
Mortality (dams): 1 death in control group on GD 28. Clinical signs on GD 27 included: a reduction in activity, hemorrhaging from the vagina, a reduction in body weight and elimination of food uptake on GD 27.

Clinical signs (dams): Red urine was seen in 5/14 animals in the 100 mg/kg group after the completion of dosing starting from GD 22 and in 7/14 females in the 500 mg/kg group starting at GD 14. This was not considered indicative of miscarriage. Miscarriage was noted in one female in the 20 mg/kg group on day 19 and in one female in the 100 mg/kg group on day 29. Bleeding was also noted in 2 additional cases in the 20 mg/kg group, on GD 28 and 29. Red urine was seen in 5/14 animals in the 100 mg/kg group after the completion of dosing starting from GD 22 and in 7/14 females in the 500 mg/kg group starting at GD 14. This was not considered indicative of miscarriage. Red urine is a common phenomena in rabbit, and in the absence of correlating pathologies, this finding is not considered toxicologically significant.

Infertility: 1 case in control and 500 mg/kg group, 2 cases each in the 20 and 100 mg/kg groups.

Body weight (dams): No test article effects.

Food consumption (dams): No significant test article effects.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No test article related findings in the dams other than 2/14 animals in the 500 mg/kg dose group presenting black red spots in the lung. Fetus results obtained for 13, 12, 12, and 14 dams in the control, 20, 100 and 500 mg/kg dose groups, respectively. There were no test article effects on the number of corpus luteum, implantations, pre-implantation loss, early resorptions, late resorptions, number of dead and live fetuses, gender ratio, body weight or placental weights.

External examinations: No external abnormalities were observed in 112, 92, 100 and 123 animals in the 0, 20, 100 and 500 mg/kg dose groups, respectively.

Visceral organ examinations: No test article effects observed for 60, 48, 52 and 66 embryos examined for the 0, 20, 100 and 500 mg/kg dose groups, respectively.

Skeletal examinations: No toxicologically relevant effects observed.

Prenatal and postnatal development

Study title: **Reproductive and Developmental Toxicity Study on Bepotastine Besilate – Study on Oral Administration to Rats During the Perinatal and Lactation Periods**

Key study findings: NOAEL for general toxicological indices and reproductive functions of dams was 10 mg/kg/day. Deaths were observed in 8 animals in the 1000 mg/kg dams, 3 on days 21, 22 and 23 of gestation and 5 on days 0, 1, and 2 of lactation. The animals that died on day 22 and 23 died during delivery, but delivered 1-2 live newborns each. Five dams had litters whose pups were either 100 % stillborn or died shortly after birth due to poor nursing. Mydriasis was observed in 2 of the dams, while lower abdominal soiling due to urine was observed in 1 of the dams. The retention of one dead fetus (10.02 g) was observed in the uterus of the animal of the 100 mg/kg group that showed no signs of delivery even after 23 days of gestation.

NOAEL for general toxicological indices and for fetuses/pups was 10 mg/kg/day. The number of stillborns was increased in the 100 and 1000 mg/kg dose groups as compared to controls (6.01% and 10.18% vs 3.79%). There was also a decrease in the number of animals remaining alive at day 4 after birth in the 1000 mg/kg group vs controls (46.09% vs 94.10% with incidences being similar for males and females).

Also seen in the 1000 mg/kg F1 pups were a decreased four-day survival rate (-50%), a tendency toward the delay of the differentiation of pinna detachment (50% of animals), incisor eruption (~10% of animals), and eyelid separation (~10% of males needing 1 additional day); decreased righting reflex rate (20 to 30% slower than controls), suppression of weight gain (-11 to -15%), and transient decreased food consumption (days 28 to 56 after birth) as compared to controls. No effects of test article on motor coordination, learning ability, emotionality, or reproductive capacity. There were no abnormalities in the development of the F1 generation.

F_2 findings: NOAEL =100 mg/kg/day. The total number of early resorptions was slightly higher in the 1000 mg/kg dose group as compared to controls. The sex ratio of embryos (males/females) was ~ 50% lower in the 1000 mg/kg dose group as compared to controls.

Study no.: TNB-NI-13
eCTD location : 4.2.3.5.2.1
Conducting laboratory and location: (b) (4)

Date of study initiation: February 14, 1994
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot 12, 99.65 to 99.46% pure

Methods
Doses: 0, 10, 100 and 1000 mg/kg/day during perinatal and lactation periods (Day 17 of gestation through day 21 post-delivery). Dosing solutions were within 99.54 and 103.7% of targeted dose and were considered homogeneous and stable over the course of the study.

Doses were based on a 2-week preliminary study evaluating 30 to 1000 mg/kg doses of bepotastine besilate (Study No. UB21). Suppression of weight gain and reduced food consumption at the 1000 mg/kg dose were the observations leading to the choice of highest dose for the present study.

Species/strain: Sprague-Dawley rats (Crj:CD) from (b) (4).

Number/sex/group: For F0 mating: 21 animals in the 0, 10 and 100 mg/kg dose groups. 24 animals in the 1000 mg/kg dose group.
For F1 mating: 15 animals
Age: 10 week old males, 9 and 10 week old females for F1 generation production, 10 week old females for F2 generation production

Weight: 378.7 to 470.5 g males and 245.2 to 286.9 g females
Route, formulation, volume: oral gavage, 0.5% methylcellulose 1500 solution, 10 ml/kg

Results
F_0 in-life: The following observations were made in the 1000 mg/kg dose group: Mydriasis, lower abdominal soiling by urine, hypoactivity, prone position, bradypnea, dyspnea, cyanosis, tonic convulsions, reduced body temperature, and lower abdominal soiling due to bleeding during delivery were all seen at a low frequency (generally affecting 1-2 animals) in the 1000 mg/kg dose group following prolonged treatment (GD 21 through lactation day 12). For the 2 animals that died during delivery, there were no abnormal conditions observed.

No effects of the administration of the test substance were observed in the other dose groups.

Body weight (dams): The suppression of body weight gain was observed in the 1000 mg/kg group from the end of gestation until the middle of delivery, the differences being especially significant on gestation days 19 and 20 and on day 11 after delivery. No effects were seen in the 100 and 10 mg/kg groups.

Food consumption (dams): Food consumption tended to decrease in the 1000 mg/kg group from the end of the gestation period until the middle of delivery, with differences being especially significant on Days 18, 19 and 20 of gestation and on Days 2, 3, 7 and 11 after delivery. No effects were seen in the 100 and 10 mg/kg groups.

F_0 necropsy: Mortality (dams): Deaths were observed in 8 animals in the 1000 mg/kg dams, 3 on days 21, 22 and 23 of gestation and 5 on days 0, 1, and 2 of lactation. The animals that died on day 22 and 23 died during delivery, but delivered 1-2 live newborns each. Five dams had litters whose pups were either 100 % stillborn or died shortly after birth due to poor nursing. Mydriasis was observed in 2 of the dams, while lower abdominal soiling due to urine was observed in 1 of the dams.

One dam with all dead newborns was observed on Day 2 after delivery in the control group, and 1 dam with all dead newborns was observed on Days 1 and 3 after delivery in the 100 mg/kg group (2 dams total). These deaths were ascribed to poor nursing behavior. There was one dam in the 100 mg/kg group that showed no signs of delivery even after 23 days of gestation. No premature deliveries or abortions were observed in any of the groups.

Sixteen dead fetuses were observed in the uterus of the animal that died on Day 21 of gestation and 15 live fetuses were observed in the uteruses of both animals that died on Days 22 and 23 of gestation, but no external abnormalities were observed in these fetuses.

There were no toxicologically relevant findings at necropsy of the dams, F1 fetuses or 4 day old pups.

F1 offspring:
Increased stillborn incidence (+6.3%), decreased four-day survival rate (-50%), a tendency toward the delay of the differentiation of pinna detachment (50% of animals), incisor eruption (~10% of animals), and eyelid separation (~10% of males needing 1 additional day), decreased righting reflex rate (20 to 30% slower than controls), suppression of weight gain (-11 to -15%), and transient decreased food consumption (days 28 to 56 after birth) were seen in the 1000 mg/kg pups as compared to controls. No effects of test article on motor coordination, learning ability, emotionality, or reproductive capacity, and no abnormalities were observed with regard to the development of the F1 pups. Stillborn incidence in 100 mg/kg group was +2% above controls. No effects of the administration of the test substance were observed in the groups administered 10 mg/kg or less.

The sex ratio (male/female offspring) was approximately 40% lower in the 10 and 1000 mg/kg/day groups as compared to controls, but this was not considered toxicologically significant due to the absence of a dose response.

F_1 physical development: No test article effects observed.

F_1 behavioral evaluation: No test article effects observed.

F_1 reproduction: No test article effects observed.

F_2 findings: The total number of early resorptions was slightly higher in the 1000 mg/kg dose group as compared to controls. The sex ratio of embryos (males/females) was ~ 50% lower in the 1000 mg/kg dose group as compared to controls.

Study title: **TAU-284 Pharmacokinetics Study (3rd Report) – Relating to the Internal Pharmacokinetics of Betotastine Besilate (TAU-284): Transfer into the Fetus and into Milk in Rats, and Distribution and Excretion During Repeated Administration** (Note paper published by Tsukimoto M., et al., in Pharmacokinetics, 12(5): 439-459 (1997))

Key study findings: Radiolabeled bepotastine besilate quickly distributed following oral administration of a single 3 mg/kg dose to both males and females, with maximal plasma levels being achieved within 30 minutes of dosing. In Long Evans male rats, bepotastine besilate was found to associate with melanin pigmented tissue, but dispersed with time, reaching undetectable levels by 30 days after dosing. Bepotastine besilate did show some potential to accumulate with multiple doses (3 mg/kg/day for 21 days), with systemic levels being approximately 9% higher on day 21 than on day 1. Bepotastine besilate was excreted primarily in the feces and urine in a near 60:40 ratio.

Following treatment on Gestation Day 12, distribution of bepotastine besilate to the yolk sac was similar to that in maternal plasma. Fetal levels of bepotastine besilate were found to reach approximately 27% of plasma levels at 30 minutes after dosing and 52% of plasma levels by 4 hours after dosing. Treatment on Gestation day 18 resulted in fetuses achieving ~ 7 to 33% of maternal plasma concentrations, with levels in fetal livers being nearly equivalent to that of maternal plasma. Concentrations did disperse with time, with the only detectable levels remaining in the brain and liver of the fetus 24 hours after dosing (5.9% and 3.1% of maximal plasma concentrations).

Bepotastine besilate was found to be excreted in milk of lactating dams, with the maximal level being 0.56 µg*eq/ml at 1 hour after administration and falling to below levels of detection by 48 hours after dosing. Concentrations in milk were 1.2 to 2.2 times greater than the plasma concentration of bepotastine besilate.

Study no.: TNB-HE-02
eCTD location : 4.2.3.5.3.1
Conducting laboratory and location: (b) (4)

Date of study initiation: March 14, 1991
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: radiolabeled bepotastine besilate, lot CP-1266, 92-04-18-A01, radiological purity of at least 95%

Methods
Doses: 3 mg/kg (approximately 20 µCi/kg)

Species/strain: SD-SPF from (b) (4)

Number/sex/group: male Long Evans rats

Age: SD-SPF rats: 7 weeks males; Females 9 weeks old at time of mating; Long Evans rats 7-8 weeks old

Weight: SD-SPF males 236 – 361 g, females 217 to 328 g; Long Evans rats 180 to 267 g
Route, formulation, volume: oral gavage, 0.5% methylcellulose 1500 solution, 0.6 mg/ml solution given 5 ml/kg

Special study design: Male Long Evans rats were sacrificed at 24, 72, and 168 hours and 30 days after test article administration. Distribution of radiolabeled test article was determined by whole body autoradiography. Female SP-SD rats were dosed on either Day 12 or Day 18 of gestation or on Day 11 post-delivery.

There was also a repeated dose portion of the study in which male rats were treated for 21 days with radiolabeled bepotastine besilate to evaluate excretion in urine and feces and to measure body distribution using whole body autoradiograms. Animals in the repeat dose study were collected ater the 1st, 7th, 14th and 21st dosing events, with collections being at 1, 4, 8 and 24 hours post-dose for all days, and a 72 hour timepoint being added for the 21st day collection point. Tissues measured in the repeat dose study included blood plasma, whole blood, cerebrum, cerebellum, pituitary gland, eyes, Harderian gland, thyroid gland, submandibular gland, thymus gland, heart, trachea, lungs, liver, kidneys, adrenal gland, spleen, pancreas, white fat (surrounding the kidneys), brown fat, skeletal muscle (femoral), skin (flank), bone marrow, artery, testes, epididymis, bladder, stomach, small intestine, intestinal cecum, and large intestine.

For females dosed during gestation, whole body autoradiograms were prepared to determine level of placental transfer. Tissue concentration measurements were also done. Females dosed on Day 12 of gestation were collected at 30 minutes and 4 hours after dosing. Females dosed on Day 18 of gestation were collected at 30 minutes, 4 hours and 24 hours post dose. Radioactivity was measured in blood plasma, whole blood, cerebrum, heart, trachea, lungs, liver, kidneys, adrenal gland, uterus, ovaries, placenta, and amniotic fluid in the dams and the fetuses. For the Day 18 fetuses, fractional measurements were also made for whole blood, brain, heart lungs, liver and kidneys.

Measurement of the concentration of the milk and blood of lactating dams was done at 0.5, 1, 2, 4, 8, 24, and 48 hours after administration.

Results
Distribution in male Long Evans rats: Whole body autoradiograms demonstrated radioactivity in melanin pigmented tissue, but was not sequestered there as concentrations dispersed by 30 days after dosing.

Following administration of radiolabeled bepotastine besilate to female rats on Day 12 of gestation, radioactivity was found equally distributed in the placenta, yolk sac, and maternal blood at 30 minutes postdose, with radioactivity accumulating in the placenta and yolk sac compared to blood at 4 hours postdose. In the fetus, radioactivity levels were approximately 27% of plasma levels at 30 minutes

postdose and ~52% of plasma levels at 4 hours postdose. Only 5.9% and 3.1% of maximal plasma bepotastine besilate concentrations were detected in the brain and liver of the fetus at 24 hours postdose.

Following administration of radiolabeled bepotastine besilate to female rats on Day 18 of gestation, radioactivity was equally distributed to the placenta and fetal membrane and maternal blood at 30 minutes postdose. At 4 hours postdose, radioactivity was shown to slightly accumulate in the fetal membrane with levels higher than that in maternal blood. The level of radioactivity in the placenta and fetus was similar.

Following administration of radiolabeled bepotastine besilate to female rats on Day 11 after delivery, the concentration in the milk was maximal at 1 hour after administration (0.56 µg * eq/ml) and was below the limit of detection at 48 hours postdose. When comparing milk to plasma concentrations a the same timepoints, milk concentrations of bepotastine besilate were 1.2 to 2.2 times higher than that in plasma.

Administering multiple doses of radiolabeled bepotastine besilate to male rats for 21 days indicated that test article did accumulate systemically with multiple dosing such that blood levels were ~9% higher on day 21 than on day 1. Bepotastine besilate was excreted in both the urine and feces at rates of 34.9% and 41.6% in urine on Days 1 and 21 and at rates of 61.6% and 56.0% in feces on Days 1 and 21. Whole body autoradiograms showed high levels of radioactivity in the bile, stomach and intestinal contents and in bladder urine 1 hour after administration. High levels of radioactivity were also observed in kidneys, liver and nose.

2.6.6.7 Local tolerance

2.6.6.8 Special toxicology studies

Study title: Skin Sensitization Test with TAU-284 Ophthalmic Solution in Guinea Pigs (Maximization Test Method)

Key study findings: No sensitization was seen with isotonic sodium chloride or 1.0 or 1.5% bepotastine besilate opthalmic solutions. Sensitization was observed for the positive control DCNB (1-chloro-2,4-dinitrobenzene).

Study no.: **SNJ-NI-6**
eCTD location: 4.2.3.6.1
Conducting laboratory and location: Senju Pharmaceutical Co., Ltd. Kobe Creative Center, Nagano, Japan
Date of study initiation: 25 December 2001
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate, lot 1Y28, 100.08 to 100.16% pure
DCNB (1-chloro-2,4-dinitrobenzene, lot KSN7104) was used as the positive control
Formulation/vehicle: Ophthalmic solution

Methods

Doses: 1.0 and 1.5% bepotastine besilate ophthalmic solutions, isotonic sodium chloride solution for negative control and 0.1% DNCB for positive control. Test article was considered stable and homogeneous for the study.

Note: The top dose of bepotastine besilate was chosen based on the observation of anterior eye irritation with 2.0% bepotastine besilate solution in a pretest conducted at Senju Pharmaceutical Co., Ltd.

Species/strain: Slc: Hartley guinea pigs, from (b) (4)
Number/sex/group: 2 groups of 10 and 2 groups of 5 animals, only males assessed
Age: 6 weeks
Weight: 347 to 439 g
Route, formulation, volume: skin painting, ophthalmic solution, 0.1 ml at primary and secondary sensitization and 0.2 ml at induction

Study design:

Primary sensitization, intradermal administration of test solutions applied to 4x6 cm shaved area above the shoulder blade.

Secondary sensitization 7 days after primary sensitization, transdermal administration to shaved skin in 2 x 4 cm sections, covered by occlusive plasters for 48 hours.

Induction 2 weeks after secondary sensitization, challenge with 24 hour treatment.

Evaluation 24, 48 and 72 hours after induction.

Results:
1.0 and 1.5% bepotastine besilate solutions did not cause skin sensitization in guinea pigs.

Study title: Antigenicity Study on Bepotastine Besilate in Guinea Pigs – Active Systemic Anaphylaxis (ASA) Reactions, Homologous Passive Cutaneous Anaphylaxis (PCA) Reactions, and Enzyme-linked Immunosorbent Assay (ELISA) Method in Guinea Pigs

Key study findings: Bepotastine besilate did not demonstrate antigenicity potential in Guinea pigs at doses up to 4 mg/kg.

Study no.: TNB-NI-15
eCTD location: 4.2.3.7.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: Not indicated
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate (TAU-284), Lot #11, 99.34 to 99.54% pure
Formulation/vehicle: 0.5% methylcellulose 1500

Methods

Doses: TAU-284, TAU-284-KLH or TAU-284-OVA for oral route 0.4 and 4 mg/kg based on intended clinical dose and 10 times higher, 10 mg/animal TAU-284 and 1 mg/animal TAU-protein conjugate were used for s.c. dose based on typical dose for application with FCA and on preliminary studies which evaluated the balance between the natural antihistamine activity of TAU-284 and its potential to serve as a challenge. A 5 mg/animal dose demonstrated antihistamine activity, while TAU-284 doses of 0.05, 0.1, 0.2, 0.5 and 1 mg/animal did not. Dose solutions were within 99.22 to 105.5% of targeted dose, and were homogeneous and stable for the course of the study.

(OVA = TAU-284 coupled to ovalbumin, KLH is TAU-284 coupled to keyhole limpet hemocyanin, FCA = Freund's complete adjuvant)

Species/strain: Hartley guinea pigs (Crj: Hartley, (b) (4)

Number/sex/group: only females studied, 5-10 animals/group (see tables in study design section for specific details)

Age: 6 weeks sensitization animals, passive animals were 7-8 weeks old
Weight: 360 to 434 g for sensitization and 316 to 498 for passive anaphylaxis animals
Route, formulation, volume: skin painting, ophthalmic solution, 0.1 ml at primary and secondary sensitization and 0.2 ml at induction

Study design: Sensitization by oral and subcutaneous routes were evaluated. Oral sensitization was done by 3 cycles of 5 days dosing, 2 days off for a total of 15 doses. Subcutaneous administration was by 3 weekly injections.

Active systemic anaphylaxis (ASA) was evaluated 14 days after last sensitization.

	Sensitization			ASA reaction			
Group	**Antigen**	**Dose**	**Route**	**Number of animals**	**Challenging antigen**	**Dose (mg/animal)**	**Route**
I	**TAU-284**	**0.4 mg/kg X 15 TIMES**	**p.o.**	**5**	**TAU-284**	**1**	**i.v.**
				5	**TAU-284-BSA**	**1**	**i.v.**
II	**TAU-284**	**4 mg/kg X 15 times**	**p.o.**	**5**	**TAU-284**	**1**	**i.v.**
				5	**TAU-284-BSA**	**1**	**i.v.**
III	**TAU-284 + FCA**	**10 mg/animal X 3 times**	**s.c.**	**5**	**TAU-284**	**1**	**i.v.**
				5	**TAU-284-BSA**	**1**	**i.v.**
IV	**TAU-284-KLH + FCA**	**1 mg/animal X 3 times**	**s.c.**	**5**	**TAU-284**	**1**	**i.v.**
				5	**TAU-284-BSA**	**1**	**i.v.**
V	**OVA + FCA**	**1 mg/animal X 3 times**	**s.c.**	**10**	**OVA**	**1**	**i.v.**
VI	**Non-treated**	**-**	**-**	**5**	**TAU-284**	**1**	**i.v.**
				5	**TAU-284-BSA**	**1**	**i.v.**

Homologous passive cutaneous anaphylaxis (PCA)
A homologous four-hour PCA reaction was performed according to Ovary's method to determine the presence of specific IgG1 antibodies and challenging antigens in guinea pig serum.

Group	Sensitization				PCA reaction			Elisa	
	Antigen	Dose	Route	# of animals	Challenging antigen	Dose (mg/animal)	Route	Antigen	Amount antigen/well
I	TAU-284	0.4 mg/kg X 14 times	p.o.	10	TAU-284	1	i.v.	TAU-284-BSA	0.2 µg
					TAU-284-BSA	1	i.v.		
II	TAU-284	4 mg/kg X 15 times	p.o.	10	TAU-284	1	i.v.	TAU-284-BSA	0.2 µg
					TAU-284-BSA	1	i.v.		
III	TAU-284 + FCA	10 mg/animal X 3 times	s.c.	10	TAU-284	1	i.v.	TAU-284-BSA	0.2 µg
					TAU-284-BSA	1	i.v.		
IV	TAU-284-KLH + FCA	1 mg/animal X 3 times	s.c.	10	TAU-284	1	i.v.	TAU-284-BSA	0.2 µg
					TAU-284-BSA	1	i.v.		
V	OVA + FCA	1 mg/animal X 3 times	s.c.	10	OVA	1	i.v.	OVA	0.2 µg
VI	Non-treatment	--	--	10	TAU-284	1	i.v.	TAU-284-BSA	0.2 µg
					TAU-284-BSA	1	i.v.		
					OVA	1	i.v.	OVA	0.2 µg

Results:
No changes in general clinical condition or ability to gain weight were seen for any of the dose groups.

ASA was observed with subcutaneous TAU-284-BSA 1 minute after challenge. Symptoms were seen in 5/5 animals, with 4 resulting in death between 5 and 9 minutes post administration.

ASA reactions were observed in all animals sensitized by subcutaneous OVA and FCA between immediately and 3 minutes after administration. Death was observed in 6/10 animals between 6 and 9 minutes after administration. Severity of effects in the remaining 4 animals involved convulsions in 1 animal and urination, defecation, dyspnea and staggering gait in the remaining 3.

Homologous PCA reactions:
PCA antibody values were 512X controls in animals following challenge by TAU-284-BSA. A similar level of reaction was seen with test serum from s.c. administration of OVA and FCA following challenge by OVA. There were no background PCA reactions noted in untreated control animals when challenged with TAU-284, TAU-284-BSA or OVA.

ELISA method:
Serum from test animals to be sensitized by s.c. administration of TAU-284 and FCA, antibody values of 10^2 were detected in 1/10 animals, all other animals were negative.

With test serum from animals to be sensitized by s.c. administration of TAU-284-KLH and FCA or OVA and FCA, antibody levels > 10^4 were seen in all animals.

Study title: A Study on Antigenicity in Mice Using Bepotastine Besilate – Rat Passive Cutaneous Anaphylactic (PCA) Reaction

Key study findings: TAU-284 did not induce antigenicity in mice at concentrations up to 4 mg/kg.

Study no.: TNB-NI-16
eCTD location: 4.2.3.7.1.1
Conducting laboratory and location: (b) (4)

Date of study initiation: March 10, 1992
GLP compliance: Yes, Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: bepotastine besilate (TAU-284), Lot #11, 99.1 to 99.34% pure
Formulation/vehicle: 0.5% methylcellulose 1500

Methods
Doses: TAU-284, TAU-284-KLH or TAU-284-OVA for oral route 0.4 and 4 mg/kg doses were used, 0.01 mg/animal doses were used for the i.p. studies. Dosing solutions were within 99.0 and 100.12% of targeted dose, considered homogeneous and stable as used in the study.

(OVA = TAU coupled to ovalbumin, KLH is TAU coupled to keyhole limpet hemocyanin, FCA = Freund's complete adjuvant)

Species/strain: Crj:C57BL/6 and Crj:C3H/He mice purchased from (b) (4) for sensitization trials

For PCA reaction trials, Crj: CD(SD) male rats from (b) (4)

Number/sex/group: only males used, N = 5 / group for mice, 3-5/group for rats

Age: 5-6 weeks mice, 9-11 weeks rats
Weight: C57BL/6 mice: 17.7 – 20.4 g, C3H/He mice: 23.5 – 27.1 g and rats : 315.3 – 434.4 g.

Route, formulation, volume: oral or i.p. sensitization, 0.5% methylcellulose 1500, 0.1 ml/10 kg for oral and 0.2 mg/animal for i.p.

Study design:
TAU-284-KLH conjugate was administered i.p. with aluminum hydroxide gel to obtain sera to assess induction of IgE antibody production.

TAU-284-BSA induced PCA reaction. No PCA reaction in response to TAU-284 or TAU-284-BSA was observed when TAU-284 was given orally or via i.p. injection.

General Condition:

All of the mice receiving i.p. injections of drug-Alum demonstrated a depressed level of activity 1 day after the injection, these signs wore off for all but 2 TAU-284 dosed animals during the sensitization period. The 2 affected animals appeared to initially recover, but went into a depressed state again 6 days after the first sensitizing dose. These animals died on days 7 and 8 and an autopsy revealed adhesive peritonitis induced by the Alum. No anomalies were observed in the TAU-284 group sensitized by oral administration. There was no effect of test article on body weight gain.

No allergenic induction of TAU-284 sensitized animals either i.p. or orally were observed following challenge with TAU-284 or TAU-284-BSA.

I.P. sensitization by TAU-284-KLH resulted in PCA reaction following challenge with TAU-284 in all animals in both strains of mice. PCA reaction following induction using TAU-284-BSA as a challenging antigen demonstrated a difference between strains, with 4/5 C57BL/6 mice showing a PCA antibody titer of 16-64X that of controls while 5/5 C3H/H3 mice showed a PCA antibody titer of 4-16X that of control levels.

When sample sera obtained from sensitization through i.p. injection of OVA + Alum in the C57BL/6 and C3H/He animals, PCA reaction following OVA induction was seen in all C57BL/6 mice at 8-64X of controls and in all C3H/He mice at 64-128X of controls.

2.6.6.9 Discussion and Conclusions

Bepreve™ (bepotastine besilate ophthalmic solution) 1.5% appears reasonably safe for the proposed indication of treatment of itching associated with signs and symptoms of allergic conjunctivitis in patients aged 3 years or older. The drug would be used by applying one drop of Bepreve™ into the affected eye(s) twice a day (BID) for relief lasting at least 8 hours. This regimen is supported by a 15X safety factor provided by the 26 week toxicology study in dog (NOAEL = 1.5% bepotastine besilate given 4X/day), due to the observation of a 20-33% decrease in electroretinogram measures of A- and B-wave amplitude in the 1.5% bepotastine besilate 8X/day group at 26 weeks compared to pretreatment values. Some small changes in A- and B-wave amplitude were seen at the 13 week timepoint, but were considered to be within the range of standard variation. The NOAEL in the 13-week ocular study in dogs was 1.5% bepotastine besilate given 8X/day. A 4 week ocular instillation study in rabbit also displayed a NOAEL = 1.5% bepotastine besilate 8X/day, although ERG was not measured in the rabbits. The sponsor also conducted a series of oral and i.v. studies that provided additional information regarding the toxicologic potential of bepotastine besilate when systemic exposures much greater than that achieved by the intended ocular route of administration for Bepreve™.

Calculation of safety factors:
The Applicant conducted a clinical study (SNJ-TO-02) assessing pharmacokinetic parameters for 1.0% and 1.5% bepotastine besilate administered 4X/day for 7 days topically to the eye. Cmax for the 1.0% and 1.5% solutions were determined to be 5.138 ± 2.503 ng/ml and 7.335 ± 1.876 ng/ml. Additional details of this study can be found in the Clinical pharmacology review of this NDA.

To determine safety factors for toxicology findings, the comparison of Cmax values established in preclinical studies were compared with that measured for 1.5% bepotastine besilate solution (the subject of this NDA) in clinical study SNJ-TO-02. In some cases, the toxicokinetics of the test article were not measured in the particular toxicology study in which findings were reported. In these cases, the systemic

exposures were estimated using data from other preclinical toxicology studies with similar dose levels when possible. The table below summarizes the safety factors applied in this review. In the case of long term treatment studies, the highest Cmax identified in the study was used to correct for the potential for induction of drug metabolizing enzymes at longer durations and causing changes in overall systemic exposure that may not hold true in humans.

Species	From what study	Dose (mg/kg/day)	Cmax (ng/ml)	Safety factor animal Cmax ng/ml / 7.335 ng/ml
Mouse	Dietary carcinogenesis study	190 to 200 nominal	2590 (value from week 52 females)	353X
Mouse	Dietary carcinogenesis study	57.0 to 60.1 nominal	1030 (value from week 52 males)	140X
Mouse	Dietary carcinogenesis study	18.7 to 19.9 nominal	440 (value from Day 1 females)	60X
Rat	Dietary carcinogenesis study	97.0 to 97.9 nominal	1460 (value from week 52)	199X
Rat	Dietary carcinogenesis study	9.58 to 9.81	130 (value from week 52 males)	18X
Rat	26 week oral toxicity study	20	680 (value from day 1 males)	93X
Rat	26 week oral toxicity study	60	7020 (value from day 1 females)	957X
Rat	26 week oral toxicity study	200	24430 (value from day 1 males)	3330X
Rat	26 week oral toxicity study	600	50710 (value from day 1 males)	6913X
Dog	Cardiac toxicity study	10 mg/kg (i.v.)	35870	4890X
Dog	26 week oral toxicity study	30	2100 (value from females at week 26	2876X
Dog	26 week ophthalmic study	4X/day 1.5% TAU-284	110 (value from week 13 females)	15X
Dog	26 week ophthalmic study	8X/day 1.5% TAU-284	229 (value from week 26 females)	31.22X

Additional information.

Pharmacology:

Mechanism of action: The sponsor claims bepotastine besilate works as a histamine h1 receptor antagonist. The pharmacology studies conducted in Guinea pigs and rabbits support this proposed mechanism although effects on H1 receptor kinetics were not directly measured.

Drug activity related to proposed indication: The sponsor conducted a series of preclinical proof of concept studies to demonstrate that bepotastine besilate was effective in preventing histamine-induced

inflammation and edema in Guinea pigs and rabbits. These data support use for the proposed indication of allergic conjunctivitis.

Safety pharmacology:

Neurological effects:

No data submitted beyond what was included in the chronic toxicology studies. No neurological effects were observed beyond mydriasis seen at the highest dose in many of the oral toxicology studies in rats (300-1000 mg/kg, safety factors of at least 3,300 X that of maximal systemic concentration based on topical ocular administration in humans). This is likely due to local effects on the parasympathetic nervous system, as bepotastine besilate was not found to significantly cross the blood brain barrier. The sponsor did conduct an additional study which determined that bepotastine besilate did not substantially cross the blood brain barrier displaying an AUC_{brain}/AUC_{plasma} ratio of 0.069 vs 16.862 for ketotifen measured 8 hours after a 3 mg/kg i.v. dose given to male Wistar rats.

Cardiovascular and nervous system effects:

Cardiovascular effects: The sponsor did however conduct a non-GLP safety pharmacology study in anesthetized dogs. The results of this study showed that bepotastine caused only slight depression in vertebral blood flow, heart rate and blood pressure in a dose dependent manner, while it did not affect QTc, QT, QRS or PR intervals at doses up to 10 mg/kg (a safety factor of ~4890X that of the anticipated human systemic concentrations for ophthalmic use. Due to the ocular instillation route of application for the proposed indication, this study is considered to provide sufficient confidence that bepotastine besilate would not pose a significant risk to cardiac effects in humans. A thorough QTc study in humans was not examined for this NDA, but would be expected for indications requiring a higher systemic exposure.

Pharmacokintics/Toxicokinetics:

A radiolabeled ocular instillation study in pigmented rabbits revealed that bepotastine besilate distributed to multiple subocular compartments with minimal systemic exposure. The ocular compartments with the highest concentrations included the conjunctiva, cornea and retina. Tmax was achieved in 0.25 hours. The half life of bepotastine besilate was between 0.5 and 2 hours. This was the only study in which subocular distribution of test article was measured, toxicokinetic assessments from the pivotal toxicology studies provided further support of the observation of low systemic exposure following ocular instillation ($AUC_{0\text{-}INF}$ Blood = 945.68 ng eq.*h/ml vs 325.62 to 186279.18 ng eq*h/ml or gram for ocular subcompartments). Systemic toxicokinetics were also evaluated in the 26 week ocular instillation dog study (Study SNJ-NI-5), with the maximum $AUC_{0\text{-}24}$ being 3310 ± 500 ng*h/ml. There was a trend for slight accumulation (approximately 50%) of bepotastine besilate over the 26 week dosing period, and may be due to some potential for melanin binding that was identified in the radiolabeled ocular instillation study. Affinity for melanin binding was further confirmed in the radiolabeled study to determine whole body distribution of bepotastine besilate, and transfer of radiolabeled compound to fetus and milk in pregnant and lactating dams (Study TNB-NI-15). This binding was transient, showing complete dispersion by 30 days after a single oral dose of radiolabeled bepotastine besilate. There were no differences between males and females in terms of exposure levels. In contrast, systemic exposures following oral dosing were generally 1000X higher than that seen with ocular instillation, with AUC ranging from 169.25 to 1707.12 µg*h/ml in week 26 of the 26 week dog oral toxicity study. These results provided additional support that systemic exposure following ocular dosing was low and did not accumulate with multiple dosing, although in the case of the dogs, the Tmax was generally achieved between 6-8 hours post-dose.

In metabolism studies, bepotastine besilate did not appear to be a substrate for human liver microsome metabolism or inhibit CYP 3A4, 2C9 or 2C19 metabolism of classic substrates at concentrations up to 200 μM. In female mice, 2 weeks oral administration of bepotastine besilate increased CYP450 protein content 2.1 X that of controls, with specific induction of Cyp2b, Cyp2c, Cyp2d, Cyp2a and Cyp1a1/2, with the effects on Cyp2b being particularly strong achieving 3.5 to 6.5 fold that of controls. Two week oral dosing in rats demonstrated induction of Cyp2b1/2, Cyp3a1/2, Cyp2a1 and likely Cyp2c11. These data support the interpretation that the positive liver tumor findings in female mice in the 2 year carcinogenicity study were a species specific event and not a relevant indicator of human risk.

The sponsor submitted several study reports that evaluated pharmacokinetics/toxicokinetics of bepotastine besilate following oral administration in guinea pig, rat and dog. These data indicate that bepotastine besilate does not significantly accumulate in the body, with Tmax being achieved by 0.5 hours and an average half life of ~4 hours. Bepotastine besilate appears to be renally excreted in dogs, via glomerular filtration. In a radiolabeled study in rats (TNB-NI-15), bepotastine besilate was excreted in an approximate 60:40 fashion in feces and urine, respectively. There is a small tendency for bepotastine besilate to accumulate with multiple dosing, as seen in rats dosed with 3 mg/kg/day for 21 days. Plasma concentrations were ~9% higher on Day 21 than on Day 1.

Bepotastine besilate was transferred to the yolk sac/placenta at levels nearly equivalent to maximal plasma concentration, ~33-55% of bepotastine besilate was transferred to the developing fetus. At 24 hours following a single oral administration of 3 mg/kg, ~5.9% and 3.1% of maximal plasma bepotastine besilate concentrations were detected in the brain and liver of the fetus at 24 hours postdose. Bepotastine besilate was also noted to be transferred to milk in lactating rats, with milk concentrations being 1.5 to 2 times maximal plasma concentrations by 1 hour postdose and reaching levels below the limit of detection by 48 hours postdose.

Oral toxicity studies:
The NOAEL was 30 mg/kg/day in a 26 week oral toxicity study in dogs and was 20 mg/kg/day in a 26 week oral toxicity study in rats, resulting in safety factors of 2876X and 93X, respectively when comparing systemic exposures to anticipated maximal concentrations seen with human topical ophthalmic use. The primary clinical signs of toxicity seen in these oral toxicity studies were vomiting and salivation in dogs and liver pathologies associated with metabolic induction in the rats. The sponsor also submitted a single dose intravenous study in rats in which the LD_{50} was determined to be 130 mg/kg for males and 126 mg/kg for females. Systemic exposure following the oral and intravenous dosing routes were far above what occurs after ocular administration of bepotastine besilate.

Ocular toxicity studies:
Several short term ocular toxicity studies were conducted, but the pivital study for the proposed indication was a 26 week study in dogs using 4 and 8X per day dosing with the 1.5% bepotastine besilate solution. The 4X/day dosing paradigm was determined to be the NOAEL, due to the observation of decreases in A and B wave amplitude in electroretinogram examinations in the 8X/day dose group. When considering systemic exposures seen in this study, the identified NOAEL provides an 15X safety factor over that of anticipated systemic exposures seen with topical ocular use in humans.

Mutagenicity/Genotoxicity/Carcinogenesis potential:
Carcinogenicity: Bepotastine besilate was mixed in feed and administered *ad libitum* to mice and rats to evaluate carcinogenic potential. Oral administration of bepotastine besilate was found to induce hepatocellular carcinomas/tumors in female mice at the highest dose tested (nominal dose 190 mg/kg/day) for 21 months which represents a safety factor approximately 353 times that anticipated that of systemic concentration seen with topical ocular application in humans. The Applicant concluded that the liver neoplasms in the female mice were drug related, but the incidence of these neoplasms were not statistically significant according to criteria applied by CDER. There were no similar tumor findings in the rat study which assessed doses up to 97.9 mg/kg/day for 24 months (provide a relative safety factor approximately 200 times that anticipated for systemic concentration seen with topical ocular application in humans. Given the ocular dose of the use of this drug, bepotastine besilate is not believed to pose a risk for cancer induction in humans.

Genetic toxicology:
Bepotastine besilate did not demonstrate mutagenic/genotoxic potential in any of the tests conducted. This supports the suggestion that liver tumors seen in mice were due to enzyme induction rather than due to a genotoxic mechanism. The details of the genetic toxicology studies are bulleted below:

- negative; Ames assay, concentrations up to 5000 µg/plate for both with and without metabolic activation
- negative; chromosome aberration assay, concentrations up to 2100 µg/ml for 24 hour direct mutagenicity, up to 1000 µg/ml for the 48 hour direct mutagenicity and up to 5500 µg/ml in the metabolic activation study
- negative; unscheduled DNA synthesis in primary mouse hepatocyte cultures concentrations up to 1000 µg/ml
- negative; in vivo micronucleus test in mice, doses up to 1000 mg/kg x 2 days (lethal dose was 2000 mg/kg)

Reproductive toxicology:
Overall view:
As an antihistamine, bepotastine besilate, may eventually be used for other indications besides ocular. Pregnancy Category C is recommended for the label, based on the observation of a rare skeletal abnormality (an increase in pre-sacral vertebra and lumbar rib seen in 11.1% of litters in the 1000 mg/kg dose group vs none in controls) seen in the rat study evaluating fertility and development through day 7 of gestation (TNB-NI-10). The NOAEL for this observation was 200 mg/kg/day. The sponsor did not measure toxicokinetics values for the reproductive toxicology studies. A safety factor of 3330X was estimated by referring to systemic concentrations seen in the 200 mg/kg/day group in the 26 week oral toxicology study in rats on day 1, to the anticipated human systemic concentration seen with topical ocular use.

NOAEL for pregnant female toxicities was 10 mg/kg for rats and rabbits due to the observation of mydriasis at the next higher dose in the different studies. Additional toxicities were seen with the higher doses. Mortality was seen at 1000 mg/kg dose levels. There were no effects on organ development observed in rats or rabbits. There was an increase incidence of stillborns and survival at day 4 post-birth in 100 and 1000 mg/kg dosed rats. Developmental delays were also seen in the 1000 mg/kg dose group. Due to the anticipated topical ocular use of this product, the potential risk for reproductive effects in humans is considered low.

Additional details for each study are provided in the text below:

Fertility and early pregnancy (through Gestation Day 7): Sprague-Dawley Rats, bepotastine besilate doses of 0, 8, 40, 200 and 1000 mg/kg/day examined.

Parent body toxicities: Oral administration of bepotastine besilate displayed a NOAEL of 40 mg/kg/day due to the observation of mydriasis in 100% of animals on at least one occasion in the 200 and 1000 mg/kg/day dose groups. Two males in the 1000 mg/kg/day group died on days 49 and 57 of dosing.

Fertility: NOAEL for reproductive capacity was 200 mg/kg/day for both males and dams, based on observation of 20% infertility (both males and dams) in the 1000 mg/kg/day dose group. There were no pathologies seen at necropsy to explain this, and there were no assessments done on sperm motility or morphology.

NOAEL for embryotoxicity and teratogenic potential through gestation day 7 was 200 mg/kg/day due to increased preimplantation loss (approximately 2-fold higher) in the 1000 mg/kg/day group as compared to controls. There may have also been a slight ability to serve as a teratogen with 3/132 fetuses (2.27% or 11.1% on a per litter basis) in the 1000 mg/kg/day group displaying an increase in pre-sacral vertebra and lumbar rib, with none seen in any of the control fetuses. This was also seen in 1/238 fetuses (0.004%) in the 40 mg/kg/day group, but this was considered to be an incidental finding. According to a 1993 publication from Charles River Laboratories, this is considered a very rare finding. It was this finding that led to the application of Pregnancy Category C to the label.

Developmental toxicity: Fetal Organ formation (Gestation Days 7 to 17) in Sprague-Dawley Rats; TAU-284 doses 0, 10, 100 and 1000 mg/kg/day

Parent body toxicity: NOAEL 10 mg/kg/day for dams due to mydriasis seen at the 100 and 1000 mg/kg/day dose levels.

Developmental toxicity (organ development stage):
NOAEL was 1000 mg/kg/day, the highest dose examined in this study. There were no apparent test article effects on either the F1 or F2 generations.

Developmental toxicity: Fetal Organ formation (Gestation days 6 to 18) in NZW Rabbits; bepotastine besilate doses 0, 20, 100 and 500 mg/kg/day

Parent body toxicity: A NOAEL was not established but is considered to be < 20 mg/kg/day. Miscarriage was noted in one 20 and one 100 mg/kg/day dam on gestation days 19 and 29, respectively, although these deaths were not clearly test article related. Bleeding was noted in 2 additional dams in the 20 mg/kg group on Gestation days 28 and 29. Red urine was seen in 5/14 animals in the 100 mg/kg group after the completion of dosing, starting on Gestation day 22 and in 7/14 females in the 500 mg/kg group starting on gestation day 14. This was not considered indicative of miscarriage but may be due to a species specific metabolite.

NOAEL for the organogenesis stage of development was considered to be 500 mg/kg/day. There were no apparent test article effects on the F1 generation.

Perinatal and Lactation period toxicity (dams treated Gestation day 17 through Day 21 post-delivery): Developmental and reproductive toxicity Sprague-Dawley rats; bepotastine besilate, 0, 10, 100 and 1000 mg/kg/day

Parent body toxicity: NOAEL = 10 mg/kg/day due to the retention of a dead fetus in the uterus of one dam in the 100 mg/kg/day group. Other observations were made in the 1000 mg/kg/day dose group included deaths of several dams, and a higher incidence of still births. Mydriasis and soiling of lower abdomen was also seen in this high dose group.

F1 generation effects: NOAEL = 10 mg/kg/day due to increased incidence of stillborns in the 100 and 1000 mg/kg dose groups as compared to controls (6.01% and 10.18% vs 3.79%). There was also a decrease in the number of animal surviving from birth to post-delivery day 4 in the 1000 mg/kg dose group as compared to controls (46.09% vs 94.10%). There was no difference in distributions of effects between male and female offspring.

Also seen in the 1000 mg/kg dose group were a tendency toward the delay of the differentiation of pinna detachment (50% of animals), incisor eruption (~10% of animals), and eyelid separation (~10% of males needed 1 additional day). A 20-30% decrease in righting reflex rate, suppression of weight gain (-11 to -15%), and transient decreased food consumption between days 28 to 56 after birth were seen in the 1000 mg/kg pups as compared to controls. No effects of test article on motor coordination, learning ability, emotionality, or reproductive capacity, and no abnormalities were observed with regard to the development of F1 fetuses.

F2 effects: NOAEL = 100 mg/kg/day, due to the observations of slightly higher total number of early resorptions seen in the 1000 mg/kg dose group as compared to controls. The sex ratio of embryos (males/females) was ~50% lower in the 1000 mg/kg dose group as compared to controls.

Special toxicology:
Bepotastine besilate is not expected to cause skin sensitization or antigenicity based on studies conducted in Guinea pigs and mice.

2.6.6.10 Tables and Figures

All tables and figures relevant to this NDA have been included in other sections of this review.

2.6.7 TOXICOLOGY TABULATED SUMMARY

The sponsor did not provide an appropriate summary table in the application.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Bepotastine besilate in the Bepreve™ formulation appears reasonably safe for the proposed indication of bacterial conjunctivitis in pediatric and adult patients. It did not cause ocular inflammation or histopathologic changes in rabbits or dogs. Given the apparent species specificity for hepatocellular

tumors in mice and the large safety margin the 2 year dietary carcinogenicity study provides for the ocular route of administration, Bepreve™ is not considered to significantly increase one's risk to liver tumors. A safety factor of approximately 353X was calculated from maximal systemic exposure in the female mice receiving a nominal dose of 190 mg/kg/day compared to the maximal systemic exposure anticipated for topical ocular use in humans. There was no increase in hepatocellular tumors seen in the 24 month study in rats evaluating nominal doses up to 97 mg/kg/day, representing a systemic exposure approximately 200 times that anticipated with human ophthalmic use. Bepotastine besilate did not exhibit genotoxic/mutagenic potential based on a standard set of in vitro and in vivo studies.

Bepreve™ also did not cause strong hypersensitivity reactions with repeated use.

As an antihistamine, bepotastine besilate, may eventually be used for other indications besides ocular. The Pregnancy category C is recommended for this product due to the observation of a rare skeletal malformation seen in the fertility/early embryo development study in rats at the 1000 mg/kg dose. The approximate safety factor for the 200 mg/kg/day NOAEL identified in this study was 3330X that of anticipated human systemic exposure with topical ocular use. In rats given oral doses of 100 mg/kg/day, an increased incidence of stillborns were observed. At the 1000 mg/kg/day dose level in this same study, an increase in stillborns, decreased survival and decreased rate of development were observed in pups. There were no effects observed in rats treated with 10 mg/kg/day (representing a maximal systemic concentration less than 93 times that anticipated for topical ocular use in humans).

From a radiolabeled study in pregnant rats, it is recognized that bepotastine besilate can rapidly distribute to the yolk sac/placenta and to the fetus. Bepotastine besilate was transferred to the yolk sac/placenta at levels nearly equivalent to maximal plasma concentration, ~33-55% of bepotastine besilate was transferred to the developing fetus. At 24 hours following a single oral administration of 3 mg/kg, ~ 5.9% and 3.1% of maximal plasma bepotastine besilate concentrations were detected in the brain and liver of the fetus at 24 hours postdose. Bepotastine besilate was also noted to be transferred to milk in lactating rats, with milk concentrations being 1.5 to 2 times maximal plasma concentrations by 1 hour postdose and reaching levels below the limit of detection by 48 hours postdose.

Although bepotastine besilate appears to be a substrate for Cyp450 metabolism in rodents, it does not appear to be a target/inhibitor of human CYP450 enzymes. In both rats and dogs, test article is primarily excreted in feces and urine.

The pharmacology/toxicology reviewer has no objection to the approval of this NDA.

Unresolved toxicology issues (if any): None

Recommendations: There are no recommendations for additional nonclinical studies.

Suggested labeling:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogensis, Mutagenesis and Impairment of Fertility

Long term dietary studies in mice and rats were conducted to evaluate the carcinogenic potential of bepotastine besilate. Bepotastine besilate did not significantly induce neoplasms in mice receiving a

nominal dose of up to 200 mg/kg/day for 21 months or rats receiving a nominal dose of up to 97 mg/kg/day for 24 months. These dose levels represent systemic exposures approximating 353 and 200 times that anticipated for human topical ocular use. The no observable adverse effect level for bepotastine besilate based on nominal dose levels in these carcinogenicity tests were 18.7 to 19.9 mg/kg/day in mice and 9.58 to 9.8 mg/kg/day in rats (representing exposure margins of 60 and 17.7 times the systemic exposure anticipated for human topical ocular use).

There was no evidence of mutagenicity in the Ames test, in CHO cells (chromosome aberrations), in mouse hepatocytes (unscheduled DNA synthesis) or in the mouse micronucleus test.

When oral bepotastine was administered to male and female rats at doses up to 1,000 mg/kg/day, there was a slight reduction in fertility index and surviving fetuses. Infertility was not seen in rats given 200 mg/kg/day oral bepotastine besilate (approximately 3330 times the maximum systemic concentration anticipated for topical ocular use in humans).

8.1 Pregnancy
Pregnancy Category C: Teratogenicity studies have been performed in animals. Bepotastine besilate was not found to be teratogenic in rats at oral doses up to 200 mg/kg/day (representing a maximal systemic concentration approximately 3330 times that anticipated for topical ocular use in humans), but did show some potential for causing skeletal abnormalties at 1000 mg/kg/day. There were no effects seen in rabbits at oral doses up to 500 mg/kg/day (level of systemic exposure in the rabbits was not determined).

Evidence of infertility and conceptus loss was seen in rats given oral bepotastine besilate 1000 mg/kg/day; however, no evidence of infertility was observed in rats given 200 mg/kg/day (representing a maximal systemic concentration approximately 3330 times that anticipated for topical ocular use in humans). The concentration of radiolabeled bepotastine besilate was similar in fetal liver and maternal blood plasma following a single 3 mg/kg oral dose. The concentration in other fetal tissues was one-third to one-tenth the concentration in maternal blood plasma. There are no adequate and well-controlled studies of bepotastine besilate in pregnant women. Because animal reproduction studies are not always predictive of human response, Brepreve™ (bepotastine besilate ophthalmic solution) 1.5% should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

8.2 Labor and Delivery In rats given oral doses of 100 mg/kg/day, an increased incidence of stillborns were observed. At the 1000 mg/kg/day dose level in this same study, an increase in stillborns, decreased survival and decreased rate of development were observed in pups. There were no effects observed in rats treated with 10 mg/kg/day (representing a maximal systemic concentration less than 93 times that anticipated for topical ocular use in humans). There are no adequate and well-controlled studies in pregnant women. Brepreve™ (bepotastine besilate ophthalmic solution) 1.5% should be used during labor and delivery only if the potential benefit justifies the potential risk do the fetus.

8.3 Nursing Mothers
Reduced nursing was observed in lactating rats given an oral dose of 1,000 mg/kg/day. A reduction in nursing was not observed in rats given 100 mg/kg/day (representing a maximal systemic concentration slightly higher than 957X times that anticipated for topical ocular use in humans). Following a single 3 mg/kg oral dose of radio-labeled bepotastine besilate to nursing rats 11 days after delivery, the maximum concentration of radioactivity in milk was 0.40 μg eq/mL 1 hour after administration; at 48 hours after administration the concentration was below detection limits. The milk concentration was higher than the maternal blood

plasma concentration at each time of measurement. It is not known if bepotastine besilate is excreted in human milk. Caution should be exercised when **Bepreve™** (bepotastine besilate ophthalmic solution) 1.5% is administered to a nursing woman.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Histopathology inventory (optional)

Study	TNB-NI-25	TNB-NI-22R	TNB-NI-23	SNJ-NI-3	SNJ-NI-5	TNB-NI-05	TNB-NI-07	TNB-NI-04	TNB-NI-06	TNB-NI-21	TNB-NI-20	TNB-NI-24R
Species	90 day mice oral	14 day rats oral	90 day rats oral	4 week Dutch rabbits ocular	26 week Dogs ocular	4 week Beagle dogs oral	26 week Beagle dogs oral	4 week rats oral	26 week rats oral	24 month mouse oral	24 month rat oral	14 day mouse oral
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X*	X	X	X*
Aorta	X	X	X	X	X		X			X	X	
Appendix	X	X	X	X	X		X	X	X	X	X	
Arteries						X		X	X			
Bladder	X	X	X		X	X	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X	X	X	X	X	X	X
Bone	X	X	X	X	X	X	X	X	X	X	X	X
Brain	X*	X*	X*	X*	X*	X*	X*	X*	X*	X	X	X*
Cecum						X						X
Cephalic region	X											
Cervix												
Cholescyst					X							
Coagulating glands	X	X*	X							X	X	X*
Colon	X	X	X	X	X	X	X	X	X	X	X	X
Dermis	X			X								
Duodenum	X	X	X	X	X	X	X	X	X	X	X	X
Epididymis	X	X	X	X	X*	X*	X*	X*		X	X	X
Epithelial body					X							X
Esophagus	X	X	X		X	X	X	X	X	X	X	
Eye	X	X	X	X	X	X	X	X	X	X	X	X
Fallopian tube												
Gall bladder				X		X*	X*					
Glandula parotidea					X							

Gross lesions												
Gullet				X								
Harderian gland	X	X	X	X				X	X	X	X	X
Head region		X	X									
Heart	X	X*	X	X*	X*	X*	X*	X*	X*	X	X	X*
Ileum	X	X	X	X	X	X	X	X	X	X	X	X
Injection site												
Jejunum	X	X	X	X	X	X	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X	X*	X*	X*	X	X	X*
Knee joint	X	X	X									X
Lachrymal gland				X	X							
Larynx	X	X	X		X							X
Liver	X*	X*	X*	X*	X*	X	X*	X*	X*	X	X	X*
Lungs	X	X*	X	X*	X*	X*	X*	X*	X*	X	X	X*
Lymph nodes, cervical	X	X	X							X	X	X
Lymph nodes submandibular						X	X	X	X			
Lymph nodes, mesenteric	X	X	X	X	X	X	X	X	X	X	X	X
MammaryGland (females only)	X	X	X	X	X		X	X	X	X	X	X
Nasal cavity												
Nictitans gland						X	X					
Optic nerves				X	X	X	X	X	X			
Ovaries	X*	X*	X*		X*	X*	X*	X*	X*	X	X	X*
Palpebral conjunctiva				X	X							
Pancreas	X	X	X	X	X*		X*	X	X	X	X	X
Paraorchis									X*			
Parathyroid	X	X*	X	X		X*	X*	X*	X*	X	X	
Parotid gland				X		X	X	X	X			
Peripheral nerve												
Pharynx	X	X	X	X								X
Pituitary	X	X*	X	X*	X*	X*	X*	X*	X*	X	X	X*
Prostate	X	X*	X	X	X	X*	X*	X*	X*	X	X	X*
Rectum	X	X	X	X	X	X	X	X	X	X	X	X
Retropharyngeal lymph nodes					X							
Salivary gland	X	X*	X	X*						X	X	X*
Sciatic nerve	X	X	X		X	X	X	X	X	X	X	X
Seminal vesicles	X	X*	X	X				X*		X	X	X*
Skeletal muscle				X	X	X	X	X	X	X	X	X
Skin		X	X		X	X	X	X	X	X		X
Spinal cord	X	X	X		X	X	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*	X*	X*	X*	X	X	X*
Sternum	X	X	X				X		X	X	X	X
Stomach	X	X	X	X	X	X	X	X	X	X	X	X
Sublingual glands				X		X	X					
Submandibular glands				X			X*					
Submaxillary gland					X*	X*		X*	X*			
Testes	X*	X*	X*	X*	X*	X*	X*	X*	X*	X	X	X*

Thymus	X	X*	X	X*	X*	X*	X*	X*	X*	X	X	X*
Thyroid	X*	X*	X	X*	X*	X*	X*	X*	X*	X	X	X*
Tongue	X	X	X	X	X	X	X	X	X	X	X	X
Trachea	X	X	X	X	X	X	X	X	X	X	X	X
Triceps,inferior medullary			X									
Triceps surae	X											
Urinary bladder				X								
Uterus	X	X*	X		X	X*	X*	X*	X*	X	X	X*
Vagina	X	X	X		X	X	X	X	X	X	X	X
Vena cava												X
Vertebra	X	X	X							X	X	X
Zymbal gland												

X, histopathology performed
*, organ weight obtained

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/s/

Theresa Allio
7/21/2009 03:41:29 PM
PHARMACOLOGIST

Wendelyn Schmidt
7/21/2009 03:47:21 PM
PHARMACOLOGIST
I concur with Dr. Allio's assessment of the data,
in terms of the scope, completeness, and interpretation.
The label accurately and adequate reflects these findings.

DIVISION OF ANTI-INFECTIVE AND OPHTHALMOLOGY PRODUCTS
PHARMACOLOGY/TOXICOLOGY REVIEW AND MEMO TO FILE: NDA 22-288

DATE: 08/12/09
TO: File, 22-288
FROM: Theresa Allio, Ph.D.
Pharmacologist, DAIOP
NDA submission: 0000/November 14, 2008/original application

Sponsor: ISTA Pharmaceuticals, Inc., Irvine, CA
Compound: Bepreve™, bepotastine besilate
Therapeutic Class: histamine H_1 receptor antagonist
Target indication: allergic conjunctivitis in patients ages 3 years and older

THROUGH: Wendelyn Schmidt, Ph.D., Supervisory Pharmacologist, DAIOP

RE: correction to DNA review regarding segment 3 reproductive toxicology study assessment

Background Information: Bepreve™ (1.5% bepotastine besilate), is intended to be administered by ocular instillation two times daily. The sponsor submitted a complete series of reproductive toxicology studies in rats and rabbits. In the pharmacology review for the original NDA filing, the NOAEL for fetal/neonatal effects in the rat Segment 3 study (TNB-NI-13) was noted to be 10 mg/kg/day based on the observation of a decreased fetal survival rate at the 100 and 1000 mg/kg/day dose levels (6.01 and 10.18% respectively) as compared to 3.79% seen in controls. During the labeling review this data was reassessed and determined that the values seen at the 100 mg/kg/day dose level were not outside the range of normal deviation. Furthermore, the value for fetal survival in the 1000 mg/kg/day dose group did not reach statistical significance compared to controls, while the value for postnatal survival was considered significant.

Thus, the NOAEL for fetal/neonatal effects in this study is considered to be 100 mg/kg/day, with the observation of decreased growth, development and survival of neonates seen in the 1000 mg/kg/day dose group. This is the information that is captured in the pregnancy and lactation section of the final label approved for Bepreve™, and should be applied to corresponding pages in the original Pharmacology review for NDA 22-288 (pages 4, 5, 45, 46, 111, 113, 125, 127, 128, 129).

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22288	ORIG 1		BEPOTASTINE BESILATE OPHTHALMIC SOLUTION

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

THERESA C ALLIO
08/14/2009

WENDELYN J SCHMIDT
08/17/2009

DIVISION OF ANTI-INFECTIVE AND OPHTHALMOLOGY PRODUCTS
PHARMACOLOGY/TOXICOLOGY REVIEW AND MEMO TO FILE: NDA 22-288

DATE: 5/22/09

TO: File, NDA 22-288 (Bepotastine besilate)
Attn: Shrikant Pagay, Ph.D.

FROM: Theresa Allio, Ph.D.
Pharmacologist, DAIOP

Date of Consultation request: 12 May 2009

THROUGH: Wendelyn Schmidt, Ph.D.
Supervisory Pharmacologist, DAIOP

RE: Toxicological review of organic volatile and semi-volatile leachables identified in drug product under various storage conditions.

Background Information: Sponsor submitted NDA 22-288 for Bepotastine besilate, an ophthalmic solution for allergic conjunctivitis. Stability of the drug product was tested in 10.0 ml fill, 10cc bottle (sublot 218053) and 1.0 ml fill, 7.5 cc bottle (sublot 218054), stored at both 25ºC/40% relative humidity and 40ºC/40% relative humidity in the horizontal position. These fill conditions were believed to estimate worst case scenarios for fill volume and bottle size, while the horizontal position provided the maximum surface area to drug product. The following compounds were identified as leachables.

(b) (4)

All of the above compounds were identified as leachables in the drug product when stored under various conditions. There was an unknown leachable that was found only in (b) (4) samples stored at the accelerated storage conditions (40 ºC) for 6 months, and was not identifiable. The values for (b) (4) and (b) (4) are both within acceptable limits according to ICH Q3C. The remaining leachables, including the unknown leachable, were all detected at levels below the reporting threshold (0.1%) identified in ICH Q3B.

Reviewer recommendation

Although all of the identified compounds have been listed as ocular irritants in their respective MSDS profiles, they are not considered to pose a risk due to the extremely low levels detected in drug product.

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/s/

Theresa Allio
6/19/2009 08:27:33 PM
PHARMACOLOGIST

wendy, this is the chemistry consult

Wendelyn Schmidt
6/25/2009 02:12:14 PM
PHARMACOLOGIST

Executive CAC
Date of Meeting: April 27, 2009

Committee: David Jacobson-Kram, Ph.D., OND-IO, Chair
Abigail Jacobs, Ph.D., OND-IO, Member
Paul Brown, Ph.D., OND IO, Member
Al DeFelice, Ph.D., DCRP, Alternate Member
Wendelyn Schmidt, Ph.D., DAIOP, Supervisor
Theresa Allio, Ph.D., DAIOP, Presenting Reviewer

Author of Draft: Theresa Allio, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 22-288
Drug Name: Bepreve™
Sponsor: ISTA Pharmaceuticals

Background: Bepreve™ (bepotastine besilate) is a histamine H1 receptor antagonist being developed by ISTA Pharmaceuticals as a 1.5% ophthalmic solution for the treatment of allergic conjunctivitis in patients aged 3 years and older. Labelled use allows for administration 2 times a day with effectiveness lasting up to 8 hours. The applicant submitted NDA 22-288 on November 12, 2008 with a PDUFA goal date of September 12, 2009.

The protocols for the carcinogenesis studies had not received prior approval by CDER Exec CAC, but the doses used were considered adequate for evaluating carcinogenicity potential of the proposed topical ocular route of administration. Doses used in the rat and mouse carcinogenicity studies were selected on the basis of 14 and 90 day dose range finding studies conducted in each species. Bepotastine besilate did not display mutagenic/genotoxic activity in any of the studies conducted (Ames, mammalian chromosome aberration, mouse micronucleus and unscheduled DNA synthesis in mouse liver).

Rat Carcinogenicity Study
Test article was administered in animal feed, with doses estimated based on average daily food intake.

RAT ESTIMATED DOSE LEVELS (mg/kg/day):
- Low Dose: males 9.58, females 9.81
- Middle Dose: males 29.2, females 29.5
- High Dose-1: males 97.0, females 97.9

There were no test article related neoplasms observed.

Mouse Carcinogenicity Study
Test article was administered in animal feed, with doses estimated based on average daily food intake.

MOUSE ESTIMATED DOSE LEVELS (mg/kg/day):
- Low Dose: males 19.9, females 18.7
- Middle Dose: males 60.1, females 57.0
- High Dose-1: males 200, females 190

Positive hepatocellular carcinomas were seen in high dose females. This was considered species specific, due to metabolic activation in the liver, and not of human relevance. The sponsor submitted a separate study confirming metabolic activation in the mouse liver following bepotastine besilate administration.

Executive CAC Recommendations and Conclusions:

The Committee's recommendations and conclusions are proffered pending receipt of individual animal tables and additional data to assure the GLP quality of the study.

Rats:

The Committee agreed that the study was adequate.

The Committee concurred that there were no drug-related neoplasms.

Mouse:

The Committee agreed that the study was adequate.

The Committee concurred that there were no drug-related neoplasms.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DAIOP
/WSchmidt, DAIOP
/TAllio, DIAOP
/RRodriguez DAIOP
/ASeifried, OND-IO

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/s/

David Jacobson-Kram

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