CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20706

CHEMISTRY REVIEW(S)

DIVISION OF ANALGESICS, ANTIINFLAMMATORY AND OPHTHALMIC DRUG PRODUCTS (HFD-550)

Review of Chemistry, Manufacturing, and Controls

NDA #:	CHEM. REVIEW#:	REVIEW DATE:
20-706	01	3 October 1996

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Orig	~~26~~ 22 Mar 96	~~27~~ 26 Mar 96	29 Apr 96

NAME & ADDRESS OF APPLICANT:
Susan H. Caballa
817 568 6296

Alcon Laboratories Inc.
6201 South Freeway
Fort Worth, Texas 76134

DRUG PRODUCT NAME
PROPRIETARY: Emadine 0.05% Ophthalmic Solution
NONPROPRIETARY/USAN: Emedastine Difumarate
CHEM. TYPE/THER. CLASS: 1S

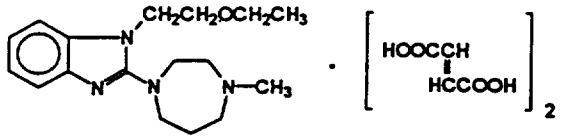
PHARMACOL. CATEGORY/INDICATION: relief of signs and symptoms of allergic conjunctivitis

DOSAGE FORM: Topical

STRENGTH(S): 0.05%

ROUTE OF ADMINISTRATION: Topical (ocular)
DISPENSED: [X] Rx Otc

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepin-1-yl)- 1H-benzimidazole difumarate
CAS Registry Number:
Emedastine: 87233-61-2
Emedastine Difumarate: 87233-62-3
Molecular Formula: Emedastine: $C_{17}H_{26}N_4O$
Fumaric Acid: $C_4H_4O_4$
Emedastine Difumarate: $C_{25}H_{34}N_4O_9$
Molecular Weight: Emedastine Difumarate: 534.57



SUPPORTING DOCUMENTS:

REMARKS/COMMENTS:

The label states that the drug can be stored at 4 °C - 30 °C with an expiration date of 1 year.
Test methods for release and stability required additional development. Individual test methods for a stability indicating assay and related substances has been requested. The method validation package for a stability indicating assay, related substances , and benzalkonium chloride is incomplete.
The applicant has not presented clear evidence that particulates are monitored using the proposed release specifications. This information is essential for the approvability of the application.

CONCLUSIONS & RECOMMENDATIONS:

Orig. NDA 20-706
HFD-820/S.A. Gilman/03 October 1996/Date Revised: 14 October 1996
HFD-551/Project Management/J.Holmes
HFD-830/Teamleader/H.PATEL
HFD-30 E.SHEININ [#1 ONLY]
R/D INIT BY : Hasmukh B. Patel 11-8-96

S.A.GILMAN, REVIEW CHEMIST
filename: NDA20706.LWP

Division of Anti-inflammatory, Analgesic and Ophthalmic Drugs
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-706

REVIEW # 2 **DATE REVIEWED:** 6/17/97

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
	2 (Date changed by Sue Chen 12/11/97)	6 5+2	
ORIGINAL	3-26-96	3-27-96	4-29-96
AMENDMENT	1-03-97	1-06-97	1-10-97
AMENDMENT	3-11-97	3-12-97	3-14-97

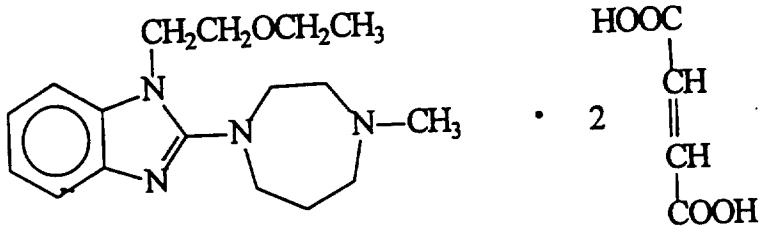
NAME & ADDRESS OF APPLICANT:
Alcon Laboratories Inc.
6201 South Freeway
Fort Worth, Texas 76134

DRUG PRODUCT NAME

Proprietary:	Emadine 0.05% Ophthalmic Solution
Established:	emedastine difumarate
Chem.Type/Ther.Class:	1S

PHARMACOL. CATEGORY:	antihistamine
DOSAGE FORM:	Ophthalmic Solution
STRENGTHS:	0.05%
ROUTE OF ADMINISTRATION:	ophthalmic
DISPENSED:	X Rx __ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA AND WEIGHT:
1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-1H-benzimidazole difumarate



REMARKS:

Drug Substance:
The DMF holder

Drug Product:
The applicant has not satisfactorily responded to the deficiencies in release and stability specifications. Based on the stability data presented, the current limit , , for total related substances should be
Sterility testing is mandatory for every lot at release, not just for stability batches.
Extension of expiration period to 24 months is not acceptable. The submitted stability data justify an 18 month expiration dating period only.

CONCLUSIONS & RECOMMENDATIONS:

The CMC section of this NDA is approvable pending satisfactory response to the chemist's draft deficiencies.

cc:
Orig. NDA 20-706
HFD-550/Division File
HFD-550/Lin
HFD-550/LoBianco
HFD-830/C Chen

Sue-Ching Lin, 6/9/97, Revised 10/22/97
Sue-Ching Lin, M.S., R.Ph.
Chemist, HFD-550/830

Hasmukh B. Patel 10-27-97
Hasmukh Patel, Ph.D.
Chemistry Team Leader, HFD-550

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Division of Anti-inflammatory, Analgesic and Ophthalmic Drugs
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-706

REVIEW # 3 **DATE REVIEWED:** 11-Dec-97

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL[1]	22-Mar-96	26-Mar-96	29-Apr-96
AMENDMENT[2]	03-Jan-97	06-Jan-97	10-Jan-97
AMENDMENT[2]	11-Mar-97	12-Mar-97	14-Mar-97
AMENDMENT[3] (AZ)	24-Oct-97	27-Oct-97	06-Nov-97
AMENDMENT[3] (BC)	28-Oct-97	03-Nov-97	06-Nov-97

[1] submission covered in chemistry review #1
[2] submissions covered in chemistry review #2
[3] submissions covered in this review

NAME & ADDRESS OF APPLICANT:
Alcon Laboratories Inc.
6201 South Freeway
Fort Worth, Texas 76134

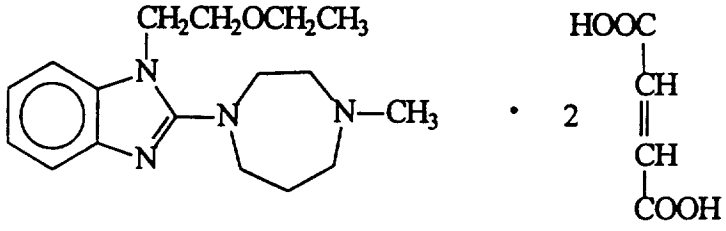
DRUG PRODUCT NAME

Proprietary:	Emadine 0.05% Ophthalmic Solution
Established:	emedastine difumarate
Chem.Type/Ther.Class:	1S

PHARMACOL. CATEGORY: antihistamine
DOSAGE FORM: Ophthalmic Solution
STRENGTHS: 0.05%
ROUTE OF ADMINISTRATION: ophthalmic
DISPENSED: _X_ Rx __ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA AND WEIGHT:

1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-1H-benzimidazole difumarate



SUPPORTING DOCUMENTS:

REMARKS:

These amendments responded to the chemistry deficiencies listed in chemistry review #2, which were conveyed to the sponsor by facsimile on October 23, 1997. The applicant has adequately addressed the deficiencies.

CONCLUSIONS & RECOMMENDATIONS:

The CMC section of this NDA is acceptable. This NDA may be **approved**. The letter to the applicant should include the standard methods validation paragraph and a statement that the submitted stability data support an eighteen (18) month expiration dating period for the 2.5 mL size drug product and a twenty-four (24) month expiration dating period for the drug product in 5 mL, 10 mL, and 15 mL sizes.

cc:
Orig. NDA 20-706
HFD-550/Division File
HFD-550/Lin
HFD-550/LoBianco
HFD-830/C Chen

Sue-Ching Lin, 12/11/97

Sue-Ching Lin, M.S., R.Ph.
Chemist, HFD-550/830

Hasmukh Patel, Ph.D.
Chemistry Team Leader, HFD-550

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CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20706

ENVIRONMENTAL ASSESSMENT AND/OR FONSI

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

NDA 20-706

EMADINE™

(emedastine 0.05% ophthalmic solution)

REVIEW DIVISION: HFD-550

Division of Anti-Inflammatory, Analgesic, and Ophthalmologic Drug Products

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-706

EMADINE™

(emedastine 0.05% ophthalmic solution)

REVIEW DIVISION: HFD-550

Division of Anti-Inflammatory, Analgesic, and Ophthalmologic Drug Products

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for EMADINE™, Alcon Laboratories has prepared an abbreviated environmental assessment in accordance with 21 CFR 25.31a(B)(3)(attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Emedastine fumarate is a chemically synthesized drug which is administered as a 0.05% ophthalmic solution in the treatment of allergic conjunctivitis. The drug substance is manufactured by a confidential third party pharmaceutical manufacturing facility. The drug product will be manufactured by the applicant in Fort Worth, Texas. The finished drug product will be used by physicians and patients in a hospital, clinic or home. Emedastine may enter the environment from excretion by patients, from disposal of pharmaceutical waste or from emissions from manufacturing sites.

Disposal of the drug may result from out of specification lots, discarding of unused or expired product, and user disposal of empty or partly used product and packaging. Returned or out-of-specification drug substance and rejected or returned drug product will be disposed of by permitted disposers at authorized sites. No treatment of waste is necessary or required on site. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic regulations. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills,

incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

12/18/96 P G Vincent
DATE

Prepared by
Phillip G. Vincent, Ph.D
Environmental Scientist
Center for Drug Evaluation and Research

12/18/96 Nancy B Sager
DATE

Concurred
Nancy Sager
Acting Supervisor/Team Leader
Environmental Assessment Team
Center for Drug Evaluation and Research

Attachments: Environmental Assessment
Material Safety Data Sheet (drug substance)

AUG 29 1996

SEP 13 1996

REVIEW FOR DIVISION OF
ANALGESIC, ANTI-INFLAMMATORY, AND OPHTHALMIC DRUG PRODUCTS, HFD-550
OFFICE OF NEW DRUG CHEMISTRY, MICROBIOLOGY STAFF, HFD-805
MICROBIOLOGIST'S REVIEW NO. 1
AUGUST 27, 1996

Reviewing Microbiologist: Carol K. Vincent, HFD-805

A. 1. **NDA NO.:** NDA 20-706

PRODUCT NAME: Emadine (emedastine difumarate) solution 0.05%

APPLICANT and MANUFACTURING SITE: Alcon Laboratories, Inc.
6201 South Freeway
Fort Worth, TX 76134-2099

2. **DOSAGE FORM AND ROUTE OF ADMINISTRATION:**

Sterile Ophthalmic Solutions, topical; 5, 10, and 15 mL fill in 5, 10, and 15 mL Drop-Tainer dispensers, respectively.

3. **METHOD(s) OF STERILIZATION:**

4. **PHARMACOLOGICAL CATEGORY AND / OR PRINCIPAL INDICATION:**

The drug substance in Emadine (emedastine difumarate) solution 0.05% is a selective, topical, histamine H_1 antagonist indicated for signs and symptoms of allergic conjunctivitis.

5. **DRUG PRIORITY CLASSIFICATION:** 1 S

B. 1. **INITIAL APPLICATION DATE:** 03-22-96

2. **RECEIVED FOR REVIEW:** 04-08-96

C. **CONCLUSION and RECOMMENDATION:** We recommend approval of NDA 20-706, Emadine (emedastine difumarate) solution 0.05% for sterility assurance based on the provided information.

cc:
Orig. HFD-550 NDA 20-706
HFD-160/Consult/CKVincent [HFD-805]
HFD-550/Chambers/Tso/Holmes
Drafted by: CKVincent/08-13-96
Revised by: CKVincent/08-23-96/08-27-96
R/D Init by: PHCooney/08-29-96

Carol K. Vincent 8-27-96

D Thursam for PHC 8-29-96

NDA20706

CENTER FOR DRUG EVALUATION AND RESEARCH

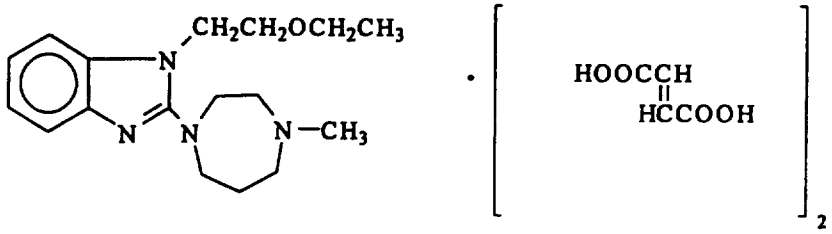
APPLICATION NUMBER: 20706

PHARMACOLOGY REVIEW(S)

NOV 18 1996

DIVISION OF ANTI-INFLAMMATORY, ANALGESIC AND OPHTHALMOLOGIC DRUG PRODUCTS
PHARMACOLOGY AND TOXICOLOGY REVIEW

NDA	**20-706**
DRUG:	Emadine™ (Emedastine Difumarate) 0.05%
OTHER NAMES:	ALØ3432A; KG-2413; LY188695
SPONSOR:	Alcon Laboratories, Inc. 6201 South Freeway Fort Worth, TX 76134
SUBMISSION DATE:	March 22, 1996
TYPE OF SUBMISSION:	Original Full Application
DATE COMPLETED:	September 16, 1996
REVIEWER:	W. C. Josie Yang, Ph.D.
CDER STAMP DATE:	March 26, 1996
DATE RECEIVED IN HFD-550:	April 3, 1996
DATE ASSIGNED TO REVIEWER:	April 9, 1996
DRUG CATEGORY:	Histamine H_1 Antagonist
FORMULA:	$C_{25}H_{34}N_4O_9$; 1H-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2); MW=534.57



INDICATION:	Relief of the Signs and Symptoms of Allergic Conjunctivitis
DOSAGE FORM:	0.05% Emedastine Difumarate Ophthalmic Solution
RELATED DRUG/INDs/NDAs/DMFs:	

TABLE OF CONTENTS (i)

PRECLINICAL/LABORATORY STUDIES:

PHARMACOLOGY

I. Pharmacologic Actions Relevant to the Proposed Used

i. *In Vitro* H_1 Receptor Antagonist Activity (Alcon Technical Report Nº 004:39930:0893)

- Emedastine was a potent H_1 antagonist with a K_i value of 1.3 nM.
- Emedastine inhibited histamine-induced phosphoinositide (PI) turnover in human trabecular meshwork cells, human cornea fibroblasts and human conjunctival epithelial cells with IC_{50} values of 1.44 ± 0.3 nM, 0.5 ± 0.1 nM, and 1.8 ± 0.7 nM, respectively.
- Emedastine was demonstrated to have antihistaminic activity on isolated ileum with an IC_{50} value of 6.1 nM.
- Emedastine antagonized histamine-induced contractions of guinea pig ileum, aorta, and trachea with pK_B values of 9.9, 9.9, and 9.2, respectively.

ii. *In Vivo* Systemic Antihistaminic Activity

- Emedastine, administered orally 1 hr before histamine challenge, prevented histamine-induced mortality with an ED_{50} value of 4.4 μg/kg, po. It was 3, 39, 68, and 780x more potent than ketotifen, chlorpheniramine, clemastine, and diphenhydramine, respectively.
- Emedastine, administered orally 1 hr before histamine challenge, antagonized histamine-induced increases in vascular permeability in the skin of fasted Hartley guinea pig (10/group) with an ED_{50} of 3.7 μg/kg, po. It was shown to be ≈4 and 27x more potent than ketotifen and chlorpheniramine.
- Emedastine, administered orally 1 hr before histamine challenge, suppressed histamine-induced increases in total pulmonary impedance with an ED_{50} of 3 μg/kg, po. It was shown to be 2 and 10x more potent than ketotifen and chlorpheniramine in preventing histamine-induced increases in airway resistance in guinea pigs.

iii. Topical Ocular Antihistaminic Activity of Emedastine in Guinea Pig (Alcon Technical Report No. 012:39900:0493 & Alcon Technical Report Nº 034:39900:0893).

- Emedastine (0.1-0.001%, w/v), administered topically either 5 or 30 min before antigen challenge, significantly reduced the allergic response (passive conjunctival immediate hypersensitivity) in guinea pig (5-8/group) with calculated ED_{50} values of 0.0046 and 0.00022%, respectively.
- Topical administration of Emedastine 1 min, 3 min, 2, 4, or 8 hr prior to subconjunctival histamine challenge caused a dose (%, w/v) -dependent inhibition of histamine-induced vascular permeability response, with calculated ED_{50} values of 0.0002%, 0.000035%, 0.0029%, 0.019% and 0.19% (w/v), respectively.
- Single topical ocular administration of 0.05% or 0.1% Emedastine (clinical formulation) suppressed histamine-induced conjunctival vascular permeability by 54% and 67%,

respectively, through 16 hrs.

- Emedastine, administered topically 1 min before histamine challenge, was 3, 8, and 17x more potent than ketotifen, pheniramine, and antazoline, respectively, and approximately equipotent to pyrilamine in prevention of histamine-induced vascular permeability response in the conjunctiva.
- Emedastine, administered topically 30 min before histamine challenge, was 7, 7, 10, 10, 100, and 3333x more potent than bromopheniramine, chlorpheniramine, clemastine, pyrilamine, levocabastine and diphenhydramine, respectively, and approximately equipotent to pyrilamine in interfering of histamine-induced vascular permeability response in the conjunctiva.

iv. *In Vivo* Systemic Antiallergic Effects of Emedastine

- Emedastine, administered orally at doses of 0.005-0.04 mg/kg 1 hr prior to challenge, markedly inhibited lethal anaphylaxis induced by rabbit IgG in ♂ Hartley guinea pig (10/group) with an ED_{50} value of 0.023 mg/kg.
- Emedastine, at doses >0.003 mg/kg po, significantly inhibited the homologous 48 hr PCA reactions in guinea pigs, with complete inhibition occurring doses ≥0.1 mg/kg.
- Emedastine, administered orally 1 hr prior to challenge, suppressed both IgE-mediated homologous PCA and passive anaphylactic bronchoconstriction in guinea pigswith ED_{50} values of 1.7 and 22 μg/kg, respectively.
- Emedastine, administered orally 1 hr prior to challenge, inhibited the antigen-induced increases in airway resistance in passively sensitized guinea pigs with an ED_{50} of 12 μg/kg.

II. General Pharmacology

i. Demonstration of H_1-Receptor Selectivity

In Vitro Receptor Binding Profile (Alcon Technical Reports Nº 004:39930:0893 and 010:39900:0392)

The H_1 receptor selectivity of Emedastine was assess using radioligand binding assays . The K_i values for Emedastine's binding to the H_1, H_2, and H_3 histamine receptors were 1.3, 49067 and 12430 nM, respectively. Emedastine, at a concentration of 10 μM, did not interact with adenosine 1 & 2, α_1, α_2, β, dopamine 1 & 2, serotonin 1 & 2, nicotinic, leukotriene B_4, leukotriene D_4, and thromboxane A_2 receptors.

Selective Effect of Emedastine on Smooth Muscle Preparations *In Vitro*

The selectivity of Emedastine for the histamine H_1 receptor was further evaluated by using a variety of smooth muscle preparations from rat and guinea pig. Emedastine was shown to have weak anticholinergic and antibradykinin properties with IC_{50} values of 31 μM and 340 μM, respectively.

In Vivo Effect of Topically Applied Emedastine on Platelet Activating Factor (PAF)- and/or Serotonin (5HT)-Induced Vascular Permeability in Rat Conjunctiva (Alcon Technical Report Nº 012:39900:0493)

Emedastine (0.1%), applied onto the eye 30 min before challenge, failed to prevent either platelet-activating-factor (PAF) or serotonin (5-HT) induced vascular permeability changes in conjunctiva. The results are listed in the following table.

Stimulus	Compound	Conc.. (%, w/v)	Wheal Area (Intensity)	%Change
PAF	NaCl	0.9	365 ± 41	-
	Emedastine	0.1	404 ± 60	+10
5-HT	NaCl	0.9	248 ± 40	-
	Emedastine	0.1	225 ± 43	-9
	Cyproheptadine	0.01	141 ± 29*	-43

*p<0.01, Dunnett's t Test

ii. Neuropharmacological Effects of Emedastine

The effects of Emedastine on the central nervous system were assessed in ♂ ddY mice.

- Emedastine, at a high dose of 100 mg/kg po, inhibited locomotor activity and acetic acid-induced writhing.
- Emedastine, at the levels of 10-100 mg/kg po, had no effects on muscle tone, various experiment-induced convulsions, oxotremorine-induced tremor, physostigmine-induced mortality, or hexobarbital-induced sleep.

Studies in ♂ Wistar rats showed that

- Emedastine, at a dose of 20 mg/kg iv, did not affect spontaneous EEG;
- Emedastine, at a dose of 100 mg/kg po, did not alter a condition avoidance response, rectal temperature, nor did have effects on monosynaptic and polysynaptic reflexes in lamectomized rats.

- Emedastine at a concentration of 4.0% (w/v), administered topically in to the conjunctival sac, caused no local anesthetic effect on corneal reflex in ♂ Hartley guinea pigs.

iii. Effects of Emedastine on the Cardiovascular System

The effects of Emedastine on blood pressure, heart rate, electrocardiogram (ECG) and respiration in anesthetized mongrel dogs were measured. The drug was introduced (15 second infusion) through a cannula inserted into a femoral vein or the duodenum. A dose-dependent ↓ in blood pressure and heart rate were observed over the dose range of 0.3 - 3.0 mg/kg, iv, and at 30 mg/kg, i.d., but ECG was not altered at any of the doses tested.

The effect of Emedastine on the isolated right atria of guinea pigs *in vitro* was also studied. Right atria preparations were suspended in an organ bath and the contractile force and heart

rate were recorded. Emedastine, at the 10^{-4} g/ml, a decrease in contractile force of isolated right atria was observed.

iv. Effects of Emedastine on the Respiratory System

The effect of Emedastine on respiration was examined in anesthetized guinea pigs. At a high dose (10 mg/kg, iv) the rate and volume of respiration increased. However, no effect on respiration was noted with Emedastine administered intraduodenally at a dose (30 mg/kg) 2500 times greater than the dose required to inhibit antigen-induced bronchoconstriction.

The effect of Emedastine on the volume and viscosity of respiratory tract secretions was also studied in rabbits. An intravenous dose of 3.0 mg/kg did not alter the amount or viscosity of respiratory secretions. No effect on tracheal ciliary movement was detected following intravenous doses (0.3 to 3.0 mg/kg) of Emedastine.

Emedastine, administered intravenously at a dose of 3 mg/kg, a transient suppression of respiration was noted in anesthetized dogs. Lower doses (0.3 and 1.0 mg/kg) were without effects. Similar transient effects on respiration were noted with chlorpheniramine and ketotifen at doses of 1.0 mg/kg.

v. Effects of Emedastine on the Gastrointestinal System

Emedastine's effects on intestinal transport were evaluated using fasted male ddY mice. Emedastine at a dose of 100 but not ≤30 mg/kg po, administered 30 min prior to oral administration of charcoal powder, caused an inhibition in intestinal peristalsis.

The effects of Emedastine on the secretion of gastric juices and bile were assessed in fasted Wistar male rats following intraduodenal administration. Emedastine, at 100 mg/kg administered 4 hr prior to collection of gastric juices, slightly lowered gastric juice volume. Emedastine at the dose range of 10-100 mg/kg induced a dose-dependent increase in biliary secretion. In contrast, reference compounds, ketotifen and chloropheniramine at 10 and 100 mg/kg, respectively, administered intraduaodenally decreased biliary secretion.

vi. Other Effects of Emedastine

The effect of Emedastine on urine volume, urinary electrolyte excretion, blood clotting and blood sugar levels was evaluated in fasted water-deprived male Wistar rats. Emedastine, at doses of 30 and 100 mg/kg po, had no effects on urine volume and urinary electrolytes. Administered 60 min before blood withdrawal, Emedastine (10-100 mg/kg, p.o.) demonstrated no significant effects on coagulation or blood sugar levels in rats.

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

I. Ocular Uptake and Distribution

i. Ocular Tissue Distribution of Radioactivity Following a Single Dose of ^{14}C-AL03432A (Emedastine), Alcon Technical Report Nº 024:38570:0695. (Vol. 11, p 5-0890 - 5-0928)

The ocular tissue distribution and systemic concentrations of radioactivity following a single unilateral topical dose of ^{14}C-Emedastine (0.1%) was evaluated in the ♂ New Zealand white rabbit. Ocular tissues were collected at 0.5, 1, 2, 4, 6, 8, 10, 24 hr and 48 hr post-dosing. Radioactivity decreased rapidly in ocular tissues except lens and cornea and plasma with $t_{½}$ of ≤2 hr. The C_{max} values in ocular tissues were: cornea > conjunctiva > iris-ciliary body > aqueous humor > choroid > retina > lens > vitreous humor. Results are presented in the following table.

Tissue	C_{max} (µg eq/g) Dosed Eye	T_{max} (hr) Dosed Eye	$t_{½}$ (hr) Dosed Eye	C_{max} (µg eq/g) Undosed
Aq. Humor	0.310 ± 0.124	0.5	0.7	0.0009
Cornea	3.28 ± 1.26	0.5	16.7	0.003
ICB	0.669 ± 0.148	0.5	1.0	0.019
Lens	0.016 ± 0.008	1.0	12.5	BLQ
Vit. Humor	0.001 ± 0.000	0.5	2.0	0.0006
Retina	0.021 ± 0.003	0.5	0.7	0.013
Choroid	0.060 ± 0.025	1.0	0.6	0.015
Conjunctiva	0.809 ± 0.335	0.5	1.1	0.006
Whole Blood	0.006 ± 0.001	0.5	1.0	-
Plasma	0.007 ± 0.001	0.5	1.2	-

BLQ = Below limit of quantitation (< 0.2 to 0.5 ng equiv./g for a tissue weight range of 0.27 to 0.52 grams)
ICB=iris-ciliary body

ii. Ocular Tissue Distribution of Radioactivity Following a Single 0.1% Dose of ^{14}C-AL-3432A (Emedastine) to Male Dutch Belted Rabbits, Alcon Technical Report 027:38570:0695. (Vol. 11, p 5-0929 - 5-0967)

The uptake and elimination of radioactivity from ocular tissue after single application of 0.1% ^{14}C-Emedastine was determined in ♂ Dutch Belted (DB) rabbits. The pharmacokinetic parameters of ^{14}C-Emedastine radioactivity in DB Rabbit ocular tissues and plasma are listed as follows:

Tissue	Cmax (µg eq./g)	Tmax (Hours)	$t_{½}$
Aqueous Humor	0.281 ± 0.067	1	0.8 hours
Cornea	3.32 ± 0.16	1	1.1 hours
ICB	10.2 ± 2.8	10	23.0 days
Retina	0.207 ± 0.082	0.5	Not Determined*
Choroid	0.770 ± 0.137	3	Not Determined*
Conjunctiva	0.824 ± 0.169	0.5	1.1 hours
Plasma	0.009 ± 0.004	1	1.0 hour

Half-life could not be reliably determined in these tissues due to inter-animal variability in the data. However, the general trend in the data indicated a prolonged half-life similar to ICB.

The results showed that C_{max}, T_{max} and $T_{½}$ in non-pigmented ocular tissues (aqueous humor, cornea, and conjunctiva) were similar to the values obtained from New Zealand white (NZW) rabbits. **However, C_{max} values for retina, choroid, and iris-ciliary body (ICB) were ≥10x higher than the corresponding values measured in NZ rabbits suggesting that Emedastine-melanin binding might have occurred. Prolonged $T_{½}$ (23 days) and higher C_{max} noted in ICB of DB rabbits implied that accumulation of Emedastine in ocular tissues containing high levels of melanin may occurr after a long term treatment. Potential ocular accumulation and toxicity of Emedastine in non-albino rabbits should be explored further.**

II. Systemic Pharmacokinetics, Absorption, Bioavailability, Systemic Tissue Distribution, Metabolism, and Excretion

Absorption Excretion & Bioavalability

i. Sakai, T., Hamada, T., Awata, N., Watanabe, J., Interspecies Differences in Pharmacokinetics of an Antiallergic Agent, 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1,4-diazepine-1-yl)benzimidazole Difumarate (KG-2413) After Intravenous Administration to Rats, Guinea Pigs and Dogs. *Chem. Pharm. Bull.* 37:753-756 (1989).

The pharmacokinetics of Emedastine were assessed in rats, guinea pigs, and dogs following a single dose of 2 mg/kg iv administration. The mean PK parameters are displayed in the following table. The elimination of Emedastine was rapid with $t_{½}$ of 1.1, 0.7, and 1.9 hr for the rat, guinea pig and dog, respectively.

Parameter	Rat	Guinea Pig	Dog
$AUC_{0-\infty}$ (ng•hr/mL)	218 ± 30	421± 140	369 ± 80
$t_{½}$(hours)	1.1 ± 0.2	0.69±0.08	1.9 ± 0.6
Vd_{ss} (L/kg)	7.92 ± 1.75	2.66 ± 0.94	8.21 ± 3.11
CL_t	72.7 ± 10.0	64.0 ± 21.3	44.8 ± 9.7

Each value represents the mean ± standard deviation.

ii. Sakai, T., Hamada, T., Awata, N., Watanabe, J., Pharmacokinetics of an Antiallergic Agent, 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepine-1-yl)-1H-benzimidazole Difumarate (KG-2413) After Oral Administration: Interspecies Differences in Rats, Guinea Pigs and Dogs. *J. Pharmacobio-Dyn.* 12:530-536 (1989).

Pharmacokinetic parameters of unchanged Emedastine following oral administration to rats, guinea pigs and dogs are depicted in the following table. Comparison of the iv and oral data exhibited moderate absolute bioavailabitlity in guinea pig. In contrast, very low bioavailability was seen in either the dog (5.2%) and rats (3.6%) inferring that a strong first pass effect might take place in these two species.

Parameter	Rat (20 mg/kg) (n=8)	Guinea Pig 2 mg/kg (n=7)	Dog 2 mg/kg (n=7)
T_{max} (hr)	0.39 ± 0.14	0.54 ± 0.06	0.85 ± 0.32
C_{max} (ng/mL)	57.3 ± 12.2	148 ± 9	4.78 ± 0.45
C_{max}/dose (ng•mg/mL•kg)	2.87 ± 0.61	74.2 ± 4.3	2.39 ± 0.23
$t_{1/2}$ (hr)	0.71 ± 0.69	0.69 ± 0.04	2.16 ± 0.19
AUC (ng•hr/mL)	79.0 ± 20.6	208 ± 16	19.1 ± 2.4
AUC/dose (ng•hr•mg/mlL•kg)	3.95 ± 1.03	104 ± 8	9.53 ± 1.20

Each value represents the mean ± SD.

iii. Sakai, T., Takahashi, H., Hamada, T., Awata, N., Watanabe, J. The Biological Fate of 1-(2-Ethoxyethyl)-2-(4-methyl-1-homopiperazinyl) benzimidazole Difumarate (KB-2413) I. Absorption and Excretion After Oral Administration to Rats and Guinea Pigs. *Xenobio. Metabol. and Dispos.* 2:123-131 (1987).

The absorption and excretion of oral Emedastine was conducted in rats and guinea pigs. Radiolabeled ^{14}C-Emedastine was administered to rats at 1 mg/kg and to guinea pigs at 2 mg/kg for both oral and iv studies. Rats received 1, 4, or 8 mg/kg ^{14}C-Emedastine orally for plasma pharmacokinetic studies. Concentrations of radioactivity in plasma, urine, feces, intestinal and bile samples were measured using liquid scintillation counting. Mean pharmacokinetic parameters of the radioactivity following oral or intravenous administration of ^{14}C-Emedastine to rats and guinea pigs are shown as follows. There was a dose-proportional increase in Cmax and $AUC_{0-\infty}$ values in rats receiving 1, 4, or 8 mg/kg ^{14}C-Emedastine po.

Dose (mg/kg)	Route of Administration	T_{max} (h)	C_{max} (ng eq/mL)	C_o (ng eq/mL)	$t_{1/2(9-24h)}$ (h)	$AUC_{0-\infty}$ (ng eq•h/mL)
Rat						
1	p.o. (n=3)	0.25	46.3 ± 3.6	-	8.6 ± 3.5	432 ± 86
4	p.o. (n=3)	0.25	251 ± 33	-	12.8 ± 3.6	1930 ± 320
8	p.o. (n=3)	0.25	408 ± 37	-	11.7 ± 2.0	4090 ± 1010
1	i.v. (n=4)	-	-	370 ± 36	5.8 ± 2.0	574 ± 65
Guinea pig						
2	p.o. (n=4)	0.5	398 ± 84	-	6.2 ± 2.0	846 ± 180
2	i.v. (n=3)	-	-	966 ± 179	8.3 ± 1.1	1200 ± 210

Each value represents the mean ± S.D.

By the comparison of iv and oral $AUC_{0-\infty}$, it appeared that the absolute oral bioavailability in the two species was similar at approximately 75 and 70% for the rat and guinea pig, respectively. In rats, the $AUC_{0-\infty}$ and C_{max} increased in proportion to the oral dose. After oral administration, the dose-normalized C_{max} in guinea pigs was about 4 times higher than that in rats. This was due to a secondary peak plasma concentration in rats at 4 to 6 hours after oral dosing. Within 96 hours after oral and iv administration of ^{14}C-Emedastine, nearly all of the administered doses were recovered in the excreta of both species. Radioactivity in feces was approximately 50-65% of the total dose administered by either route. In rats, radioactivity in expired air was negligible. When ^{14}C-Emedastine was *in situ* injected into GI loops (stomach, duodenum, jejunum, and ileum) (n=4), about 80% of the dose was absorbed from the small intestine in 60 minutes with 56% of the dose absorbed

from the duodenum in 15 minutes and only 1% absorbed from the stomach. In rats with indwelling bile duct cannulae, cumulative excretion of radioactivity from the bile totaled 77.3, 88.5 and 89.6% of the oral dose at 4, 24 and 48 hours after dosing, respectively. Urinary radioactivity excretion was 10.9% after 24 hours. **Therefore, excretion of radioactivity was near completion during this period.** For the enterohepatic study, a 1 ml aliquot of a 6-hour collection of radioactive bile was deposited into the duodenum of other rats with indwelling bile duct cannulae. Bile in these rats contained 9.3, 57.8 and 72.5% of the administered radioactivity at 6, 12 and 24 hours after dosing, respectively. These results implied that a significant amount of bile radioactivity was reabsorbed from the intestinal tract. *In vitro* studies found 81% binding of radioactivity to guinea pig plasma proteins at a concentration of 60 ng/ml of ^{14}C-Emedastine. In rats, binding was only 39% under the same conditions.

iv. Wada, Y., Hamada, T., Sakai, T., Kawashima, T., Awata, N., Absorption, Metabolism and Excretion of an Antiallergic Agent, 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepine-1-yl)-1H-benzimidazole Difumarate (KG-2413), in Dogs. *Xenobio. Metabol. and Dispos.* 4:471-480 (1989) (Alcon TR 082:38570:0995).

The absorption, metabolism and excretion of Emedastine in dogs were measured after oral and iv administration of 2 mg/kg ^{14}C-Emedastine. T_{max} was 60 min after oral administration. The $AUC_{0-\infty}$ and urinary excretion rate of radioactivity were similar for both routes. These results suggest that Emedastine was well absorbed from the intestinal tract in dogs, as seen with guinea pigs and rats. After oral administration, unchanged Emedastine accounted for only 1% of plasma radioactivity in dogs. Emedastine undergoes an extensive first-pass metabolism in this species. Immediately after iv dosing, most of the radioactivity was as unchanged drug. Based on quantitative analysis of plasma metabolites and urinary excretion products, the main metabolites in dogs by both routes were the N-oxide and 6-hydroxyemedastine (free and conjugated). The 5-hydroxy metabolite found in the plasma and urine of both rats and guinea pigs was below the detection limit in dogs (< 10 ng/mL). After both oral and iv dosing, 97% of administered radioactivity was excreted into urine and feces within 96 hours. For both routes of administration, approximately 70% of the total dose was found in the urine with a 30% fecal recovery, suggesting that fecal excretion occurs *via* bile. The relative amounts of the main metabolites in dogs differ from those in rats and guinea pigs.

Tissue Distribution

v. Sakai, T., Takahashi, H., Hamada, T., Awata, N., Watanabe, J., The Biological Fate of 1-(2-Ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole Difumarate (KB-2413) II. Distribution After Single and Multiple Oral Administration to Rats. *Xenobio. Metabol. and Dispos.* 2:133-145 (1987).

Tissue distribution of radioactivity following single and multiple oral dose of ^{14}C-Emedastine (1 mg/kg/day) was assessed in the ♂ Wistar rats. For the single dose study,

animals were sacrificed for tissue collections at 0.25, 2, 6, 24, 48, 192, and 480 hours post administration of ^{14}C-Emedastine. For whole body autoradiography (WBA), rats were dosed with 1.5 mg/kg po and were sacrificed at 0.25, 2, 6, and 48 hr post dosing. Results from the WBA study showed high levels of radioactivity in the liver, pancreas, stomach content, urine, kidney, Harder's gland, salivary gland, lung and spleen. Concentrations of radioactivity were low in the eye, CNS and fat. The maximal levels of radioactivity in most tissues were measured at 0.25 hr post dosing, with the highest concentrations observed in the liver, stomach, and intestine. The levels of radioactivity in all tissues declined to <0.5% of the dose within 24 hr post dosing. Liver and Kidney had the highest levels of radioactivity at 24 hr. The data obtained from single dose tissue distribution are summarized in the following table.

Tissue	Radioactivity (ng eq. of Emedastine base/g)						
	0.25 hr (a)	2 hr	6 hr	24 hr	2 Days	8 Days	20 Days
Plasma	77.6 ± 20.7	17.8 ± 2.5	23.3 ± 3.7	4.95 ± 2.27	0.912 ± 0.459	N.D. (b)	N.D.
Blood	44.1 ± 8.6	10.9 ± 1.4	12.1 ± 1.5	2.80 ± 1.20	0.990 ± 0.270	N.D.	N.D.
Brain	4.57 ± 1.61	1.68 ± 0.22	1.50 ± 0.50	0.867 ± 0.640	N.D.	N.D.	N.D.
Hypophysis	104 ± 47	54.4 ± 1.8	38.6 ± 5.5	N.D.	N.D.	N.D.	N.D.
Eye	13.9 ± 3.3	6.29 ± 0.43	6.40 ± 0.89	1.89 ± 0.90	N.D.	N.D.	N.D.
Harder's Gland	33.7 ± 6.5	18.9 ± 3.9	12.7 ± 1.1	6.91 ± 4.50	1.35 ± 0.20	N.D.	N.D.
Thyroid	88.4 ± 3.1	48.4 ± 23.8	34.2 ± 3.68	N.D.	N.D.	N.D.	N.D.
Parotid Gland	45.3 ± 14.2	31.9 ± 4.0	19.3 ± 6.8	10.3 ± 5.5	1.30 ± 0.22	N.D.	N.D.
Submaxillary, Sublingual Gland	72.5 ± 23.6	37.4 ± 5.5	18.6 ± 2.3	3.14 ± 1.61	0.617 ± 0.080	N.D.	N.D.
Thymus	38.1 ± 9.5	16.1 ± 0.9	8.50 ± 0.47	4.33 ± 2.32	0.861 ± 0.110	N.D.	N.D.
Lung	146 ± 12	49.1	31.5 ± 3.2	14.8 ± 9.1	1.16 ± 0.28	N.D.	N.D.
Heart	58.9 ± 4.9	22.6 ± 1.4	9.23 ± 1.90	2.85 ± 1.59	N.D.	N.D.	N.D.
Liver	3650 ± 1250	409 ± 212	184 ± 15	65.7 ± 16.5	25.9 ± 11.9	7.48 ± 2.41	1.30 ± 0.30
Pancreas	469 ± 108	48.1 ± 15.7	17.4 ± 1.5	3.48 ± 1.76	0.646 ± 0.020	N.D.	N.D.
Spleen	91.8 ± 29.6	28.1 ± 10.0	21.2 ± 1.8	5.15 ± 1.76	1.56 ± 0.35	0.884 ± 0.083	N.D.
Adrenal	90.1 ± 8.0	38.7 ± 14.4	18.7 ± 2.9	5.95	N.D.	N.D.	N.D.
Kidney	321 ± 84	147 ± 18	126 ± 67	25.6 ± 9.6	8.55 ± 0.46	4.30 ± 0.43	1.31 ± 0.40
Testis	7.76 ± 1.03	6.68 ± 0.78	6.85 ± 0.72	9.52 ± 5.28	3.01 ± 0.40	1.38 ± 0.05	N.D.
Skin	28.7 ± 10.2	12.2 ± 2.4	9.57 ± 4.03	3.34 ± 1.58	0.746	N.D.	N.D.
Muscle	26.4 ± 5.6	10.5 ± 0.7	6.17 ± 0.11	1.56 ± 0.77	N.D.	N.D.	N.D.
Stomach	3600 ± 2920	157 ± 88	26.8 ± 7.5	3.67 ± 0.65	0.991 ± 0.556	N.D.	N.D.
Duodenum	5950 ± 1030	228 ±62	87.2 ± 10.2	9.06 ± 4.37	1.36 ± 0.64	N.D.	N.D.
Jejunum	6510 ± 5950	565 ± 340	71.4 ± 11.3	16.2 ± 6.2	2.87 ± 2.68	N.D.	N.D.

Each value represents the mean ± S.D. of three animals or the mean of two animals.
(a)Time after administration. (b) Less than two times of back ground.

For the multiple dose experiments, non-fasted rats were orally dosed with ^{14}C-Emedastine 1 mg/kg/day for up to 14 days. The levels of radioactivity in plasma and tissues reached to steady-state by the 7th dose. Approximate 98% of the cumulative dose was excreted in the urine and feces within 96 hr post 14-day dosing. The highest radioactivity levels were seen in the liver and kidney with the brain exhibiting very low levels.

Tissue	Radioactivity (ng eq. of Emedastine base/g)				
	1 Dose		7 Doses	14 Doses	
	24 Hours	96 Hours	24 Hours	24 Hours	96 Hours
Plasma	5.48 ± 2.33	0.184 ± 0.076	5.10 ± 0.57	10.8 ± 1.2	0.617 ± 0.023
Blood	3.05 ± 0.89	0.198 ± 0.060	3.01 ± 0.54	7.59 ± 0.45	1.70 ± 0.12
Brain	1.44 ± 1.01	N.D. (a)	0.600 ± 0.091	0.889 ± 0.170	0.294 ± 0.087
Hypophysis	N.D.	N.D.	14.8 ± 7.6	25.4 ± 1.4	N.D.
Eye	1.89 ± 0.66	N.D.	2.24 ± 0.28	3.29 ± 0.56	0.912 ± 0.396
Harder's Gland	7.25 ± 4.25	N.D.	4.93 ± 0.73	6.17 ± 0.45	1.29 ± 0.07
Thyroid	18.1 ± 5.1	N.D.	47.5 ± 8.3	103 ± 10	101 ± 21
Parotid Gland	6.63 ± 3.51	N.D.	6.12 ± 0.85	9.86 ± 1.53	1.65 ± 0.32
Submaxillary, Sublingual Gland	2.90 ± 1.33	N.D.	2.88 ± 0.46	6.23 ± 1.08	1.10 ± 0.19
Thymus	3.96 ± 2.42	1.06 ± 1.74	2.34 ± 0.48	4.88 ± 1.36	2.37 ± 1.98
Lung	16.0 ± 11.5	1.99 ± 2.96	6.34 ± 0.28	13.8 ± 4.9	4.49 ± 4.16
Heart	2.70 ± 1.61	0.361 ± 0.396	2.07 ± 0.35	4.28 ± 0.54	1.50 ± 0.44
Liver	56.6 ± 19.8	11.3 ± 2.6	195 ± 29	280 ± 69	82.7 ± 16.4
Pancreas	4.70 ± 3.35	0.347 ± 0.259	3.55 ± 1.59	5.78 ± 1.19	1.65 ± 0.28
Spleen	4.49 ± 1.97	1.02 ± 0.52	8.78 ± 0.74	21.2 ± 1.4	15.5 ± 2.0
Adrenal	N.D.	N.D.	6.00 ± 1.87	10.8 ± 1.1	8.04 ± 1.76
Kidney	35.9 ± 11.6	7.99 ± 4.53	69.7 ± 4.5	124 ± 11	59.5 ± 6.8
Testis	14.2 ± 7.6	2.85 ± 1.06	20.1 ± 1.4	29.1 ± 1.2	19.7 ± 1.4
Skin	2.93 ± 1.31	0.536 ± 0.385	3.46 ± 0.25	6.63 ± 0.74	1.73 ± 0.30
Muscle	1.28 ± 0.58	N.D.	1.69 ± 0.20	2.64 ± 0.44	0.935 ± 0.164
Stomach	4.56 ± 3.31	N.D.	5.05 ± 2.82	7.87 ± 0.68	1.73 ± 0.70
Duodenum	14.6 ± 11.6	0.261 ± 0.075	14.2 ± 4.3	24.3 ± 1.2	1.68 ± 0.03
Jejunum	18.6 ± 7.0	0.310 ± 0.165	30.8 ± 15.3	38.3 ± 22.8	2.14 ± 0.18

Each value represents the mean ± standard deviation of 3 or 4 animals or the mean of two animals.
(a) Less than 2 times background.

vi. Sakai, T., Takahashi, H., Hamada, T., Awata, N., Watanabe, J. The Biological Fate of 1-(2-Ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole Difumarate (KB-2413) III. Transfer to Fetus and Milk in Rats. *Xenobio. Metabol. and Dispos.* 2:147-154 (1987).

Placental and lactal transfer of Emedastine and its metabolites was evaluated in the following oral administration of 1 mg/kg ^{14}C-Emedastine to pregnant (Gestation Day 19) and nursing Wistar rats (Day 11 postpartum). Blood samples were collected for the determination of radioactivity and whole body autoradiography was performed on pregnant rats at 2, 6, 24 hr post dosing. Maximum plasma levels in pregnant rats occurred at 30 min after dosing, with a secondary peak at 6 hours. The maximum level of radioactivity (≈1% of total dose) in fetal tissue occurred at 2 hr after dosing, with a concentration comparable to that in maternal plasma. Fetal radioactivity had declined to < 0.1% of dose by 48 hours. Radioactivity was transferred to milk and reached a peak concentration of 26.2 ± 8.8 ng equiv/ml at 8 hours after dosing. Lactal concentrations then declined with a half-life of approximately 16 hours with milk/plasma radioactivity concentration ratio increasing from 0.21 at 1 hour to 4.55 at 24 hours. **Significant sex difference was noted** by comparison of the data from both pregnant and non-pregnant female rats with previous data from previous study in males (see Nº v in the ADME section II). Both pregnant and nonpregnant female rats had higher systemic levels of radioactivity following an oral ^{14}C-Emedastine dose than those measured in the ♂. For a single dose of Emedastine, 1 mg/kg, mean C_{max}

values were 347 and 221 ng/ml for nonpregnant and pregnant ♀ rats, respectively, versus 46.3 ng/ml for ♂. Similarly, mean $AUC_{0-\infty}$ values for nonpregnant and pregnant rats were 658 and 910 ng•hr/ml, respectively, compared to 432 ng•hr/ml for males.

Metabolism (*In Vivo & in Vitro*)

vii. Awata, N., Takahashi, H., Noumi, K., Sakai, T., Hamada, T. Identification of the Metabolites of an Antiallergic Agent, 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepine-1-yl)-1H-benzimidazole Difumarate (KG-2413), in Rats. *Yakugaku Zasshi* 109:318-328 (1989)(Alcon TR 083:38570:0995).

Urinary and biliary metabolites of Emedastine in rats were identified. Fasted rats were given oral doses of either unlabeled Emedastine (8 to 50 mg/kg) or ^{14}C-Emedastine (1 mg/kg). Rats were then placed in metabolism cages for urine collection over 24 hours. For collection of bile, a cannula was placed in the bile duct and bile collected for 24 hours. The mean urinary excretion of radioactivity over 24 hours was 24.5 ± 3.3% of the dose. Metabolites conjugated with glucuronic acid represented >50% of the Emedastine excretion into urine. Little amount of Emedastine in the urine was unconjugated. Biliary excretion accounted for 89.4 ± 5.8% of the administered dose, with metabolites present mainly as conjugates. Isolation, purification and identification of individual metabolites were performed, using ^{14}C-labeled suspected metabolite standards as markers, by analysis with HPLC, GC, ^{1}H nuclear magnetic resonance, mass spectrometry and gas chromatography/mass spectrometry (GC/MS). The primary metabolites in rats were the 5-hydroxy and 6-hydroxy metabolites, formed by hydroxylation of the benzimidazole ring. Other important metabolic pathways in rats were: (1) N-oxidation and N-demethylation of the 1,4-diazepine ring; (2) oxidation of the α-carbon of the 1,4-diazepine ring (lactam formation); and (3) O-deethylation on the N-ethoxyethyl side chain. Plasma metabolite profiles in rats following a single oral dose are listed in the following table.

Metab.	15 Min. Free	15 Min. Conj.	60 Min. Free	60 Min. Conj	360 Min. Conj
Parent	0.61 ± 0.07	N.D.	0.67 ± 0.08	N.D.	N.D.
5/6-OH-5'oxo (M2a,b)	2.48 ± 0.42	N.D.	4.26 ± 1.14	N.D.	N.D.
5'oxo + M4 (unidentified)	3.25 ± 0.15	N.D.	2.38 ± 0.48	N.D.	N.D.
6-OH (M5a)	1.32 ± 0.19	8.23 ±2.76	1.05 ± 0.33	7.66 ± 1.03	7.46 ± 1.24
5-OH (M5b)	0.37 ± 0.10	33.99 ± 1.92	0.31 ± 0.05	30.91 ± 3.28	62.16 ± 2.45
N-desmethyl (M6)	0.30 ± 0.04	N.D.	0.29 ± 0.12	N.D.	N.D.
O-desethyl-5'-oxo-N-desmthr (M7)	0.43 ± 0.12	N.D.	1.14 ± 0.33	N.D.	N.D.
O-desethyl (M8)	0.15 ± 0.01	N.D.	0.13 ± 0.04	N.D.	N.D.
N-oxide (M9)	0.33 ± 0.05	N.D.	0.22 ± 0.08	N.D.	N.D.
5/6-OH-N-desmeth (M10a,b)	N.D.	4.41 ± 1.15	N.D.	3.81 ± 0.50	N.D.
5/6-OH-O-desethyl (M11a,b)	0.35 ± 0.03	1.99 ± 0.92	N.D.	5.25 ± 0.447	N.D.

5/6-OH-N-oxide n.d. except 1.59 ± 0.34 conj at 360 min; N.D. = Not Detected (< 0.1%)

viii. Wada, Y., Takahashi, H., Hamada, T., Sakai, T., Kawashima, T., Awata, N. Metabolism of an Antiallergic Agent, 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepine-1-yl)-1H-benzimidazole Difumarate (KG-2413), in Rats and Guinea Pigs. *Xenobio. Metabol. and Dispos.* 4:459-470 (1989) (Alcon TR 084:38570:0995).

Emedastine and its metabolites in the urine, bile, plasma and liver of rats and in the urine of guinea pigs were identified. Fasted rats and guinea pig received ^{14}C-Emedastine orally at 1 mg/kg and 2 mg/kg, respectively. Urine and bile were collected from bile duct cannulated rats for 24 hours. Urinary and biliary metabolites were fractionated according to established methods and quantified using thin-layer chromatography and autoradiograms. This study confirmed the presence of similar metabolites in liver and plasma. The major urinary and biliary metabolites of Emedastine in rats were the 5-hydroxylated compound, the 6-hydroxylated compound and their corresponding conjugates. The major metabolite in guinea pig urine was the N-oxide. The 5-hydroxy and 6-hydroxy metabolites were present as conjugates in amounts approximately six-fold lower than the N-oxide. The metabolic pathways of Emedastine are basically similar for rats and guinea pigs but the relative amounts of the various metabolites were different between the two species. Cumulative (24 hr) urinary excretion of Emedastine and metabolites in various species are shown as followings.

Compound	% Dose Rat	% Dose G. Pig	% Dose Dog	% Dose Man
6-OH (M5a)	2.07/1.21	0.18/0.45	4.61/3.66	10.0/16.0
5-OH (M5b)	1.43/8.23	0.43/1.19	0.48/0.22	4.2/7.5
Parent Drug	0.10/N.D.	3.67/N.D.	0.75/N.D.	3.6/N.D.
5 and 6-OH-5'-oxo (M2a,b)*	0.13/N.D.	N.D./N.D.	N.D./N.D.	2.4/N.D.
5 and 6-OH, N-desmeth. (M10 a,b)	0.15/0.08	N.D./0.13	N.D./N.D.	N.D.
N-Oxide (M9)	0.07/N.D.	12.63/N.D.	10.40/N.D.	0.4/N.D.
N-desmeth-5'-oxo-O-deethyl (M7)*	0.34/N.D.	0.46/0.15	0.59/N.D.	N.D.
N-desmethyl (M6)	0.09/N.D.	0.56/N.D.	1.21/N.D.	N.D.
Total	13.9	19.8	21.9	44.1

Each pair of numbers = free/conjugated; N.D. = Not Detected (< 0.1%);
*Cross-reactivities for these compound were not reported. However, the cross-reactivity of 5'-oxo-Emedastine was < 0.01%.

ix. Hamada, T., Kawashima, T., Awata, N. Cross-reactivities of Active Metabolites in the Radioreceptor Assay of an Antiallergic Agent, 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepine-1-yl)-1H-benzimidazole Difumarate (KG-2413). *Yakugaku Zasshi* 109:474-479 (1989).

The cross reactivity of active metabolites of Emedastine with histamine H_1 receptors were determined via the radioreceptor binding assay (RRA) against known metabolites. The eight main metabolites of Emedastine were tested for extraction efficiency in the aqueous NaOH/benzene extraction procedure as well as cross-reactivity with the parent compound for histamine H_1 receptors in the RRA method. The results are shown in the following table.

Metabolite	Extraction Efficiency at pH 10	RRA Cross-Reactivity (%)*
N-desmethyl	78	29.0
O-desethyl	24	0.8
N-oxide	0	0.6
5-hydroxy	26	21.0
6-hydroxy	18	1.7
4-hydroxy	Not Reported	1.9
7-hydroxy	Not Reported	2.9
5'-oxo	Not Reported	≤ 0.01

*Relative to parent compound

The 5-hydroxy metabolite showed significant cross-reactivity (21%), but low extraction efficiency (26% at pH 10). The N-desmethyl metabolite had high extraction efficiency (78% at pH 10) and cross-reactivity of 29%. The other metabolites showed either low cross-reactivity or low extraction and therefore did not significantly interfere with Emedastine determination. Following oral administration of 2 mg/kg Emedastine to guinea pigs, plasma concentrations of the desmethyl metabolite paralleled those of parent drug, ranging from to 21 to 32% on a relative basis.

x. Hamada, T., Wada, Y., Awata, N. Influence of 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1,4-diazepine-1-yl)benzimidazole Difumarate (KG-2413) on the Liver Microsome Drug-Metabolizing Enzyme System in Rats. Internal Report, Pharmaceuticals Research Center, Kanebo, Ltd. (Alcon TR 087:38570:0995).

The effects of high dosages of Emedastine on liver microsomal enzymes were assessed in rats. Young male Wistar rats were given once daily oral doses of Emedastine 0, 50 or 250 mg/kg/day for 1, 3, 6, 10 or 14 days or positive control phenobarbital sodium 80 mg/kg/day ip for three days. The liver was removed and microsomal suspensions were quantified for levels of aminopyrine N-demethylase and aniline p-hydroxylase activity, cytochrome P-450 content and microsomal protein 24 hr after the final dosing. Rats at 50 mg/kg/day Emedastine showed no significant increase in liver weight, microsomal protein content/g of liver, or cytochrome P-450 content. Aniline p-hydroxylase and aminopyrine N-demethylase activities were slightly increased (≈ 15%) in these animals. Absolute liver weight in the 250 mg/kg/day group was not significantly increased. However, a significant increase in relative liver weight as the result of suppression of body weight gains was seen. Microsomal protein content increased significantly after 10 and 14 days of administration. In the 250 mg/kg/day group, aminopyrine N-demethylase activity, aniline p-hydroxylase activity and cytochrome P-450 content were about 1.9, 1.8, and 1.5 times those of controls, respectively, after 14 days of dosing. These results exhibited that repeated administration of Emedastine at a high dosage caused the induction of liver microsomal drug-metabolizing enzymes.

xi. Wada, Y., Hamada, T., Kawashima, T., Awata, N. *In Vitro* Metabolism of an Antiallergic Agent, Emedastine Difumarate, in Rats and Guinea Pigs. *Yakugaku Zasshi* 110:40-48 (1990) (Alcon TR 085:38570:0995).

Microsomes (S9) fraction of liver, kidney, lung and small intestine from rats and guinea pigs were evaluated to determine the metabolic pathways responsible for the differences and the activities of Emedastine hydroxylase, N-oxidase and N-demethylase in the rat and guinea pig. The disappearance rates of Emedastine from rat kidney, lung and small intestinal 9000 x g supernatants were very low relative to that from the hepatic microsomal fraction, indicating that the liver was the primary site of metabolism of Emedastine. In guinea pig liver S9, the rate of N-oxidation was 5 to 11 times higher than that of hydroxylation. These findings were consistent with *in vivo* data. There was also a high N-oxidation activity in rat liver S9 that was comparable to the 5-hydroxylation activity. The N-oxidation activity in guinea pigs was about 4 times greater than that in rats. In rats, the Emedastine N-oxide reductase activity was nearly equal to the N-oxidase activity, whereas in guinea pigs, the reductase activity was lower than the N-oxidase activity. Both microsomal and soluble liver fractions contained reductase activity in rats. In guinea pigs, N-oxide reduction was mostly catalyzed by microsomal enzymes. Enzyme activities were found to be of the following order in rats: 5-hydroxylase ≥ N-oxidase > 6-hydroxylase > N-demethylase. In guinea pigs, the order was: N-oxidase > N-demethylase > 6-hydroxylase > 5-hydroxylase. Therefore, differences in Emedastine metabolism between guinea pigs and rats appeared to be the result of differences in the rates of N-oxidation of the tertiary amine in the 1,4-diazepine ring and the reduction of N-oxide once it is formed. Whether the relative rates of oxidase and reductase activities observed *in vitro* reflected those *in vivo* remained unknown.

III. In Vitro Plasma Binding

i. Sakai, T., Hamada, T., Awata, N., Watanabe, J. Binding of 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1,4-diazepine-1-yl)benzimidazole Difumarate (KG-2413) to Serum or Plasma Proteins in Man, Guinea Pig and Rat. *J. Pharmacobio-Dyn.* 11:262-267 (1988).

Binding of ^{14}C-Emedastine in human serum, rat plasma, guinea pig plasma, and protein solutions containing human α_1-acid glycoprotein (α_1-AG), human serum albumin (HSA), guinea pig serum albumin (GSA) or rat serum albumin (RSA) was used to characterize via equilibrium dialysis. Scatchard plots of the protein solution data showed saturable binding with two classes of binding sites for human α_1-AG and GSA, while HSA and RSA showed nonsaturable binding, indicating only one binding site. Profound interspecies differences were noticed with the binding affinity being high in guinea pig plasma, intermediate in human serum and low in rat plasma. Binding capacity of human serum for Emedastine was higher than that of guinea pig and rat plasma. In isolated protein solution, the binding of Emedastine to human α_1-AG was 7x greater than that to HSA. The primary binding site for Emedastine free base in human serum was likely to be α_1-AG by the evidence of comparable dissociation constant at the high affinity binding site (K_{d1}) for human serum and isolated α_1-AG. Results from rat and guinea pig experiments suggested low levels of Emedastine binding to serum albumin. Therefore, the interspecies differences in Emedastine binding may be due to species differences in the binding affinity or capacity, or both, of α_1-AG.

ii. Sakai, T., Hamada, T., Kawashima, T. and Awata, N. Interactions Between 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4,-diazepin-1-yl)-1H-benzimidazoledifumarate (KG-2413) and Other Concomitant Drugs in Binding to Serum Proteins. Alcon Technical Report 086:38570:0995.

The potential of plasma free Emedastine to interact with potential concomitant drugs was determined using *in vitro* serum protein binding of ^{14}C-Emedastine in the presence of potential concomitant drugs. Five representative compounds, isoproteronol HCl (β-receptor stimulant), theophylline (xanthine derivative), prednisolone (adrenocortical steroid), bromhexine HCl (expectorant) and dihydrocodeine phosphate (antitussive), were tested. Each concomitant drug was tested at a concentration of approximately 10x (range 8.7-11.1x) its reported plasma maximum concentration following a therapeutic dose. The results showed no significant changes in binding of Emedastine to serum proteins for any of the concomitant drugs tested.

TOXICOLOGY

I. Ocular Toxicity

i. Three-month Topical Ocular Irritation and Systemic Toxicity Evaluation of Emedastine Ophthalmic Solution (ALØ3432A) in Rabbits (One Month Interim Report). Technical Report Nº 028:38520:0893, Alcon Laboratories, Inc.

Report Nº: 028:38520:0893
Study Aim: To determine the ocular irritation potential of Emedastine (ALØ3432A) Ophthalmic Solution resulting from QID topical ocular administration to New Zealand White rabbits for three months (One Month Interim Report).
Compound: 0.1% (Lot Nº 93-5769), 0.3% (Lot Nº 93-5767), or 1.0% (Lot Nº 93-5765) Emedastine (as the base) Ophthalmic Solution
Control Vehicle: 0.01%Benzalkonium Cl + 0.01% Disodium Edetate + 0.85% NaCl + 0.5% Hydroxypropyl Methylcellulose, pH 7.4 (Lot Nº 93-5855)
Dose & Route: 2 drops/topical ocular instillation, QID
Animal: New Zealand White rabbits, weighing 2.3-2.7 kg, 3/sex/group

Treatment Group	Nº of Animals	Treatment Volume (μl)	Treatment/Day	Duration (Days)
1. Untreated Control	3/Sex/Group	-	-	30
2. Vehicle Control	3/Sex/Group	70	4	30
3. 0.1% Emedastine	3/Sex/Group	70	4	30
4. 0.3% Emedastine	3/Sex/Group	70	4	30
5. 1.0% Emedastine	3/Sex/Group	70	4	30

Study Location: Alcon Laboratories, Inc., Fort Worth, TX.
Compliance with GLP/QAU: Yes

Results:
Each animal was observed twice daily for morbidity/mortality, general well being and for overt pharmacotoxic signs. Animals were weighed and slit-lamp biomicroscopic

examinations were performed before the initiation of the treatment. During the one-month treatment phase these parameters were evaluated at weekly intervals. Indirect ophthalmoscopic examinations and pachymetry measurements were performed at the prescreen and again at the one-month evaluation. A complete necropsy was performed on Day 31 of the study on three rabbits/sex/group. All animals demonstrated an overall positive weight gain during the one-month treatment period. Slit-lamp biomicroscopic examination of the study animals did not reveal any treatment-related findings. Conjunctival congestion (hyperemia) of minimal severity was noted in all treatment and control groups. Indirect ophthalmoscopic evaluations revealed that all the animals remained within normal limits during the one-month treatment period. Pachymetry data was unremarkable. Unremarkable findings were noted at postmortem examinations. Microscopic changes were not observed in any organ attributable to topical ocular application of Emedastine Ophthalmic Solution for 30 days. Therefore, 1.0% ALØ3432A did not produce any significant ocular irritation and systemic toxicity following one-month of daily topical ocular administration in New Zealand White rabbits.

ii. Three-Month Topical Ocular Irritation and Systemic Toxicity Evaluation of Emedastine Ophthalmic Solution (ALØ3432A) in Rabbits. Technical Report Nº 006:38520:0194, Alcon Laboratories, Inc.

Report Nº: 006:38520:0194
Study Aim: To determine the ocular irritation potential of Emedastine (ALØ3432A) Ophthalmic Solution resulting from QID topical ocular administration to New Zealand White rabbits for three months.
Compound: 0.1% (Lot Nº 93-5769), 0.3% (Lot Nº 93-5767), or 1.0% (Lot Nº 93-5765) Emedastine (as the base) Ophthalmic Solution
Control Vehicle: 0.01%Benzalkonium Cl + 0.01% Disodium Edetate + 0.85% NaCl + 0.5% Hydroxypropyl Methylcellulose, pH 7.4 (Lot Nº 93-5855)
Dose & Route: 2 drops/topical ocular instillation in to right eye, QID for 93 days
Animal: New Zealand White (NZW) rabbits, weighing 2.3-2.7 kg, 4/sex/group
Study Location: Alcon Laboratories, Inc., Fort Worth, TX.
Compliance with GLP/QAU: Yes
Study Design:

Treatment Group	Nº of Animals	Treatment Volume (µl)	Treatment/Day	Duration (Days)
1. Untreated Control	4/Sex/Group	-	-	93
2. Vehicle Control	4/Sex/Group	70	4	93
3. 0.1% Emedastine	4/Sex/Group	70	4	93
4. 0.3% Emedastine	4/Sex/Group	70	4	93
5. 1.0% Emedastine	4/Sex/Group	70	4	93

- Mortality and Clinical Signs - 2x/day;
- Physical Examination - 1x/day for wks 1 & 2 and 2x/wk thereafter;
- Body Weights - Days 0, 7, 14, 21, 28, 42, 56, 70, 84 and 94;
- Slit-lamp Biomicroscopic Examinations - Days 0, 7, 14, 21, 28, 35, 49, 63, 77, & 91;
- Indirect Ophthalmoscopic Examinations - Days 0, 28, and 91;
- Pachymetry Measurements - Days -1, 30 and 91;

- Clinical Chemistry and Hematology Analysis - Day 93;
- Plasma Drug Levels - Days 29 and 90;
- Necropsy & Histology Examinations - Day 94.

Results:

- Mortality and Clinical Signs - No deaths occurred. Clinical findings were remarkable. Isolated occasions of ocular discharge were seen in the treated eyes (1♂ @vehicle control, 1♂ & 1♀ @ 0.3% Emedastine and 1♀ @ 1.0% Emedastine).
- Weight Gains - Normal.
- Slit-lamp Biomicroscopic Examination - Minimal conjunctival congestion (hyperemia) was noted in all treatment and control groups. One incidence of severe conjunctival congestion was noted in the left eye of 1♂ @ 0.3% Emedastine on day 14. A single occasion of minimal conjunctival swelling was seen 1♀ @ 0.3% Emedastine on day 49. One ♀ @ 0.3% Emedastine had minimal corneal cloudiness in the left eye from days 14 - 93 and right eye from days 28-93.
- Indirect Ophthalmoscopic Examinations - Within normal limit.
- Pachymetry Measurements - Normal.
- Clinical Laboratory Analysis - No significant treatment-related changes were noted.
- Plasma Emedastine Levels - The mean peak plasma levels (±SD) of Emedastine on Day 90 were higher than those on Day 29, an indicative of possible accumulation following repeated dosing.

Dose Level (%)	Sampling Day	C_{max} (ng/ml, N=8)
0.1	Day 29	0.70 ± 0.25
	Day 90	1.65 ± 1.16
0.3	Day 29	1.56 ± 0.89
	Day 90	2.92 ± 3.11
1.0	Day 29	3.67 ± 2.29
	Day 90	7.93 ± 3.38

- Necropsy & Histology Examinations - The gross observations at necropsy were generally unremarkable. Microscopic changes were not observed in the eyes and adnexa or in any organ which were attributable to topical ocular application of Emedastine for three months.

In conclusion, ALØ3432A (1.0%, 0.3% or 0.1%) did not cause any significant ocular irritation and systemic toxicity in New Zealand White rabbits following a 3-month daily QID topical ocular treatment.

iii. Six-Month Topical Ocular Irritation and Systemic Toxicity Evaluation of Emedastine Ophthalmic Solution (ALØ3432A) in Rabbits. Technical Report Nº 047:38520:0595, Alcon Laboratories, Inc.

Report Nº: 047:38520:0595

Study Aim: To determine the ocular irritation potential of Emedastine (ALØ3432A) Ophthalmic Solution resulting from QID topical ocular administration to New Zealand White rabbits for 6 months.

Compound: 0.1% (Lot Nº94-10832), 0.3% (Lot Nº94-10833), or 1.0% (Lot Nº94-10834) Emedastine (as the base) Ophthalmic Solution
Control Vehicle: 0.01%Benzalkonium Cl + 0.5% Tromethamine + 0.71% NaCl + 0.25% Hydroxypropyl Methylcellulose, pH 7.4 (Lot Nº 94-10835)
Dose & Route: 2 drops/topical ocular instillation to the right eye, QID for 190 days
Animal: New Zealand White rabbits, 8/sex/group
Study Location: Alcon Laboratories, Inc., Fort Worth, TX.
Compliance with GLP/QAU: Yes
Study Design:

Treatment Group	Nº of Animals	Treatment Volume (µl)	Treatment/Day	Duration (Days)
1. Untreated Control	8/Sex/Group	-	-	190
2. Vehicle Control	8/Sex/Group	60	4	190
3. 0.1% Emedastine	8/Sex/Group	60	4	190
4. 0.3% Emedastine	8/Sex/Group	60	4	190
5. 1.0% Emedastine	8/Sex/Group	60	4	190

- Mortality and Clinical Signs - 2x/day;
- Physical Examination - 2x/wk;
- Body Weights - Days 0, 7, 13, 21, 28, 49, 77, 105, 147, 168 and 190 (♂)/191 (♀);
- Slit-lamp Biomicroscopic Examinations - Days 0, 7, 13, 21, 28, 77, 105, 147, 168, & 189;
- Indirect Ophthalmoscopic Examinations - Days 0 and 189;
- Pachymetry Measurements - Days 0 and 188;
- Clinical Chemistry and Hematology Analysis - Day 188(♂)/189 (♀);
- Plasma Drug Levels - Days 1, 90, and 181;
- Necropsy & Histology Examinations - Day 190 (♂)/191 (♀).

Results:

- Mortality and Clinical Signs - No deaths occurred. Clinical findings were not remarkable. Isolated occasions of ocular discharge were seen in the treated eyes (2♂ @ vehicle control, 1♀ each @ 0.1%, 0.3%, and 1.0% Emedastine). Malocclusion of upper and lower teeth was seen in one nontreated ♂ and 1♂ at 1.0% Emedastine. One ♀ in vehicle control group had swollen urogenital area on days 55 & 58.
- Weight Gains - Normal.
- Slit-lamp Biomicroscopic Examination - Minimal conjunctival congestion (hyperemia) was noted in all treatment and control groups. One incidence of severe conjunctival congestion was noted in the left eye of 1♂ @ 0.3% Emedastine on day 14. A single occasion of minimal conjunctival swelling was seen 1♀ @ 0.3% Emedastine on day 49. One ♀ @ 0.3% Emedastine had minimal corneal cloudiness in the left eye from days 14 - 93 and right eye from days 28-93.
- Indirect Ophthalmoscopic Examinations - Within normal limit.
- Pachymetry Measurements - Normal.
- Clinical Laboratory Analysis - No significant treatment-related changes were noted.
- Necropsy & Histology Examinations - The gross observations at necropsy were generally unremarkable. Microscopic changes were not observed in the eyes and adnexa or in any organ which were attributable to topical ocular application of

Emedastine for three months.

- Plasma Emedastine Levels - The peak plasma levels for topical 1.0% Emedastine on days 90 and 181 were higher than those on day 1. This suggested that accumulation of Emedastine occurred at the high dose (1.0%), following repeated ocular instillation.

Dose Level (%)	Sampling Day	C_{max} (ng/ml, N=8)
0.1	Day 1	2.10 ± 0.76
	Day 90	1.51 ± 0.46
	Day 181	1.69 ± 0.88
0.3	Day 1	2.33 ± 0.70
	Day 90	2.32 ± 0.32
	Day 181	2.75 ± 0.63
1.0	Day 1	8.58 ± 2.47
	Day 90	14.1 ± 3.00
	Day 181	12.0 ± 2.70

Based on the data obtained from the current study, ALØ3432A in concentrations up to 1.0% did not produce any significant ocular irritation and systemic toxicity in New Zealand white rabbits following a daily QID topical ocular administration for 190 days.

II. Systemic Toxicity

ACUTE TOXICITY STUDIES

i. Acute Toxicity Study of Emedastine Difumarate (KG-2413). Pharmacometrics, 39(3):209-214, Pharmaceutical Research Center, Kanebo, Ltd

The acute (oral intravenous, and subcutaneous) toxicity of Emedastine difumarate (KG-2413, ALØ3432A) was analyzed in mice, rats and dogs. The LD_{50} values for each species with various administration routes are summarized in the following table. Acute toxic signs in mice and rats were decreased spontaneous movements, increased startle-reaction, ataxic gait and convulsions. In dogs, the signs of toxicity were tachypnea, increased startle-reaction, ataxic gait and convulsions. The cause of death for the all animals that died during the study was severe respiratory failure. All mortalities occurred on the day of dosing for all species. In addition, discharge, inflammatory edema and hair loss were noted at the sites of subcutaneous injection.

Species (Strain)	Route	Dose Range (mg/kg)	Approximate LD_{50}(mg/kg) [95% CI]	
			♂	♀
Mouse (Jcl:SD)	Oral	625 - 5000	2,547 [1689-3840]	2,206 [1675-2906]
	SQ	420 - 1000	712 [615-824]	609 [551-673]
	IV	71 - 142	101[95-108]	93 [87-98]
Rat (Jcl:ICR)	Oral	840 - 2378	2,151[1745-2651]	1,854 [1478-2326]
	SQ	420 - 1189	666 [585-758]	643 [560-739]
	IV	42 - 119	72 [62-83]	77 [67-89]
Dog (Beagle)	Oral	100 - 1000	193 mg/kg	

ii. Acute Intravenous Toxicity of 5-Hydroxy KG-2413 (a Metabolite of KG-2413) in Rats. Study Nº SBL 24-14, Pharmaceutical Research Center, Kanebo, Ltd.

iii. Acute Intravenous Toxicity of 6-Hydroxy KG-2413 (a Metabolite of KG-2413) in Rats. Study Nº SBL 24-13, Pharmaceutical Research Center, Kanebo, Ltd

Acute intravenous toxicity of 5-hydroxy KG-2413 & 6-hydroxy KG-2413, metabolites of KG-2413, were evaluated in Jcl:SD rats. Rats received a single iv dose of 5-hydroxy KG-2413 (80, 100, 125, 156, 174, 195 or 218 mg/kg) or 6-hydroxy KG-2413 (30, 51, 87, 147 or 251 mg/kg). Dose-related death occurred immediately after administration in males and females at ≥174 mg/kg 5-hydroxy KG-2413, the ♂ at ≥87 mg/kg 6-hydroxy KG-2413 and in the ♀ at ≥51 mg/kg 6-hydroxy KG-2413. The survival animals were in a prone position and had clinical signs of altered respiration, tremors, piloerection, convulsions, salivation or staggering. No abnormal changes in body weights were noted and no abnormal changes were present at necropsy. The LD_{50} values for 5-hydroxy KG-2413 & 6-hydroxy KG-2413, with 95% confidence limits, for both males and females are presented in the following table.

Compound	Route	Dose Range (mg/kg)	Approximate LD_{50} (mg/kg) [95% CI]	
			♂	♀
5-Hydroxy KG-2413	IV	80 - 218	184 [169-210]	119 [109-130]
6-Hydroxy KG-2413	IV	30 - 251	91[60-137]	90 [59-137]

REPEATED-DOSE (SUBACUTE & CHRONIC) STUDIES

iv. Subacute Toxicity Study of Emedastine Difumarate (KG-2413) in Rats. Pharmacometrics, 39(3):215-230 (1990), Pharmaceutical Research Centre, Kanebo, Ltd.

The purpose of this study was to determine the subacute oral toxicity potential of KG-2413 (ALØ3432A) in rats. Groups of Jcl:SD rats (14/sex/group), ≈5 weeks old, were orally dosed (by gavage) either 0, 10, 50, 250, or 1250 mg ALØ3432A/kg/day once daily for three months.

All animals were observed daily for pharmacotoxic signs. Body weights, food consumption and water intake were determined weekly. Urinalysis was examined at 1.5 and three months of study and clinical chemistries and hematology examined at the three-month period. Ophthalmological examinations, including appearance, mydriatic reflex, examination of the fundus oculi and ERG examination, and an aural reflex examination were performed at the three-month period. Necropsy was done on all animals at the end of the treatment period and histopathologic evaluations was performed. Electron microscopy was performed on the liver and kidney of 3 male and 3 female rats in the 1250 mg/kg group that were sacrificed at 1.5 months.

Results:

- Clinical Signs - No remarkable signs were seen in animals at ≤50 mg/kg. Salivation and behavior of creeping into bedding were noted occasionally in animals at 250 mg/kg. The major clinical symptoms observed in the 1250 mg/kg group included mydriasis, reduced locomotor movements, exaggerated reaction and clonic or tonic

convulsions that occurred sporadically and resulted in the death of 7 ♂ and 11 ♀ by 6 weeks of treatment. The remaining animals in the 1250 mg/kg group were sacrificed at 1.5 months of treatment. One ♀ @ 0 and one ♂ @ 250 mg/kg died during the study due to urinary calculus and dosing error, respectively.

- Body Weight, Water Intake & Food Consumption - Increased water intake was noted for ♂ and ♀ @ 250 mg/kg and for ♀ @ 50 mg/kg. Body weight gain and food consumption were normal for animals at ≤250 mg/kg/day. Marked ↓ in the body weight gain and food consumption were observed in both ♂ & ♀ @ 1250 mg/kg/day.
- Ophthalmological and Aural Examinations - Unremarkable.
- Clinical Chemistry & Hematology - Some slight changes were noted in the hematological parameters (↓RBC in ♀ @ 50 & 250 mg/kg; ↓PCV in ♀ @ 250 mg/kg; ↑WBC in ♂ @ 50 & 250 mg/kg and ♀ @ 250 mg/kg; ↓RBC & ↑WBC in ♂ & ♀ @ 1250 mg/kg), however, none of these changes suggested specific blood toxicity. No abnormality was observed in the PT & APTT tests. Statistically significant ↑ in ALP, Albumin, A/G ratio, BUN, Ca, and P levels were noted in the ♂ @ 250 mg/kg group, however, most changes were within the physiological values. Significant ↑ in BUN, Ca, P, and ALP were seen in ♀ @ 250 mg/kg. Increases in ALP, BUN, Ca, P and K were also observed in the 1250 mg/kg group, and their values were slightly higher than those obtained in the 250 mg/kg group. Serum cholinesterase activities were statistically significantly ↓ for the ♀ @ 50 and 250 mg/kg/day. A slight ↓ in urine pH was noted for the ♀ @ ≥250 mg/kg.
- Necropsy & Histopathology - Significant decreases in absolute spleen and thymus weights and an increase in absolute liver weight were noted in the 250 mg/kg group. Although a few animals in the low and intermediate dose groups showed changes in both actual and relative organ weights, no dose-effect relationship was observed. In the 1250 mg/kg group necropsied at the 1.5 month period, a trend in increased weights of the liver, kidneys and adrenal glands and a trend in decreased weights of the thymus, spleen, ovary and uterus were observed. The main histopathologic findings included swelling of the hepatocytes in the 1250 mg/kg dose groups, and centrilobular hepatocyte swelling, hyalin degeneration, and eosinophilic degeneration in the 250 mg/kg group. The electron microscopic (EM) examination on the liver of animals @ 1250 mg/kg revealed dilation of biliary canaliculus, lingual shape welling, abnormal and reduced numbers of microvilli, a ↑ in lysosome and appearance of myelin body. Eosinophilic degeneration and/or vacuolar degeneration in tubular epithelium, and tubular calcification were observed in the kidneys of animals @ ≥250 mg/kg. The EM examination of kidneys of animals @ 1250 mg/kg showed an increase in glomerular epithelium. Other microscopic changes were:
  - atrophy of thymus (medulla) in some animals at 1250 mg/kg;
  - atrophy of lymph node (follicle) in animals @ ≥250 mg/kg;
  - focal necrosis of pancreatic parachyma and pancreatic ductitis in 1♂ @ 250 mg/kg;
  - focal tubular atrophy in the testis and prostate epithelial atrophy in ♂ @ 1250 mg/kg;
  - swelling of zona fasciculate in the adrenal glands of all rats @ 1250 mg/kg and

some rats at other dosages.

These pathological changes suggested that the main effects of ALØ3432A were on the liver and kidney. Based upon the study results, the maximum no-effect dose of ALØ3432A in rats following three months of oral dosing was 10 mg/kg.

v. Subacute Toxicity Study of Emedastine Difumarate (KG-2413) in Dogs. Pharmacometrics, 39(3):231-268 (1990), Pharmaceutical Research Centre, Kanebo, Ltd.

Dogs (6/sex/group), ≈8- month old, were orally administered either 0, 3, 15, or 75 mg ALØ3432A/kg/day in a gelatin capsule once daily for three months. The study also incorporated a one-month recovery period for 2/group. All animals were observed daily for pharmacotoxic signs. In addition, body weights, food consumption and water intake were determined once per week. Urinalysis, hematology and clinical chemistry evaluations were performed monthly throughout the treatment period and again at the end of the recovery period. Ophthalmological and electrocardiographic examinations were also performed. At the end of the treatment or recovery period, animals were subjected to a necropsy. Organ weights were determined and histopathologic evaluations performed. Electron microscopy was performed on the liver and kidney of selected animals.

Results:

- Clinical Observation and Mortality - Acute symptoms such as gasping, failure of muscular coordination, decreased spontaneous movements and convulsions were observed 0.5 to 6 hours after the start of administration in the 75 mg/kg group. Three ♂ and 4 ♀ of this group died of convulsions and dyspnea.
- Body Weight H_2O & Food Consumption - Decreased body weight gain and ↓ in food and water intake were observed in dogs @ 75 mg/kg.
- Ophthalmologic and Electrocardiographic Examinations - Normal.
- Hematology & Clinical Chemistry - Dogs @ 75 mg/kg had a slight ↑ in ALP and a slight ↓ in triglyceride levels. No treatment-related changes in the urinalysis were noted.
- Necropsy & Histopathology - In the 15 mg/kg and 75 mg/kg groups, ↑ liver weights and proliferation of the smooth endoplasmic reticulum were observed. In addition, the high dose group demonstrated a slight ↓ of prostatic epithelial granules. None of the above changes were noted in the animals after the one-month recovery period and no additional observations were noted.

Based on the results of this study, the maximum no adverse-effect dose of ALØ3432A in dogs following three months of oral dosing was 15 mg/kg day.

vi. Chronic Toxicity Study of Emedastine Difumarate (KG-2413) in Rats. Pharmacometrics, 39(3):269-284 (1990), Pharmaceutical Research Centre, Kanebo, Ltd.

Five groups of Jcl:SD rats (24/sex/group), ≈5 weeks old were orally administered (by gavage) either vehicle, 1, 3, 10, or 50 mg ALØ3432A/kg/day once daily for one year. All animals were observed daily for pharmacotoxic signs. In addition, body weights, food

consumption and water intake were determined. Urinalysis, hematology and clinical chemistry evaluations were performed and an ophthalmological examination and an auditory examination were conducted. At the end of the treatment period, all animals were subjected to a necropsy. Organ weights were determined and histopathologic evaluations performed. Electron microscopy was performed on the liver and kidney of 3 male and 3 female rats in the control and 50 mg/kg groups.

Results:

- Clinical Observations and Mortality - Individual data for this study were not submitted. Total of 17 rats died or were sacrificed. The sponsor stated that dosing error (3♂ & 6♀) and spontaneous pituitary tumor (2♂ & 6♀) were the major causes of death.
- Body Weight, H_2O Intake & Food Consumption - A significant ↓ in weight gain was observed in the ♂ receiving 50 mg/kg/day. Food consumption and H_2O intake were not affected.
- Ophthalmologic and Auditory Examinations - Unremarkable.
- Clinical Pathologic Examinations - Significant ↓ in RBC, Hb, and PCV were found in ♀ @ 50 mg/kg. A significant higher in WBC with an exceptional high standard deviation value was seen in ♀ @ 3 mg/kg. Clinical chemistry analysis revealed that cholinesterase (ChE) activity decreased approximately 25% in females @ 3 or 50 mg/kg following one year of treatment. At the 6-month urinalysis, results revealed that bilirubin was detected more often in ♂ @ ≥10 mg/kg. Ketone body and bilirubin were detected more frequently in ♀ at 50 mg/kg/day. No significant differences existed between the ALØ3432A treated groups and the control group at the 12-month urinalysis.
- Necropsy & Histopathology - The relative liver weights increased in ♀ @ 50 mg/kg/day. Histopathologic examination on these organs revealed only centrilobular hepatocyte swelling. The centrilobular hepatocyte swellings, increased relative liver weights and decreased ChE activity in the 50 mg/kg females were thought to be the effect of ALØ3432A on the liver.

Based on the results of this study, the no-effect dose of orally administered ALØ3432A for one year in rats is 10 mg/kg/day.

vii. Chronic Toxicity Study of Emedastine Difumarate (KG-2413) By One-Year Oral Administration in Beagle Dogs. Pharmacometrics, 39(3):285-318 (1990).

Groups of beagle dogs (4/sex/group), ≈8 months of age, were orally dosed with either 0, 1, 3, 15, or 45 mg ALØ3432A/kg/day in a gelatin capsule once daily for one year. All animals were observed daily for pharmacotoxic signs. In addition, body weights and food consumption were determined throughout the study. Urinalysis, hematology and clinical chemistry evaluations were performed at 1, 3, 6, 9 and 12 months of study. Ophthalmological and electrocardiographic examinations were also performed. At the end of the treatment period, animals were subjected to a necropsy. Organ weights were determined and histopathologic evaluations performed. Electron microscopy was

performed on the liver and kidneys of the control and high dose animals.

Results:

- Clinical Signs and Mortality - No death occurred. Clinical observations revealed acute symptoms such as tachypnea, hyperactivity, muscle spasms and involuntary movement of the tongue lasting for several hr from the first hr after dosing throughout the treatment.
- Body Weight & Food Consumption - An inhibition of weight gain was seen in the ♂ & ♀ @ 45 mg/kg/day. There was no treatment-related effect on food consumption.
- Ophthalmologic and Electrocardiographic Examinations - Normal.
- Hematology, Clinical Chemistry, & Urinalysis - The clinical pathology results displayed that animals in the 45 mg/kg group had an increase in ALP activity (♂ & ♀), elevated GPT (♂) at the 12-month analysis and decreased sperm in the urinary sediment. A positive occult blood test was seen in one ♂ each @ 15 & 45 mg/kg/day. Both dogs were shown to have vesicle calculus at necropsy.
- Gross & Microscopic Pathology - In the 45 mg/kg group, the ♂ had an increased liver weight. This increase correlated with the proliferation of the smooth endoplasmic reticulum. In addition, the high dose group showed a decreased testicular weight and atrophy of the prostatic epithelium. Local deciduation of the epithelium of the seminiferous tubules was noted in 2 or 3 animals from all ALØ3432A treated groups but was not seen in the controls. This change in the high dose group was local and sometimes unilateral.

viii. One Year Chronic Toxicity Study of KG-2413 by the Oral Administration in Beagle Dogs Followed by Recovery Period. Study Nº GTX 305, Pharmaceutical Research Centre, Kanebo, Ltd.

An additional one-year study in dogs similar to the above was conducted with a three-month recovery period for the 45 mg/kg group to evaluate the effects on the testes. Results from this study revealed that the focal loss of seminiferous tubule epithelia, prostatic atrophy and decrease in prostatic weight that were observed in the first study were not reproducible. In addition, the change in the area of the testicular horizontal section was a reversible change. Based on the results of these two chronic dog studies, the maximum no adverse-effect dose of ALØ3432A in dogs following one year of oral dosing was 15 mg/kg/day.

III. Reproductive Toxicity

i. A Ten-Week Basic Fertility Study of Administration in the Diet to Fischer 344 Rats. Study Nº RO2285,

The study was performed to assess the effects of (KG-2413; ALØ3432A) on the reproductive performance of Fischer 344 rats. The test article, ALØ3432A, was administered to female rats in the diet at concentrations of 0, 0.01, 0.05 or 0.25% that were

in equivalent of average daily doses of approximately 0, 6.2, 30.4, or 135.8 mg/kg, respectively, during the premating and gestation periods. Male rats were exposed to the same dietary levels as the ♀ for 10 weeks prior to mating. Then, the male rats (10/group) were cohabited with treated females of the same treatment groups for two weeks. At the time of cohabitation, the females (10/group) had received treatment for two weeks. Females were allowed to deliver naturally and rear their offsprings through postpartum day 21. After the two-week mating period, males (10/group) were placed on a control diet and immediately cohabited, for 12 days with adult untreated female Fischer 344 rats. The remaining males (10/group) were continued on the test diets and cohabited overnight with untreated sexually-receptive Fischer 344 females. Untreated females were sacrificed in midgestation and examined for evidence of pregnancy.

Results:

No maternal deaths or treatment-related clinical signs of toxicity were noted. Body weight and weight gain were reduced, and ↓ food consumption during gestation and lactation periods in the ♀ at the 0.25%. Estrous cycles were not affected. Mating performance was significantly ↓ for both ♂ & ♀ in the 0.25% group. However, mating performance of the treated ♂ was not altered once the treatment ended. Fertility indices were not affected. In untreated females examined in mid-gestation, no effects on the number of implantations, resorptions, preimplantation loss, or postimplantation loss were noted. In treated females allowed to deliver, no effects were observed on gestation length, gestation survival, or the numbers of live birth. Offspring body weight was ↓ in the 0.25% group, but survival, sex distributions and physical condition were not influenced by the parental treatment. In conclusion, maternal toxicity was shown by the evidence of reduced body weight gain and food consumption in ♀ @ 0.25%. Treatment-related developmental toxicity was exhibited by body weight depression in offspring of animals treated with diets containing 0.25% ALØ3432A.The no-effect level for reproductive performance in this study was 0.05% (equivalent to a dose of approximately 30 mg/kg/day) for males and nonlactating females.

ii. Reproduction Study of Emedastine Difumarate (KG-2413) Fertility Study in Rats. Pharmacometrics, 39(3):319-328 (1990),

This study was performed to assess the effects of KG-2413 (ALØ3432A) on the reproductive performance of Jcl: SD (24 rats/sex/group) rats. The test article, at doses of 0, 10, 40, or 140 mg/kg/day, was orally dosed to ♂ rats from 9 weeks before mating, throughout cohabitation for 99/100 days, and to ♀ rats from 14 days before mating until day 7 of gestation. Female rats with unsuccessful copulation during this mating period were remated with male rats which had a confirmed copulation within the same group or with untreated ♂ rats. Male rats were sacrificed on Day 99/100 of treatment. The pregnant female rats were sacrificed on gestation day 21. The numbers of corpora lutea, implantations, dead fetuses at an early stage, dead fetuses at late stage and live fetuses were enumerated. For both ♂ & ♀, at necropsy, the thoracicoabdominal viscera were macroscopically examined and the heart, liver, spleen, kidneys, adrenals, and gonads were weighed. Live fetuses were weighed and examined for sex and external anomalies. About

two thirds of the live fetuses in all litters were examined for skeletal anomalies, variations and degree of ossification. The remaining third of the fetuses were fixed and examined for cephalic and internal anomalies.

Results:
Salivation was noted in the high dose group. Body weights and food consumption in ♂ and ♀ rats were unaffected. No abnormalities were found in the reproductive parameters. Necropsy of the male and female rats showed no abnormalities and the organ weights remained normal. Treatment of ALØ3432A had no effects on preimplantation loss, fetal growth, or fetal mortality. No treatment-related abnormalities in the external appearance, internal organs and skeletons were noted during the examination of fetuses. Based upon the reported data, the no-effect dose for reproductive function and for fetal development was 140 mg/kg.

iii. Reproduction Study of Emedastine Difumarate (KG-2413) - Teratological Study in Rats. Pharmacometrics, 39(3):329-342 (1990), Pharmaceutical Research Centre, Kanebo, Ltd.

KG-2413 (ALØ3432A) at doses of 0, 10, 40 or 140 mg /kg/day was administered orally to Jcl:SD rats (34 per group) on Gestation Days 7 -17 to determine its effects on dams, fetuses and offsprings. A cesarean section was performed on Day 21 (22 rats/group). The remaining 12 rats/group were permitted to deliver. The dams were observed daily for pharmacotoxic signs. Body weights and food consumption were monitored during gestation. For animals allowed to deliver, these conditions were monitored up to Postpartum Day 21 . The dams of the cesarean section group were sacrificed on Gestation Day 21 and the fetuses were examined. For the groups allowed to deliver, the parturition conditions (duration of gestation, incidence of total resorptions) and nursing conditions (pup survival through the lactation period) were observed and the length of gestation was recorded. At necropsy, the major organs in the thoracicoabdominal region were macroscopically inspected and selected organ weights obtained. Live fetuses from the cesarean section dams were weighed and examined for sex and external abnormalities. Two thirds of the live fetuses in each litter were examined for skeletal anomalies, variations and the degree of ossification. The remaining fetuses were examined for cephalic and internal anomalies. The litters of the dams allowed to spontaneously deliver were examined for the number of pups delivered live or stillbirths and for external abnormalities. On Postpartum Day 4, the litters were culled to eight pups. The culled pups were examined for external, visceral, or skeletal anomalies. During the nursing period, the pups were observed for incisor eruption, pinna detachment, and eye opening. The F_1 were also subjected to the neurological examined (righting reflex, Preyer's reflex, corneal reflex, pain response, pinna reflex and grip strength) and behavioral and learning ability tests. Reproductive function tests were conducted on selected animals. F_1 males and females from litters were allowed to mate (avoiding brother/sister matings). Males used for mating were necropsied after mating. The copulated F_1 females' body weights were monitored during gestation. On Gestation Day 21, the F_1 females were sacrificed and laparotomized for the observations of the fetuses in the uterus in the same manner as that for the F_0 dams.

Results:

F_0 Observation: Mild mydriasis and salivation were observed in a few of the high dose F_0 dams. A slight ↓ in body weight gain was noted in the 40 and 140 mg/kg groups. Food consumption was not affected. The relative weights of the liver, kidneys, adrenals and ovaries of the 140 mg/kg F_0 females in the cesarean section group were significantly increased, but this was not observed in the dams allowed to deliver. The parturition condition, the nursing condition and necropsy findings did not show any treatment- related effects.

F_1 Fetal & Offspring Observations: A significant ↑ in fetal mortality and a significant ↓ in the number and body weight of live fetuses were seen in the 140 mg/kg group. In addition, a significant ↓ in the numbers of phalanges and the caudal vertebrae were noted in this group at the skeletal examination, an indicative of delayed ossification. These findings suggested that at the dose of 140 mg/kg, ALØ3432A exhibited lethal and suppressive effects on fetal development. However, the increasing incidence of external, visceral and skeletal anomalies was dose-dependently. Observations of the F_1 pups during the nursing period indicated a significant decrease in the number of pups born and in the birth index in the 140 mg/kg group. Skeletal examination of the culled pups revealed a significant ↑ in the incidence of dysplasia of the caudal vertebrae in this same group. No effects were noted in the necropsy findings of the culled pups, in the viability, body weights, postnatal differentiation or general function during the nursing period. After weaning, the body weight gain was slightly ↓ in both ♂ and ♀ of the 140 mg/kg group. No effects were noted in pup viability, general conditions and sex maturation after weaning. Necropsy at three weeks of age revealed dysplasia of the caudal vertebrae in one animal in the 140 mg/kg group, however the necropsy at six weeks of age revealed no ALØ3432A treatment effects. Behavioral and learning ability tests did not demonstrate any treatment-related effects. In the reproductive function tests, no abnormalities were seen in the copulation and pregnancy parameters in all groups.

F_2 Fetal Observations: No remarkable findings were noted in skeletal and visceral examinations of the F_2 fetuses. No evidence of a drug-related teratogenic effect was observed during this study.

Based on the presented results, the no-effect dose of ALØ3432A was 10 mg/kg for the maternal toxicity and 40 mg/kg for the fetal and developmental toxicities.

iv. A Teratology Study of [redacted] Administered Orally to Dutch Belted Rabbits. Study Nº BO3785.

ALØ3432A (0, 3, 15 or 75 mg/kg/day po) was administered orally to Dutch Belted rabbits (25/group) on Gestation Days 7 - 17 to determine its effects on dams and fetuses. The dams were observed daily for pharmacotoxic signs. Body weights and food consumption was monitored during gestation. On Gestation Day 28, a cesarean section was performed and a gross examination was performed. In addition, the number and distribution of implantations, live and dead fetuses and resorptions were recorded. Live fetuses were individually weighed and examined for sex and external, visceral and skeletal anomalies.

Results:
Maternal toxicity was exhibited in the 75 mg/kg group by the evidence of one death and one abortion on Gestation Days 18 and 22, respectively. Weight loss and ↓ food consumption were noted in these animals. Decreased food consumption with normal weight gain, but the two previously mentioned, was observed in the high dose group. Examination of the fetuses revealed no treatment-related findings. The no-effect level in this study for maternal toxicity, embryo/fetal toxicity, and teratogenicity in the rabbit was 15, 75 and 75 mg/kg/day, respectively. Based on these data, the fetal no-effect level exceeds the maternal no-effect level in the rabbit.

v. Reproduction Study of Emedastine Difumarate (KG-2413) - Perinatal and Postnatal Study in Rats. Pharmacometrics, 39(3):343-354 (1990), Pharmaceutical Research Centre, Kanebo, Ltd.

KG-2413 (ALØ3432A) at doses of 0 (control), 10, 40, or 140 mg/kg/day was administered orally to female Jcl:SD rats (23/group) during the perinatal and postnatal periods (Gestation Day 17 to Postpartum Day 20) to investigate its effects on the dams and the postnatal growth of the offspring. The dams were observed daily for signs of pharmacotoxicity. Body weights and food consumption was also monitored. All dams in each group were allowed to deliver naturally and their parturition and nursing conditions were observed. On Postpartum Day 21, all dams were necropsied and the number of implantation sites were determined. The major thoracicoabdominal organs were examined and weighed. The pups were observed throughout the 21-day nursing period. The number of live and stillbirths were recorded. The litters were culled on Postpartum Day 4 to 8 pups. Body weights and postnatal development and differentiation were monitored. On Postpartum Day 19 , all pups were subjected to the general function tests (righting reflex, Preyer's reflex, corneal reflex, pain response, pinna reflex and grip strength). On Postpartum Day 20, one ♂ & one ♀ per litter were randomly selected for each course of tests: skeletal examination, the measurement of organ weights, behavioral and learning ability tests and reproductive function.

Results:
Transient salivation was observed occasionally in the dams in the 140 mg/kg dose group. Body weight gain was ↓ in the first half of lactation in ♀ @ ≥ 40 or 140 mg/kg, however, it steadily returned to normal. Food consumption was not affected. No treatment-related adverse effects were noted on parturition or nursing. Necropsy and organ weights displayed no pathologic findings. Observations at birth revealed no treatment-related findings in any parameter examined. During the nursing period, a significant ↓ in body weight gain was noted in the 140 mg/kg group. However, no effects were noted in the viability of the pups, their postnatal differentiation and general functions. In the body weight evaluation after weaning, slight, statistically significantly ↓ weights were seen at up to six weeks of life in ♂ and up to five weeks in ♀ of the 140 mg/kg group, as compared with controls. The change in body weight gain inclined to return to normal after this period. Sex maturation and motor coordination were not affected. Necropsy and organ weights of the F_1 sacrificed

at 3 and 6 weeks of age were not remarkable. No effects were noted in the behavioral or learning ability tests that began at six weeks of age. For reproductive testing, selected F_1 ♂ and ♀ from the same dose group were mated avoiding brother/sister matings. No effects of ALØ3432A were seen on the copulation and the pregnancy indices. Body weight was unaffected in the F_1 pregnant dams. Observations of the F_2 fetuses revealed no treatment-related effects. This study explicated that the no-effect dose of ALØ3432A was 10 mg/kg for dams and 40 mg/kg for offsprings.

IV. Mutagenicity

i. The Effects of _ on the Induction of Reverse Mutations in *Salmonella Typhimurium* Using the Ames Test. Study Nº 840813AMS2327,

Study Nº: 840813AMS2327
Study Aim: To determine whether induced point mutation in *Salmonella.*
Bacteria: *Salmonella* typhimurium: TA1535, TA1537, TA1538, TA98 and TA100
Compound: 1H-Benzimidazole,1-(2-ethoxyethyl)-2-(hexahydro-4methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2) (Lot Nº A55-8Y5-125) in DMSO: 250, 500, 1000, 2500, and 5000μg/plate
Positive Control:
+ S-9: 2-Aminoanthracene (2AA), 2.5 and 1.25 μg/plate
-S-9: 2-Nitrofluorene (2NF), 5, 0.5 μg/plate; 9 amino acridine (9 AmAc), 100 and 50 μg/plate; M-methyl-N'-nitrosoguanidine (MNNG), 5 and 2.5 μg/plate
Solvent Control: DMSO
Duration of Exposure: 48 hrs
Study Location:

Compliance with GLP/QAU: Yes
Study Date: 8/13/84 to 8/17/84

Results: The results suggested that at the dose levels up to 5000 μg/plate with or without S-9 metabolic activation was not mutagenic under the present testing condition.

ii. The Effect on the Induction of Bacterial Mutation Using a Modification of the Ames Test. Study Nº 8405329GPA2327,

Study Nº: 8405329GPA2327
Study Aim: To determine whether induced point mutation in *Salmonella* and *E. Coli.*
Bacteria: *Salmonella typhimurium* (histidine auxotrophs) LT-2: G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, and TA98
E. Coli (tryptophan auxotrophs): WP2 and WP2uvrA⁻

Compound: 1H-Benzimidazole,1-(2-ethoxyethyl)-2-(hexahydro-4methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2) (Lot Nº A55-8Y5-125) in DMSO: gradients 0.1-1, 1-10, 10-100, and 100-1000 μg/plate.
Positive Control:
+ S-9: 2-Aminoanthracene (2AA), 0.01-10 μg/plate.
- S-9: M-methyl-N'-nitrosoguanidine (MNNG), 0.01-100 μg/plate.
Solvent Control: DMSO
Duration of Exposure: 48 hrs
Study Location:

Compliance with GLP/QAU: Yes
Study Date: 5/29/84 to 6/1/84

Results: The results suggested that at the dose levels up to 1000 μg/plate with or without S-9 metabolic activation was not mutagenic under the present testing condition.

iii. Analysis of Metaphase Chromosomes Obtained from CHL Cells cultured *In Vitro* and Treated With KG-2413. Study Nº KNE 26/891489, Pharmaceutical Research Centre, Kanebo, Ltd.

Study Nº: KNE 26/891489
Study Aim: To investigate the effects of KG-2413 on the chromosomes of a mammalian cell line cultured *in vitro*.
Cell Line: Chinese hamster lung (CHL), strain JCRB 0030
Compound: KG-2413, 1H-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4 methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2) (Lot Nº 702005) in ddH_2O: 7.6, 15.2, 30.4, 60.9, 122, 243, 487, 974, 1950, and 3900 μg/ml
Positive Control:
+ S-9: Cyclophosphamide, 10 μg/ml
- S-9: Mitomycin C, 0.1 & 0.2 μg/ml
Solvent Control: ddH_2O
Duration of Exposure: 6, 24, and 48 hrs
Study Location:

Compliance with GLP/QAU: Yes
Study Date: 3/28/89 - 9/21/89

Results: The effects of KG-2413 on the mitotic index (data expressed as % of control culture) of CHL cells are shown in the following table.

Concentration (μg/ml)	Treatment Time (hr)	% Mitotic Index + S-9	% Mitotic Index - S-9	Treatment Time (hr)	% Mitotic Index - S-9	Concentration (μg/ml)	Treatment Time (hr)	% Mitotic Index - S-9
974		19.3	158.9		102.3	243		65.6
1950	6	28.1	135.1	24	29.1	487	48	45.8
3900		29.8	17.5		0	974		12.5

iv. The Effect of on the Induction of Forward Mutation at the Thymidine Kinase Locus of Mouse Lymphoma Cells. Study Nº 840522MLA2327 and 841030MLA2327,

Study Nº: 840522MLA2327 & 841030MLA2327
Study Aim: To investigate the potential of to induce mammalian cell point-mutation in the $TK^{+/-}$ mouse lymphoma cell assay with or without metabolic activation.
Cell Line: $TK^{+/-}$ cells (TK3.7.2C)
Compound: 1H-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4 methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2) (Lot Nº A55-8Y5-125) in DMSO: 0.1-1000μg/ml
Positive Control:
+ S-9: EMS (Ethylmethanesulfonate), 620 μg/ml
- S-9: 3MC (3-Methylccholanthrene), 2 μg/ml
Solvent Control: DMSO (1%)
Duration of Exposure: 4 hrs
Study Location:

Compliance with GLP/QAU: Yes
Study Date: 5/22/84 - 5/24/84 & 10/30/84 - 11/12/84

Results: Results showed that was mutagenic to $TK^{+/-}$ mouse lymphoma cells in the presence or absence of S9 metabolic activation mixture.

v. The Effect of on the Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes. Study Nº 840705UDS2327 and 840710UDS2327,

Study Nº: 840705UDS2327 & 840710UDS2327
Study Aim: To investigate the potential of to induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes.
Cell Line: Primary cultures of adult ♂ Fischer 344 rat hepatocytes
Compound: 1H-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4 methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2) (Lot Nº A55-8Y5-125) in DMSO: 0.5-1000μg/ml
Positive Control:
MNNG (N-Methyl-N'-nitro-N-nitrosoguanidine), 1, 5, 10, and 20 μg/ml

2AAF (2-Acetylaminofluorene), 0.05, 0.1, 0.5, and 1 μg/ml
Solvent Control: DMSO (1%)
Duration of Exposure: 20 hrs
Study Location:

Compliance with GLP/QAU: Yes
Study Date: 7/5/84 - 7/6/84 & 7/10/84 - 7/11/84

Results: Results showed that was not active to induce UDS in adult rat primary hepatocyte cultures.

vi. The Effect of on the *In Vivo* Induction of Sister Chromatid Exchange (SCE) in Bone Marrow of Chinese Hamsters. Study Nº 850429SCE2327,

Study Nº: 850429SCE2327
Study Aim: To evaluate the potential of to induce sister chromatid exchange (SCE) in bone marrow of Chinese hamster.
Compound: 1H-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4 methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2) (Lot Nº A55-8Y5-125), 500, 250, 125, and 62.5 mg/kg, in H_2O, 10 ml/kg po
Positive Control:
Cyclophosphamide: 50 mg/kg in H_2O, 10 ml/kg po
Induction of SCE: Skin implantation of BrdUrd (Bromodeoxyuridine), 20-30 mg agar coated tablets, for 5 hrs
Vehicle Control: Sterile H_2O
Animals: ♀ Chinese hamster (*Cricetulus griseus*), 3/ each dose of LY188695, 2/vehicle control and 1/Cyclophosphamide.
Duration of Exposure: 19 hrs
Study Location:

Compliance with GLP/QAU: Yes
Study Date: 4/29/85 - 4/30/85

Results: Results revealed that did not induce SCE in vivo in bone marrow of Chinese hamster.

vii. Mouse Micronucleus Test on KG-2413. Study Nº KNE 27/891016, Pharmaceutical Research Centre, Kanebo, Ltd.

Study Nº: KNE 27/891016
Study Aim: To investigate the potential mutagenic effect of KG-2413 following a single oral administration to mice.
Compound: KG-2413, 1H-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4 methyl-1H-

1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2) (Lot Nº 702005) in ddH_2O: 36, 108, and 360 mg/kg po
Positive Control: Mitomycin C, 12 mg/kg po
Vehicle Control: ddH_2O
Animals: CD-1 mice, ≈35 days old, weighing 22-24 g, 15/sex/group
Duration of Exposure: 24, 48 and 72 hrs
Study Location

Compliance with GLP/QAU: Yes
Study Date: 5/2/89 - 6/27/89

Results: Based on reported results, KG-2413, following a single oral dose of 36, 108, or 360 mg/kg to mice, did not show to be mutagenic.

V. Carcinogenicity

i. Oncogenicity Study in Mice with 1-(2-Ethoxyethyl)-2-(4-Methyl homopiperazinyl) Benzimidazole Difumarate (KG-2413). Study Nº 366-102, Pharmaceutical Research Centre, Kanebo, Ltd.

Study Nº: HLA Nº 366-102
Study Aim: To identify potential carcinogenic effects of Emedastine when administered orally via diet to mice for ≥104 weeks.
Compound: Emedastine (KG-2413, Lot Nº HN-6-3), 0.01, 0.03 and 0.1% in the diet
Animals: B6C3F1 mice, ≈7 weeks of age, weighting 18.5-25.4 g for the ♂ and 13.4-20.7 g for the ♀, 50/sex/group

Group	Nº of Animals	Dietary Levels (%)	Equivalent Oral Dose (mg/kg/day) ♂	♀
1	50/sex/group	0	0.00	0.00
2	50/sex/group	0.01	15.23	16.85
3	50/sex/group	0.03	44.42	56.91
4	50/sex/group	0.1	170.47	218.50

Study Location:

Study Date: 4/27/87-5/4/89
Compliance with GLP/QAU: Yes
Experimental Design:

- Mortality and Clinical Signs - 2x/day;
- Detailed Physical Examination - 1x/week;
- Body Weights & Food Consumption - 1x/week for wks 1-16 and 1x/4 weeks thereafter;
- Clinical Pathology - prior to scheduled and unscheduled sacrifice;
- Necropsy & Microscopic Examinations - died or killed in extremis and scheduled sacrifice (wk 104).

Results:

- Mortality and Clinical Signs - No treatment-related effects on survival or clinical signs were observed. Alopecia, sores and small moveable tissue mass were major clinical signs that occurred in all groups of animals. The mortality at wk 105 and causes for the unscheduled deaths are shown in the following two tables.

Dietary Levels (%)	Survivors (%)		Non-Survivors		Removed from Study	
	♂	♀	♂	♀	♂	♀
0	43 (86)	37 (74)	5	12	2	0
0.01	38 (76)	37 (74)	12	12	0	1
0.03	40 (80)	34 (68)	9	16	1	0
0.1	43 (86)	43 (86)	7	7	0	0

Unscheduled Deaths								
Death Comments	♂				♀			
	0	0.01%	0.03%	0.1%	0	0.01%	0.03%	0.1%
N° of Animals	5[a]	12[c]	9[a]	7	13[a]	12[a]	16	7
Remove from Study	2	0	1	0	0	1	0	0
Accidental	0	0	1	0	0	0	0	0
Undetermined	0	1	0	2	5	0	4	0
Hepatocellular Neoplasms	1	5	5	0	0	0	0	1
Hemangiosarcoma	1	2	2	1	1	2	0	0
Alveolar/Bronchiolar Adenoma/Carcinoma	2	1	0	0	0	0	0	0
Fibrosarcoma/Neurofibrosarcoma	0	0	0	0	1	2	1	0
Malignant Histiocytic/Lymphoblastic/Lymphocytic/Mixed Lymphoma	0	1	1	0	4	5	6	6
Granulocytic Leukemia	0	0	0	0	0	0	1	0
Carcinoma, Uterus/Hardarian Gland/Adrenal Cortex/Basal Cell	0	0	0	0	1	1	2	0
Pheochromocytoma/Thymoma/Osteosarcoma/Liposarcoma	0	1	0	1	0	1	1	0
Necrosis, Liver	0	0	0	0	1	0	0	0
Suppurative Pyelitis	2	0	0	0	0	0	0	0

[a] One animal had more than one death comment.
[b] Two animals had more than one death comment.
[c] Three animals had more than one death comment.

- Body Weights & Food Consumption - The high dose groups had significant lower mean body weight and weight gain values from week 14 throughout the treatment as compared with the control values. Body weights for the ♀ in low- and mid-dose groups were significantly increased as compared with vehicle controls. Body weights for the ♂ at high dose were not statistically different from controls. In contrast, body weights in the ♀ at high dose were lower than control groups with 7.23% less relative to the controls. The mean body weight gains for each group and the difference (%) from the controls are presented in the following table. Mean weekly food consumption was comparable in all groups.

Dietary Dose Level (%)	♂				♀			
	Mean B. Wt.		% Difference from Control	Gain relative to Control (%)	Mean B. Wt.		% Difference from Control	Gain relative to Control (%)
	wk104	wk 0	wk 0-104	wk 0-104	wk 104	wk 0	wk 0-104	wk 0-104
Control	37.0	21.7	-	-	31.8	18.0	-	-
0.01	36.5	21.8	-1.35	-3.92	33.1	18.1	4.09	8.69
0.03	37.1	22.1	0.27	-1.96	32.9	18.1	3.46	7.25
0.1	35.3	22.0	-4.59	-13.07	29.5	18.2	-7.23	-18.12

- Clinical Pathology - There were no significant findings in the hemogram or leukogram for treated or control animals. The following clinical chemistry parameters were evaluated: glucose, BUN, creatinine, ALT, ALP, and total bilirubin. Evaluation of the clinical chemistry data revealed significantly increased serum alkaline phosphatase in the Group 4 females, a possible indication of mild hepatic cholestasis. No other treatment-related changes in the clinical pathology findings were noted.
- Necropsy & Microscopic Examinations - No treatment-related effects with respect to gross pathology or organ weights were apparent. Histopathologic non-neoplastic and neoplastic alterations of all tissues examined were randomly distributed between control and treated animals. The observed type and frequency of tumors were normally seen for this strain of mouse. The neoplastic incidences of microscopic changes were summarized in the following table.

Neoplastic Incidence (All Animals)						
Tissue	Type of Tumor	Sex	Group			
			Control	0.01%	0.03%	0.1%
Pituitary	Adenoma/Carcinoma	♂	0	0	0	0
		♀	5	6	7	5
Lung	Alveolar/Bronchiolar Adenoma/Carcinoma	♂	11	13	17	9
		♀	2	2	5	2
Spleen	Hemangioma/Hemangiosarcoma	♂	4	2	6	2
		♀	3	4	0	0
Liver	Hepatocellular Adenoma/Carcinoma	♂	14	20	19	12
		♀	6	2	2	8
Adrenal Cortex	Adenoma/Carcinoma	♂	2	0	0	1
		♀	1	0	1	0
Hematopoietic System	Malignant Histiocytic/Mixed/Lymphocytic Lymphoma, Histiocytic Sarcoma, and Granulocytic Leukemia	♂	2	6	0	5
		♀	10	12	13	12
Harderian Gland	Adenoma/Carcinoma	♂	6	5	4	7
		♀	5	2	4	2
Tumor Bearing Mice		♂	32	34	34	29
		♀	32	33	29	31

In conclusion, KG-2413 appears to have no oncogenic potential in male or female B6C3F1 mice when administered in the diet at dose levels of 0.01, 0.03 and 0.1% for 104 weeks

ii. Oncogenicity Study in Rats with 1-(2-Ethoxyethyl)-2-(4-Methyl homopiperazinyl) Benzimidazole Difumarate (KG-2413). Study Nº 366-103, Pharmaceutical Research Centre, Kanebo, Ltd.

Study Nº: HLA 366-103
Study Aim: To identify potential carcinogenic effects of Emedastine when administered orally via diet to rats for 104 weeks.
Compound: Emedastine (KG-2413, Lot Nº HN-6-3), 0.01, 0.03 and 0.1%

Group	Nº of Animals	Dietary Levels (%)	Equivalent Oral Dose (mg/kg/day)	
			♂	♀
1	50/sex/group	0	0.00	0.00
2	50/sex/group	0.01	5.36	6.50
3	50/sex/group	0.03	16.14	19.55
4	50/sex/group	0.1	53.55	67.31

Animals: Fischer CDF® (F344), ≈6.5 weeks of age, 50/sex/group
Study Location:

Study Date: 4/9/87-4/17/89
Compliance with GLP/QAU: Yes
Experimental Design:

- Mortality and Clinical Signs - 2x/day;
- Detailed Physical Examination - 1x/week;
- Body Weights & Food Consumption - 1x/week for weeks 1-16 and 1x/4 weeks thereafter;
- Clinical Pathology - prior to scheduled and unscheduled sacrifice;
- Necropsy & Microscopic Examinations - died or killed in extremis and scheduled sacrifice (wk 105).

Results:

- Mortality and Clinical Signs - No effects on survival and no clinical signs were observed. Alopecia, urine stains, lacrimation, chromodacryorrhea, and small movable tissue masses were the most commonly observed clinical signs in rats among each group. The most frequent cause of death or morbidity was mononuclear cell leukemia (Fischer rat leukemia). The causes for the unscheduled deaths were summarized as followings.

Unscheduled Deaths								
Death Comments	♂				♀			
	0	0.01%	0.03%	0.1%	0	0.01%	0.03%	0.1%
Nº of Animals	15	15	15	11	16	14	17	12
Unidentifiable	0	3	0	0	1	0	2	0
Chronic Progressive Nephropathy	3	2	3	0	0	1	1	0
Inflammatory Process and Genitourinary Tract Inflammatory/Obstruction	1	1	0	1	0	0	1	2
Mononuclear Cell Leukemia	6	4	8	2	11	6	9	5
Pituitary Adenoma	1	0	0	3	0	3	2	0
Malignant Mesothelioma	1	1	1	1	0	0	0	0
Fibroma/Fibrosarcoma/Neurofibrosarcoma	0	0	0	2	1	0	0	0
Malignant Lymphoma	0	0	0	0	0	0	0	1
Sarcoma, Endothelial Stromal/Histiocytic/Myxosarcoma	0	2	0	0	1	1	2	2
Carcinoma, Squamous Cell/Uterine/Thymic/Pancreatic Acinar Cell/ "C" Cell	2	0	2	0	0	2	0	1
Malignant Pheochromocytoma/Chordoma/Mammary Gland Fibroadenoma/Endometrial Adenoma	0	2	1	1	2	0	1	1

The mortality for each group at wk 105 is presented in the following table.

Dietary Levels (%)	Survivors (%)		Non-Survivors	
	♂	♀	♂	♀
0	35 (70)	34 (68)	15	16
0.01	35 (70)	36 (82)	15	14
0.03	35 (70)	33 (66)	15	17
0.1	39 (78)	38 (76)	11	12

- Body Weights & Food Consumption - Mean body weights, mean body weight gains, and growth were significantly decreased at all weeks for the Group 4 ♂ and ♀ and in some weeks for the Group 3 ♀. The mean body weight gains for each group and the difference (%) from the controls are presented in the following table.

Dietary Dose Level (%)	♂				♀			
	Mean B. Wt. (g)		% Difference from Control	Gain relative to Control (%)	Mean B. Wt (g).		% Difference from Control	Gain relative to Control (%)
	wk104	wk 0	wk 0-104	wk 0-104	wk 104	wk 0	wk 0-104	wk 0-104
Control	402.6	126.7	-	-	296.6	96.9	-	-
0.01	387.0	127.7	-3.87	-6.02	289.3	96.0	-2.46	-3.20
0.03	385.2	127.2	-4.32	-6.49	279.2	95.6	-5.87	-8.06
0.1	368.4	127.0	-8.49	-12.50	246.7	95.2	-16.8	-24.14

Total food consumption was decreased in the Group 4 ♂ and ♀ from initiation through wks 14, 24, 52, 76 and 104. Mean total food consumption (g) for different periods were summarized in the table as follows.

Week	♂ Group - Dietary Dose Level (l%)				♀ Group - Dietary Dose Level (l%)			
	Control	0.01	0.03	0.1	Control	0.01	0.03	0.1
1-14	1736.8	1778.6	1766.6	1650.9	1345.9	1389.6	1349.5	1249.1
1-24	2229.9	2274.0	2266.4	2127.2*	1716.0	1761.6	1710.0	1588.7*
1-52•	3138.5	3190.8	3191.6	3004.5*	2385.4	2443.9	2362.4	2208.6*
1-76•	3912.0	3966.9	3975.7	3748.8*	2983.2	3042.3	2933.0	2745.4*
1-104•	4795.9	4874.1	4893.6	4605.7*	3755.7	3828.6	3688.2	3461.6*

* Significant different from control value, $p \leq 0.05$.
• Significant negative trend for food consumption, $p \leq 0.05$.

- Clinical Pathology - Increases in mean leukocyte, corrected leukocyte and absolute lymphocyte counts were noted in Group 4 ♂ and Group 2 and 3 ♀ as results of spontaneous occurrence of lymphocytic leukemia. Microscopic examinations confirmed the findings of this disease. **Slight to moderate elevation of mean ALT with high standard deviation values was noted for Emedastine-treated ♂ and ♀ rats. Clinical pathology data for the each individual animal were not submitted. The sponsor stated that the increases in ALT, ALP and/or total bilirubin seen in some animals from each group were due to the progressiveness of spontaneous liver disease. Again, histopathology reports for each individual were not submitted. Therefore, based upon submitted evidence the review pharmacologist could not conclude that Emedastine treatment did not result in insults in the liver. However, results from one-year chronic toxicity study showed that microscopic lesion in the liver (centrilobular hepatocyte swelling) and increased liver weights were characterized in rats receiving 50 mg/kg/day. Thus, liver could be the target organ.**
- Necropsy & Microscopic Examinations - The greatest change was a dose-related decrease (≈50%) in absolute and relative thyroid/parathyroid weights in Group 3 and 4♂, but not in ♀. The incidences for gross/microscopic non-neoplastic findings were comparable in all groups. The following table shows the incidence rates for the most frequent microscopic neoplastic events in all animals. It appeared no treatment-

related increases in tumor incidence rates.

Neoplastic Incidence (All Animals)						
Tissue	Type of Tumor	Sex	Group			
			Control	0.01%	0.03%	0.1%
Pituitary	Adenoma/Carcinoma	♂	15	25	15	18
		♀	21	23	24	24
Thyroid	"C" Cell Adenoma/Carcinoma	♂	16	10	8	16
		♀	9	14	13	11
	Follicular Cell Adenoma/Carcinoma	♂	2	0	1	2
		♀	1	1	1	0
Testis	Benign Interstitial Cell Tumor	♂	46	41	44	47
Mammary Gland	Fibroadenoma	♂	5	1	1	4
		♀	10	10	5	5
Hematopoietic System	Mononuclear Cell Leukemia	♂	23	18	20	30
		♀	20	19	26	16
Uterine	Endometrial Stromal Polyps	♀	15	8	6	13
Rats with One or More Neoplasm		♂	50	49	49	40
		♀	46	43	46	46

VI. Special Studies

i. The Effects of KG-2413 on LH, FSH and Prolactin in Blood of Castrated Male Rats. Pharmaceutical Research Centre, Kanebo, Ltd.

KG-2413 (ALØ3432A), 0, 50 or 500 mg/kg, was administered orally to castrated Jcl:SD rats (6/group) either once or once daily for one week to examine the effects of ALØ3432A on the hypothalamic-pituitary gland by assaying LH, FSH and prolactin levels in the blood. Two rats, in the 7-day study, receiving 500 mg/kg died on day 2 and the dose for this group was reduced to 250 mg/kg for the remainder of the study.

Results: LH, FSH and prolactin levels in blood were not affected following oral administration of ALØ3432A. Therefore, Emedastine did not exhibit any effects of on the rat hypothalamic-pituitary gonadotrophin in castrated rats.

ii. The Effects of Emedastine (KG-2413) on Testosterone, LH and FSH in Beagle Dogs. Pharmaceutical Research Centre, Kanebo, Ltd.

Since effects on the testes had been observed in Beagle dogs treated with Emedastine (ALØ3432A), this study was conducted to ascertain if ALØ3432A had any effects on testosterone, LH, or FSH in the blood. Beagle dogs (4/sex/group for 0.3, 1, and 3 mg/kg/day groups and 6/sex/group for control and 45 mg/kg/day groups) were orally administered ALØ3432A for one year with dose levels of 0, 0.3, 1, 3, or 45 mg/kg. The extra two dogs per sex in the control and 45 mg/kg/day groups were allowed to have a three month recovery period. Blood testosterone, LH and FSH levels were determined.

Results: **Data were poorly presented and equivocal.** A transient lower level of

testosterone in the blood was noted in the 45 mg/kg group at wk 2 when compared with the levels of pre-treatment. Administration of Emedastine caused little or no effects on the secretions of LH and FSH.

iii. Effect of KG-2413 and its Main Metabolites on 5α-reductase, Androgen Receptor and Dopamine Receptor. Pharmaceutical Research Centre, Kanebo, Ltd.

The effects of KG-2413 (ALØ3432A) and its main metabolites at concentrations of 3 μM - 100 μM on 5α-reductase, androgen, and dopamine receptors were studied using rat prostate and striatum homogenate. No effects on these parameters were noted. Therefore, ALØ3432A and its main metabolites, 5-Hydroxy ALØ3432A and 6-hydroxy ALØ3432A were considered to have no influence on androgen activity and central nervous and endocrine systems.

iv. Studies on the Dependent Liability of KG-2413 in Rats. Study Nº SBL 24-15, Pharmaceutical Research, Kanebo, Ltd.

Study Nº : SBL 24-15
Compound, Dose & Route:
KG-2413 (ALØ3432A) (Lot Nº 702005): 50 mg/kg/day, or 100 mg/kg/day for wks 1-2, and 250 mg/kg/day for wks 3-7 po;
Morphine: 10 mg/kg/day sc for wks 1 & 2, 25 mg/kg/day sc for wks 2 & 3, and 50 mg/kg/day sc for wks 4-10;
Phenobarbital: 25 mg/kg/day po for wks 1 & 2, 50 mg/kg/day po for wks 2 & 3, and 100 mg/kg/day po for wks 4-10;
Naloxone: 2 mg/kg sc on day 56.
Animals: ♂ Crj:Wistar rats, 5-week of age, weighing 154-171 g , 10/group.
Compliance with GLP/AUC: Yes
Study Site:

Study Date: 11/16/88 - 7/25/89
Study Design:
General Condition - 1x/day;
Observation of Withdraw Symptoms (24 hrs for morphine group and 48 hrs for other groups) -
Natural Withdraw: on Days 28, 35 and 70;
Cross-Withdraw: Days 42/43 & 49/50;
Naloxone-induced Withdraw: Day 56.
Body Weight - 2, 4, 8, 24 & 48 hr after withdraw

Results: A significant decrease in the body weight gain from wks 1-10 was observed in all morphine-treated animals with clinical signs of accelerated irritability and muscular rigidity. In the natural withdrawal test, a significant decrease in body weight was observed

as a withdrawal symptom in the phenobarbital and morphine groups on days 28, 35 and 70. Clinical signs of accelerated irritability and teeth chattering were also noted in the morphine treated animals. ALØ3432A administration did not affect body weight and exhibited no withdrawal symptoms. ALØ3432A failed to alleviate withdrawal symptoms caused by the phenobarbital and morphine in the cross-dependence liability test. Administration of naloxone exhibited no symptoms and signs of withdraw in animals receiving ALØ3432A. Therefore, ALØ3432A did not form dependence liability under the experimental conditions of this study.

v. Antigenicity Study of KG-2413. Study Nº STX 088, Pharmaceutical Research Centre, Kanebo, Ltd.

The antigenic potential of KG-2413 (ALØ3432A) was conducted in guinea pigs and mice. Guinea pigs were sensitized with ALØ3432A (or a reaction mixture of ALØ3432A and BSA) with or without FCA. The following systems were used to determine the possibility of production of antibodies against ALØ3432A: 1) systemic anaphylaxis test; 2) active cutaneous anaphylaxis test; 3) agar gel precipitation test; and 4) passive latex agglutination test. No production of antibodies was found in any of the four tests. When animals were challenged with 10 mg/ml Emedastine, mild irritation was observed in the active cutaneous anaphylaxis test. Similarity, no IgE antibody was detected by the passive cutaneous anaphylaxis test in rats using serum obtained from mice sensitized with ALØ3432A or the reaction mixture of ALØ3432A and BSA, in combination with alum.
The possible influence of the antiallergic action of ALØ3432A on allergic reactions in the present *in vivo* experiments was investigated using a mixture of BSA and ALØ3432A as eliciting antigen for BSA-sensitized animals. In each experiment, a slight inhibitory effect was found but the positive reaction was retained.
Based on the above findings, it was concluded that ALØ3432A has little, if any, antigenic potential.

APPEARS THIS WAY
ON ORIGINAL

SUMMARY AND EVALUATION

I. PHARMACOLOGY

Emedastine [1-(2-ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)-benzimidazole difumarate] was demonstrated to be a potent, selective histamine H_1 antagonist. The calculated K_i values for histamine H_1, H_2, and H_3 receptors were 1.3 nM, 49,067 nM, and 12,430 nM, respectively. Emedastine antagonized histamine-induced phosphoinositide turnover in ocular cells (IC_{50} values of 0.5-1.8 nM). These *in vitro* radioligand binding and functional potency data were confirmed by the studies using a number of smooth muscle preparations. Emedastine suppressed on histamine-induced contractions of ileum, aorta and trachea, with pK_B values of 9.9, 9.9 and 9.2 respectively. *In vivo* systemic antihistaminic activity of Emedastine following oral and intravenous administration had also been reported. Emedastine was 3, 39 and 780 times more potent than ketotifen, chlorpheniramine and diphenhydramine, respectively, at preventing histamine-induced death in guinea pigs and 4 and 27 times more potent than ketotifen and chlorpheniramine at hindering histamine-induced increases in vascular permeability.

Topical ocular antihistaminic activity of Emedastine was evaluated in histamine and antigen stimulated conjunctivitis models. Topical ocular administration of Emedastine caused a concentration dependent suppression of histamine induced vascular permeability changes occurring in the conjunctiva between and ranged from 1 min to 8 hr before histamine challenge. The calculated ED_{50} values obtained using intervals of 1 min, 30 min, 2, 4 and 8 hrs were 0.0002%, 0.00004%, 0.0029%, 0.019% and 0.19% (w/v), respectively. Emedastine, given topically 30 min prior to challenge, was equipotent to ketotifen, and 7, 7, 10, 100, 357, 3333, and 5813 times more potent than brompheniramine, chlorpheniramine, pyrilamine, levocabastine, pheniramine, diphenhydramine, and antazoline, respectively. Emedastine (0.1%) failed to reduce either serotonin or platelet-activating-factor induced vascular permeability changes, indicating specific activity at the histamine receptor. In a guinea pigs passive conjunctival anaphylaxis model, significant inhibition was observed following topical ocular administration of Emedastine 5 min or 30 min prior to antigen challenge with ED_{50} values of 0.0046% and 0.00022%, respectively.

There was no significant interaction between Emedastine (10 mM) and α adrenergic, dopamine D_2, serotonin S_2, and numerous other receptors. Studied with a variety of smooth muscle preparations from the rat and guinea pig, exhibited that Emedastine did not antagonize the effects of leukotriene D_4, bradykinin, prostaglandin $F_{2\alpha}$, acetylcholine, and norepinephrine. Other pharmacological effects of Emedastine are summarized in the following table.

Animal Species	Dose (mg/kg)	Route	Effects
NERVOUS SYSTEM			
♂ ddY Mouse	100	po	↓ locomotive activity and acetic acid-induced writhing
	10-100	po	↔ muscle tone, various experimental convulsion, oxotremorine-induced tremor, physostigmine-induced mortality, or hexobarbital-induced sleep
♂ Wistar Rat	20	iv	↔ spontaneous EEG
	100	po	↔ condition avoidance response, monosynaptic and polysynaptic reflexes in lamectomized rats
♂ Hartley Guinea Pig	4.0%	Topical	↔ cornea reflex
Bullfrog	0.5 mM	Bath	↔ action potential of sciatic nerve
CARDIOVASCULAR SYSTEM			
♂ & ♀ Mongrel Dog	0.3-3.0	iv	↓ blood pressure and heart rate dose-dependently; ↔ ECG
	30	id	
♂ Hartley Guinea Pig	0.1 mg/ml	bath	↓ atria contractile force
RESPIRATORY SYSTEM			
♂ Hartley Guinea Pig	1.0-10	iv	≤3.0 mg/kg: ↑ rate and volume of respiration at; 10 mg/kg: ↓ respiration
	30	id	↔ respiration
	0.3-3.0	iv	↔ tracheal ciliary movement
♂ NZW Rabbit	3.0	iv	↔ the volume and viscosity of respiratory secretion and tracheal ciliary movement
♂ & ♀ Mongrel Dog	3.0	Iv	↓ respiration
GI SYSTEM			
♂ ddY Mouse	100	po	↓ intestinal peristalsis
♂ Wistar Rat	100	id	↓ gastric secretion
	10-100	id	↑ biliary secretion dose-dependently
URINARY SYSTEM			
♂ Wistar Rat	30, 100	po	↔ urine volume and urinary electrolytes (Na^+ & Cl^-)
SMOOTH MUSCLES			
♀ Wistar Rat	1-10 μg/ml	bath	↔ spontaneous movement of isolated uteri from virgin or pregnant rats
♂ Hartley Guinea Pig	10^{-4} & 10^{-5} g/ml	bath	↓ $BaCl_2$ and nicotine-induced contractile responses of isolated ileum; ↑ vas deferens contractile response induced by epinephrine
♂ NZW Rabbit	10^{-4} & 10^{-5} g/ml	bath	↔ spontaneous movement of isolated duodenum, jejunum and ileum
OTHER EFFECTS			
♂ NZW Rabbit	10^{-4} & 10^{-5} g/ml	bath	↓ platelet aggregation slightly
♂ Wistar Rat	10-100 mg	po	↔ blood sugar level, blood clotting system
	100	po	↓ carrageenin-induced paw edema

↑: increase; ↓: decrease; ↔: no effect.

Based upon the data from preclinical pharmacological studies, it clearly proves that Emedastine is a potent histamine H_1 antagonist.

II. ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

i. Ocular ADME

The ocular distribution and plasma pharmacokinetics of radioactivity (^{14}C-Emedastine) were studied in male NZW rabbits and male pigmented (DB) rabbits following a single

unilateral topical dose of 0.1% Emedastine. Summary of PK parameters are shown in the following table.

Tissue	DB Rabbits			NZW Rabbits		
	C_{max} (μg eq./g)	T_{max} (hr)	$t_{½}$ (hr)	C_{max} (μg eq./g)	T_{max} (hr)	$t_{½}$ (hr)
Aqueous Humor	0.281 ± 0.067	1	0.8 hr	0.310 ± 0.124	0.5	0.8
Cornea	3.32 ± 0.16	1	1.1 hr	3.28 ± 1.26	0.5	0.8
ICB	10.2 ± 2.8	10	23 days	0.669 ± 0.148	0.5	0.8
Retina	0.207 ± 0.082	0.5	Not Determined	0.021 ± 0.004	0.5	0.7
Choroid	0.770 ± 0.137	3	Not Determined	0.060 ± 0.025	1	0.9
Conjunctiva	0.824 ± 0.169	0.5	1.1 hr	0.809 ± 0.335	0.5	0.7
Plasma	0.009 ± 0.004	1	1 hr	0.007 ± 0.001	0.5	1.2

For Dutch Belted retina and choroid, half-lives could not be reliably determined due to variability is the data. However, the general trend in both tissues indicated a prolonged half-life similar to ICB.

The results showed that C_{max}, T_{max} and $T_{½}$ in non-pigmented ocular tissues (aqueous humor, cornea, and conjunctiva) were similar to the values obtained from New Zealand white (NZW) rabbits. However, C_{max} values for retina, choroid, and iris-ciliary body (ICB) were ≥ 10x higher than the corresponding values measured in NZW rabbits, an indicative of Emedastine-melanin binding. **Prolonged $T_{½}$ (23 days) and higher C_{max} noted in ICB of DB rabbits implied that accumulation of Emedastine in ocular tissues containing high levels of melanin may occurred followed by a long term treatment. Potential ocular accumulation and toxicity of Emedastine in non-albino rabbits should be explored further.**

ii. Systemic ADME

Pharmacokinetic parameters of Emedastine following oral or iv administration to rats, guinea pigs, and dogs are summarized in the following table. The values for C_{max} & AUC were highly variable among different study reports.

Dose (mg/kg)	Route	T_{max} (h)	C_{max} (ng eq/mL)	C_o (ng eq/mL)	$t_{½}$ (h)	$AUC_{0-\infty}$ (ng eq·h/mL)	Report Nº
Rat							
1	p.o. (n=3)	0.25	46.3 ± 3.6	-	8.6 ± 3.5*	432 ± 86	*Xenobio. Metabol. and Dispos.* 2:123, 1987.
4	p.o. (n=3)	0.25	251 ± 33	-	12.8 ± 3.6*	1930 ± 320	
8	p.o. (n=3)	0.25	408 ± 37	-	11.7 ± 2.0*	4090 ± 1010	
1	i.v. (n=4)	-	-	370 ± 36	5.8 ± 2.0*	574 ± 65	
20	p.o. (n=8)	0.39 ± 0.14	57.3 ± 12.2	-	0.71 ± 0.69	79.0 ± 20.6	*J. Pharmacobio-Dyn.* 12:530, 1989.
2	i.v. (n=27)	-	-	-	1.1 ± 0.2	218 ± 30	*Chem. Pharm. Bull.* 37:753, 1989.
Guinea pig							
2	p.o. (n=4)	0.5	398 ± 84	-	6.2 ± 2.0*	846 ± 180	*Xenobio. Metabol. and Dispos.* 2:123, 1987.
2	i.v. (n=3)	-	-	966 ± 179	8.3 ± 1.1*	1200 ± 210	
2	p.o. (n=7)	0.54 ± 0.06	148 ± 9.0	-	0.69 ± 0.04	208 ± 16	*J. Pharmacobio-Dyn.* 12:530, 1989.
2	i.v. (n=27)	-	-	-	0.69 ± 0.08	421 ± 140	*Chem. Pharm. Bull.* 37:753, 1989.
Dogs							
2	p.o. (n=3)	1.0	462 ± 127	-	1.2 ± 0.2	2573 ± 392	*Xenobio. Metabol. and Dispos.* 4:471, 1989. (Alcon TR 082:38570:0995)
	i.v. (n=3)	-	-	-	1.4 ± 0.1	2973 ± 112	
2	p.o. (n=7)	0.85 ± 0.32	4.78 ± 0.45	-	2.16 ± 0.19	19.1 ± 2.4	*J. Pharmacobio-Dyn.* 12:530, 1989.
2	i.v. (n=27)	-	-	-	1.9 ± 0.6	369 ± 80	*Chem. Pharm. Bull.* 37:753, 1989.

*: $T_{½(9-24h)}$

Tissue distribution of radioactivity following single and multiple oral dose of ^{14}C-Emedastine (1 mg/kg/day) was assessed in the ♂ Wistar rats. Results from single dose study showed high levels of radioactivity in the liver, pancreas, stomach content, urine, kidney, Harder's gland, salivary gland, lung and spleen. Concentrations of radioactivity were low in the eye, CNS and fat. The maximal levels of radioactivity in most tissues were measured at 0.25 hr post dosing, with the highest concentrations observed in the liver, stomach, and intestine. The levels of radioactivity in plasma and tissues reached to steady-state by the 7th dose following orally dosed with ^{14}C-Emedastine 1 mg/kg/day for up to 14 days. Approximate 98% of the cumulative dose was excreted in the urine and feces within 96 hr post 14-day dosing. The highest radioactivity levels were seen in the liver and kidney with the brain exhibiting very low levels.

Maximal plasmal levels in pregnant (Gestation Day 19) rats following an oral administration of 1 mg/kg ^{14}C-Emedastine was 30 min after dosing, with a secondary peak at 6 hours. The maximum level of radioactivity (≈1% of total dose) in fetal tissue occurred at 2 hr after dosing, with a concentration comparable to that in maternal plasma. Radioactivity was transferred to milk and reached a peak concentration of 26.2 ± 8.8 ng equiv./ml at 8 hours after dosing. Lactal concentrations then declined with a $T_{½}$ value of approximate 16 hours. **Significant sex difference was noted by comparison of the data from both pregnant and non-pregnant female rats with data from the study in males.** Both pregnant and nonpregnant female rats had higher systemic levels of radioactivity following an oral ^{14}C-Emedastine (1 mg/kg) dose than those measured in the ♂ (see the following table).

Parameter	♂	♀	
		pregnant	nonpregnant
C_{max} (ng/ml)	46.3	221	347
$AUC_{0-\infty}$ (ng·hr/ml)	432	910	658

Emedastine and its metabolites in the urine, bile, plasma and liver of rats and in the urine of guinea pigs were identified. The metabolic pathways of Emedastine are basically similar for rats and guinea pigs but the relative amounts of the various metabolites were different between the two species The major urinary and biliary metabolites of Emedastine in rats were the 5 & 6-hydroxylated compounds and their corresponding conjugates. The major metabolite in guinea pig urine was the N-oxide. The 5-hydroxy and 6-hydroxy metabolites were present as conjugates in amounts approximately six-fold lower than the N-oxide. Cumulative (24 hr) urinary excretion of Emedastine and metabolites in various species are shown as followings.

Compound	% Dose Rat	% Dose G. Pig	% Dose Dog	% Dose Man
6-OH (M5a)	2.07/1.21	0.18/0.45	4.61/3.66	10.0/16.0
5-OH (M5b)	1.43/8.23	0.43/1.19	0.48/0.22	4.2/7.5
Parent Drug	0.10/N.D.	3.67/N.D.	0.75/N.D.	3.6/N.D.
5 and 6-OH-5'-oxo (M2a,b)*	0.13/N.D.	N.D./N.D.	N.D./N.D.	2.4/N.D.
5 and 6-OH, N-desmeth. (M10 a,b)	0.15/0.08	N.D./0.13	N.D./N.D.	N.D.
N-Oxide (M9)	0.07/N.D.	12.63/N.D.	10.40/N.D.	0.4/N.D.
N-desmeth-5'-oxo-O-deethyl (M7)*	0.34/N.D.	0.46/0.15	0.59/N.D.	N.D.
N-desmethyl (M6)	0.09/N.D.	0.56/N.D.	1.21/N.D.	N.D.
Total	13.9	19.8	21.9	44.1

Each pair of numbers = free/conjugated; N.D. = Not Detected (< 0.1%);
*RRA activity for this compound not reported. However, the cross-reactivity of 5'-oxo-Emedastine was < 0.01%

Following a 14-day oral dosing at 250 mg/kg/day, Emedastine caused a significant increase in aminopyrine N-demethylase activity, aniline p-hydroxylase activity and cytochrome P-450 content.

Results from an *in vitro* plasma-Emedastine binding study showed profound interspecies differences.

- Binding Affinity - guinea pig plasma >human serum > rat plasma.
- Binding Capacity - human serum > guinea pig > rat plasma.

III. TOXICOLOGY

i. Ocular Toxicity

Reports from three-month (with a one-month interim report) and six-month ocular toxicities studied in the rabbits were submitted. The toxicokinetic analyses of plasma drug levels showed that repeated ocular application of Emedastine may lead to accumulation.

Six-month ocular toxicity in the monkey was recently completed and no data from this study were included in the present NDA.

ii. Systemic Toxicity

ACUTE TOXICITY STUDIES

The acute toxicity studies for Emedastine were conducted by Kanebo Pharmaceutical Ressearch and the LD_{50} values for the mouse, rat and dog are summarized in the following table.

Species (Strain)	Route	Dose Range (mg/kg)	Approximate LD_{50}(mg/kg) [95% CI]	
			♂	♀
Mouse (Jcl:SD)	Oral	625 - 5000	2,547 [1689-3840]	2,206 [1675-2906]
	SQ	420 - 1000	712 [615-824]	609 [551-673]
	IV	71 - 142	101[95-108]	93 [87-98]
Rat (Jcl:ICR)	Oral	840 - 2378	2,151[1745-2651]	1,854 [1478-2326]
	SQ	420 - 1189	666 [585-758]	643 [560-739]
	IV	42 - 119	72 [62-83]	77 [67-89]
Dog (Beagle)	Oral	100 - 1000	193 mg/kg	

The LD_{50} values for metabolites 5- & 6-hydroxy KG-2413 in the rat are shown as followings.

Compound	Species	Route	Dose Range (mg/kg)	Approximate LD_{50} (mg/kg) [95% CI]	
				♂	♀
5-Hydroxy KG-2413	Rat Jcl:SD	IV	80 - 218	184 [169-210]	119 [109-130]
6-Hydroxy KG-2413		IV	30 - 251	91[60-137]	90 [59-137]

REPEATED-DOSE (SUBACUTE & CHRONIC) STUDIES

Series of systemic repeated-dose oral toxicity studies in the rat and dog with various durations conducted by Kanebo Laboratories. The study design and dose distributions are summarized in the following table.

Species	Strain	Nº Animals/ Group	Doses (mg/kg/day)	Duration	NOEL (mg/kg/day)	Report Number
Rat	Jcl:SD	14/sex	0, 10, 50, 250, 1250	3 months	10	Pharmacometrics, 39(3):215-230 (1990)
Dog	Beagle	6/sex	0, 3, 15, 75	3 months	15	Pharmacometrics, 39(3):231-268 (1990)
Rat	Jcl:SD	24/sex	0, 1, 3, 10, 50	1 year	10	Pharmacometrics, 39(3):269-284 (1990)
Dog	Beagle	4/sex	0, 1, 3, 15, 45	1 year	-	Pharmacometrics, 39(3):285-318 (1990)
Dog	Beagle	4/sex; 6/sex for control and high dose	0, 1, 45	1 year with recovery period	15	GTX 305

- Three-month Subacute Toxicity:

Rat: Groups of 14/sex Jcl:SD rats were orally (by gavage) dosed with Emedastine at doses of 0, 10, 50, 250 or 1250 mg/kg/day for 3-month. Mortality was noted in rats @ 1250 mg/kg. The major clinical signs observed in this group consisted of mydriasis, reduced locomotor movements, exaggerated reaction and clonic or tonic convulsions. Salivation and behavior of creeping into bedding were occasionally noted in rats @ 250 mg/kg. Rats @ 1250 mg/kg had marked ↓ body weight gain and food consumption. Significant ↑ in BUN, Ca, P, ALP were identified in the rats @ ≥250 mg/kg. Female rats @ 50 and 250 mg/kg had significantly ↓ serum cholinesterase activity. Histopathological examinations showed that kidney and liver were the target organs. The main microscopic changes in the liver included swelling of hepatocyte with hyalin and/or eosinophilic degeneration. The microscopic lesions in the kidney were eosinophilic degeneration and/or vacuolar degeneration in tubular epithelium, and tubular calcification. The electron microscopic (EM) examination on the liver and kidney obtained from animals @ 1250 mg/kg revealed dilation of biliary canaliculus, lingual shape welling, abnormal and reduced numbers of microvilli, a ↑ in lysosome and appearance of myelin body in the liver and an increase in glomerular epithelium in the kidney.

Dog: Begle dogs, 6/sex/group, were orally given Emedastine capsule at doses of 0, 3, 15, or 75 mg/kg/day for 3-month. Gasping, failure of muscular coordination, decreased spontaneous movements and convulsions were observed 0.5 to 6 hours after the start of administration in the 75 mg/kg group. Seven deaths (3 ♂ & 4 ♀) were observed in this group as results of convulsions and dyspnea. Dogs @ 75 mg/kg also had ↓ body weight gain and ↓ in food and ↓water intake with a slight ↑ in ALP and a slight ↓ in triglyceride levels. Increased liver weights and proliferation of the smooth endoplasmic reticulum were noted in dogs @ ≥15 mg/kg. In addition, the high dose group displayed a slight ↓ of prostatic epithelial granules. These changes were not seen in the animals after the one-month recovery period.

- 12-month Chronic Toxicity:

Rat: Jcl:SD rats, 24/sex/group, were dosed (by oral gavage) with Emedastine (0, 1, 3, 10, or 50 mg/kg/day) for 12-month. Total of 17 rats died or were sacrificed. As the sponsor indicated that dosing error (3♂ & 6♀) and spontaneous pituitary tumor (2♂ & 6♀) were the major causes of death. A significant ↓ in weight gain but not food consumption and H_2O intake was observed in the ♂ receiving 50 mg/kg/day. Significant ↓ in RBC, Hb, and PCV were found in ♀ @ 50 mg/kg. Cholinesterase (ChE) activity ↓ approximately 25% were noted in females @ 3 or 50 mg/kg. The target organ was the liver by the evidence that centrilobular hepatocyte swellings, increased relative liver weights and decreased ChE activity were characterized in the macro and microscopic examinations.

Dog: Two studies were conducted in the groups of 4-6/sex beagle dogs. Dogs were orally given with 0, 1, 3, 15, or 45 mg/kg Emedastine in a gelatin capsule once daily for one year. In one study dogs @ 45 mg/kg/day were allowed to have a 3-month recovery phase after the last dosing. No mortality was noted. Acute symptoms of tachypnea, hyperactivity, muscle spasms and involuntary movement of the tongue lasting for several hours from the first hr after dosing throughout the treatment were observed. Dogs @ 45 mg/kg /day had ↓ weight gain, ↑ ALP activity and ↑ GPT. The ♂ @ 45 mg/kg had an increased liver weight with microscopic lesions of proliferation of the smooth endoplasmic reticulum. A ↓ testicular weight and atrophy of the prostatic epithelium with local deciduation of the epithelium of the seminiferous tubules were characterized in ♂ @ 45 mg/kg. These changes in the testes of the high dose ♂ were local and sometimes unilateral, and were not noted in the dogs with a 3-month recovery period after the last doing.

iii. Reproductive Toxicity

Totals of 5 studies were performed to evaluate the effects of Emedastine on the reproductive performance and embryo development.

Species	Strain	N°Animals/ Group	Mode of Administration	Doses (mg/kg/day)	Treatment Duration (Wks)	NOEL (mg/kg/day)	Report Number
Rat (Seg I)	Fischer 344	10♂, 10♀	Diet (0.01, 0.05, or 0.25%)	0, 6.2, 30.4, 135.8	Females: 2 wks PM-LD21 Males: 10 wks PM-sacrifice	30.4	R02285
Rat (Seg I)	Jcl:SD	24♂, 24♀	Oral Gavage	0, 10, 40, 140	Female: 2 wks PM-GD7 Males: 9 wks PM-sacrifice	140	Pharmacometrics, 39(3):319-328 (1990)
Rat (Seg II)	Jcl:SD	34♀	Oral Gavage	0, 10, 40, 140	Gestation Days 7-17	10/maternal 40/fetal & offspring	Pharmacometrics, 39(3):329-342 (1990)
Rabbit (Seg II)	Dutch Belted	20♀	Oral Gavage	0, 3, 15, 75	Gestation Days 6-18	15/maternal 75/fetal	B03785
Rat (Seg III)	Jcl:SD	24♀	Oral Gavage	0, 10, 40, 140	Gestation Days 17-LD20	10/maternal 40/offspring	Pharmacometrics, 39(3):343-354 (1990)

- Segment I Studies: The test article, ALØ3432A, was administered to female rats in the diet at concentrations of 0, 0.01, 0.05 or 0.25% that were in equivalent of average daily doses of approximately 0, 6.2, 30.4, or 135.8 mg/kg, respectively, during the premating and gestation periods. Body weight and weight gain were reduced, and ↓ food consumption

during gestation and lactation periods in the ♀ at the 0.25%. Mating performance was significantly ↓ for both ♂ & ♀ in the 0.25% group. However, mating performance of the treated ♂ was not altered once the treatment ended. Treatment-related developmental toxicity was exhibited by body weight depression in offspring of animals treated with diets containing 0.25%. In another study, Emedastine, at doses of 0, 10, 40, or 140 mg/kg/day, was orally dosed to ♂ rats from 9 weeks before mating, throughout cohabitation for 99/100 days, and to ♀ rats from 14 days before mating until day 7 of gestation. No abnormalities were noted in the examined reproductive and fetal parameters.

- Segment II Studies:
  Rat: Emedastine at doses of 0, 10, 40 or 140 mg /kg/day was administered orally to Jcl:SD rats (34 per group) on Gestation Days 7 -17. Mild mydriasis and salivation were observed in a few of the high dose F_0 dams. A slight ↓ in body weight gain was noted in the 40 and 140 mg/kg groups. The relative weights of the liver, kidneys, adrenals and ovaries of the 140 mg/kg F_0 females in the cesarean section group were significantly increased, but this was not observed in the dams allowed to deliver.
  A significant ↑ in fetal mortality and a significant ↓ in the number and body weight of live fetuses were seen in the 140 mg/kg group. In addition, a significant ↓ in the numbers of phalanges and the caudal vertebrae were noted in this group at the skeletal examination, an indicative of delayed ossification. Dose-dependent increasing incidences of external, visceral and skeletal anomalies were noted. Observations of the F_1 pups during the nursing period indicated a significant decrease in the number of pups born and in the birth index in the 140 mg/kg group. Skeletal examination of the culled pups revealed a significant ↑ in the incidence of dysplasia of the caudal vertebrae in this same group. After weaning, the body weight gain was slightly ↓ in both ♂ and ♀ of the 140 mg/kg group. Necropsy at three weeks of age revealed dysplasia of the caudal vertebrae in one animal in the 140 mg/kg group, however the necropsy at six weeks of age revealed no Emedastine treatment effects. Behavioral and learning ability, and reproductive functions were not affected. No remarkable findings were noted in skeletal and visceral examinations of the F_2 fetuses. No evidence of a drug-related teratogenic effect was observed during this study.

  Rabbit: Emedastine (0, 3, 15 or 75 mg/kg/day) was administered orally by gavage to Dutch Belted rabbits (25/group) on Gestation Days 7 - 17. Maternal toxicity was exhibited in the 75 mg/kg group by the evidence of one death and one abortion on Gestation Days 18 and 22, respectively. Decreased food consumption with normal weight gain was observed in the high dose group. Examination of the fetuses revealed no treatment-related findings.

- Segment III Study: Emedastine at doses of 0 (control), 10, 40, or 140 mg/kg/day was administered orally to female Jcl:SD rats (23/group) during the perinatal and postnatal periods (Gestation Day 17 to Postpartum Day 20). Transient salivation was observed occasionally in the dams in the 140 mg/kg dose group. Body weight gain was ↓ in the first half of lactation in ♀ @ ≥ 40 or 140 mg/kg, however, it steadily returned to normal. A significant ↓ in body weight gain was noted in pups the 140 mg/kg group at birth and after

weaning. Sex maturation and motor coordination in the F_1 were not affected. Necropsy and organ weights of the F_1 sacrificed at 3 and 6 weeks of age were not remarkable. No effects were noted in the behavioral or learning ability and reproductive indices in the F_1. F_2 fetuses were not affected.

iv. Mutagenicity

Emedastine was shown to be not mitogenic or clastogenic in a panel of *in vitro* and *in vivo* studies that included Ames bacterial mutation tests, mouse lymphoma forward mutation assay, chromosome aberration assay in Chinese hamster lung culture, induction of DNA repair synthesis in primary adult rat hepatocytes, in vivo induction of sister chromatid exchange in Chinese hamster bone marrow, and mouse micronucleus test.

v. Carcinogenicity

Two studies were conducted and the design of each study is presented in the following table.

Species	Animals/ Group	Route	Dietary Doses (%)	Equivalent Oral Dose (mg/kg/day)	Duration	Report Nº
Mouse B6C3F1	50♂	Diet	0, 0.01, 0.03, 0.1	0, 15.23, 44.42, 170.47	104 wks	366-102
	50♀			0, 16.85, 56.91, 218.50		
Rat FischerCDF	50♂	Diet	0, 0.01, 0.03, 0.1	0, 5.36, 16.14, 53.55	104 wks	366-102
	50♀			0, 6.65, 19.55, 67.31		

(1) Mouse Study:
There were significant decreases in body weight gains for the ♂ and ♀ mice in the high dose group (0.1%) with values of 13.1 and 18.1%, respectively. Survival distribution in the high dose females was not different from the controls. It could be concluded that the MTD might have reached.

(2) Rat Study:
Profound decreases in body weight gains were observed for the ♂ and ♀ rats in the high dose group (0.1%) with values of 12.5 and 24.1%, respectively. No difference in the survival distribution between control group and Emedastine-treated groups for both ♂ and ♀ was noted. Based on body weight gains and food consumption observed in the present study, MTD was reached for both male and female rats in the high dose groups. Although high occurrence in pituitary gland adenoma/adenocarcinoma, mononuclear cell leukemia and thyroid "C" cell adenoma/carcinoma was reported, there was no significant increase attributable to the treatment. There were no significant drug-induced increases in the tumor incidences.

vi. Special Studies

Series of special studies were conducted and the study designs plus results are presented as followings.

Assay	Species	Nº of Animals	Route	Dose	Results	Report Nº
Hormonal Effect	Rat (Jcl:SD)	6♂/time/group	po	0, 50, 500 mg/kg (Single dose, or 1 dose daily for 1 wk)	No effect on LH, FSH or Prolactin	Investigational
Hormonal Effect	Dog (Beagle)	4♂, 4♀ or 6♂, 6♀ (Control & High Dose Groups)	po	0, 0.3, 1, 3, 45 mg/kg/day (1 year)	No effect on LH, FSH or Testosterone up to 3 mg/kg	Investigational
Receptor Effect	Rat (Wistar ♂)	Isolated Tissue	In Vitro	3-100 μM	No effect on 5α-reductase, androgen receptor or dopamine receptor	Investigational
Dependent Liability	Rat (Wistar)	10♂/group	po	50, 100/250 mg/kg (10 wks)	No dependence	SBL 24-15
Antigenic/ Sensitization Potential	Guinea Pig (Hartley)	15/group	Induction: po, SQ or IM; Challenge: IV or intradermal	3, 30 mg/head	No antigenicity	STX 088
	Mouse (C3H/He, BALB/c)	5/group	IP induction	1, 10, 100 μg/mouse		

RECOMMENDATION:

Binding of Emedastine to melanin was observed. In addition, elimination of Emedastine in the pigmented ocular tissues is rather slow. Although the 6-month ocular toxicity studies have been done in the albino rabbits and monkeys, only data from the rabbit study were presented in the present submission. Therefore, the sponsor should be advised to submit a full study report (including individual animal data) for the 6-month ocular toxicity study in the monkey as soon as possible. Potential ocular accumulation and toxicity of Emedastine in the color eyes is unknown. Commitment of conducting post-marketing surveillance is highly encouraged.
Based upon available preclinical information, the approval of this application is recommended.

Josie Yang 9/16/96
W.C. Josie Yang, Ph.D.

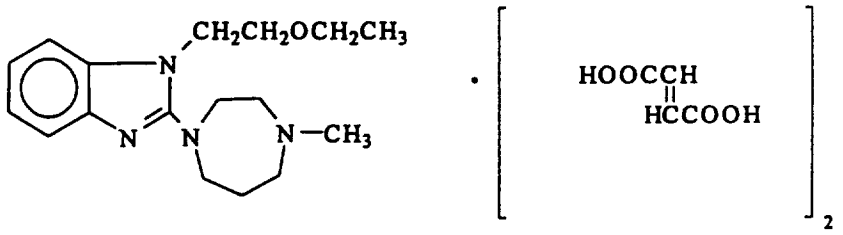
Concur by team leader: Yes ☒ No ☐ Conrad H. Chen 11-8-96
Conrad Chen, Ph.D.

cc:
NDA 20-706
HFD-550/Division File
/JYang
/ELudwig
/JHolmes
HFD-345
HFD-024
F/T by JYang, September 16, 1996

FEB 5 1997

DIVISION OF ANTI-INFLAMMATORY, ANALGESIC AND OPHTHALMOLOGIC DRUG PRODUCTS
PHARMACOLOGY AND TOXICOLOGY REVIEW

NDA	**20-706**
DRUG:	Emadine™ (Emedastine Difumarate) 0.05%
OTHER NAMES:	ALØ3432A; KG-2413; LY188695
SPONSOR:	Alcon Laboratories, Inc. 6201 South Freeway Fort Worth, TX 76134
SUBMISSION DATE:	October 31, 1996 and December 16, 1996
TYPE OF SUBMISSION:	Amendment
DATE COMPLETED:	February 3, 1997
REVIEWER:	W. C. Josie Yang, Ph.D.
CDER STAMP DATE:	November 1 and November 24, 1996
DATE RECEIVED IN HFD-550:	November 1 and November 24, 1996
DATE ASSIGNED TO REVIEWER:	November 7, 1996 and January 8, 1997
DRUG CATEGORY:	Histamine H_1 Antagonist
FORMULA:	$C_{25}H_{34}N_4O_9$; 1H-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4methyl-1H-1,4-diazepin-1-yl)-, (E)-2-butenedioate (1:2); MW=534.57



INDICATION:	Relief of the Signs and Symptoms of Allergic Conjunctivitis
DOSAGE FORM:	0.05% Emedastine Difumarate Ophthalmic Solution
RELATED DRUG/INDs/NDAs/DMFs:	

PRECLINICAL STUDIES:

TOXICOLOGY STUDY

Six Month Topical Ocular Irritation and Systemic Toxicity Evaluation of Emedastine Ophthalmic Solution (AL03432A) in Primates

Report Nº: 081:38520:0596

Study Aim: To determine the ocular irritation potential of Emedastine (ALØ3432A) Ophthalmic Solution resulting from q.i.d. topical ocular administration to cynomolgus monkeys for 6 months.

Compound: 0.05% (Lot Nº 95-13772) or 0.1% (Lot Nº95-13771) Emedastine (as the base) Ophthalmic Solution

Control Vehicle: 0.01%Benzalkonium Cl + 0.5% Tromethamine + 0.71% NaCl + 0.25% Hydroxypropyl Methylcellulose, pH 7.4 (Lot Nº 95-13770)

Dose & Route: 2 drops/topical ocular instillation to the right eye, QID for 190 days

Animal: 28 young adult cynomolgus monkey, weighing 3.8-7.1 kg for the ♂ and 2.7-4.4 for the ♀, 2 or 4/sex/group

Study Location: Alcon Laboratories, Inc., Fort Worth, TX.

Compliance with GLP/QAU: Yes

Study Design:

Treatment Group	Nº of Animals	Treatment Volume (µl)	Daily Dose (mg)	Treatment/Day	Duration (Days)
1. Untreated Control	2/Sex/Group	-	-	-	-
2. Vehicle Control	4/Sex/Group	120	0	4	192/193
3. 0.05% Emedastine	4/Sex/Group	120	0.24	4	192/193
4. 0.10% Emedastine	4/Sex/Group	120	0.48	4	192/193

- Mortality and Clinical Signs - 2x/day;
- Physical Examination - 2x/wk;
- Body Weights - Days 0, 7, 13, 21, 28, 49, 77, 105, 147, 168 and 190 (♂)/191 (♀);
- Slit-lamp Biomicroscopic Examinations - Weeks 1, 3, 5, 6, 9, and 11, and then 1x/month thereafter.
- Indirect Ophthalmoscopic Examinations - Days 0 and 190;
- Specular Microscopy - Days 0 and 190;
- KOWA Flare Cell Meter - Days -4, 83 and 186;
- Clinical Chemistry and Hematology Analysis - Days -1 and 182;
- Plasma Drug Levels - Days 30, 90, and 178 on all animals in groups 2, 3 and 4 at 10 min before dosing and 30 min postdose;
- Ocular Tissue Drug Levels - Day 178 on 2 animals/dose group;
- Necropsy & Histology Examinations - Day 193 (♂)/194 (♀).

Results:

- Mortality and Clinical Signs - No mortality was reported. Unremarkable clinical observations were noted. Loose stools were seen in one monkey @ 0.1% on Days 55, 62, 65-86, 121, and 132.
- Body Weights - Mean body weights and body weight gains were comparable between treated and control groups.

- Biomicroscopic Examinations - No significant ocular irritation, conjunctival congestion, swelling, discharge, flare, iritis, impaired light reflex or abnormal lenses were observed.
- Specular Microscopic and KOWA Flare Cell Measurements - There were no differences in specular microscopic measurements (cell density, coefficient of variation, and % hexagons) on Days 0 and 182 between treated and non-treated groups. Similar cell counts were obtained in Emedastine-treated and non-treated groups. However, the flare cell counts in ♀ @ 0.1% were higher than controls on Day 83 and were lower than controls on Day 186.
- Clinical Pathology - Data from clinical chemistry and hematology analyses showed non-treatment related changes. Although significant↓ in hematocrit and hemaglobulin were noted in treated monkeys, yet these values were within normal range established by the sponsor.
- Post-Mortem Observations -

  Organ Weights: Group 4 ♀ had lower heart weight but not relative heart/body weight as compared to controls.

  Gross and Microscopic Pathology: No treatment-related changes were seen in non-ocular tissues. Mild (Grade 1) to severe (Grade 4) mononuclear cell infiltration in the corneal limbus and sclera were noted in Emedastine-treated animals. Combined incidences of left and/or right corneal and scleral mononuclear cell infiltrations (/animal) are shown as follows.

Selected Ocular Lesions	Control		0.05%		0.1%	
	♂	♀	♂	♀	♂	♀
Cornea, Limbus Mononuclear Cell Infiltrate	0	0	1	0	4	1
Sclera, Mononuclear Cell Infiltrate	0	0	1	1	2	1

Infiltrates varied in size and consisted of loosely to densely packed aggregates of small and large lymphocytes. The scleral infiltrates were often located near the corneal limbus.

- Plasma and tissue Emedastine levels - Systemic exposure to Emedastine was detected following q.i.d. topical ocular administration of 0.05 and 0.1% Emedastine ophthalmic solution. Data showed that steady-state was reached by Day 30. A dose-proportional increase in systemic exposure was noted. Peak plasma Emedastine concentrations measured 30 min postdose on Days 30, 90 and 178 are given in the following table.

Samples	30 Min Peak Concentration (ng/ml)(N=8)					
	Day 30		Day 90		Day 178	
	0.05%	0.10%	0.05%	0.10%	0.05%	0.10%
Plasma	0.81 ± 0.32	2.00 ± 0.47	1.04 ± 0.43	1.67 ± 0.44	0.98 ± 0.28	2.00 ± 0.60

No dose proportionality in drug concentrations was detected in ocular tissues. Quantifiable concentrations for both dose levels were represented in pigmented tissues with highest ocular concentrations seen in ICB followed by choroid, cornea and retina. The lowest concentrations were found in aqueous humor. Substantially high levels (100-250 μg/g) of Emedastine were detected in the periocular palpebral conjunctiva. The mean concentrations of Emedastine in pigmented tissues from 2 animals of each dose group are displayed in the following table.

Dose (%)	Tissue Mean Emaedastine Levels (μg/g) (N=2)					
	Choroid	ICB	Cornea	Retina	Aqueous Humor	Periocular Palpebral Conjunctiva
0.05	66.7	27.7	13.4	3.17	0.18	200
0.1	62.9	50.9	8.65	1.24	0.13	113

SUMMARY AND CONCLUSIONS

No clinical signs of toxicity were observed in monkeys following six-month q.i.d. ocular applications of Emedastine. Histopathological examination revealed dose-dependent increases in the incidences of corneal and scleral mononuclear cell infiltrations in Emedastine-treated monkeys. Although mild (Grade 1) scleral mononuclear cell infiltrations can spontaneously occur in cynomolgus monkeys, none of these changes were identified in the control group. Therefore, the moderate (Grade 2-3) and severe (Grade 4) severity of scleral infiltrates in Emedastine-treated animals might be drug-related. Toxicokinetic analysis of blood and ocular tissues showed that Emedastine was systemically available and measurable concentrations were present in pigmented tissues with highest ocular concentrations seen in ICB followed by choroid, cornea and retina.

Ocular pathological changes were not noted in the 3-month study. Inflammatory lesions (corneal and scleral mononuclear cell infiltrations) appeared after 6-month of ocular applications. Therefore, long term ocular applications of Emedastine may results in pathological changes in pigmented ocular tissues in human. It is recommendable to conduct Phase IV study and monitor patients treated with Emedastine for more than 6-months.

Josie Yang 2/3/97
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Concur by team leader: Yes ☒ No ☐ Conrad H. Chen 2-5-97
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