

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-861

PHARMACOLOGY REVIEW(S)

INTEROFFICE MEMO

TO: NDA 21861
FROM: C. Joseph Sun, Ph. D., Supervisory Pharmacologist,
Division of Pulmonary and Allergy Products
DATE: March 6, 2008

I concur with pharmacologist's recommendation that pharmacology and toxicology of olopatadine and its inactive ingredients have been adequately studied and evaluated. Therefore, the intranasal formulation of the product not containing providone (inactive ingredient) is recommended approval from a preclinical standpoint.

Pharmacology: The action of olopatadine is a typical antihistamine that blocks H₁ receptors as evidenced by *in vitro* receptor binding studies and *in vivo* allergic bronchospasm or histamine-induced bronchoconstriction animal models.

General toxicity: Chronic oral toxicity studies have been conducted with olopatadine in rats and dogs up to 52 weeks in duration. In rats, target organs of toxicity include the kidneys, heart, liver, eyes, urinary bladder and pancreas. In dogs, target organs of toxicity included the kidneys, spleen, liver, heart, bone marrow and eyes. Original intranasal formulation of olopatadine containing the inactive ingredient (providone) did not cause any notable toxicity in the 6-month rat and 9-month dog intranasal studies. However, the formulation was only tested for the first two months of the 6-month rat study. In a subsequent 6-month intranasal bridging study of providone in rats which is to further qualify for its chronic intranasal use, olfactory epithelial degeneration and respiratory turbinate epithelial vacuolization were observed at all the doses tested in a dose-responsive manner with regard to incidence and severity. As such, the safe use of the original drug product containing providone could not be rendered. The current drug product does not contain providone and therefore no longer has any safety issue.

Reproductive toxicity: Olopatadine did impair fertility in rats. Although it was not teratogenic in rats and rabbits, fetal deaths at birth in rats and rabbits and decreased pup survival after delivery in rats were observed. Therefore, pregnancy category C is appropriate.

Geotoxicity: Olopatadine was not genotoxic in the standard battery of assays (Ames test, chromosome aberration assay in Chinese hamster lung cells and *in vivo* mouse micronucleus test).

Carcinogenicity: In two oral carcinogenicity studies in mice and rats, olopatadine did not induce any tumors.

Labeling: Carcinogenesis, mutagenesis and impairment of fertility section, animal toxicology section and pregnancy section should be revised as recommended to incorporate the above-mentioned nonclinical findings of olopatadine.

Outstanding preclinical issue: There is no outstanding preclinical safety issue regarding providone in the previous formulation as the new drug product (formulation) does not contain providone.

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

/s/

Joseph Sun
3/6/2008 04:30:13 PM
PHARMACOLOGIST



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: **21-861**
SERIAL NUMBER: **N-000-AZ**
DATE RECEIVED BY CENTER: **September 27, 2007**
PRODUCT: **PATANASE® Nasal Spray**
(olopatadine hydrochloride)
INTENDED CLINICAL POPULATION: **Adults and children 12 years of age and older with**
seasonal allergic rhinitis
SPONSOR: **Alcon Inc./Alcon Research, Ltd**
DOCUMENTS REVIEWED: **Module 1, Vol. 1.01, Module 2, Vol. 1.1-Vol 1.6,**
Module 4, Vol. 1.1- Vol 1.20
REVIEW DIVISION: **Division of Pulmonary and Allergy Products**
PHARM/TOX REVIEWER: **Jean Q. Wu, MD, PhD**
PHARM/TOX SUPERVISOR: **C. Joseph Sun, PhD**
DIVISION DIRECTOR: **Badrul Chowdhury, MD, PhD**
PROJECT MANAGER: **Miranda Raggio**

Date of review submission to Division File System (DFS): March 4, 2008

TABLE OF CONTENTS

| | |
|---|-----------|
| EXECUTIVE SUMMARY | 3 |
| 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW | 5 |
| 2.6.1 INTRODUCTION AND DRUG HISTORY..... | 5 |
| 2.6.2 PHARMACOLOGY..... | 10 |
| 2.6.2.1 Brief summary | 10 |
| 2.6.2.2 Primary pharmacodynamics | 10 |
| 2.6.2.3 Secondary pharmacodynamics | 10 |
| 2.6.2.4 Safety pharmacology | 10 |
| 2.6.2.5 Pharmacodynamic drug interactions..... | 10 |
| 2.6.3 PHARMACOLOGY TABULATED SUMMARY..... | 10 |
| 2.6.4 PHARMACOKINETICS/TOXICOKINETICS | 10 |
| 2.6.4.1 Brief summary | 10 |
| 2.6.4.2 Methods of Analysis:..... | 10 |
| 2.6.4.3 Absorption | 10 |
| 2.6.4.4 Distribution..... | 10 |
| 2.6.4.5 Metabolism | 10 |
| 2.6.4.6 Excretion..... | 10 |
| 2.6.4.7 Pharmacokinetic drug interactions..... | 10 |
| 2.6.4.8 Other Pharmacokinetic Studies..... | 10 |
| 2.6.4.9 Discussion and Conclusions | 11 |
| 2.6.4.10 Tables and figures to include comparative TK summary | 11 |
| 2.6.6 TOXICOLOGY | 11 |
| 2.6.6.1 Overall toxicology summary | 11 |
| 2.6.6.2 Single-dose toxicity | 11 |
| 2.6.6.3 Repeat-dose toxicity | 11 |
| 2.6.6.4 Genetic toxicology..... | 11 |
| 2.6.6.5 Carcinogenicity..... | 11 |
| 2.6.6.6 Reproductive and developmental toxicology..... | 11 |
| 2.6.6.7 Local tolerance | 11 |
| 2.6.6.8 Special toxicology studies | 11 |
| 2.6.6.9 Discussion and Conclusions | 11 |
| 2.6.6.10 Tables and Figures | 11 |
| 2.6.7 TOXICOLOGY TABULATED SUMMARY | 11 |
| OVERALL CONCLUSIONS AND RECOMMENDATIONS..... | 11 |
| APPENDIX 1..... | 16 |

EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: Approval
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling: See the suggested labeling changes at the end of the current review.

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings

In this NDA resubmission, no additional pharmacology and toxicology studies of the drug product were submitted.

The preclinical pharmacology and toxicology evaluation of olopatadine was reviewed in the original NDA. Repeated oral dosing studies have been conducted with olopatadine in rats and dogs for up to 52 weeks. Target organ of toxicity included the kidneys, heart, lungs, liver, eyes, urinary bladder, lymph nodes and pancreas in rats, and kidneys, liver, heart, spleen, bone marrow and eyes in dogs. Antimuscarinic effects such as mydriasis were commonly observed after treatment with this antihistaminic drug.

Olopatadine was not mutagenic or carcinogenic. It was not teratogenic in rats and rabbits. A decrease in the fertility index and reduced implantation rate was observed in rats. Based on the findings of the decrease in the number of live fetuses in rats and rabbits and the reduced viability of pups after delivery in rats, olopatadine is considered to be labeled a pregnancy category C. Olopatadine is excreted in the milk of nursing mothers in rats. It also crosses the placental barrier and distributed to the fetuses in rats.

Olopatadine in the original clinical formulation containing povidone did not cause any notable toxicity in 6-month rat and 9-month dog intranasal studies while the formulation containing povidone was tested only for the first two months of the 6-month rat study. The NOAEL identified in the 6-month rat intranasal study of olopatadine yielded a safety margin of 1 based on the AUC ratio of the NOAEL to that of the proposed human dose, and a safety margin of 2 for local effects based on the nasal surface area. The NOAEL identified in the 9-month dog intranasal study of olopatadine yielded a safety margin of 18 based on the AUC ratio of the NOAEL to that of the proposed human dose, and a safety margin of 3 for local effects based on the nasal surface area.

In the original NDA review, the preclinical safety issue relevant to clinical use was the local toxicity of the excipient, povidone, since the NOAEL could not be identified in the 6-month intranasal rat bridging study of povidone for its intranasal use. As povidone has been removed from the current clinical formulation, the preclinical safety issue is considered resolved.

B. Pharmacologic activity

Olopatadine is an antihistamine that blocks H₁ receptors competitively and possesses anti-allergic activities as evidenced by in vitro receptor binding studies and in vivo guinea pig and/or rat animal models.

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

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 21-861

Review number: 001

Sequence number/date/type of submission: 000-AZ/September 26, 2007/Resubmission

Information to sponsor: Yes (X) No ()

Sponsor and/or agent:

Alcon Inc.
P.O. Box 62
Bosch 69
CH-6331 Hunenberg, Switzerland

Alcon Research, Ltd.
6201 South Freeway
Forth Worth, TX 76234-2009

Manufacturer for drug substance:

Alcon Manufacturing, Ltd.
Cusi Manufacturing
6201 South Freeway
Forth Worth, TX 76134-2099

(b) (4)



Reviewer name: Jean Q. Wu

Division name: Division of Pulmonary and Allergy Drug Products

HFD #: 570

Review completion date: March 4, 2008

Drug:

Trade name: Patanase® Nasal Spray

Generic name: Olapatadine HCl Nasal Spray

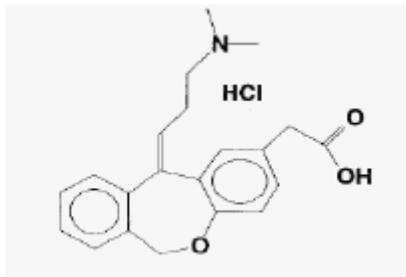
Code name: AL-4943A

Chemical name: (Z)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrobenz[b,e]-oxepine-2-acetic acid hydrochloride

CAS registry number: 140462-76-6

Molecular formula/molecular weight: C₂₁H₂₃NO₃.HCl/373.88

Structure:



Relevant INDs/NDAs/DMFs: IND (b) (4) & NDA 20-688, IND 60,116, IND 60,991, DMFs (b) (4)

Intended clinical population: Patients with seasonal allergic rhinitis at age of 12 years and above

Clinical formulation: The product is the topical aqueous nasal solution, which contains olopatadine hydrochloride 0.665% w/v or 0.6% w/b as base. The current proposed formulation and the formulation in the original NDA are listed in the table below (excerpted from Module 2, Vol. 2. Section 2.4, Page 2).

The proposed dosing will be two sprays (100 µL/spray, 0.6% olopatadine)/nostril BID resulting in a total dose of 4.8 mg olopatadine/day (800 µL/day).

Table 2.4.1-1

Compositions of PATANASE NDA Formula (FID^a: 103718) and Proposed Reformulation (FID: 109941)

| Components | NDA Formulation ((b) PVP) FID (b) (4) | Proposed Reformulation (PVP-free) FID 109941 |
|---|--|--|
| | % w/v | % w/v |
| Olopatadine Hydrochloride | 0.665 ^b | Same |
| Benzalkonium Chloride ^c | (b) (4) | (b) (4) |
| Edetate Disodium ^d | (b) (4) | (b) (4) |
| Povidone (PVP) | (b) (4) | (b) (4) |
| Sodium Chloride | (b) (4) | (b) (4) |
| Dibasic Sodium Phosphate ^e | (b) (4) | (b) (4) |
| Hydrochloric Acid and/or Sodium Hydroxide | (b) (4) | (b) (4) |
| Purified Water | (b) (4) | (b) (4) |

^aFormulation identification number.

^b0.665% w/v olopatadine hydrochloride (665 mcg/spray) is equivalent to 0.6% w/v olopatadine as base (600 mcg/spray).

^cAn equivalent amount of benzalkonium chloride solution may be used in the manufacture of the drug product.

^dEdetate disodium, dihydrate is used.

^eDibasic sodium phosphate, anhydrous is used.

Route of administration: Intranasal Spray

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

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-861 are owned by Alcon Inc. or are data for which Alcon has obtained a written right of reference. Any information or data that necessary for approval of NDA 21-861 that Alcon does not own or have a written right of reference constitutes one of the followings: 1. published literature, or 2. a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling.

Studies reviewed within this submission:

The following studies were reviewed in the Chemistry Consultation.

26-Week Repeated Subcutaneous Dose Carcinogenicity Study In p53^{+/-} Mice with A Toxicokinetic Study in C57BL/6 Mice with (b) (4) (TDOC-0002519, TDOC-0003380 TK)

26-Week Repeated Subcutaneous Dose Carcinogenicity Study In p53^{+/-} Mice with A Toxicokinetic Study in C57BL/6 Mice with (b) (4) (TDOC-0002455, TDOC-0003379TK)

28-Day Repeated Dose Mechanistic Subcutaneous Toxicity Study in C57BL/6 Mice of (b) (4) (TDOC-0002104, TDOC-0002884 TK)*

28-Day Repeated Dose Mechanistic Subcutaneous Toxicity Study in C57BL/6 Mice with a Preliminary Rangefinding Toxicity Study of (b) (4) and (b) (4) (TDOC-0001781, TDOC-0002294 TK)*

* Studies reviewed only for dose selection purpose used in the corresponding 26-week carcinogenicity studies in p53^{+/-} mice.

Studies not reviewed within this submission:

A. The following study was submitted under the original NDA 21-861. The amended study report was submitted in current submission. As the revisions (due to the omission of a single page of clinical pathology results in the final report) made in the amended report had no impact on data interpretation or study conclusion, the amended report was not reviewed further.

9-Month Intranasal Toxicity Study of (b) (4) Nasal Spray (0.1 and 0.2%) in Dogs (TDOC-0000295)

B. The following studies are either non-GLP compliant/non-relevant pilot studies, studies which were related to CMC but not requested by the CMC for consultation, or the updated method validation reports which were not reviewed originally in the previous NDA submission.

3-Month Intranasal Toxicity Study of Olopatadine Hydrochloride (0.6%) Nasal Spray Container Closure System Leachates in Rats (TDOC-0001788) [note: updated report. The original report was not reviewed in the original NDA submission.]

3-Month Intranasal Toxicity Study in Male Rats with 1-Month Recovery Period: Samples Evaluated Include Olopatadine Hydrochloride 0.6% Nasal Spray Samples Aged for 2 Years Under Several Storage Conditions and a Marketed Control (TDOC-0002456)

6-Month Intranasal Povidone (PVP) Qualification Study in Rats (TDOC-0004098).

14-Day (Non-GLP) Repeated Dose Mechanistic Oral Toxicity Study in C57BL/6 Mice With a Preliminary Rangefinding Toxicity Study of (b) (4) (TDOC-0001782, TDOC-0002376 TK)

Analytical Methods and Validation Reports (Updated with storage stability data)

Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of olopatadine (AL-4943) in rat plasma at (b) (4) (TDOC-0006430)

Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of olopatadine (AL-4943) and its N-desmethyl (M1) and N-oxide (M3) metabolites in rat plasma at (b) (4) (TDOC-0002090)

Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of AL-4943, AL-24956, AL-38244 and AL-38189 in dog plasma at (b) (4) (TDOC-0001277)

Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of (b) (4) in rat plasma at (b) (4) (TDOC-0002083)

Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of (b) (4) in mouse plasma at (b) (4) (TDOC-0002087)

Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of (b) (4) in rat plasma at (b) (4) (TDOC-0002088)

Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of (b) (4) in mouse plasma at (b) (4) (TDOC-0002089)

C. Part of the micronucleus tests in the following studies of (b) (4) and (b) (4) were not evaluated since the 26-week carcinogenic studies of (b) (4) and (b) (4) in p53^{+/-} Mice were evaluated.

28-Day Repeated Dose Mechanistic Subcutaneous Toxicity Study in C57BL/6 Mice with a Preliminary Range-finding Toxicity Study of (b) (4) and (b) (4) (TDOC-0001781, TDOC-0002457) [note: The micronucleus analysis were planned in the study but was not performed due to deviation. A subsequent study TDOC-0002457 was performed only to correct the deviation. No separate report for TDOC-0002457 was submitted.]

28-Day Repeated Dose Mechanistic Subcutaneous Toxicity Study in C57BL/6 Mice of (b) (4) (TDOC-0002104, TDOC-0002884 TK)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Refer to the original NDA 21-861 review.

2.6.2.2 Primary pharmacodynamics

Refer to the original NDA 21-861 review.

2.6.2.3 Secondary pharmacodynamics

Refer to the original NDA 21-861 review.

2.6.2.4 Safety pharmacology

Refer to the original NDA 21-861 review.

2.6.2.5 Pharmacodynamic drug interactions

N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Refer to the original NDA 21-861 review.

2.6.4.2 Methods of Analysis:

N/A

2.6.4.3 Absorption

Refer to the original NDA 21-861 review.

2.6.4.4 Distribution

Refer to the original NDA 21-861 review.

2.6.4.5 Metabolism

Refer to the original NDA 21-861 review.

2.6.4.6 Excretion

Refer to the original NDA 21-861 review.

2.6.4.7 Pharmacokinetic drug interactions

Refer to the original NDA 21-861 review.

2.6.4.8 Other Pharmacokinetic Studies

N/A

2.6.4.9 Discussion and Conclusions

N/A

2.6.4.10 Tables and figures to include comparative TK summary

N/A

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.6 TOXICOLOGY**2.6.6.1 Overall toxicology summary**

Refer to the original NDA 21-861 review.

2.6.6.2 Single-dose toxicity

Refer to the original NDA 21-861 review.

2.6.6.3 Repeat-dose toxicity

Refer to the original NDA 21-861 review.

2.6.6.4 Genetic toxicology

Refer to the original NDA 21-861 review.

2.6.6.5 Carcinogenicity

Refer to the original NDA 21-861 review.

2.6.6.6 Reproductive and developmental toxicology

Refer to the original NDA 21-861 review.

2.6.6.7 Local tolerance

Refer to the original NDA 21-861 review

2.6.6.8 Special toxicology studies

N/A

2.6.6.9 Discussion and Conclusions

Refer to the original NDA 21-861 review

2.6.6.10 Tables and Figures

N/A

2.6.7 TOXICOLOGY TABULATED SUMMARY

N/A

OVERALL CONCLUSIONS AND RECOMMENDATIONS

In this NDA resubmission, no additional pharmacology and toxicology studies of the drug product were submitted.

The preclinical pharmacology and toxicology evaluation of olopatadine was reviewed in the original NDA submission by Dr. Gary Bond (dated August 26, 2005, see Appendix 1). Repeated oral dosing studies have been conducted with olopatadine in rats and dogs for up to 52 weeks. Target organ of toxicity included the kidneys, heart, lungs, liver, eyes, urinary bladder, lymph nodes and pancreas in rats, and kidneys, liver, heart, spleen, bone marrow and eyes in dogs. Antimuscarinic effects such as mydriasis were commonly observed after treatment with this antihistaminic drug.

Olopatadine administered orally was not carcinogenic in mice and rats at doses up to 500 mg/kg/day and 200 mg/kg/day, respectively. There was no evidence of genotoxicity when olopatadine was tested in an *in vitro* bacteria reverse mutation test (Ames), an *in vitro* mammalian chromosome aberration assay or an *in vivo* mouse micronucleus test.

Based on NDA 20-688 review, olopatadine orally administered to male and female rats reduced the implantation rate and resulted in a decrease in the fertility index at a dose of 400 mg/kg/day. However, the fertility was not affected at oral dose of 50 mg/kg/day in rats. Olopatadine was not teratogenic in rabbits and rats at oral doses up to 400 or 600 mg/kg/day, respectively. However, the decrease in the number of live fetuses was observed in rabbits at doses of 25 mg/kg and above and in rats at doses of 60 mg/kg and above, which were not clearly specified in the original NDA 21-861 review and the label of Patanol®. The reduced viability of pups after delivery in rats treated with olopatadine at dose of 60 mg/kg/day and above, but was not observed at dose of 20 mg/kg/day. Therefore, due to the finding of the decrease in the number of live fetuses in rabbits and rats and a decrease in the viability of fetuses after delivery in rats, olopatadine is considered to be labeled a pregnancy category C. Olopatadine is excreted in the milk of nursing mothers in rats. It also crosses the placental barrier and distributed to the fetuses in rats.

Olopatadine in the original formulation containing povidone did not cause any notable toxicity in 6-month rat and 9-month dog intranasal studies while the formulation containing povidone was tested only for the first two months of the 6-month rat study. The NOAEL (0.4 mg/day, dosed in a single nostril) identified in the 6-month intranasal rat study of olopatadine yielded a safety margin of 1 based on the AUC ratio of the NOAEL (79 ng.h/mL) to that of the proposed recommended human dose (78 ng.h/mL), and a safety margin of 2 for local effects based on the nasal surface area (rat: 0.057 mg/cm², human: 0.03 mg/cm²). The NOAEL identified in the 9-month dog intranasal study of olopatadine yielded a safety margin of 18 based on the AUC ratio of the NOAEL (1370 ng.h/mL) to that of the proposed recommended human dose (78 ng.h/mL), and a safety margin of 3 for local effects based on nasal surface area (dog: 0.08 mg/cm², human: 0.03 mg/cm²).

In the original NDA, the preclinical safety issue relevant to clinical use was the local toxicity of the excipient, povidone, since the NOAEL could not be identified in the 6-

month intranasal rat bridging study of povidone for its intranasal use. As povidone has been removed from the current clinical formulation, the preclinical safety issue is considered resolved.

Therefore, olopatadine in the current formulation not containing povidone is considered safe for human use at the proposed recommended human dose.

Recommendations: From a preclinical perspective, approval is recommended for the application.

Suggested labeling:

1. Section 8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C: There were no teratogenic effects in either rats or rabbits at oral olopatadine dosages of up to 600 or 400 mg/kg/day, respectively (approximately 1,000 and 1,300 times the maximum recommended daily intranasal dose in adults on a mg/m² basis).

No adequate and well-controlled studies in pregnant women have been conducted. PATANASE[®] Nasal Spray should be used in pregnant women only if the potential benefit to the mother justifies the potential risk to the fetus.

Reviewer's comment: Although there were no teratogenic effects, a decrease in the number of live fetuses was observed in rabbits and rats and the reduced viability of pups after delivery was observed in rats. Based on these findings, a pregnancy category C was labeled. Therefore, the findings should be listed as suggested below.

Proposed labeling:

8.1 Pregnancy

Pregnancy Category C: No adequate and well-controlled studies in pregnant women have been conducted. Animal reproductive studies in rats and rabbits revealed treatment-related effects on fetuses or pups. Because animal studies are not always predictive of human responses, PATANASE[®] Nasal Spray should be used in pregnant women only if the potential benefit to the mother justifies the potential risk to the embryo or fetus.

A decrease in the number of live fetuses was observed in rabbits and rats at the oral olopatadine doses approximately 88 times and 100 times the MRHD and above, respectively, for adults on a mg/m² basis. In rats, viability and body weights of pups were reduced on day 4 post partum at the oral dose approximately 100 times the MRHD for adults on a mg/m² basis, but no effect on viability was observed at the dose approximately 35 times the MRHD for adults on a mg/m² basis.

2. Section 10 OVERDOSAGE

(b) (4)

Reviewer's comment: The related animal information should be added in this section when the human overdose is undetermined.

Proposed labeling:

10 OVERDOSAGE

(b) (4)

No mortality was observed in rats at intranasal dose of 3.6 mg/kg (approximately 6 times the MRHD for adults on a mg/m² basis), or in dogs at oral dose of 5 g/kg (approximately 28,000 times the MRHD for adults on a mg/m² basis). The oral median lethal dose (MLD) in mice and rats were 1,490 mg/kg and 3,870 mg/kg, respectively (approximately 1,235 times and 6,500 times the MRHD for adults on a mg/m² basis, respectively).

3. Section 12.1 Mechanism of Action

(b) (4)

Reviewer's comment: It is not appropriate to make specific statements in the label based on the literature references which were not subject to review in the NDA submission. Suggest remove the statements and the corresponding references in Section 15 References, or use the data in the NDA submission to support the statements.

Proposed labeling:

(b) (4)

4. Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Reviewer's comment: Based on the review of NDA 20-688 and the label of Patanol®, the impairment of fertility was observed in male and female rats at dose of 400 mg/kg but was not observed at dose of 50 mg/kg. Therefore, the label about impairment of fertility should be modified as proposed below.

Proposed labeling:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Olopatadine administered orally was not carcinogenic in mice and rats at doses of up to 500 mg/kg/day and 200 mg/kg/day, respectively (approximately 420 and 340 times the MRHD for adults by intranasal administration on a mg/m² basis, respectively).

There was no evidence of genotoxicity when olopatadine was tested in an *in vitro* bacteria reverse mutation test (Ames), an *in vitro* mammalian chromosome aberration assay or an *in vivo* mouse micronucleus test.

Olopatadine administered orally to male and female rats at dose of 400 mg/kg/day (approximately 680 times the MRHD for adults on a mg/m² basis) resulted in a decrease in the fertility index and reduced implantation rate. No effects on fertility were observed at dose of 50 mg/kg/day (approximately 85 times the MRHD for adults on a mg/m² basis).

5. Section 13.2 Pharmacology

(b) (4)

Reviewer's comment:

It is not appropriate to include the pharmacology information based on the literature reference in Section 13.2 under the Section 13 Nonclinical Toxicology. Suggest remove the pharmacology information in Section 13.2 and the corresponding reference in Section 15 References.

For the preclinical reproductive toxicology studies, based on the review of NDA 20-688, the decrease in the number of live fetuses was observed in rabbits at doses of 25 mg/kg and above, and in rats at doses of 60 mg/kg and above, which were not clearly specified in the original NDA 21-861 review and the label of Patanol®. Suggest add the reproductive toxicology data in this section with a modified subtitle as proposed below.

Proposed labeling:**13.2 Animal Toxicology**

Reproductive Toxicology Studies

Olopatadine was not teratogenic in rabbits and rats at oral doses of up to 400 or 600 mg/kg/day, respectively (approximately 1,400 and 1,000 time the MRHD for adults on a mg/m² basis). However, a decrease in the number of live fetuses was observed in rabbits at the oral doses of 25 mg/kg (approximately 88 times the MRHD for adults on a mg/m² basis) and above, and in rats at oral doses of 60 mg/kg (approximately 100 times the MRHD for adults on a mg/m² basis) and above. In rats, viability and body weights of pups were reduced on day 4 post partum at the oral doses of 60 mg/kg (approximately 100 times the MRHD for adults on a mg/m² basis) and above, but no effect on viability was observed at the dose of 20 mg/kg (approximately 35 times the MRHD for adults on a mg/m² basis).

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX 1

Original Review of NDA 21-861



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-861
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 12/27/2004
PRODUCT: Patanase® Nasal Spray
INTENDED CLINICAL POPULATION: Seasonal ^{(b) (4)} allergic rhinitis
(≥ 12 years old)
SPONSOR: Alcon Research, Ltd.
DOCUMENTS REVIEWED: Vol. 1-45 (module 4)
REVIEW DIVISION: Division of xxx Drug Products (HFD-570)
PHARM/TOX REVIEWER: Gary P. Bond, Ph.D., DABT
PHARM/TOX SUPERVISOR: C. Joseph Sun, Ph.D.
DIVISION DIRECTOR: Badrul A. Chowdhury, M.D., Ph.D.
PROJECT MANAGER: Anthony Zeccola
Date of review submission to Division File System (DFS): August 25, 2005

TABLE OF CONTENTS

| | |
|---|-----------|
| EXECUTIVE SUMMARY | 3 |
| 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW | 5 |
| 2.6.1 INTRODUCTION AND DRUG HISTORY..... | 5 |
| 2.6.2 PHARMACOLOGY..... | 8 |
| 2.6.2.1 Brief summary | 23 |
| 2.6.2.2 Primary pharmacodynamics | 24 |
| 2.6.2.3 Secondary pharmacodynamics | 24 |
| 2.6.2.4 Safety pharmacology | 24 |
| 2.6.2.5 Pharmacodynamic drug interactions..... | 25 |
| 2.6.3 PHARMACOLOGY TABULATED SUMMARY..... | 25 |
| 2.6.4 PHARMACOKINETICS/TOXICOKINETICS | 25 |
| 2.6.4.1 Brief summary | 25 |
| 2.6.4.2 Methods of Analysis | 26 |
| 2.6.4.3 Absorption | 26 |
| 2.6.4.4 Distribution..... | 27 |
| 2.6.4.5 Metabolism | 28 |
| 2.6.4.6 Excretion..... | 28 |
| 2.6.4.7 Pharmacokinetic drug interactions..... | 28 |
| 2.6.4.8 Other Pharmacokinetic Studies..... | 28 |
| 2.6.4.9 Discussion and Conclusions | 28 |
| 2.6.4.10 Tables and figures to include comparative TK summary | 28 |
| 2.6.5 PHARMACOKINETICS TABULATED SUMMARY..... | 28 |
| 2.6.6 TOXICOLOGY | 28 |
| 2.6.6.1 Overall toxicology summary | 28 |
| 2.6.6.2 Single-dose toxicity | 34 |
| 2.6.6.3 Repeat-dose toxicity | 36 |
| 2.6.6.4 Genetic toxicology..... | 48 |
| 2.6.6.5 Carcinogenicity..... | 48 |
| 2.6.6.6 Reproductive and developmental toxicology..... | 48 |
| 2.6.6.7 Local tolerance | 49 |
| 2.6.6.8 Special toxicology studies | 50 |
| 2.6.6.9 Discussion and Conclusions | 50 |
| 2.6.6.10 Tables and Figures..... | 55 |
| 2.6.7 TOXICOLOGY TABULATED SUMMARY | 55 |
| OVERALL CONCLUSIONS AND RECOMMENDATIONS..... | 55 |
| APPENDIX/ATTACHMENTS | 59 |

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Not approvable based on no identified NOAEL for local toxicities of excipient Povidone thereby not allowing a safety assessment of this excipient for intranasal use in humans.

B. Recommendation for nonclinical studies

Qualify the excipient Povidone by conducting a 6-month intranasal rat study to identify a NOAEL that would provide an adequate safety margin for the clinical formulation.

C. Recommendations on labeling

Suggested labeling to include animal to human dose ratios compared on a mg/m² basis in Carcinogenesis Mutagenesis and Impairment of Fertility, Pregnancy Category, and Overdosage sections of the label.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Repeated oral dosing studies have been conducted with olopatadine in rats and dogs for up to 52 weeks in duration. In rats, target organs of toxicity include the kidneys, heart, lungs, liver, eyes, urinary bladder, lymph nodes and pancreas. In dogs, target organs of toxicity include the kidneys, spleen, liver, heart, bone marrow, and eyes. Antimuscarinic effects such as mydriasis were commonly observed after treatment with this antihistaminic drug.

Olopatadine did not cause any notable toxicity in 6-month rat and 9-month dog intranasal studies although the formulation that contained the excipient Povidone was only tested for the first two months of the 6-month rat study. In the rat at the NOAEL, the highest dose tested, the AUCs ratio of the rat NOAEL dose to that of the proposed human dose is 1 with a local safety margin of 2 for intranasal effects based on dose of olopatadine per nasal surface area. In the dog at the NOAEL, the highest dose tested, the AUCs ratio of the dog NOAEL to that of the proposed human dose is 18 with a local safety margin of 3 for intranasal effects based on dose of olopatadine per nasal surface area.

Olopatadine was not mutagenic or carcinogenic. It was not teratogenic and did not impair fertility. On the basis of the decrease in the number of live fetuses in rats and rabbits and a decrease in the viability of pups after delivery in rats, olopatadine should be labeled a pregnancy category C. Olopatadine is excreted in the milk of nursing

mothers in rats. It is also crosses the placental barrier and distributed to the fetuses in rats.

In the 6-month intranasal bridging study in rats for the excipient Povidone, olfactory epithelial degeneration and respiratory turbinate epithelial vacuolation were observed at high incidence with some marked severity in Povidone treated groups in a dose-responsive manner at both doses tested [REDACTED] ^{(b) (4)}. As a result, there was no NOAEL identified. Thus, a safety assessment of intranasal use of Povidone cannot be conducted.

B. Pharmacologic activity

Olopatadine is an antihistamine that blocks H₁ receptors competitively and possesses anti-allergic activities as evidenced by *in vitro* receptor binding studies and *in vivo* guinea pig and/or rat animal models.

C. Nonclinical safety issues relevant to clinical use

Based on the results of the 6-month rat bridging study that showed the excipient caused local nasal effects and no NOAEL was identified, Povidone is not considered safe to use as an excipient in the proposed formulation of olopatadine hydrochloride nasal spray.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-861

Review number: 1

Sequence number/date/type of submission: 000/December 24, 2004/original

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: Alcon Inc., P.O. Box 62, Bosch 69, CH-6331 Hunenberg,
Switzerland
Alcon Research, Ltd., 6201 South Freeway, Fort Worth, TX
76234-2009

Manufacturer for drug substance: [REDACTED] (b) (4)

Reviewer name: Gary P. Bond, Ph.D., DABT

Division name: Division of Pulmonary and Allergy Drug Products

HFD #: 570

Review completion date: August 25, 2005

Drug:

Trade name: Patanase® Nasal Spray

Generic name: Olopatadine Hydrochloride Nasal Spray

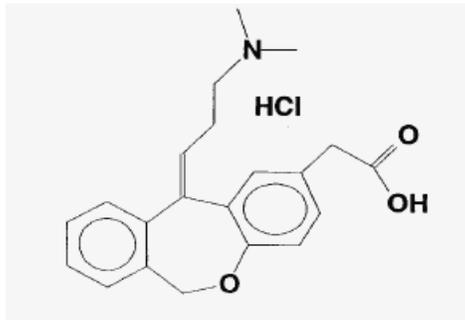
Code name: AL-4943A

Chemical name: (Z)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrobenz[b,e]-oxepine-2-acetic acid hydrochloride

CAS registry number: 140462-76-6

Molecular formula/molecular weight: C₂₁H₂₃NO₃.HCl/373.88

Structure:



Relevant INDs/NDAs/DMFs: IND (b) (4) & NDA 20-688 (Patanase®), IND 60116 (Patanol®), IND 60991, DMFs [REDACTED] (b) (4)

Drug class: antihistamine.

Intended clinical population: ≥ 12 years old for seasonal [REDACTED] (b) (4) allergic rhinitis

Clinical formulation: The topical aqueous nasal solution contains olopatadine hydrochloride (0.665% W/v; 0.6% w/v as base), benzalkonium chloride (0.01% w/v), edentate sodium (b) (4) (b) (4)/Povidone® (b) (4) w/v, sodium chloride (b) (4) dibasic sodium phosphate (b) (4) and purified water (b) (4). Sodium hydroxide and hydrochloric acid are added to adjust pH to target of (b) (4). Dosing will be two 100 ul sprays/nostril BID resulting in a total dose 800 ul with a total daily dose of olopatadine of 4.8 mg.

Route of administration: intranasal

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

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-861 are owned by Alcon or are data for which Alcon has obtained a written right of reference. Any information or data necessary for approval of NDA 21-861 that Alcon does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling.

Studies reviewed within this submission:

PHARMACOKINETICS

ABSORPTION

Hasegawa Y, Ohishi T, Kobayashi H, Kobayashi S. Disposition of (b) (4)-4679NS (6): Pharmacokinetics of (b) (4)-4679 in male rats after single administration of (b) (4)-4679 nasal formulation. English Translation of Japanese Report. (b) (4) Technical Report No. 98-477.

Hasegawa Y, Ohishi T, Kobayashi H, Kobayashi S. Disposition of (b) (4)-4679: Pharmacokinetics of (b) (4)-4679 in dogs after nasal or intravenous administration of (b) (4)-4679 nasal formulation or (b) (4)-4679. English Translation of Japanese Report. (b) (4) Technical Report No. 98-599.

Chastain JE. Plasma concentrations of olopatadine and metabolites in toxicology study N-01-059: A 9-month intranasal toxicity study of (b) (4) nasal spray (0.1 and 0.2%) in dogs. Fort Worth (TX): Alcon Research, Ltd. 2004 May. Technical Report No.: TDOC-0000228.

Chastain JE. Plasma concentrations of olopatadine and metabolites in toxicology study N-03-088: A 9-month intranasal toxicity study of olopatadine hydrochloride (0.6% and 1.5%) nasal spray in dogs. Fort Worth (TX): Alcon Research, Ltd. 2004 Oct. Technical Report No.: TDOC-0001616.

DISTRIBUTION

Roy AK. Distribution of radioactivity in the respiratory tree following intranasal administration of ^{14}C -olopatadine in dogs. Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0001741.

TOXICOLOGY

SINGLE DOSE TOXICITY

Goldenthal E. Acute intranasal toxicity study in rats. Alcon Research, Ltd. 2000 Aug. Technical Report No.: 097:30:0400.

REPEAT DOSE TOXICITY

(b) (4) Research on the safety of (b) (4) 4679: Four week, repeated dose, oral administration toxicity study in dogs. Final Report (b) (4)

(b) (4) 9-month intranasal toxicity study of (b) (4) nasal spray (0.1 and 0.2%) in dogs. Alcon Research Ltd., 2004 Jun. Technical Report No.: TDOC-0000295.

(b) (4) 9-month intra-nasal toxicity study of olopatadine hydrochloride nasal spray (up to 1.5%) in dogs. Alcon Research Ltd., 2004 Nov. Technical Report No.: TDOC-0001779.

(b) (4) 6-month intranasal bridging toxicity study in rats to qualify Povidone as an excipient in intranasal formulations. Alcon Research, Ltd., 2004 Nov. Technical Report No.: TDOC-0001794.

(b) (4) 2-week intranasal bridging study in dogs to qualify Povidone as an excipient in intranasal formulations. Alcon Research, Ltd., 2004 Sep. Technical Report No.: TDOC-0000689.

LOCAL TOLERANCE

(b) (4) Seventy two-hour ocular evaluation of olopatadine HCl nasal spray following single-dose administration in New Zealand white rabbits. Alcon Research, Ltd., 2004 Sep. Technical Report No.: TDOC-0001715.

ANTIGENICITY

(b) (4) A dermal sensitization study in guinea pigs with olopatadine HCl (maximization design). Final Report. Fort Worth (TX): Alcon Research, Ltd. 2000 Jun. Technical Report No.: 136:30:0600.

(b) (4) Toxicological study of (b) (4) 4679; antigenicity test of (b) (4) 4679. Final Report. (b) (4) 1990 July. Technical Report No.: A-90-43.

=====

Studies not reviewed within this submission: studies have been reviewed as part of NDA 20-688 (*) or IND 60116 (**), which are contained in the appendix

PRIMARY PHARMACODYNAMICS

(b) (4) Affinity and potency of (b) (4) -4679 (AL-4943A) for histamine receptor subtypes determined by receptor binding and phosphoinositide turnover techniques. Fort Worth (TX): Alcon Laboratories, Inc.; 1993 Oct. Technical Report No.: 005:39930:1093.

(b) (4) Evaluation of the anti-histaminic activity of AL-4943A clinical formulations. Fort Worth (TX): Alcon Laboratories, Inc.; 1993 Oct. Technical Report No.: 039:39900:1093.

(b) (4) Preclinical evaluation of the anti-histaminic efficacy of the proposed QD Patanol clinical formulation. Fort Worth (TX): Alcon Laboratories, Inc.; 2000 June. Technical Report No.: 033:32:0600.

(b) (4) AL-4943A (b) (4) -4679): Summary of preclinical pharmacology evaluation. Fort Worth (TX): Alcon Laboratories, Inc.; 1993 June. Technical Report No.: 017:39900:0693.

(b) (4) Biochemical characterization of (b) (4) 4679, an antihistaminic and antiallergic agent. Final Report. (b) (4) 1990 Nov. Technical Report No.: 90-131(Y).

(b) (4) Occupancy of histamine H1 receptors in guinea pigs after oral administration of (b) (4) 4679, an antiallergic agent. Final Report. (b) (4) 1989 Jan. Technical Report No. 89-72(Y).

SECONDARY PHARMACODYNAMICS

(b) (4) Olopatadine (AL-4943A): Ligand binding and functional studies on a novel, long-acting H1 selective histamine antagonist / anti-allergic agent for

use in allergic conjunctivitis. Fort Worth (TX): Alcon Laboratories, Inc.; 1995 Aug. Technical Report No.: 012:39730:0895.

(b) (4) NovaScreen AL-18876-01 and AL-24956A data. Fort Worth (TX): Alcon Laboratories, Inc.; 2001 Nov. Technical Report No.: 020:32:1101.

(b) (4) In vitro receptor binding profiles of ALO4943A (b) (4)-4679 and ALO3024 (ketotifen). Fort Worth (TX): Alcon Laboratories, Inc.; 1992 Aug. Technical Report No.: 030:39900:0892.

(b) (4) Occupancy of histamine H1 receptors in guinea pigs after oral administration of (b) (4)-4679, an antiallergic agent. Final Report. (b) (4) 1989 Jan. Technical Report No.: 89-72Y.

(b) (4) AL-18876 (olopatadine N-oxide): Effect on histamine release from human conjunctival mast cells. Fort Worth (TX): Alcon Laboratories, Inc.; 2000 Mar. Technical Report No.:012:32:0300.

(b) (4) Pharmacological properties of (b) (4)-4679 - Effects on passive cutaneous anaphylaxis in rats. Final Report. (b) (4) 1989 Aug. Technical Report No.: 89-111(Y).

(b) (4) Pharmacological properties of (b) (4)-4679 - Effects on bronchial anaphylactic reactions. (b) (4) 1989 Aug. Technical Report No.: 89-108(Y).

(b) (4). In vitro receptor binding profiles of ALO4943A (b) (4)-4679 and ALO3024 (ketotifen). Final Report. Fort Worth (TX): Alcon Laboratories, Inc.; 1992 Aug. Technical Report No.: 030:39900:0892.

(b) (4) Study of anticholinergic effect of (b) (4)-4679. Final Report. (b) (4) 1988 May. Technical Report No.: A-88-67.

(b) (4) Pharmacological properties of (b) (4)-4679 - Effects of (b) (4)-4679 on contraction of tracheal smooth muscle. Final Report. (b) (4) 1989 Aug. Technical Report No.: 89-110(Y).

(b) (4) Mouse Micronucleus Test Using (b) (4) and AL-4943A (Olopatadine). Final Report. Alcon Research, Ltd. 05-Dec-2003. Technical Report: TDOC-000653.

SAFETY PHARMACOLOGY

(b) (4) AL-4943A ((b) (4) -4679): Summary of preclinical pharmacology evaluation. Final Report. Fort Worth (TX): Alcon Laboratories, Inc.; 1993 Jun. Technical Report No.: 017:39900:0693

(b) (4) Effects of olopatadine hydrochloride on cloned hERG channels. Final Report. Alcon Research, Ltd. 2000 Aug. Technical Report No.: 137:30:0700.

(b) (4)
Pharmacological properties of (b) (4) -4679 - Effects on the central and peripheral nervous systems. Final Report. (b) (4)
1989 Sept. Technical Report No.: 89-121(Y).

(b) (4)
Pharmacological properties of (b) (4) -4679 - Effects of (b) (4) -4679 on the sleep-wakefulness cycle in cats. Final Report. (b) (4) 1990
Apr. Technical Report No.: 1156.

(b) (4)
Pharmacological properties of (b) (4) -4679 - Effects on respiratory and cardiovascular systems. Final Report. (b) (4)
1991 Mar. Technical Report No.: 91-58(Y).

(b) (4) 0. Effects of (b) (4) -4679 on cardiovascular system in dogs. Final Report. (b) (4) 1988 Jun. Technical Report No. A-88-73.

(b) (4) Effects of (b) (4) -4679 on electrocardiogram, heart rate, and blood pressure in conscious dogs. Final Report. (b) (4)
1995 Oct.

PHARMACODYNAMIC DRUG INTERACTIONS

(b) (4) Study on drug interaction (of olopatadine) in human liver microsomes. Final Report. Fort Worth (TX): Alcon Research, Ltd. 2000 Apr. Technical Report No.: 022:33:0400.

(b) (4) Effect of combination of (b) (4) -4679 and itraconazole on the ECG in conscious dogs. Final Report. (b) (4)
1999 Sept.

PHARMACOKINETICSANALYTICAL METHODS AND VALIDATION REPORTS

(b) (4) Radioimmunoassay of (b) (4)-4679 (olopatadine): Determination of (b) (4)-4679 in rat and dog plasma (b) (4) TR No., 89-103(Y)). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 067:38570:0995.

(b) (4) Radioimmunoassay of (b) (4)-4679 (olopatadine): Determination of (b) (4)-4679 and (b) (4) 13452 in rat plasma by the radioimmunoassay combined with HPLC (b) (4) TR No. 90-43(Y)). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 068:38570:0995.

(b) (4) Validation of Alcon Technical Procedure 33.8.019 for the determination of AL04943A (b) (4) 4679 in rabbit plasma by gas chromatography with mass selective detection. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Jul. Technical Report No.: 028:38570:0894.

(b) (4) Validation of Alcon Technical Procedure 33.8.019 for the determination of AL04943 (b) (4) 4679 in Cynomolgus monkey plasma by gas chromatography with mass selective detection. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 090:38570:1095.

(b) (4) Validation of an HPLC/MS/MS method for the determination of olopatadine and metabolites in dog plasma at (b) (4). Fort Worth (TX): Alcon Research, Ltd. 2000 Oct. Technical Report No.: 025:33:0600.

(b) (4) Validation of an HPLC/tandem mass spectrometry (HPLC/MS/MS) method for the determination of AL-4943, AL-24956, AL-38244 and AL-38189 in dog plasma at (b) (4) Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0001277.

(b) (4) Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of olopatadine and its N-desmethyl (M1) and N-oxide (M3) metabolites in rat plasma. Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0002090.

(b) (4) Validation of an HPLC/tandem mass spectrometry (HPLC/MS/MS) method for the determination of (b) (4) in rat plasma at (b) (4). Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0002083.

(b) (4) Validation of an HPLC tandem mass spectrometry (HPLC/MS/MS) method for the determination of (b) (4) in mouse plasma at (b) (4). Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0002087.

(b) (4) Validation of a gas chromatography mass spectrometry (GC/MS) method for the determination of (b) (4) in rat plasma at (b) (4). Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0002088.

(b) (4) Validation of a gas chromatography mass spectrometry (GC/MS) method for the determination of (b) (4) in mouse plasma at (b) (4) Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0002089.

ABSORPTION

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Pharmacokinetics of (b) (4)-4679 in rats (b) (4) Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 046:38570:0995.

(b) (4) Plasma pharmacokinetics of AL04943A following either a single topical ocular or intravenous dose to New Zealand white rabbits. Fort Worth (TX): Alcon Laboratories, Inc. 1996 Jan. Technical Report No.: 033:38570:0994.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Pharmacokinetics of (b) (4)-4679 in dogs (b) (4) Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 051:38570:0995.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Pharmacokinetics of (b) (4)-4679 in monkeys (b) (4) Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 052:38570:0995.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Comparison of (b) (4)-4679 pharmacokinetics in male and female rats (b) (4) Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 055:38570:0995.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Absorption, distribution, and excretion of C- (b) (4)-4679 in rats (b) (4) Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 043:38570:0995.

(b) (4) Pharmacokinetics of olopatadine, N-desmethyl olopatadine and olopatadine N-oxide in male beagle dogs following oral administration of olopatadine. Fort Worth (TX): Alcon Research, Ltd. 2001 Feb. Technical Report No.: 002:33:0100.

(b) (4) Pharmacokinetics of (b) (4)-4679 (olopatadine) in humans, (Phase I trial in West Germany) (b) (4) TR No. 89-30(Y)). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 071:38570:0995.

(b) (4) Retrospective toxicokinetic evaluation in support of the mammalian (mouse) erythrocyte micronucleus test of AL-4943A and (b) (4) Alcon Research, Ltd., 2004 Nov. Technical Report No.: TDOC-0001784.

DISTRIBUTION

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Absorption, distribution, and excretion of ^{14}C - (b) (4)-4679 in rats (b) (4) TR No. 94-243). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 043:38570:0995. (This report is provided in Section 4.2.2.2. Absorption).

(b) (4) Disposition of (b) (4)-4679 (olopatadine): *In vitro* protein binding of (b) (4)-4679 (b) (4) TR No. 94-644). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 056:38570:0995.

(b) (4). *In vitro* protein binding of (b) (4)-4679 (olopatadine) (b) (4) TR No. 89-88(Y)). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 069:38570:0995.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): *In vitro* protein binding of (b) (4)-4679 (b) (4) TR No. 90-73(Y)). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 070:38570:0995.

(b) (4). Disposition of (b) (4)-4679 (olopatadine): Transfer into the fetus in rats (b) (4) TR No. 93-246). Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 061:38570:0995.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Distribution of ^{14}C - (b) (4)-4679 administered in pregnant rats by whole-body autoradiography and radioimmunography (b) (4) TR No. 92-234). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 060:38570:0995.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Milk transfer of ^{14}C - (b) (4)-4679 after oral administration (b) (4) TR No. 94-106). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 062:38570:0995.

METABOLISM

(b) (4). Chromatographic profiles of radioactivity in plasma and urine following an oral dose of ^{14}C -olopatadine hydrochloride (AL-4943A) in healthy human male volunteers from study C-03-10. Fort Worth (TX): Alcon Research, Ltd. 2004 Dec. Technical Report No.: TDOC-0000808.

(b) (4) Disposition of (b) (4)-4679 (olopatadine): Effect of repeated oral administrations of (b) (4)-4679 to rats on the hepatic drug-metabolizing enzymes (b) (4) TR No. 93-3). Final Report. Fort Worth (TX): Alcon Laboratories, Inc. 1995 Dec. Technical Report No.: 059:38570:0995.

(b) (4) Study on drug interaction (by olopatadine) in human liver microsomes (b) (4) Report). Final Report. Fort Worth (TX): Alcon Research, Ltd. 2000 Apr. Technical Report No.: 022:33:0400.

OTHER PHARMACOKINETIC STUDIES

(b) (4). Olopatadine plasma concentrations from toxicology study N-00-034: A 14-day intranasal toxicity study of (b) (4) nasal spray (0.1 and 0.2%) in rats. Fort Worth (TX): Alcon Research, Ltd. 2002 Jun. Technical Report No.: 039:33:1000.

(b) (4). Olopatadine plasma concentrations from toxicology study N-01-004: A 6-month intranasal toxicity study of (b) (4) nasal spray (0.1 and 0.2%) in rats. Fort Worth (TX): Alcon Research, Ltd. 2003 Jan. Technical Report No.: 015:33:0402.

(b) (4) Plasma concentrations of olopatadine and metabolites in rat toxicology study N-03-093: A 3-month intranasal toxicity study of olopatadine hydrochloride (0.6%) nasal spray container closure system leachates. Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0001614.

(b) (4) Toxicokinetics of (b) (4) 4679 (Olopatadine) (I): Plasma concentrations of (b) (4) 4679 after repeated oral administration to rats for 7 days (b) (4) Report). Final Report. Fort Worth (TX): Alcon Research, Ltd. 2000 Jun. Technical Report No.: 019:33:0400.

(b) (4) Pharmacokinetics of olopatadine, N-desmethyl olopatadine and olopatadine N-oxide in male Beagle dogs following oral administration of olopatadine. Fort Worth (TX): Alcon Research, Ltd. 2001 Feb. Technical Report No.: 002:33:0100. (This report is provided in Section 4.2.2.2. Absorption).

(b) (4). Plasma concentrations of (b) (4) in mouse toxicology study N-04-068: Mammalian erythrocyte micronucleus test of AL-4943 and (b) (4) Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0001617.

(b) (4) Plasma concentrations of (b) (4) in mouse toxicology study N-04-079: A retrospective toxicokinetic evaluation in support of the mammalian (mouse) erythrocyte micronucleus test of AL-4943A and (b) (4). Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0001618.

(b) (4) Plasma concentrations of (b) (4) and (b) (4) in toxicology study N-03-096: 13-Week intranasal toxicity study of (b) (4) and (b) (4) in rats to support olopatadine hydrochloride 0.6% nasal spray. Fort Worth (TX): Alcon Research, Ltd. 2004 Nov. Technical Report No.: TDOC-0001615.

TOXICOLOGY

SINGLE-DOSE TOXICITY

(b) (4) Toxicological studies of (b) (4) 4679; acute oral toxicity study of (b) (4) 4679 in mice. Final Report. (b) (4) 1989 Aug. Technical Report No.: A-89-69.

(b) (4) Toxicological studies of (b) (4) 4679; acute oral and intravenous study of (b) (4) 4679 in rats. Final Report. (b) (4) 1989 Aug. Technical Report No.: A-89-68.

(b) (4) Toxicological studies of (b) (4) 4679; acute oral and intravenous toxicity study of (b) (4) 4679 in dogs. Final Report. (b) (4) 1989 Aug. Technical Report No.: A-89-62.

(b) (4) Toxicological studies of (b) (4) 4679. Single dose toxicity study of (b) (4) 4679 related compound, OXO derivative, administered orally to mice. (b) (4) 1995 Dec.

REPEAT-DOSE TOXICITY

(b) (4) A-14-day intranasal toxicity study of (b) (4) nasal spray (0.1 and 0.2%) in rats. Alcon Research, Ltd., 2003 Jan. Technical Report No.: 102:30:0400.

(b) (4) Lemke LE: 14-day intra-nasal toxicity study of 0.6 and 1.2% olopatadine hydrochloride nasal spray solution in rats. Alcon Research, Ltd., 2002 Nov. Technical Report No.: 055:30:0602.

(b) (4) Interim Report^ 6-month intranasal toxicity study of (b) (4) nasal spray (0.1 and 0.2%) in rats. Alcon Research, Ltd., 2002 Feb. Technical Report No.: 187:30:1101.

** Hilaski RJ, Lemke LE. Final Report: 6-month intranasal toxicity study of (b) (4) nasal spray (0.1 and 0.2%) in rats. Alcon Research, Ltd., 2003 Apr. Technical Report No.: 054:30:0602.

* (b) (4) Toxicological study of (b) (4) 4679; 4-week subchronic oral toxicity study of (b) (4) 4679 in rats. Final Report. (b) (4) 1988 June. Technical Report No.: A-88-80.

* (b) (4) Toxicological study of (b) (4) 4679; 13-week subacute oral toxicity study of (b) (4) 4679 in rats. Final Report. (b) (4)

(b) (4) 1989 Aug. Technical Report No.: A-89-65.

(b) (4) Toxicological study of (b) (4) 4679; 52-week chronic oral toxicity study of (b) (4) 4679 in rats. Final Report. (b) (4) (b) (4) 1990 Oct. Technical Report No.: A-90-82.

(b) (4) Toxicological study of (b) (4) 4679; 13-week subacute oral toxicity study of (b) (4) 4679 in dogs. Final Report. (b) (4) (b) (4) 1989 Aug. Technical Report No.: A-89-66.

* (b) (4) Toxicological study of (b) (4) 4679; 52-week chronic oral toxicity study of (b) (4) 4679 in dogs. Final Report. (b) (4) (b) (4) 1990 Nov. Technical Report No.: A-90-92.

leachates and impurities:

(b) (4) 13-week intranasal toxicity study of (b) (4) and (b) (4) in rats to support olopatadine hydrochloride 0.6% nasal spray. Alcon Research, Ltd., 2004 Nov. Technical Report No.: TDOC-0001793.

(b) (4) 3-month intranasal toxicity study of olopatadine hydrochloride (0.6%) nasal spray container closure system leachates in rats. Alcon Research, Ltd., 2004 Nov. Technical Report No.: TDOC-0001788.

GENOTOXICITY

parent compound – in vitro:

* (b) (4) Toxicological study of (b) (4) 4679; bacterial reverse mutation assay of (b) (4) 4679. Final Report. (b) (4) (b) (4) 1988 July. Technical Report No.: A-88-93.

* (b) (4) Toxicological study of (b) (4) 4679; chromosomal aberration test of (b) (4) 4679 on CHL cells in vitro. Final Report. (b) (4) 1988 Oct. Technical Report No.: A-88-147.

(b) (4)
Mutagenicity study of E Isomer. Final Report. (b) (4) (b) (4)
1999 July.

parent compound – in vivo:

(b) (4) Toxicological study of (b) (4) 4679; micronucleus test of (b) (4) 4679 administered orally to mice. Final Report. (b) (4) 1990 Mar. Technical Report No.: A-90-17.

(b) (4) . Mutagenicity study of E Isomer. Final Report. (b) (4) 1999 July.

impurities (not reviewed in this review, but reviewed as part of CMC consult of June 14, 2005):

(b) (4) Bacterial reverse mutation assay using (b) (4) a degradation product of AL-4943A (Olopatadine). Alcon Research, Ltd., 2003 Apr. Technical Report No.: 019:30:0203.

(b) (4). *In vitro* mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay) using (b) (4) a degradation product of AL-4943A (Olopatadine). Alcon Research, Ltd., 2003 May. Technical Report No.: 018:30:0203.

(b) (4). Exploratory dose ranging/screening assay of AR and AS in the Syrian hamster embryo cell assay. Alcon Research, Ltd., 2004 Sep. Technical Report No.: TDOC-0001870.

(b) (4) Mammalian erythrocyte micronucleus test using a combined solution containing (b) (4) and AL-4943A (Olopatadine). Alcon Research, Ltd., 2003 Dec. Technical Report No.: TDOC-0000653.

(b) (4) Salmonella-escherchia coli/mammalian-microsome reverse mutation assay preincubation method with a confirmation assay with (b) (4) a degradation product of AL-4943A (Olopatadine). Alcon Research, Ltd., 2003 Dec. Technical Report No.: TDOC-0000652.

(b) (4). *In vitro* mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay) using (b) (4) a degradation product of AL-4943A (Olopatadine). Alcon Research, Ltd., 2003 May. Technical Report No.: 017:30:0203.

(b) (4) Mammalian erythrocyte micronucleus test of (b) (4). Alcon Research, Ltd., 2004 Nov. Technical Report No.: TDOC-0001783.

CARCINOGENICITY

Long-term Studies

(b) (4) (b) (4) 4679: Oncogenicity study by dietary administration to CD-I mice for 78 weeks. Final Report. Conducted by (b) (4) (b) (4) 1993 Oct. Technical Report No.: 92/KKY009/1065.

(b) (4) (b) (4) 4679: Oncogenicity study by dietary administration to F-344 rats for 104 weeks. Final Report. Conducted by (b) (4) (b) (4) 1994 Mar. Technical Report No.: # 93/KKY008/0386.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Fertility and Early Embryonic Development

* (b) (4) Toxicological study of (b) (4) 4679; fertility study of (b) (4) 4679 in rats. Final Report. (b) (4) (b) (4) 1989 Mar. Technical Report No.: #A-89-26.

Embryo-Fetal Development

(b) (4) (b) (4) 4679; teratogenicity study of (b) (4) 4679 in rats. Final Report. (b) (4) (b) (4) 1989 June. Technical Report No.: A-89-52.

(b) (4) (b) (4) 4679; teratogenicity study of (b) (4) 4679 in rabbits. Final Report. (b) (4) (b) (4) 1989 Aug. Technical Report No.: A-89-59.

Prenatal and Postnatal Development, Including Maternal Function

(b) (4) S. Reproductive and developmental toxicology study of (b) (4) 4679 in rats by oral administration; peri- and postnatal toxicity study. Final Report. (b) (4) (b) (4) 1990 Dec. Technical Report No.: D-2525.

(b) (4) Reproductive and developmental toxicity study of (b) (4) 4679 in rats by oral administration; peri- and postnatal toxicity study. Final Report. (b) (4) (b) (4) 1993 June. Technical Report No.: D-3289.

(b) (4) Supplemental study for body weight gain of F1 pups in peri- and postnatal study of (b) (4) 4679 administered orally in rats. Final Report. (b) (4) (b) (4) Technical Report No.: A-95-07.

LITERATURE REFERENCES

Cook EB, Stahl JL, Barney NP, Graziano FM. Olopatadine inhibits TNF α release from human conjunctival mast cells. *Ann Allergy Asthma Immunol*, 84:504-508, 2000.

Department of Health, ILSI/HESI research programme on alternative cancer models: results of Syrian hamster embryo cell transformation assay. COM statement COM/02/S3, April 2002.

DEREK for Windows Report for (b) (4) Version 7.0.0.

DEREK for Windows Report for (b) (4), Version 7.0.0.

Fukuishi N, Matsuhisa M, Shimono T, Murata N, Iwanaga M, Sagara H, Matsui N, Akagi M. Inhibitory effect of olopatadine on antigen-induced eosinophil infiltration and the LFA-1 and Mac-1 expression in eosinophils. *Jpn J Pharmacol*, 88:463-466, 2002.

Gerlis LS, Gumpel JM. Isoxepac in rheumatoid arthritis: A double-blind comparison with aspirin. *Rheumatology and Rehabilitation* 1981; 20:50-53.

(b) (4) DVM, PhD, DACVP. Correspondence: (b) (4)

(b) (4) Study 03-251, 03-250, 03-245. August 2004.

Honig PK, Wortham DC, Samani K, Conner DP, Mullin JC, Cantilena LR. Terfenadine-ketoconazole interaction. Pharmacokinetic and electroretinographic consequences. *JAMA* 1993Mar;269(12):1513-8.

Honig WJ, Pelgrom R, Chadha DR. Analgesic effect of isoxepac on postmeniscectomy pain: A controlled trial. *J. Clin. Pharmacol* 1982; 22:82-88.

Ikemura T, Manabe H, Sasaki Y, Ishii H, Onuma K, Miki I, Kase H, Sato S, Kitamura S, Ohmori K. KW-4679, an antiallergic drug, inhibits the production of inflammatory lipids in human polymorphonuclear leukocytes and guinea pig eosinophils. *Int Arch Allergy Immunol*, 110:57-63,1996.

Illing HPA, Fromson JM. Species differences in the disposition and metabolism of 6,11-dihydro-1 l-oxodibenz[be]oxepin-2-acetic acid (isoxepac) in rat, rabbit, dog, rhesus monkey, and man. *Drug Metab Dispos* 1978;6(5):510-517.

Ishii KKH, Sasaki Y, Manabe H, Ikemura T, Satou H, Ichikawa S, Shiozaki S, Kitamura S, Oumori K. General pharmacology of KW-4679, a new antiallergic drug (1st report) - Effects on the central nervous system, autonomic nervous system and peripheral nervous system. *Clin Pharm Therap* 1995a;5(8):1421-40.

Ishii H, Sasaki Y, Manabe H, Sato H, Fuji M, Iketa J, Kitamura S, Ohmori T. General pharmacology of KW-4679, an antiallergic drug (2nd report) - effects on respiratory, circulatory system, urogenital system and digestive system. *Clin Pharmacol Ther* 1995b;5(12):53-71.

Ishii H, Sasaki Y, Ikemura T, Kitamura S, Ohmori K. [Pharmacological studies on KW-4679, an antiallergic drug. (1): Inhibitory effect on passive cutaneous anaphylaxis (PCA) and experimental asthma in rats and guinea pigs]. *Nippon Yakurigaku Zasshi*, 106:289-298, 1995.

ISP, Certificate of Analysis

Kaise T, Manabe H, Ohmori K. [The effect of KW-4679, an antiallergic drug, on experimental allergic rhinitis]. *Arerugi*, 44:1229-33, 1995.

Kajita J, Inano K, Fuse E, Kuwabara T, Kobayashi H. Effects of olopatadine, a new antiallergic agent, on human liver microsomal cytochrome P450 activities. *Drug Metab Dispos* 2002;30:1504-1511.

Kamei C, Ichiki C, Yoshida T, Tsujimoto S. Effect of the new antiallergic agent olopatadine on EEG spectral powers in conscious rats. *Arzneim-Forsch/Drug Res* 1996;46(II,8):789-93.

Kamei C, Sugimoto Y, Nakamura S, Zhong C. Effect of (Z)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid hydrochloride on experimental allergic conjunctivitis and rhinitis in rats and guinea pigs. *Arzneim-Forsch/Drug Res*, 45(11): 1005-08, 1995.

Kato Y, Mori T, Ohmori K, Ichimura M. Effect of terfenadine and KW-4679, a novel antiallergic compound, on action potential of guinea pig ventricular myocytes. *Jpn J Pharmacol* 1996;70:199-202.

Klimisch HJ, Deckardt K, Gemhardt C, Hildebrand B, Kuttler K, Roe FJC. Subchronic inhalation and oral toxicity of N-Vinylpyrrolidone-2. Studies in Rodents. *Food and Chemical Toxicology* 1997; 35:1061-1074.

Lassman HB, Kirby RE, Wilker JC, Me Fadden AR, Aultz DE, Hoffman D, Helsley GC, Novick, Jr WJ. Pharmacology of a new non-steroidal, anti-inflammatory agent: 6,11-Dihydro-11-Oxodibenz[b, e] Oxepin-2-Acetic Acid (HP 549). *Arch. Int. Pharmacodyn.* 1977; 227:142-154.

Mauthe RJ, Gibson DP, Bunch RT, Custer L. The Syrian Hamster Embryo (SHE) Cell Transformation Assay: Review of the Methods and Results. *Toxicologic Pathology* 2001; 29 (supplement): 138-146.

Miller S, Cook E, Graziano F, Spellman J, Yanni J. Human conjunctival mast cell responses in vitro to various secretagogues. *Ocular Immunol Inflamm*, 4:39-49, 1996.

Mohr W, Endres-Klein R. [Do polyvinylpyrrolidone (PVP) deposits still occur in internal organs at the turn of the millennium? Observations on three patients from the former USSR]. *Pathologe* 2002. 5:386-8.

Nair B. Cosmetic Ingredients Review Expert Panel, Final Report on the Safety Assessment of Polyvinylpyrrolidone (PVP), *Int. J. Toxicol.* 17 (Suppl. 4): 95-130, 1998.

Ohishi T, Magara H, Yasuzawa T, Kobayashi H, Yamaguchi K, Kobayashi S. Disposition of KW-4679 (4): Metabolism of KW-4679 in rats and dogs. *Xenobio Metab Dispos* 1995;10(5):1-18.

Ohishi T, Nishiie H, Fuse E, Kobayashi H, Kobayashi S. Pharmacokinetics of the new anti-allergy drug KW-4679 (part 2): Absorption, excretion, and distribution of ¹⁴C-KW-4679 in repeated oral administration to rats and its effect on the drug-metabolizing enzyme system. *Xenobio Metab Dispos* 1995;10(5):669-682.

Ohmori K, Hayashi K, Kaise T, Ohshima E, Kobayashi S, Yamazaki T, Mukouyama A. Pharmacological, pharmacokinetic and clinical properties of olopatadine hydrochloride, a new antiallergic drug. *Jpn JPharmacol* 2002;88:379-397.

Ohmori K, Ishii H, Sasaki Y, Ikemura T, Manabe H, Kitamura S. Effects of KW-4679, a new orally active antiallergic drug, on antigen-induced bronchial hyperresponsiveness, airway inflammation and immediate and late asthmatic responses in guinea pigs. *Int Arch Allergy Immunol*, 110:64-72, 1996.

Parent RA. Comparative Biology of the Normal Lung; *Treatise on Pulmonary Toxicology*. 1992. Volume 1, page 9.

PDR Library for INDOCIN® Capsules, Oral Suspension and (Merck) Suppositories (Indomethacin)

Pharmaceutical and Healthcare Industry News Database (PHIND), 2004, Dialog© File Number 129. Accession No. 007374: NSA1 hepatotoxicity warning. *Scrip*, Issue: 702, Page 9, June 1982. Accession No. 006833: Hoechst '81 pharms sales up 17%, *Scrip*, Issue: 696, Page 6, May 1982.

Pharmacopeial Forum: Volume No. 29 (4), Page 1064.

Plasdone® Povidone USP, Technical Profile, ISP (International Specialty Products).

Povidone, USP27-NF22 Supplement: No. 2, Page 3294.

Povidone, Inactive Ingredients Database, Center for Drug Evaluation and Research, USFDA, <http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>.

Robinson B, Sullivan F, Borzelleca J, Schwartz S. Povidone: *A critical review of the kinetics, and toxicology of polyvinylpyrrolidone (Povidone)*, Lewis Publishers, 1990 Chapters 9,10 and appendix pg 121 to 202.

Sasaki Y, Ikeda Y, Ikemura T, Okamura K, Miyake K, Ishii H, Ohmori K. Effect of the new antiallergic drug KW-4679 on histamine and leukotoleuen released by the abdominal cavity effusion cells in rats. *ClinPharm Ther*, 5(10): 1837-50, 1995b.

Sasaki Y, Ishii H, Ikemura T, Miki I, Tamura T, Kitamura S, Ohmori K. The antihistaminic effect of KW-4679, a novel antiallergic drug. *Clin Pharm Ther*, 5:1825-1835, 1995a

Sharif NA, Xu SX, Miller ST, Gamache DA, Yanni JM. Characterization of the ocular antiallergic and antihistaminic effects of olopatadine (AL-4943 A), a novel drug for treating ocular disease. *J Pharmacol Exp Ther*, 12:1252-1261, 1996a.

Sharif NA, Xu SX, Yanni JM. Olopatadine (AL-4943A): Ligand binding and functional studies on a novel, long acting HI-selective histamine antagonist and anti-allergic agent for use in allergic conjunctivitis. *JOPT*, 12:401-7, 1996.

Shobha Devi P, Polasa H. Evaluation of the anti-inflammatory drug indomethacin, for its genotoxicity in mice. *Mutation Research* 1987; 188:343-347.
Soluble Kollidone® Grades, Technical Information, BASF, September 2004.

Svendsen LB, Hansen OH, Johansen A. A comparison of the effects of HP 549 (Isoxepac), indomethacin and acetylsalicylic acid (Aspirin®) on gastric mucosa in man. *Scand. J. Rheumatology* 1981. 10:186-188.

Wangenheim J, Bolesfoldi G. Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis* 1988. 3(3): 193-205.

WHO-FAO Nutrition Meetings Report Series 40abc-090. Polyvinylpyrrolidone. Available at <http://www.inchem.org/documents/jecfa/jecmono/40abcj48.htm>

WHO-JECFA Evaluations-Polyvinylpyrrolidone. Available at http://www.inchem.org/documents/jecfa/jeceval/jec_1679.htm

WHO Food Additive Series 15-486 Introduction. Available at <http://www.inchem.org/documents/jecfa/jecmono/v15je01.htm>

WHO Food Additives Series 15-493 Polyvinylpyrrolidone. Available at <http://www.inchem.org/documents/jecfa/jecfa/jecmono/v15je08.htm>

Yanni JM, Miller ST, Gamache DA, Spellman JM, Xu S, Sharif NA. Comparative effects of topical ocular anti-allergy drugs on human conjunctival mast cells. *Ann Allergy Asthma Immunol*, 79:541-545, 1997.

Yanni JM, Stephens DJ, Miller ST, Weimer LK, Graff G, Parnell D, Lang LS, Spellman JM, Brady MT, Gamache DA. The *in vitro* and *in vivo* ocular pharmacology of olopatadine (AL-4943A), an effective anti-allergic/antihistaminic agent. *JOPT*, 12:389-400, 1996.

Yanni JM, Weimer LK, Sharif NA, Xu SX, Gamache DA, Spellman JM. Inhibition of histamine-induced human conjunctival epithelial cell responses by ocular allergy drugs. *Arch Ophthalmol*, 117:643-647, 1999.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Primary pharmacodynamic effects of olopatadine are as an antihistamine that blocks H₁ receptors competitively and possesses anti-allergic activities. Several *in vivo* and *in vitro* experiments were conducted to demonstrate its pharmacodynamic properties. The mode of action is through the end organ blockade on H₁ receptor and inhibition of histamine release from the inflammatory cells. Competitive receptor binding studies identified potency in the guinea-pig lung and tracheal smooth muscle and human conjunctival epithelial, corneal fibroblast, and trabecular cells. The *in vitro* binding studies suggest that olopatadine possesses some affinity to 5-HT₂ receptors. Olopatadine inhibited the allergic response to guinea-pig eyes *in vivo*, allergic bronchospasm in passively sensitized guinea-pigs, passive cutaneous anaphylaxis in rats. In addition, histamine-induced bronchoconstriction in guinea-pigs and capillary permeability and paw edema in rats were inhibited by olopatadine.

Secondary pharmacodynamic effects of olopatadine include weak inhibition of cyclooxygenase with no 5-lipoxygenase inhibitory activity and no inhibition of LTD₄-induced smooth muscle contraction *in vitro* at concentrations that demonstrated antihistaminic and antiallergic responses.

Safety pharmacology testing of olopatadine did not identify any effects of concern in the assessment of central nervous, cardiovascular, and respiratory systems function. Sedation was the only observed CNS effect in mice *in vivo*. Decreased blood pressure was observed after oral administration, but no effects on HR, ECG, QTc interval, and respiratory rate were observed after intravenous dosing with large doses in dogs. Increased respiratory rate and occasional vomiting were observed after large oral doses in mice and cats.

2.6.2.2 Primary pharmacodynamics

Olopatadine is an antihistamine that blocks H₁ receptors competitively and possesses anti-allergic activities. It also inhibits the release of histamine, PGD₂ and tryptase from mast cells. Several *in vivo* and *in vitro* experiments were conducted to demonstrate its pharmacodynamic properties. Olopatadine inhibited the histamine and PGD₂ release in passively sensitized rat basophil cells at 559 to 736 uM concentrations. The K_i for inhibition of ³H-pyrimilamine binding in the guinea-pig lung and tracheal smooth muscle was between 16-45 nM. While the metabolites of olopatadine N-mono-desmethyl and N-di-desmethyl olopatadine showed a competitive displacement of radioligand in guinea-pig cerebellum and lung tissues at K_i values that were approximately half or less than that for olopatadine, these metabolites do not constitute major metabolites and their contributions to the efficacy is considered to be minimal. Olopatadine also inhibited H₁ receptor transduction at 9.5 to 39.5 nM in human conjunctival epithelial, human corneal fibroblast, and human trabecular cells. The *in vitro* binding studies suggest that olopatadine possesses some affinity to 5-HT₂ receptors. The ratio of IC₅₀ for the 5-HT₂ and H₁ receptor was about 20-100 fold. On the basis of the selectivity it is concluded that the pharmacodynamic response of olopatadine will be primarily due to the antihistaminic and anti allergic responses. The anti-allergic response was also demonstrated by the inhibition of allergic bronchospasm in passively sensitized guinea-pigs at 0.011 to 0.05 mg/kg doses. However, a dose of 12.1 mg/kg orally was necessary for preventing the bronchospastic effect in actively sensitized guinea-pigs. Olopatadine inhibited passive cutaneous anaphylaxis at 0.11 mg/kg orally in rats. Olopatadine showed *in vivo* antihistaminic activity in guinea-pig eyes at 0.002% concentration when applied topically. The 0.1 and 0.2% clinical formulations also showed antihistaminic effect in guinea-pigs when applied topically. The anti-allergic response was observed within 30 minutes and sustained for 4-8 hours. Histamine-induced bronchoconstriction in guinea-pigs, capillary permeability and paw edema in rats were inhibited at 3-100 ug/ml iv and 0.014 mg/kg oral doses, respectively.

2.6.2.3 Secondary pharmacodynamics

The effect of olopatadine in LTD₄-induced smooth muscle contractions was not inhibited at concentrations between 10⁻⁶ and 1.4 x 10⁻⁴ M *in vitro*. It did not show lipoxygenase inhibitory activity up to 100 uM concentrations. Olopatadine showed about 26% inhibition of PG cyclooxygenase at 100 uM concentration. Olopatadine did not show local anesthetic activity in the guinea-pig corneal reflex experiments. As olopatadine showed weak inhibition of cyclooxygenase without 5-lipoxygenase inhibitory activity at concentrations that demonstrated antihistaminic and antiallergic responses, it should be considered an antiallergic compound rather than a mast cell stabilizer.

2.6.2.4 Safety pharmacology

Neurological effects:

The CNS effect of olopatadine was not evident at oral doses lower than 300 mg/kg in mice where sedation, but no anticonvulsant or other neurologic effects were observed.

Cardiovascular effects:

Olopatadine showed antihypertensive effect in dogs in a dose dependent manner at 20, 50, & 100 mg/kg (59% decrease at HD) with decreased total peripheral resistance. At <5mg/kg iv there are no effects on HR, ECG & respiratory rate were observed. At <30mg/kg iv there are no effects on QTc. The IC₅₀ for HERG channel is 1000x greater than for terfenadine. In studying the effect of combination of olopatadine and itraconazole (to block CYP3A4) on the ECG in conscious dogs, olopatadine alone causes a greater increase in HR and mBP (in contrast to the earlier experiment where olopatadine caused hypotension) than when administered along with itraconazole, while QT tended to be less affected. These data suggest that olopatadine may not elicit QT prolongation even when co-administered with the CYP 3A4-inhibitor itraconazole. In another study on the effects of olopatadine HCl on cloned hERG channels, olopatadine blocked hERG channels with an IC₅₀ of 1.1 mM. This block showed no use or time dependence.

Pulmonary effects:

Acute clinical signs of increased in the respiratory rate and occasional vomiting were observed at 300 and 30 mg/kg/oral doses in mice and cats, respectively.

Renal effects: NA

Gastrointestinal effects: NA

Abuse liability: NA

Other: NA

2.6.2.5 Pharmacodynamic drug interactions - NA**2.6.3 PHARMACOLOGY TABULATED SUMMARY - NA****2.6.4 PHARMACOKINETICS/TOXICOKINETICS****2.6.4.1 Brief summary**

Intranasally administered olopatadine is rapidly absorbed with systemic bioavailability comparable in rats and humans with 20-30% more bioavailable in dogs. Plasma pharmacokinetics after intranasal administration was linear in rats and dogs in the dose ranges tested. Elimination half lives ranged from 3 hours in rats to 10 hours in humans. Olopatadine distributes widely throughout the body, with the highest concentrations in the GI tract, excretory organs and dose sites, including nasal turbinates. Most or all of radioactivity in the respiratory tissues was derived from the systemic circulation and not from direct contact with dose material. In both animals and humans, olopatadine is not

extensively metabolized and unchanged olopatadine is the major constituent in plasma and excreta. In general, metabolism is a minor means of olopatadine elimination compared to renal excretion of unchanged drug. There appears to be a limited potential for accumulation of olopatadine and a low probability for drug-drug interactions.

2.6.4.2 Methods of Analysis – NA

2.6.4.3 Absorption

Following intranasal administration, olopatadine is rapidly absorbed in rats (T_{max} 5 minutes) and less rapidly in dogs (T_{max} 0.4 to 0.8 hour) and humans (T_{max} 1 hour). Systemic bioavailability was about 50% in rats, 80 to 100% in dogs and 60% in humans. Elimination half-lives of olopatadine intranasal dose ranged from 3 hours in rats to 10 hours in humans. Intranasal studies in rats and dogs at 0.1 to 1.5% olopatadine suggested that olopatadine was reasonably well absorbed with plasma concentrations comparable to those following oral doses at similar doses. Concentrations of nasally administered olopatadine increased in a dose-proportional manner. No accumulation of olopatadine or its main metabolites was evident.

In a pharmacokinetic radiolabeled absorption study in rats, plasma concentrations of olopatadine were measured after a single intranasal dose in fasting male Wistar rats at doses of 0.03, 0.1, & 0.2% at a volume of 10 μ l (3, 10, & 20 μ g dose) in the left naris. Blood samples were collected from the caudal vein at 10 minutes and 0.25, 0.5, 1, 2, 4, 6, 8, 12, & 24 hours after dosing. Plasma pharmacokinetics was linear in the dose range tested (see table). In comparison to other studies, bioavailability by intranasal route is approximately 50% compared to 60% for the oral route.

Table Pharmacokinetic parameters of (b) (4) 4679 after nasal administration of (b) (4) 4679NS to non-fasting male rats

| Nasal formulation | Dose (μ g/head) | | T_{max} (hr) | C_{max} (ng/mL) | $AUC_{0-4 \text{ hr}}$ (ng-hr/mL) | AUC_{0-t} (ng-hr/mL) | $T_{1/2}$ (hr) | $AUC_{0-\infty}$ (ng-hr/mL) | MRT (hr) |
|-------------------|----------------------|------|----------------|-------------------|-----------------------------------|------------------------|--------------------|-----------------------------|--------------------|
| 0.03 % | 3 | Mean | 0.083 | 7.96 | 6.56 | 7.03 | 3.79 ^{a)} | 7.35 ^{a)} | 4.49 ^{a)} |
| | | S.D. | 0.000 | 1.87 | 1.79 | 1.59 | | | |
| 0.1 % | 10 | Mean | 0.083 | 26.48 | 18.22 | 20.54 | 2.63 ^{b)} | 19.03 ^{b)} | 2.18 ^{b)} |
| | | S.D. | 0.000 | 12.34 | 5.61 | 4.68 | 0.92 | 1.48 | 0.52 |
| 0.2 % | 20 | Mean | 0.083 | 51.07 | 43.00 | 49.62 | 4.67 | 52.05 | 2.71 |
| | | S.D. | 0.000 | 5.72 | 8.58 | 9.06 | 1.74 | 8.40 | 1.30 |

$AUC_{0-4 \text{ hr}}$: Area under the plasma concentration-time curve from time 0 to 4 hr after dosing
n=5

a) n=1

b) n=4

In a pharmacokinetic radiolabeled absorption study in dogs, plasma concentrations of olopatadine were measured after a single intranasal dose in non-fasted male Beagle dogs at doses of 0.05, 0.1, & 0.2% at a volume of 15 μ l (7.5, 15, & 30 μ g dose) in the left naris or a single intravenous dose of 15 μ g/kg. Blood samples were collected from the caudal

vein at 10 minutes and 0.25, 0.5, 1, 2, 4, 6, 8, 12, & 24 hours after dosing. Plasma pharmacokinetics was linear in the dose range tested (see table). Bioavailability by the intranasal route ranged from 80-100%.

Table 2. Pharmacokinetic parameters of (b)(4)-4679 after nasal administration of (b)(4) 4679NS to non-fasting male dogs

| Nasal formulation ($\mu\text{g}/\text{kg}$) | Dose | | T _{max} (hr) | C _{max} (ng/mL) | T _{1/2} (hr) | AUC _{0-∞} (ng·hr/mL) | Cl/F (L/hr/kg) | MRT (hr) |
|---|------|------|-----------------------|--------------------------|-----------------------|-------------------------------|----------------|----------|
| 0.05 % | 7.5 | Mean | 0.83 | 6.79 | 4.88 | 23.90 | 0.318 | 3.98 |
| | | S.D. | 0.29 | 0.82 | 1.08 | 3.17 | 0.045 | 0.49 |
| 0.1 % | 15 | Mean | 0.39 | 12.52 | 3.20 | 43.06 | 0.349 | 3.44 |
| | | S.D. | 0.19 | 3.04 | 0.92 | 2.28 | 0.018 | 0.41 |
| 0.2 % | 30 | Mean | 0.75 | 21.84 | 5.28 | 76.94 | 0.391 | 4.79 |
| | | S.D. | 0.43 | 3.57 | 1.55 | 5.36 | 0.026 | 1.22 |

n=3

2.6.4.4 Distribution

A quantitative whole-body autoradiography study in dogs administered ¹⁴C-olopatadine hydrochloride 0.6% nasal spray solution, demonstrated that olopatadine-related radioactivity distributed widely throughout the body, with the highest concentrations in the GI tract, excretory organs and dose sites, including nasal turbinates. The very high concentrations observed in the GI tract, particularly the esophagus and stomach, were indicative of ingestion of a substantial portion of the dose. The much lower levels in the respiratory tree relative to GI levels, along with the low tissue:plasma concentration ratios in these tissues, indicated that most of the radioactivity in the respiratory tissues was derived from the systemic circulation and not from direct pulmonary deposition. Protein binding of olopatadine was moderate at approximately 55% in human sera and independent of drug concentration at levels normally found in animals and humans following oral and intranasal doses. Olopatadine was bound predominately to serum albumin, and this binding was not affected by warfarin, diazepam, or digitoxin.

In a radiolabeled distribution study in dogs, whole body distribution of olopatadine was measured after a single intranasal dose in male Beagle dogs less than 3 months old at a dose of 0.6% at a volume of 2x 100 ul (2.4 mg total dose) in each naris. Two dogs each were sacrificed at 0.5, 4, 8, 24, & 48 hours after dosing for blood, plasma, red blood cells (cellular fraction) and carcass (quantitative whole body radiography) evaluation. Maximal concentrations occurred in blood and plasma at 0.5 hours (T_{max}) with a half life of 3 hours with approximately total removal by 24-48 hours after dosing. The blood:plasma and cellular fraction:plasma ratios were less than one at all times, indicating minimal association of olopatadine with cellular components of blood. Radioactivity was widely distributed throughout the body with the majority being confined to the GI tract and excretory organs. High levels of radioactivity in bile and urine were consistent with elimination of olopatadine by the liver and kidney. Low levels of radioactivity were observed in the lungs, bronchi, and, trachea with a maximum level the same as that of plasma indicating respiratory tree exposure through the systemic

circulation of the drug absorbed by ingestion after intranasal dosing. Whole body elimination of drug was essentially complete by 24-48 hours after dosing.

2.6.4.5 Metabolism

Across species, the metabolic pathways of olopatadine involve N-demethylations and N-oxidation of the dimethylamino-propylidene side chain, hydroxylation of dihydrodibenzo[*b,e*]oxepin ring at C-8, and sulfate conjugation of the C-8 hydroxyl. N-demethylation and N-oxidations are the main pathways and are the only identified routes observed in humans. In general, metabolism is a minor means of olopatadine elimination compared to renal excretion of unchanged drug, and all of olopatadine metabolites are found at minor levels relative to unchanged drug. The metabolites formed in animals and humans are similar but do differ in the relative proportions of each. In summary, the pharmacokinetic and disposition of olopatadine in animals are unremarkable and indicate a limited potential for accumulation and low probability for drug-drug interactions.

2.6.4.6 Excretion

In both animals and humans, olopatadine is not extensively metabolized and unchanged olopatadine is the major constituent in plasma and excreta.

Pharmacokinetic drug interactions

Even though the potential exists for inhibition of olopatadine metabolism by specific inhibitors of P450 and flavin-containing monooxygenase isozymes, drug-drug interactions are not likely to result in significant changes in either pharmacokinetics because olopatadine is not extensively eliminated by metabolism compared to renal excretion.

2.6.4.8 Other Pharmacokinetic Studies - NA

2.6.4.9 Discussion and Conclusions - NA

2.6.4.10 Tables and figures to include comparative TK summary - NA

2.6.5 PHARMACOKINETICS TABULATED SUMMARY - NA

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Single Dose Toxicity:

Male and female rats exposed intranasally to 0.6 mg (3.6 mg/kg) olopatadine exhibited only a trace to slight decrease in goblet cell mucin on day 2 after treatment but not 15 days after treatment. The intranasal minimal lethal dose is >3.6 mg/kg. Acute toxicity of olopatadine was investigated in mice. The oral median lethal dose (MLD) was 1.15 g/kg in male and 1.83 g/kg in female mice. Clinical signs were reduction in the spontaneous activity, abnormal gait, tremors, convulsions, hypothermia and dyspnea. Ocular toxicity was mydriasis and inflamed eyelids. The target organ of toxicity was kidney and the eye. The data on acute toxicity of rats following oral and iv doses showed kidney toxicity associated with hydronephrosis and papillary necrosis, mydriasis, hemorrhage and vasodilatation in the iris and lacrimation in eyes. Peripheral irritation is expected due to the fact that some of the animals showed writhing movement after oral dosing. The MLD for rats was more than 5 g/kg orally for male rats, 3.87 g/kg orally for female rats, 127.5 mg/kg iv for male rats and 142.8 mg/kg iv for female rats.

Olopatadine was tolerated up to 150 mg/kg iv and 5 g/kg orally in beagle dogs in the acute toxicity study. Clinical signs were vomiting, hypothermia, convulsions, mydriasis, protrusion of the nictitating membrane and abnormal EKG. The activity of CPK and LDH was increased. This may reflect ischemia of the heart. However, there were no histological changes in the heart in animals sacrificed on day 14 after the single dose. It is interesting to note that kidney was the target organ of toxicity in rodents. However, dog hearts may be the target organ of toxicity following acute oral dosing.

Repeat Dose Toxicity:

Durations of repeat dose oral studies with olopatadine in rats were 4 weeks, 13 weeks, and 52 weeks. In a 4 week oral study, male and female rats received doses of 0, 20, 200, & 600 mg/kg day. At 600 mg/kg, mydriasis, decreased body weight gain (19% males, 27% females), inflammatory changes in the liver, urinary bladder, and parasternal lymph nodes, and hyperplasia of pancreatic ducts were observed. Associated liver function enzymes were consistent with liver toxicity. In a 13-week oral study, male and female rats received doses of 0, 6, 25, 100, & 400 mg/kg/day. At 400 mg/kg, mortality was observed during weeks 6-8 of the study with lung congestion and alveolar congestion being observed. Abnormal breathing, lacrimation, and blepharoptosis were observed in the 100 & 400 mg/kg groups. Decreased body weight gain occurred in the 100 mg/kg males (11%), 400 mg/kg males (26%) and 400 mg/kg females (9%). Elevated liver function enzymes and alkaline phosphatase were observed in males at 100 & 400 mg/kg and females at 400 mg/kg. Histological changes included myocardial degeneration in males and females at 400 mg/kg with assorted, but not gender consistent, inflammation of several other organs. The NOAEL was 6 mg/kg. In a 52-week oral study, male and female rats received doses of 0, 1, 10, & 100 mg/kg. Mydriasis was observed at 10 (females only) and 100 mg/kg. Blood alkaline phosphatase levels were increased at 6 months in 100 mg/kg females and phosphatase and transaminase (GOT) levels were increase in males and females after one year in the 100 mg/kg group. At 6 months,, myocardial fibrosis was observed in males and dilation of the uterus in females at 100

mg/kg. At 1 year, regeneration of kidney tubule epithelium and interstitial myocarditis were observed to increase in a dose-responsive manner in males and females. In dead animals, predominantly males in the 100 mg/kg group, myocardial fibrosis, degeneration of the myocardium, myocarditis, kidney tubular epithelia regeneration, glomerular capillary wall thickening, and focal necrosis of hepatocytes were observed. The kidney and heart were the primary target organs with no NOAEL being identified.

Durations of repeat dose oral studies with olopatadine in dogs were 4 weeks, 13 weeks, and 52 weeks. In a 4 week oral study, male and female dogs received doses of 0, 2, 10, 50, & 250 mg/kg day. Clinical symptoms observed in all dogs at 50 and 250 mg/kg included vomiting, mydriasis, and xerosis of the muzzle with high dose dogs also exhibiting increased salivation. Congestion of the spleen, liver bile duct vacuolar degeneration and hepatocyte fatty degeneration, kidney inflammation, and hypoplastic bone marrow was treatment related based on increase incidence and/pr severity. The NOAEL was 10 mg/kg based on clinical symptoms, body weight changes, and organ histopathology. In a 13-week oral study, male and female dogs received doses of 0, 0.6, 10, 40 & 160 mg/kg/day. Death in one high dose female was associated with bloody vomiting, conjunctival hyperemia, and anorexia. Mydriasis was observed, but only at 10 mg/kg. Kidney (clarified cytoplasm of tubular epithelium) and EKG changes were observed with the NOAEL of 10 mg/kg. In a 52-week oral study, male and female dogs received doses of 0, 0.6, 5, & 40 mg/kg/day. Vomiting, diarrhea, conjunctival hyperemia, and dryness of the mouth were observed at 40 mg/kg. Body weight gain in male dogs was decreased by 49 & 60% in males dosed with 5 & 40 mg/kg, respectively. EKG changes and histological changes in the heart were observed at 5 and 40 mg/kg in both sexes. The NOAEL was 0.6 mg/kg the heart and kidney were the primary target organs of toxicity.

Rats were treated intranasally 4 times daily for 6 months with either vehicle with benzalkonium chloride (BAC), vehicle w/o BAC, 0.2 mg/d (0.1%) or 0.4 mg/d (0.2%) of olopatadine nasal spray. The initial 8 weeks of the study were conducted using a vehicle containing PEG 400 and Povidone (b) (4) with a (b) (4). The remainder of the study was conducted using a vehicle with no Povidone or PEG 400 and of unknown pH. Nasal inflammation was observed in the HD at the 8 week sacrifice associated with Povidone-containing dose solutions. The 8-week NOAEL was the low dose for local nasal effects of Povidone and the high dose for systemic toxicity of olopatadine. Terminal (6 month) histopathologic changes included testicular hypertrophy and aspermatogenesis in HD males and increased mammary lobular hyperplasia in HD females. Low dose animals were not examined for these changes. Although, no NOAEL was identified, these systemic effects were not confirmed by the 52-week and 2-year carcinogenicity studies. At these dose levels, mean combined male and female $AUC_{0 \rightarrow 24}$ values for olopatadine were 38 ng.h/mL at 0.2 mg/day and 79 ng.h/mL at 0.4 mg/day.

In a 9-month intranasal study at 0.1% (1.6 mg/day) and 0.16% (2.6 mg/day) with olopatadine in dogs, there were no treatment related effects identified and there were no differences in treated groups from vehicle controls. Povidone was not contained in any of the dosing solutions, treated or control. On this basis, a NOAEL for olopatadine of 2.6

mg/day in dogs was identified in this 9-month intranasal study. Mean combined male and female $AUC_{0 \rightarrow 24}$ values for olopatadine were 89 ng.h/mL at 1.6 mg/day and 151 ng.h/mL at 2.6 mg/day.

In another 9-month intranasal study in dogs treated with 0.6% (7.2 mg/day) and 1.5% (18 mg/day) olopatadine with (b) (4) Povidone (b) (4), there were no treatment related effects and no difference between treated and vehicle controls. On this basis, the NOAEL for olopatadine was the high dose of 18 mg/day in dogs in vehicle containing (b) (4) Povidone (b) (4). Mean combined male and female $AUC_{0 \rightarrow 24}$ values for olopatadine were 453 ng.h/mL at 7.2 mg/day and 1370 ng.h/mL at 18 mg/day.

A 2-week intranasal bridging study in rats was performed in order to identify the most appropriate species for testing to qualify Povidone as an excipient in intranasal formulations. Rats were dosed intranasally for 14 days with 0.9 & 1.8 mg/day (0.6% and 1.2%) olopatadine hydrochloride. Povidone was contained in the dosing solutions at (b) (4) resulting in a dose of (b) (4). The NOAEL for this study is the HD of (b) (4) mg/day olopatadine along with (b) (4) of nasal tissue) of Povidone based on lack of notable changes.

A 2-week intranasal bridging study in dogs was performed in order to identify the most appropriate species for testing to qualify Povidone as an excipient in intranasal formulations. No treatment related effects were observed in any treatment groups where dogs were administered intranasal aerosols containing (b) (4) (b) (4) mg/day) or (b) (4) mg/day) Povidone for 2 weeks. Some nasal inflammation and epithelia cell necrosis was observed in all animals of vehicle and Povidone treated groups administered the dosing solutions. On this basis, the NOAEL is 108 mg/day (0.49 mg/cm² of nasal tissue) of Povidone compared to vehicle control.

A 6-month intranasal bridging toxicity study was conducted in rats to qualify Povidone as an excipient in intranasal formulations at doses of 0, (b) (4) Povidone (b) (4) mg/day) and (b) (4) mg/day). Olfactory epithelial degeneration and respiratory turbinate epithelial vacuolation were observed at high incidence (30-90% of treated animals, 0% in vehicle control) with some marked severity in Povidone treated groups in a dose-responsive manner. As a result, there was no NOAEL identified in a 6 month intranasal study in rats with Povidone as Povidone caused local toxicity at the only doses tested of (b) (4) (b) (4) of intranasal tissue surface area) and (b) (4) of intranasal tissue surface area).

Genetic toxicology:

Olopatadine was not mutagenic or genotoxic in the standard battery of assays. Olopatadine did not cause mutagenicity *in vitro* in the Ames bacterial mutation test in *Salmonella typhimurium* tester strains TA 98, 100, 1535, & 1537 and *E. coli* WP2 with or without metabolic activation up to the limit dose of 5000 ug/plate. In the *in vitro* Chinese hamster lung cell assay, olopatadine did not cause chromosomal aberrations at doses selected based on cytotoxicity at a treatment time up to 48 hours. In an oral *in vivo*

mouse micronucleus test in which 400 mg/kg was the highest dose whether administered as a single dose or 4 daily doses of 100 mg/kg, olopatadine did not induce chromosome damage in the form of micronuclei or changes in the spindle function.

Carcinogenicity:

A 78-week carcinogenicity study of olopatadine was conducted in CD-1 mice. The doses were 50, 160 and 500 mg/kg/day given in the diet mixtures. The histopathology data suggest that nonneoplastic changes were present in the heart, liver, lung, ovary, and prostate at 500 mg/kg dose. However, survival of mice was not affected in the presence of above mentioned nonneoplastic changes. For the neoplastic changes, increase incidences of adenoma and carcinoma of liver were observed in male mice at 500 mg/kg dose, but not at a statistically significant level. Considering the hepatic adenoma and carcinoma as common tumors, it is unlikely that the finding would be of any significance. Overall, it appears that mice did not show any carcinogenic potential to olopatadine. Olopatadine did not show carcinogenic lesions at 50 mg/kg (150 mg/m²), 160 mg/kg (480 mg/m²) and 500 mg/kg (1500 mg/m²) doses in mice treated for 78 weeks when the drug was administered orally in the diet.

A 104-week carcinogenicity study was conducted in F-344 rats at 20, 65 and 200 mg/kg doses given in the diet mixture. Long term oral dosing of olopatadine in rats showed increased incidences of keratitis at the low dose in male and female rats in the left eye. The right eye showed more susceptibility to keratitis for male rats. Pancreatic islet cells showed an increase in the incidences of adenoma and carcinoma, but not at a statistically significant level. Based on the findings, it can be concluded that olopatadine given in the diet did not show carcinogenic potential at 20 mg/kg (118 mg/m²), 65 mg/kg (383.5 mg/m²), or 200 mg/kg (1180 mg/m²).

No intranasal carcinogenicity study was required to be conducted for olopatadine based on the lack of carcinogenicity in the oral studies and lack of preneoplastic lesions in the chronic rat and dog intranasal studies.

Reproductive toxicology:

Fertility was evaluated in male and female rats at oral doses of 0, 6, 50, & 400 mg/kg. Olopatadine did not affect fertility at a 50 mg/kg oral dose. At the highest dose, three males of twenty two died. Mydriasis was observed in all olopatadine treated males. A decrease in the body weight gain was observed in male and female animals at 400 mg/kg. Fertility was affected at 400 mg/kg dose with decreased fertility rate, mean implantation, corpora lutea, and number of live fetuses/litter.

The oral teratogenicity study in rats was conducted at doses of 0, 60, 200, & 600 mg/kg. Mydriasis and death was observed at the high dose. Olopatadine treated rats had slightly higher incidences of post implantation loss of 2.9, 7.2, 6.3, & 7.2% at 0, 60, 200, & 600 mg/kg, respectively. Correspondingly, the percent of live fetuses was slightly decreased with percent live fetuses at 97, 92.8, 93.5, & 92.8 for control, 60, 200, & 600 mg/kg

groups, respectively. Decreased fetal body weights were observed at the maternally toxic dose of 600 mg/kg. In rabbits treated with 0, 25, 120, and 400 mg/kg orally in a teratogenicity study, decreased number of live fetuses and increased late fetal deaths but no teratogenicity was observed at 400 mg/kg.

In a peri- and post-natal reproductive study, olopatadine was tested in rats at doses of 60, 200 and 600 mg/kg orally. The 600 mg/kg dose was toxic as an increase in mortality was observed in 6 of 25 dams. Dam body weight gain was reduced in a dose responsive manner through the post partum dosing period with the high dose group reductions starting at post partum day 4 and with no effect at the low dose. Results of the study suggest that olopatadine showed postnatal toxicity to F₁ rats at 60 mg/kg and higher doses. Viability of F₁ pups was reduced in a dose-responsive manner with viability on day 4 post partum of 93, 82, 71, & 48% for control, low, mid, and high doses, respectively. The experiment was repeated at 4, 6 and 20 mg/kg doses. However, body weight gain of the F₁ pups was reduced at these doses even though F₀ rats appeared to be normal. Olopatadine is excreted in the milk of nursing mothers in rats. It also crosses the placental barrier and distributed to the fetuses.

On the basis of the decrease in the number of live fetuses in rabbits and a decrease in the viability of fetuses after delivery in rats, olopatadine should be labeled a pregnancy category C.

Local tolerance:

The potential for ocular toxicity after ocular exposure with olopatadine nasal spray was evaluated in rabbits. The nasal spray (0.6% olopatadine, (b) (4) Povidone) was tested at a single dose of 100 ul in rabbit eyes followed by a 4 day observation period to assess potential for eye irritation. Olopatadine hydrochloride did not elicit any biologically relevant signs of ocular or systemic toxicity that included biomicroscopic and pachymetric evaluation of the eyes.

Special toxicology:

Although small molecular weight compounds such as olopatadine are not usually antigenic, several tests for antigenicity were conducted in the event that olopatadine formed a hapten as a result of protein binding or some other similar event biological event. Antigenicity was evaluated in rats and guinea pigs *in vivo* for dermal contact sensitization, passive cutaneous and active systemic anaphylaxis, and *ex vivo* for passive hemagglutination with sheep red blood cells.

Olopatadine hydrochloride is not a contact sensitizer in guinea pigs. In a dermal sensitization study of maximization design, male and female guinea pigs received intradermal injections of olopatadine hydrochloride (HCl) in propylene glycol, Freund's Complete Adjuvant (FCA), or olopatadine HCl in FCA. One week later, animals received topical challenge with 75% or 100% olopatadine HCl in propylene glycol or propylene glycol alone. After two additional weeks, a challenge was performed with

75% or 100% olopatadine HCl in propylene glycol to previously treated or naïve, not previously treated animals. No dermal responses were indicated in any treatment groups whether previously treated or otherwise.

The low antigenic potential of olopatadine was also evidenced in studies using mice, rats, and guinea pigs in which mice and rats were sensitized with olopatadine in combination with Freund's Complete Adjuvant and/or Bovine Serum Albumin (BSA) for 2-3 weeks orally and intraperitoneally or intramuscularly. The resulting sera were used for several antibody-based antigenic reactions. Mouse-rat heterologous and guinea pig-guinea pig homologous Passive Cutaneous Anaphylaxis reactions were not caused by olopatadine sensitized sera as intradermally injected sera did not elicit an Evans' blue dermal reaction or an increase in antibody titer related to olopatadine treatment. Olopatadine sensitized guinea pig sera did not cause Active Systemic Anaphylaxis in guinea pigs as no systemic anaphylactic clinical symptoms were observed after penile vein injection with sensitized sera. Olopatadine treatment did not result in sheep red blood cell antibody-based hemagglutination reaction in a Passive Hemagglutination test *ex vivo*.

2.6.6.2 Single-dose toxicity

Study title: Acute Intranasal Toxicity Study in Rats

Key study findings:

- Body weight gains were decreased in treated rats over the course of the study (9% males, 29% females)
- Transient decrease in mucin in goblet cells of treated rats on day 2
- No significant toxicity observed after intranasal dosing with 0.6 mg olopatadine

Study no: TR 097:30:0400

Volume #, and page #: 10 of 65

Conducting laboratory and location: (b) (4)

Date of study initiation: August 11, 1997 (April 10, 2000 report date)

GLP compliance: yes (USFDA)

QA report: yes (x) no ()

Drug, lot #, radiolabel, and % purity:

Formulation/vehicle: 0.1% olopatadine in 0.9% NaCl

Methods

Dosing:

Species/strain: Sprague-Dawley [CrI:CD(BR)]

#/sex/group or time point (main study): 6/sex/group

Satellite groups used for toxicokinetics or recovery: 2/sex/group sacrificed on day

2 after dosing
Age: 7 weeks
Weight: 243-270 g (males) & 176-207 g (females)
Doses in administered units: 0 & 0.6 mg
Route, form, volume, and infusion rate: intranasal, 50 uL in the right naris,
12x/day with 30 minutes between doses
- 0.1% olopatadine = 0.1 mg/100 uL or 0.05 mg/50 uL; x 12 = 0.6 mg total
dose

Observations and times:

Clinical signs: 3, 6 & 24 hr post dose & daily thereafter
Body weights: pretreatment and then daily
Food consumption: daily
Ophthalmoscopy: none
EKG: none
Hematology: day 2 (all rats) and day 15
- blood collected via orbital sinus
Clinical chemistry: day 2 (all rats) and day 15
Urinalysis: none
Gross pathology: full external and internal exam on all rats
Organs weighed: adrenal, brain, liver, ovary, spleen, testis, thyroid (with
parathyroid),
Histopathology: heart, kidney, larynx, liver, lung, nasal tissues (4 levels), spleen,
trachea, and trachea bifurcation examined for all animals
- full complement of organs and tissues, including any gross lesions,
collected and fixed
Toxicokinetics: none

Results:

Mortality: none
Clinical signs: nothing remarkable (day 2)
nothing remarkable (days 3-15)
- scabbed area of right/dosed naris in 2/5 of treated males
days 3-15
Body weights: mean rat body weights were decreased for all groups, control and
treated, on day 2
body weight gains were decreased for treated males (9%) and
females (29%) over the course of the treatment period
Food consumption: nothing remarkable
Hematology: nothing remarkable
Clinical chemistry: nothing remarkable
Organ weights: nothing remarkable
Gross pathology: nothing remarkable
Histopathology: trace to mild decrease in nasal goblet cell mucin in 2 male and 2
females of treated group on day 2, which was not observed
for animals sacrificed on day 15

2.6.6.3 Repeat-dose toxicity

Study title: Four-week, repeated-dose, oral administration toxicity study in dogs
(with (b) (4) 4679 – olopatadine)

Key study findings:

- treatment-related increases in vomiting, mydriasis, and xerosis of the muzzle with all animals being affected at two highest dose groups; increased salivation at 250 mg/kg
- treatment-related histology (increased incidence and/or severity) for spleen, kidneys, bone marrow, and liver at 50 and 250 mg/kg
- NOAEL for olopatadine of 10 mg/kg for 4 weeks based on clinical symptoms, body weight changes, and histopathology

Study no.: TR No.: KH-4W-D

Volume # and page #: 42 of 65, pages 1-60

Conducting laboratory and location: (b) (4)

Date of study initiation: July 1997

GLP compliance: not reported

QA report: yes () no (x)

Drug lot # and % purity: P-002, purity not reported

Methods

Doses: 0, 2, 10, 50, & 250 mg/kg

Species/strain: male and female Beagle dogs

Number/sex/group or time point (main study): 4/sex

Route, formulation, volume, and infusion rate: oral, gelatin capsules, daily for 4 weeks

Satellite groups used for toxicokinetics or recovery: none

Age: 6 months

Weight: 7.0-10.4 kg (males), 6.2-8.9 kg (females)

Observations and times:

Mortality: 2x/day

Clinical signs: 2x/day

Body weights: weekly

Food consumption: weekly

Water consumption: weekly

Ophthalmoscopy: pre-dosing and at 4 weeks

EKG: 2/sex/group pre-dosing and at 4 weeks (24 hours post dosing)

Hematology: pre-dosing and 4 weeks (fasting state)

Clinical chemistry: pre-dosing and 4 weeks (fasting state)

Urinalysis: pre-dosing and 4 weeks (during fasting)

Gross pathology: termination

Organ weights: brain, pituitary, thymus, thyroid, heart, lungs, liver, spleen, kidneys, adrenals, pancreas, testes, prostate, ovaries, uterus

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

Peer review: yes (), no (x)

Results

Mortality: none

Clinical signs: vomiting (single animals at ≤ 10 mg/kg; all animals at 50 and 250 mg/kg)
mydriasis (sporadically in 1 male at 2 mg/kg and 2 males and 1 female at 10 mg/kg, frequently in all animals at 50 and 250 mg/kg)
xerosis of the muzzle (all animals at 50 and 250 mg/kg)
salivation (3/sex at 250 mg/kg)

Body weights: males in the 250 mg/kg group lost weight over the course of the study;
nothing else remarkable

Food consumption: decreased for the 50 and 250 mg/kg males and females

Water consumption: decreased for the 50 and 250 mg/kg males and females

Ophthalmoscopy: nothing remarkable

EKG: nothing remarkable

Hematology: nothing remarkable

Clinical chemistry: nothing remarkable

Urinalysis: nothing remarkable

Gross pathology:
gall bladder – enlarged (3 males and 1 female at 250 mg/kg)
spleen – mild congestion (2 males at 250 mg/kg; 1 female in control, 2 and 10 mg/kg, 2 at 50 mg/kg, 1 at 250 mg/kg)
- atrophy (mild in 2 females at 250 mg/kg)
thymus – atrophy (mild in 1 control and 2 mg/kg male, moderate in 1 250 mg/kg male)

Organ weights: nothing remarkable

Histopathology:
spleen – congestion (moderate in 2 males at 250 mg/kg)
thymus – lobules atrophy (mild in 1 control male and moderate in 1 250

mg/kg male; mild in 1 female at 50 mg/kg))
kidneys – inflammation (males – mild in 1 at 2, 50, and 250 mg/kg;
females – mild in 2 females in control and 1 at 50 and 250 mg/kg)
liver – vacuolar degeneration of bile duct (mild in 4 males at 250 mg/kg)
– fatty degeneration of hepatocytes (males - mild in 1 control, mild
in 2 at 2 mg/kg, mild in 1 at 250 mg/kg, moderate in 1 at
250 mg/kg; female – mild in 1 at 250 mg/kg)
bone marrow – hypoplastic (males - mild in 2 at 250 mg/kg)

=====

Study title: 9-Month Intranasal Toxicity Study of (b) (4) Nasal Spray (0.1 and 0.2%) in Dogs

Key study findings:

- 9-month intranasal study at 0.1% (1.6 mg/day) and 0.16% (2.6 mg/day) olopatadine in dogs identified no treatment related effects and no difference from vehicle controls whether benzalkonium chloride was present in vehicle control or not
- 9-month intranasal study NOAEL for olopatadine of 2.6 mg/day in dogs

Study no.: Alcon TDOC-0000295

Volume # and page #: 36 & 37

Conducting laboratory and location: (b) (4)

Date of study initiation: May 31, 2001 (December 15, 2003 amended report date)

GLP compliance: yes (US FDA)

QA report: yes (x) no ()

Drug lot # and % purity: 0.1% olopatadine (01-28631 & 01-29523-1)
0.2% olopatadine (01-28632 & 01-29524-1)

Methods

Doses: 5 groups - 0.1% (1.6 mg/day) and 0.16% (2.6 mg/day) olopatadine; olopatadine vehicle with benzalkonium chloride (BAC); olopatadine vehicle without BAC; no treatment
- olopatadine dosing solutions contain 0.1 or 0.16% olopatadine, 0.01% BAC, (b) (4) sodium chloride, (b) (4) dibasic sodium, adjusted to pH (b) (4) and (b) (4) with purified water

Species/strain: beagle dogs

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: intranasal, 2x 100 uL per nostril, 4x/day (2 hours apart), total volume 400 uL/dose – 1600 uL/day, dosing for 39 consecutive weeks

Satellite groups used for toxicokinetics or recovery: TK on main study dogs
- data from Alcon TDOC-0000228

Age: 6-8 months

Weight: 6.95-9.00 kg (males), 5.99-7.69 kg (females)

Unique study design or methodology (if any): none

Observations and times

Mortality: at least 2x/day

Clinical signs: at least 2x/day; complete physical exam pretest, week 19 and prior to termination

Body weights: prior to randomization, day -1, and weekly

Food consumption: measured daily, reported weekly

Ophthalmoscopy: pretest, week 19, prior to termination

EKG: pretest, week 19, prior to termination

Hematology: pretest, week 19, prior to termination – overnight fast; jugular vein

Clinical chemistry: pretest, week 19, prior to termination – overnight fast; jugular vein

Urinalysis: pretest, week 19, prior to termination – collected during overnight fast

Gross pathology: full examination at termination

Organ weights: adrenals, brain, ovaries, testes, heart, kidneys, liver, lung, pituitary, prostate, mandibular salivary glands, spleen, thymus, thyroids/parathyroids, uterus/cervix

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

Peer review: yes (), no (x)

- all groups being evaluated plus all masses and gross lesions

Toxicokinetics: for treated groups, blood collected in unfasted on day 1, weeks 19 and 39 prior to last dose of day and at 0.5, 1, 2, & 4 hours after last dose from jugular vein

Results

Mortality: none

Clinical signs: nothing remarkable for clinical signs and physical examinations

Body weights: nothing remarkable

Food consumption: nothing remarkable

Ophthalmoscopy: nothing remarkable

EKG: nothing remarkable

Hematology: nothing remarkable

Clinical chemistry: nothing remarkable

Urinalysis: nothing remarkable

Gross pathology: nothing remarkable

Organ weights: nothing remarkable

Histopathology: nothing remarkable

Toxicokinetics: AUC increased in an approximate dose proportional manner (see table).

| Plasma AUCs (ng.h/mL) of Olopatadine in 9 Month Dog Intranasal Study | | | | |
|--|-------------|-----------|-----------|-----------|
| Dose group | sample time | Males | Females | Combined |
| 0.1% | day 1 | 93.7±9.1 | 99.9±27.8 | 96.8±19.4 |
| | week 19 | 96.6±24.3 | 68.0±24.5 | 82.3±27.3 |
| | week 39 | 75.8±18.3 | 99.4±6.8 | 87.6±18.0 |
| 0.2% ^a | day 1 | 160±62 | 218±53 | 189±61 |
| | week 19 | 164±62 | 145±65 | 154±60 |
| | week 39 | 114±37 | 106±31 | 110±32 |

a – assayed amount was 0.2% at beginning of study and 0.16% at end of study

Study title: 9-Month Intranasal Toxicity Study of Olopatadine Hydrochloride Nasal Spray (up to 1.5%) in Dogs

Key study findings:

- 9 month intranasal study at 0.6 (7.2 mg/day) and 1.5% (18 mg/day) olopatadine in dogs identified no treatment related effects and no difference from vehicle controls which contained (b) (4) Povidone (b) (4)
- Mean combined male and female AUC₀₋₂₄ values for olopatadine were 453 ng.h/mL at 7.2 mg/day and 1370 ng.h/mL at 18 mg/day.
- 9 month intranasal study NOAEL for olopatadine of 18 mg/day in dogs in vehicle containing (b) (4) Povidone (b) (4)

Study no.: Alcon TDOC-0001779

Volume # and page #: volumes 38 & 39

Conducting laboratory and location: (b) (4)

Date of study initiation: September 19, 2003 (November 2, 2004 report date)

GLP compliance: yes (US FDA)

QA report: yes (x) no ()

Drug lot # and % purity: 0.6% Olopatadine Hydrochloride (03-34584-1)
1.5% Olopatadine Hydrochloride (03-34585-1)

Methods

- Doses: 4 groups - 0.6 (7.2 mg/day) & 1.5% (18 mg/day) olopatadine;
oloapatadine vehicle; no treatment
- olopatadine dosing solutions contain:
- 0.6 % olopatadine, 0.01% benzalkonium chloride, (b) (4) Povidone (b) (4) % sodium chloride, (b) (4) dibasic sodium, (b) (4) disodium EDTA, adjusted to pH (b) (4) and (b) (4) with purified water
 - 1.5 % olopatadine, 0.01% benzalkonium chloride, (b) (4) Povidone (b) (4) % sodium chloride, (b) (4) dibasic sodium, (b) (4) % disodium EDTA adjusted to pH (b) (4) and (b) (4) with purified water
 - vehicle of 0.01% benzalkonium chloride, (b) (4) Povidone (b) (4) sodium chloride, (b) (4) dibasic sodium, (b) (4) disodium EDTA adjusted to pH (b) (4) and (b) (4) with purified water
 - Povidone dose was (b) (4) mg/day in noted groups

Species/strain: beagle dog

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: intranasal, 2x 100 uL per nostril, 3x/day (2 hours apart), total volume 400 uL/dose – 1200 uL/day, dosing for 39 consecutive weeks

Satellite groups used for toxicokinetics or recovery: TK on main study dogs
- data from Alcon TDOC-0001616

Age: 6 months

Weight: 7.83-10.24 kg (males), 6.19-9.10 kg (females)

Unique study design or methodology (if any): none

Observations and times:

Mortality: at least 1x/day

Clinical signs: at least 1x/day; detailed clinical exam weekly

Body weights: at randomization, weekly for first 13 weeks, and monthly thereafter;
fasted body weight prior to termination

Food consumption: not measured

Ophthalmoscopy: pretest and near end of study

EKG: pretest and near end of study

Hematology: pretest and prior to termination – overnight fast; jugular vein

Clinical chemistry: pretest and prior to termination – overnight fast; jugular vein

Urinalysis: pretest and prior to termination – collected during overnight fast

Gross pathology: full examination at termination

Organ weights: adrenals, brain, ovaries, testes, heart, kidneys, liver, pituitary,
spleen, thyroids/parathyroids

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

Peer review: yes (), no (x)

- vehicle control and high dose plus suspected target organs for low dose (kidneys, larynx, liver, lungs with bronchi, nasopharyngeal tissues, pharynx, spleen, trachea, and urinary bladder)
- untreated control not evaluated

Toxicokinetics: for treated groups, blood collected on day 1, weeks 19 and 26 prior to last dose of day and at 0.5, 1, 2, & 4 hours after last dose from jugular vein

Results

Mortality: none

Clinical signs: nothing remarkable

Body weights: female vehicle control, low dose and high dose group body weights were 5, 15, and 12% lower than untreated control group at the end of the study

Food consumption: not measured

Ophthalmoscopy: nothing remarkable

EKG: nothing remarkable

Hematology: nothing remarkable

Clinical chemistry: nothing remarkable

Urinalysis: nothing remarkable

Gross pathology: nothing remarkable

Organ weights:

- increase in mean absolute thyroid weights of 7% & 40% in LD & HD males compared to vehicle controls, respectively
- increase of 12.5% in relative spleen weights HD males compared to vehicle control
- increase of 132, 144, & 146% in mean absolute spleen weights in vehicle control, LD, & HD females above untreated control, respectively
- increase of 157, 167, & 171% in relative spleen weights in vehicle control, LD, & HD females above untreated control, respectively

Histopathology:

- nothing remarkable for spleen and thyroids
- trace-slight chronic inflammation in nasopharyngeal tissue for vehicle control, LD and HD animals (untreated control not evaluated)

| Histopathology of Nasal Pharyngeal Tissue in Dog 9 Month Study* | | | | | |
|--|-----|-------|-----------------------------|------------|-----------|
| | | | Incidence/4 (mean severity) | | |
| effect | sex | level | Vehicle control | 7.2 mg/day | 18 mg/day |
| chronic inflammation | M | 1 | 3 (0.75) | 4 (1) | 4 (1) |
| | | 2 | 3 (0.75) | 3 (0.75) | 4 (1) |
| | | 3 | 4 (1) | 3 (0.75) | 3 (1) |
| | | 4 | 3 (0.75) | 4 (1.5) | 4 (1.25) |
| | F | 1 | 4 (1) | 3 (1.25) | 4 (1) |
| | | 2 | 3 (0.75) | 2 (0.5) | 4 (1) |
| | | 3 | 4 (1) | 2 (0.5) | 4 (1) |
| | | 4 | 4 (1.25) | 3 (0.75) | 3 (1) |
| Intraepithelial neutrophils | M | 1 | 4 (1) | 4 (1) | 4 (1) |
| | F | 1 | 3 (0.75) | 3 (0.75) | 4 (1.25) |

* - untreated control not examined, only vehicle control

Toxicokinetics: AUC increased in an approximate dose proportional manner (see table).

| Plasma AUCs (ng.h/mL) of Olopatadine in 6 Month Dog Intranasal Study | | | | |
|--|-------------|----------|----------|----------|
| Dose group | sample time | Males | Females | Combined |
| 0.6% | day 28 | 447±88 | 500±245 | 473±172 |
| | Day91 | 469±84 | 531±102 | 500±93 |
| | Day 182 | 363±20 | 409±50 | 386±43 |
| 1.5% | day 28 | 1630±400 | 1430±620 | 1530±490 |
| | Day91 | 1430±300 | 1370±320 | 1400±290 |
| | Day 182 | 1140±250 | 1210±170 | 1180±200 |

Study title: 2-Week Intranasal Bridging Study in Dogs to Qualify Povidone as an Excipient in Intranasal Formulations

Key study findings:

- No Povidone-related effects were observed in any treatment groups where dogs were administered intranasal aerosols containing vehicle without Povidone, (b) (4) Povidone or (b) (4) Povidone for 2 weeks
- NOAEL of 108 mg/day of Povidone compared to vehicle control
- Nasal inflammation and epithelia cell necrosis in all animals of vehicle and PVP treated groups with the pH (b) (4) dosing solutions

Study no.: Alcon TDOC-0000689

Volume # and page #: 63, pages 1-232

Conducting laboratory and location: (b) (4)

Date of study initiation: March 13, 2003 (November 14, 2003 report date)

GLP compliance: yes (USFDA) – Sponsor conducted all characterizations (e.g., purity, stability, homogeneity) of bulk test material, vehicle, and dosing formulations.

QA report: yes (x) no ()

Drug lot # and % purity: (b) (4) Povidone (PVP) – lot # 03-33167
(b) (4) PVP – lot # 03-33168
vehicle – lot # 03-33169

Methods

Doses: 0, (b) (4) & (b) (4) Povidone (PVP)
- (b) (4) PVP – (b) (4) PVP, 0.01% benzalkonium chloride, (b) (4) disodium EDTA, (b) (4) % sodium chloride, and (b) (4) dibasic sodium phosphate (b) (4) with purified water and adjusted to pH (b) (4)
(b) (4) PVP (b) (4) PVP, 0.01% benzalkonium chloride, (b) (4) disodium EDTA, (b) (4) sodium chloride, and (b) (4) dibasic sodium phosphate (b) (4) with purified water and adjusted to pH (b) (4)
- vehicle - 0.01% benzalkonium chloride, (b) (4) disodium EDTA, (b) (4) sodium chloride, and (b) (4) dibasic sodium phosphate (b) (4) with purified water and adjusted to pH (b) (4)

Species/strain: Beagle dogs

Number/sex/group or time point (main study): 2/sex/group

Route, formulation, volume, and infusion rate: intranasal, aerosol, 3 100ul sprays/nare, 4x/day (separated by 2 hours), 7 days/week for 14 consecutive days (total dose 2400 ul)

Satellite groups used for toxicokinetics or recovery: none

Age: 4-5 months

Weight: 7.10-8.53 kg the day after receipt

Unique study design or methodology (if any): none

Observations and times:

Mortality: daily

Clinical signs: daily; detailed physical examination weekly

Body weights: 2x weekly; fasted prior to terminal sacrifice

Food consumption: provided 400 grams of food daily; consumption not measured

Ophthalmoscopy: prior to treatment and prior to termination

EKG: prior to treatment and prior to termination

Hematology: prior to treatment and prior to termination (fasted) via jugular vein

Clinical chemistry: prior to treatment and prior to termination (fasted) via jugular vein

Urinalysis: during pre-termination fast

Gross pathology: at termination

Organ weights: adrenals, brain, heart, kidneys, liver, pituitary, spleen, testes, thyroid/parathyroids, ovaries

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

Peer review: yes (), no (x)

- control and high dose group; target organs (esophagus, kidneys, larynx, liver,

lungs with bronchi, nasopharyngeal tissue, pharynx, spleen urinary bladder) in low dose group

Results

Mortality: none

Clinical signs: nothing remarkable

Body weights: nothing remarkable

Food consumption: not measured (in general, all dogs ate most of their daily rations)

Ophthalmoscopy: nothing remarkable

EKG: nothing remarkable

Hematology: nothing remarkable

Clinical chemistry: nothing remarkable

Urinalysis: nothing remarkable

Gross pathology: nothing remarkable – local nasal inflammation and epithelial cell necrosis in all groups

Organ weights: nothing remarkable

Histopathology: nothing remarkable – local nasal inflammation and epithelial cell necrosis in all groups
- severity grades range similar for control and PVP treated groups for subacute inflammation (grades 1-3) and epithelial necrosis (grades 1-1.5)

=====

Study title: 6-Month Intranasal Bridging Toxicity Study in Rats to Qualify Povidone as an Excipient in Intranasal Formulations

Key study findings:

- olfactory epithelial degeneration and respiratory turbinate epithelial vacuolation observed at high incidence (30-90% of treated animals, 0% in vehicle control) with some marked severity in Povidone treated groups in a dose-responsive manner
- in a 6 month intranasal study, Povidone causes local toxicity at the only doses tested of [REDACTED]^{(b) (4)}/day in rats, thereby not identifying a NOAEL

Study no.: AlconTDOC-0001794

Volume # and page #: volumes 61 & 62 pages 1-427

Conducting laboratory and location: (b) (4)

Date of study initiation: July 2, 2003 (October 1, 2003 report date)

GLP compliance: yes (USFDA)

QA report: yes (x) no ()

Drug lot # and % purity: (b) (4) Povidone (PVP) – lot # 03-34033-1

(b) (4) PVP – lot # 03-34035-1

olopatadine vehicle – lot # 03-34034-1

Methods

Doses: 0, 0 (vehicle), (b) (4) (2.7 mg/day), & (b) (4) (6.8 mg/day) Povidone

(PVP)

(b) (4) PVP – (b) (4) PVP, 0.01% benzalkonium chloride, (b) (4) disodium EDTA, (b) (4) sodium chloride, and (b) (4) dibasic sodium phosphate (b) (4) with purified water and adjusted to pH (b) (4)

(b) (4) PVP – (b) (4) PVP, 0.01% benzalkonium chloride, (b) (4) disodium EDTA, (b) (4) sodium chloride, and (b) (4) dibasic sodium phosphate (b) (4) with purified water and adjusted to pH (b) (4)

- vehicle - 0.01% benzalkonium chloride, (b) (4) disodium EDTA, (b) (4) sodium chloride, and (b) (4) dibasic sodium phosphate (b) (4) with purified water and adjusted to pH (b) (4)

- untreated control

Species/strain: Sprague-Dawley derived rats [CrI:CD®(SD)IGS BR)

Number/sex/group or time point (main study): 20/sex/group; 10/sex/untreated group

Route, formulation, volume, and infusion rate: intranasal, 50 ul in right naris (left naris as contralateral control), 3x/day, 7 days/week for 182-183 consecutive days – total dose volume of 150 ul/day

Satellite groups used for toxicokinetics or recovery: none

Age: 4-5 weeks

Weight: 79.0-108 g (males), 61.6-90.2 g (females)

Unique study design or methodology (if any): none

Observations and times:

Mortality: 2x/day (weekdays), at least 1x/day (weekends)

Clinical signs: 2x/day (weekdays), at least 1x/day (weekends); detailed clinical exam weekly

Body weights: randomization, weekly (first 13 weeks), biweekly thereafter, fasted body weight at termination

Food consumption: concurrent with body weight determinations

Ophthalmoscopy: prior to treatment initiation and prior to termination

EKG: none

Hematology: after overnight fasting prior to termination

Clinical chemistry: after overnight fasting prior to termination

Urinalysis: urine collected during overnight fasting

Gross pathology: at termination

Organ weights: adrenals, brain, heart, kidneys, liver, pituitary, spleen, testes, thyroid/parathyroids, ovaries, thymus

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

Peer review: yes (), no ()

- vehicle control and high dose group; target organs (kidneys, larynx, liver, lungs with bronchi, nasopharyngeal tissue, pharynx, spleen, urinary bladder, trachea) in low dose group; untreated control not examined (tissues collected but not processed)

Results

Mortality: nothing remarkable (2 negative control males died)

Clinical signs: nothing remarkable

Body weights: increased HD males weeks 3, 5, 7, 8, 9, 11

Food consumption: increased males (LD – weeks 3-8; HD – study) – similar to untreated control

Ophthalmoscopy: nothing remarkable

Hematology: nothing remarkable

Clinical chemistry: nothing remarkable

Urinalysis: nothing remarkable

Gross pathology: nothing remarkable

Organ weights: nothing remarkable

Histopathology: olfactory epithelial degeneration and epithelial vacuolation of respiratory turbinates at high incidence and up to marked severity (see tables)

| Histopathology of Nasal Tissue in Rat 6 Month Study with Povidone (PVP) | | | | | |
|--|-----|------------|-------------------------------|-------------|-------------|
| | | | Incidence/20 (mean severity)* | | |
| effect | sex | nose level | negative control | (b) (4) PVP | (b) (4) PVP |
| olfactory epithelium degeneration | M | 3 | 0 (0) | 13 (1.26) | 16 (1.95) |
| | | 4 | 0 (0) | 7 (0.65) | 8 (0.95) |
| | F | 3 | 0 (0) | 15 (1.63) | 18 (1.85) |
| | | 4 | 0 (0) | 12 (1.15) | 6 (0.4) |
| turbinate epithelium vacuolation | M | 1 | 0 (0) | 11 (0.65) | 14 (1.10) |
| | F | 1 | 9 (0) | 15 (1.05) | 18 (1.40) |
| | | | severity range (range 0-4)** | | |
| effect | sex | nose level | negative control | (b) (4) PVP | (b) (4) PVP |
| olfactory epithelium degeneration | M-F | 3-4 | 0 | 0-3 | 0-4 |
| turbinate epithelium vacuolation | M-F | 1 | 0 | 0-2 | 0-3 |

* severity is mean severity/group that includes 0 severity score for unaffected animals

** - grade 1 = minimal, grade 2 = mild, grade 3 = moderate, grade 4 = marked

| Severity of Nasal Tissue Histopathology in Rat 6 Month Study with Povidone (PVP) | | | | | | | |
|---|-------------|-------|----------------------------------|----|---|---|---|
| | | | # of animals with severity grade | | | | |
| effect | sex – dose* | level | 0 | 1 | 2 | 3 | 4 |
| olfactory epithelium degeneration | M – LD | 3 | 7 | 5 | 5 | 3 | - |
| | | 4 | 13 | 3 | 2 | 2 | - |
| | M – HD | 3 | 4 | 5 | 4 | 4 | 3 |
| | | 4 | 12 | 1 | 3 | 4 | - |
| turbinate epithelium vacuolation | F - LD | 3 | 5 | 5 | 6 | 2 | 2 |
| | | 4 | 8 | 4 | 5 | 3 | - |
| | F - HD | 3 | 2 | 7 | 3 | 8 | - |
| | | 4 | 14 | 5 | - | 1 | - |
| turbinate epithelium vacuolation | M - LD | 1 | 9 | 9 | 2 | - | - |
| | | 1 | 6 | 8 | 4 | 2 | - |
| | F - LD | 1 | 5 | 9 | 6 | - | - |
| | | 1 | 2 | 10 | 6 | 2 | - |

* M = males; F = female; LD = (b) (4) PVP; HD = (b) (4) PVP

2.6.6.4 Genetic toxicology - see review in NDA 20-688 in appendix

2.6.6.5 Carcinogenicity - see review in NDA 20-688 in appendix

2.6.6.6 Reproductive and developmental toxicology - see review in NDA 20-688 in appendix

2.6.6.7 Local tolerance

Study title: Seventy Two-Hour Ocular Evaluation of Olopatadine HCl Nasal Spray following Single-Dose Administration in New Zealand White Rabbits

Key study findings:

- Olopatadine nasal spray (0.6% olopatadine, (b) (4) Povidone) administered to the rabbit eye at a single dose of 100 ul did not elicit any biologically relevant signs of ocular or systemic toxicity over a 4 day observation period that included biomicroscopic and pachymetric evaluation of the eye.

Study no.: TDOC-0001715 (TR-N-03-159)

Volume # and page #: 56 of 65, pages 1-73

Conducting laboratory and location: Alcon Research Ltd., 6201 S. Freeway, Fort Worth, TX 76134

Date of study initiation: May 25, 2004

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: vehicle (lot # 03-34583-1), 0.6% spray as drops (lot # 03-34584-1), 1.5% spray as drops (lot # 03-34585-1), 0.6% spray as spray (lot # 03-34584-1)

Formulation/vehicle: (b) (4) Povidone, 0.01% benzalkonium chloride, (b) (4) sodium chloride, (b) (4) dibasic sodium phosphate, sodium hydroxide/hydrochloric acid for pH adjustment to (b) (4) with purified water.

Methods

Doses: nasal spray as eye drops (0, 0.6, & 1.5%) and as nasal spray (0.6%) at a dose volume of 100 ul. The excipient Povidone was present in all dosing solutions at (b) (4)

Study design: New Zealand White rabbits (12 males, 3/group) exposed to olopatadine in 1 eye with the contralateral eye as control. Animals were observed twice daily for morbidity, morbundity, general well being, and signs of pain. Eyes of all animals were examined biomicroscopically (including pachymetry analysis for corneal thickness) at prescreen, approximately 1 hour post-dose, and on study days 2, 3, & 4. Body weights were obtained on day 4 prior to euthanasia. Necropsy performed with collection and fixation of both eyes/adnexa and the nasal lachrymal tissues.

Results: No mortality or morbundity observed. No effects on body weight. Statistically significant decrease (9% from vehicle control 2.5% from own predosing value) in central corneal thickness on day 4 in 1.5% olopatadine eye drop group not

considered biologically relevant as within normal variability. Nothing remarkable with maximum score of 1 (minimal) in all groups for conjunctival congestion. Minimal fluorescein staining in one 1.5% animal on day 2, but not day 4. Per sponsor's description, as no in-life ocular findings, histologic evaluation of ocular tissues was not performed.

Based on a single dose study, olopatadine 0.6% nasal spray administered to the rabbit eye did not elicit any biologically relevant signs of ocular or systemic toxicity

2.6.6.8 Special toxicology studies

Study title: A Dermal Sensitization Study in guinea Pigs with Olopatadine HCl (Maximization Design)

Key study findings:

- Olopatadine hydrochloride is not a contact sensitizer in guinea pigs using a maximization test protocol

Study no.: TR 136:30:0600

Volume #, and page #: 57 of 65, pages 1-96

Conducting laboratory and location: (b) (4)

Date of study initiation: April 5, 2000 (June 2, 2000 report date)

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: (b) (4) lot # 9521, 99+%

Formulation/vehicle: propylene glycol

Methods

Doses and study design: Young adult, male and female Hartley guinea pigs were used. Animals observed for clinical signs and weighed on day prior to dosing then euthanized without further examination after treatment and scoring completed.

topical range finding study – 4 shaved animals; 25, 50, 75, or 100% olopatadine HCl in propylene glycol to 1 of 4 sites on the sides of the animals in (b) (4) chambers, wrapped with elastic wrap for 24 hours then graded for irritation at 24 and 48 hours after unwrapping

intradermal range finding study - 4 shaved animals; 0.1, 1, 3, or 5% olopatadine HCl in propylene injected into to 1 of 4 sites on the sides of the animals (0.1 mL) then graded for irritation at 24 and 48 hours after injection

sensitization study

- intradermal induction – 10 shaved animals/sex injected at 2 of 6 injection sites with either 0.1 mL of Freund's Complete Adjuvant (FCA) emulsion, 0.1 mL of 3% olopatadine HCl in propylene glycol, or 0.1 ml of 3% olopatadine HCl in FCA emulsion
- topical induction – shaved animals treated with 0.5 mL of 10% sodium lauryl sulfate in petrolatum on day 5 after dosing
 - on day 6: 0.8 mL of 75% olopatadine HCl (test group), 100% propylene glycol (challenge control), or 100% propylene glycol (rechallenge control) was administered under Webril patches and wrapped with elastic tape and secured with adhesive tape. Removal occurred at 48 hours after dosing.
- challenge – shaved animals from sensitization study challenged on day 20 with 75% (0.3 mL) or 100% olopatadine HCl with (b) (4) chambers being wrapped for 24 hours; 5/sex previously untreated animals treated similarly to challenge animals. Test sites graded for irritation at 24 and 48 hours after dosing.

Results:

- nothing remarkable to report for clinical observations and body weights
- topical range finding study identified 75 & 100% olopatadine HCl as appropriate for topical induction (i.e., not irritating), but 75% gave better skin contact
- intradermal range finding study indicated 3% olopatadine HCl to be appropriate for intradermal induction
- sensitization study results with olopatadine HCl at 75 or 100% resulted in the same dermal irritation scores of 0 for the treated animals compared to the challenge control group.
- conducting lab provided historical positive control data (study conducted within 6 months of this study) that indicated susceptibility of test system for identification of dermal sensitization using this methodology

=====

Study title: Antigenicity Test of (b) (4)-4679/Olopatadine Hydrochloride (Passive Cutaneous Anaphylaxis, Passive Hemagglutination, & Active Systemic Anaphylaxis Tests)

Key study findings:

- Olopatadine did not cause Passive Cutaneous Anaphylaxis reactions in rats treated with sera from olopatadine sensitized mice or guinea pigs treated with sera from olopatadine sensitized guinea pigs.
- Olopatadine did not cause Active Systemic Anaphylaxis reactions in guinea pigs treated with sera from olopatadine sensitized guinea pigs.
- Olopatadine did not cause Passive Hemagglutination of sheep red blood cells with sera from olopatadine sensitized guinea pigs.

Study no.: TR A-90-43

Volume #, and page #: 57 of 65, pages 1-31

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: September 11, 1989 (May 7, 1991 report date)

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: couldn't read from report

Formulation/vehicle: distilled water was the vehicle for all dosing solutions

Methods

Study design and Results:

- 1) Mouse-rat Passive Cutaneous Anaphylaxis (PCA) test – male Balb/c CrSlc mice were used for the sensitization and male Slc:Wistar rats used as challenge recipients
 - mice were divided into 7 groups of 5 mice for sensitization as listed in the table and dosed for three weeks (3 times weekly for three weeks – oral groups; once weekly for 3 weeks – intraperitoneal groups) with blood collection 1 week after the final dose:
 - on the day of challenge, 50 ul of sensitized mouse serum in physiologically saline was injected intradermally into the backs of shaved rats
 - rats were divided into groups of 5 with each receiving iv challenge doses 24 hours after the intradermal serum treatments (see table) that also included an equal volume of 1% Evans' blue solution
 - animals were sacrificed 30 minutes after challenge and the skin of the back was peeled off inspected for a dyed spot at the intradermal injection site. A spot of 5 mm in diameter was considered positive
 - Results – olopatadine was not associated with any dyed spots and no increased antibody titer unless associated with the positive control BSA (see table)

| 24 Hour Heterologous Passive Cutaneous Anaphylaxis Test of Rats with Sera from Olopatadine Treated/Sensitized Mice | | | |
|--|--|--|--------------------|
| <u>Mice Sensitization Groups</u> Olopatadine Dose | <u>Rat Challenge Groups</u> Olopatadine Dose (iv) | PCA Reaction (antibody titer – dilution ratio) | # animals reacting |
| None | 10 ug | <5 | 5/5 |
| | 10 ug plus 1 mg OVA | <5 | 5/5 |
| 20 ug po | 10 ug | <5 | 5/5 |
| | 10 ug plus 1 mg OVA | <5 | 5/5 |
| 200 ug po | 10 ug | <5 | 5/5 |
| | 10 ug plus 1 mg OVA | <5 | 5/5 |
| 20 ug plus AH gel ip | 10 ug | <5 | 5/5 |
| | 10 ug plus 1 mg OVA | <5 | 5/5 |
| 200 ug plus AH gel ip | 10 ug | <5 | 5/5 |
| | 10 ug plus 1 mg OVA | <5 | 5/5 |
| 200 ug plus AH gel plus 0.1 mg BSA ip | 10 ug | <5 | 5/5 |
| | 10 ug plus 1 mg OVA | <5 | 5/5 |
| | 1 mg BSA | 80 | 4/5 |
| | 1 mg OVA | <5 | 5/5 |
| 0.1 mg BSA plus AH ip | 1 mg BSA | 40 | 3/5 |
| | | 80 | 1/5 |
| | | 160 | 1/5 |
| | 1 mg OVA | <5 | 5/5 |

AH – aluminum hydroxide gel; OVA – ovalbumin; BSA – bovine serum albumin

- 2) Guinea pig-guinea pig PSA test – male Slc:Hartley guinea pigs were used for the sensitization and as recipients of PCA.
- guinea pigs were divided into 7 groups of 9 animals for sensitization, except for negative control (12) and positive BSA control (6) and dosed 3 times weekly for 2 weeks (oral groups) and once weekly for 2 weeks (intramuscular groups) with blood collection from three animals/group on the 13th day after the final dose
 - on the day of challenge, 50 ul of sensitized serum in physiologically saline was injected intradermally into the backs of shaved animals
 - rats were divided into groups of 5 with each receiving iv challenge doses 4 hours after the intradermal serum treatments as listed in the table that also included an equal volume of 1% Evans' blue solution
 - animals were sacrificed 30 minutes after challenge and the skin of the back was peeled off inspected for a dyed spot at the intradermal injection site. A spot of 5 mm in diameter was considered positive

- Results – olopatadine was not associated with any dyed spots and increased antibody titer unless associated with the positive control BSA

| 4 Hour Homologous Passive Cutaneous Anaphylaxis Test of Guinea Pigs Treated with Sera from Olopatadine Treated/Sensitized Guinea Pigs | | | |
|---|---|---|--------------------|
| Sensitization Groups Olopatadine Dose | Challenge Groups Olopatadine Dose (iv) | PCA Reaction (antibody titer – dilution ratio) | # animals reacting |
| None | 1 ug | <5 | 3/3 |
| | 1 ug plus 1 mg OVA | <5 | 3/3 |
| 0.4 mg po | 1 ug | <5 | 3/3 |
| | 1 ug plus 1 mg OVA | <5 | 3/3 |
| 4 mg po | 1 ug | <5 | 3/3 |
| | 1 ug plus 1 mg OVA | <5 | 3/3 |
| 0.4 mg plus FCA im | 1 ug | <5 | 3/3 |
| | 1 ug plus 1 mg OVA | <5 | 3/3 |
| 4 mg plus FCA im | 1 ug | <5 | 3/3 |
| | 1 ug plus 1 mg OVA | <5 | 3/3 |
| 4 mg plus FCA plus 1 mg BSA im | 1 ug | <5 | 3/3 |
| | 1 ug plus 1 mg OVA | <5 | 3/3 |
| | 1 mg BSA | 320 | 1/3 |
| | | 640 | 1/3 |
| | | ≥ 1280 | 1/3 |
| 1 mg OVA | <5 | 3/3 | |
| 1 mg BSA plus FCA im | 1 mg BSA | ≥ 1280 | 3/3 |
| | 1 mg OVA | <5 | 3/3 |

OVA – ovalbumin; BSA – bovine serum albumin; FCA – Freund’s Complete Adjuvant

3) Guinea pig Active Systemic Anaphylaxis (ASA) test

- guinea pigs were sensitized as for the PSA test with all the same 7 groups
- two weeks after the final sensitization dose, the challenge solution was injected into the penile vein
 - challenge solutions were the same as for the PCA test except for the 4 mg plus FCA plus 1 mg BSA im group which did not receive BSA or OVA alone and the 1 mg BSA plus FCA im group which only received 1 m BSA challenge.
- systemic anaphylactic reactions were observed for 1 hour after challenge
- no reactions were observed in any of the guinea pigs except for the positive control BSA only group of which 3 of 3 animals died
- Results - olopatadine was not associated with an ASA reaction

4) Sensitized guinea pig serum Passive Hemagglutination (PHA) test

- guinea pigs were sensitized as for the PSA test with all the same 7 groups
- sheep red blood cell suspensions were prepared with either olopatadine or BSA then incubated with sensitized serum for 2 hours at 37oC and allowed to stand overnight at 4oC before evaluation of hemagglutination
- Results – olopatadine was not associated with a PHA reaction

2.6.6.9 Discussion and Conclusions

Olopatadine did not cause any notable toxicity in 6-month rat and 9-month dog intranasal studies although the formulation that contained the excipient Povidone was only tested for the first two months of the 6-month rat study. In the rat at the NOAEL, the highest dose tested, the AUCs ratio of the rat NOAEL dose to that of the proposed human dose is 1 with a local safety margin of 2 for intranasal effects based on dose of olopatadine per nasal surface area. In the dog at the NOAEL, the highest dose tested, the AUCs ratio of the dog NOAEL to that of the proposed human dose is 18 with a local safety margin of 3 for intranasal effects based on dose of olopatadine per nasal surface area.

In the 6-month intranasal bridging study in rats for the excipient Povidone, olfactory epithelial degeneration and respiratory turbinate epithelial vacuolation were observed at high incidence with some marked severity in Povidone treated groups in a dose-responsive manner at both doses tested ((b) (4)). As a result, there was no NOAEL identified. Thus, a safety assessment of intranasal use of Povidone cannot be conducted.

2.6.6.10 Tables and Figures - NA

2.6.7 TOXICOLOGY TABULATED SUMMARY - NA

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Systemic toxicity has been well studied with chronic oral studies in rats and dogs up to 52 weeks in duration. In rats, target organs of toxicity include the kidneys, heart, lungs, liver, eyes, urinary bladder, lymph nodes and pancreas. In dogs, target organs of toxicity include the kidneys, spleen, liver, heart, bone marrow, and eyes. Antimuscarinic effects such as mydriasis were commonly observed after treatment with this antihistaminic drug.

Olopatadine hydrochloride is not genotoxic or carcinogenic. However, based on decreases in number of live fetuses in rabbits and in pup viability within 4 days of delivery in rats, olopatadine should be labeled pregnancy category C. Olopatadine is

excreted in the milk of nursing mothers in rats. It is also crosses the placental barrier and distributed to the fetuses in rats.

Safety determinations after intranasal dosing include the active ingredient olopatadine and the excipient Povidone. In a 6-month chronic study where rats were dosed intranasally with olopatadine at 0.2 mg/day (0.014 mg/cm² of intranasal tissue) and 0.4 mg/day (0.029 mg/m² of intranasal tissue) with the first 2 months of exposure including the excipient Povidone at (b) (4), the results identified no apparent NOAEL as there were reported testicular effects and female mammary effects in the high dose group and the low dose group tissue samples were not evaluated histologically. These intranasal dosing results were compared to rat oral studies where a dose of 100 mg/kg was tested for 52-weeks and a dose of 200 mg/kg was tested in a 2-year carcinogenicity study with no comparable testicular and mammary results. Systemic absorption and metabolism were considered comparable by intranasal and oral exposure routes, so the effects observed, dose routes, and doses employed were considered in regard to systemic exposure with the chronic oral studies resulting in considerably larger doses. In a weight of evidence assessment, the effects observed in the high dose rats in the 6-month intranasal study, but not in the 52-week and 2-year oral studies, are considered incidental and the high dose was considered a NOAEL. At the NOAEL, the mean combined AUC₀₋₂₄ values for olopatadine was 79 ng.h/mL. Compared to the proposed human dose level AUC of 78 ng.h/mL, the systemic safety margin is 1. For local intranasal effects at the rat NOAEL dose of 0.057 mg/m² nasal surface area, the safety margin is 2 compared to the proposed human intranasal dose of 0.03 mg/cm².

The interim, 8-week sacrifice data from the 6-month rat intranasal study where Povidone was administered during the first 8 weeks where no local toxicity was observed, was used to support long-term clinical trials in adults (IND 60116 - teleconference minutes of February 14, 2003). Additional consideration for this decision was given to Povidone's use by other routes of administration and indication of no irritation potential when applied to mucus membranes. For the local intranasal effects of Povidone in the 8-week interim sacrifice with a NOAEL at the low dose (0.029 mg/m²), the safety margin is 1.

In two 9-month intranasal dog studies, no toxicity was observed and the highest dose tested was the NOAEL (18 mg/day - 0.08 mg/cm² of intranasal tissue). The safety margin for systemic effects for olopatadine based on the NOAEL (AUC of 1370 ng.h/mL) and the human AUC ratio of 78 ng.h/mL at the proposed human dose is 18. For local toxicity, the animal NOAEL for olopatadine is 0.08 mg/cm² with the proposed human dose of 0.03 mg/cm², resulting in a safety margin of 3. For local toxicity of Povidone, the animal NOAEL is 0.098 mg/cm² with the proposed human dose of (b) (4) with the resulting safety margin 1.

The results of the 2-week rat and dog studies with Povidone, in which no Povidone treatment-related systemic or local toxicity was observed, identified local nasal NOAELs for Povidone of (b) (4) of nasal tissue) in rats and of (b) (4) of nasal tissue) in dogs. At the human dose of Povidone of (b) (4) the local nasal effects safety margins are 2 for rats and 5.4 for dogs. The

rat was considered the more appropriate species for a 6 month bridging study with the excipient Povidone. This decision was based on the lower doses used in rats in the study and that no local nasal effects were exhibited in both rat and dogs studies. Doses of Povidone tested in rats in the 6-month intranasal study were (b) (4) of intranasal tissue surface area) and (b) (4) of intranasal tissue surface area). Neither of these doses were NOAELs as olfactory epithelial degeneration and respiratory turbinate epithelial vacuolation were observed at high incidence (30-90% of treated animals, 0% in vehicle control without Povidone) with some marked severity in a dose-responsive manner at both doses. These observed local effects were also consistent with observations in two 3-month intranasal rat studies that included Povidone-treated groups (studies of genotoxic impurities and leachates – studies TDOC-0001793 & 0001788). On this basis, the safety of the intranasal formulation cannot be assessed and the proposed human dosing level of Povidone of (b) (4) of intranasal tissue surface area contained in the olopatadine drug formulation is not considered safe to administer to humans.

Conclusions: In summary, the active ingredient olopatadine has been well studied. Intranasally, it is considered safe for human use at the proposed human dose as compared to chronic rat and dog studies in regard to local intranasal toxicity and systemic toxicity. The excipient in the drug formulation, Povidone, is not considered safe to use in the proposed formulation as no NOAELs for local intranasal effects in rats in the 6-month intranasal bridging study could be identified and the safety cannot be assessed.

Unresolved toxicology issues: lack of safety for intranasal use of excipient Povidone at proposed human formulation

Recommendations:

- 1) NDA is not approvable based on lack of safety of excipient Povidone in the intranasal formulation due to its local toxicity.
- 2) A 6-month intranasal study in rats is required to qualify Povidone in order to identify a NOAEL that would provide an adequate safety margin for the clinical use of the formulation.
- 3) The proposed labeling should be revised as suggested.

Suggested labeling:

Proposed

Carcinogenesis, Mutagenesis, Impairment of Fertility: Olopatadine administered orally was not carcinogenic in mice and rats in doses up to 500 mg/kg/day and 200 mg/kg/day, respectively. (b) (4)

(b) (4)

[Redacted] (b) (4)

Suggested

Carcinogenesis, Mutagenesis, Impairment of Fertility: Olopatadine administered orally was not carcinogenic in mice and rats in doses up to 500 mg/kg/day and 200 mg/kg/day, respectively (approximately 420 and 340 times the maximum human dose, MHD, by intranasal administration on a mg/m² basis).

[Redacted] (b) (4)

Olopatadine administered orally to male and female rats at oral doses of 400 mg/kg/day resulted in a decrease in the fertility index and reduced implantation rate (approximately 680 times the MHD on a mg/m² basis). No effects were observed on fertility at 50 mg/kg/day (approximately 85 times the MHD on a mg/m² basis).

Proposed

[Redacted] (b) (4)

Suggested

Pregnancy: Pregnancy Category C: [Redacted] (b) (4)
[Redacted] . No effect on viability was observed at 20 mg/kg (approximately 35 times the MHD on a mg/m² basis). There are, however, no adequate and well controlled studies in pregnant women. Because animal studies are not always predictive of human responses, this drug should be used in pregnant women only if the potential benefit to the mother justifies the potential risk to the embryo or fetus.

Proposed

(b) (4)

Suggested

(b) (4)

No mortality was observed after a single intranasal dose of 3.6 mg/kg in rats (approximately 6 times

(b) (4)

Signatures (optional):

Reviewer Signature Gary P. Bond, Ph.D., DABT

Supervisor Signature C. Joseph Sun, Ph.D. Concurrence Yes x No

APPENDIX/ATTACHMENTS: NDA 20-688 Pharmacology/Toxicology review, IND 60116 Pharmacology/Toxicology reviews (#3 & #6), and Animal to Human Dose Conversion Table to follow.

Pharmacology Review

NDA 20-688

Sponsor: Alcon Laboratories
Texas

Date Submitted: Jan 26, 1996

Date Received by CDR: Jan 29, 1996

Date received by HFD-550: Jan 31, 1996

Date assigned: Feb. 17, 1996

Date of Review: July 16, 1996

Drug: Opatanol 0.1% ophthalmic solution

Category: H₁ antagonist

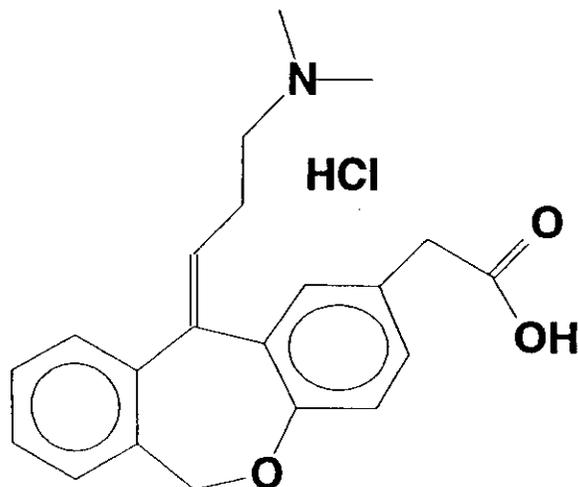
Indications: conjunctival allergy

Introduction:

Olopatadine is a H₁ antagonist and prevents the release of mediators from the conjunctival mast cells in eyes. It was conceived that the product would be effective for the symptomatic relief as well as prophylactic uses in the allergic conditions. Olopatadine does not have affinity to adrenergic, muscarinic, and serotonin receptors at the topical doses. Its systemic bioavailability in human subjects following topical administration in eyes is limited. Therefore, olopatadine is not expected to induce systemic toxicities. The maximum concentration of olopatadine in plasma following a two-week treatment with 0.15% ophthalmic solution in eyes was 1.3 ng/ml. The level is considered to be lower than that reached at tolerable oral doses. The plasma levels of olopatadine at the tolerable oral doses were between 9-7770 ng/ml following single or multiple doses. Clinical safety and efficacy of opatanol 0.1% solution were evaluated for six weeks in patients who were at least three years of age and older. The drug was administered three times a day in eyes in the six-week study. On the basis of the clinical and preclinical safety reports, the NDA is submitted for the use in allergic conjunctivitis in the pediatric and adult populations. A b.i.d. dosing regimen and unlimited length of treatment have been recommended. An oral dosage form of 10 mg tablets b.i.d. is under development in Japan. However, no marketing applications have been filed in any country.

Chemistry:

Olopatadine HCL is soluble in water. Molecular weight is 373.88. The chemical name is (Z)-11-[3-(Dimethyl amino)propylidene]-6-11-dihydrobenz[b,e]-oxepine-2-acetic acid hydrochloride. The chemical structure is shown below.



Inactive ingredients are, benzalkonium chloride 0.01%, dibasic sodium phosphate, sodium chloride, HCL or NaOH for adjusting the pH. The inactive ingredients do not have safety concerns.

Pharmacology:

Antihistaminic and antiallergic properties:

Page 5-0013 vol. 2, report 017:39900:0693:

Passive anaphylaxis in the conjunctiva were investigated in guinea-pigs by sensitizing animals with anti-ovalbumin serum injected subconjunctivally in one eye. Twenty four hours after the sensitization, animals were challenged with the antigen either by topical application to the eye or by i.v. injections. Evans blue dye was injected for the assessment of permeability of microvasculature. Thirty minutes before the challenge, animals were treated with 20 μ L of olopatadine or saline topically. In another set of experiment the pretreatment time was 4 and 8 hours. The ED₅₀ was determined on the basis of the scores for swelling, congestion and discharges from eyes. Following table shows the ED₅₀ of olopatadine.

| Antigen challenge | pretreatment time | ED50 %, w/v |
|-------------------|-------------------|-------------|
| i.v | 30 min | 0.0067 |
| i.v. | 8 hours | 0.2690 |
| topical | 30 min | 0.0170 |
| topical | 4 hours | 0.0529 |
| topical | 8 hours | 0.100 |

The data suggest that olopatadine prevents allergic response in eyes in a dose dependant manner and the response sustained more than 8 hours.

The *in vitro* antiallergic response of olopatadine was investigated in the rat basophil leukemic cell line. Cells were immunized passively by bovine serum albumin-dinitro phenol antibodies. The release of histamine was evoked by the DNP challenge. Olopatadine inhibited the histamine release from the RBL cells when preincubated for 15 minutes. The IC₅₀ value was 803 μ M. The experiment was repeated with human conjunctival mast cells. Cells were challenged with anti-human IgE. The release of histamine, tryptase and PGD₂ was assayed following preincubation

of cells at varying concentrations of olopatadine for 15 minutes. The IC_{50} for olopatadine was 559 μM for histamine release and 736 μM for PGD_2 release, respectively. Olopatadine also inhibited tryptase release. The effect was compared with ketotifen ($IC_{50}=24 \mu M$).

The *in vivo* anti-allergic response of olopatadine was investigated using pulmonary dynamics following antigen challenge to passively sensitized guinea-pigs. The ED_{50} for IgG and IgE mediated responses was 0.011 and 0.031 mg/kg/oral, respectively, when guinea-pigs were pretreated for one hour before the challenge. The ED_{50} of olopatadine for preventing anaphylactic bronchospasm in actively sensitized rats was 12.1 mg/kg/oral. The antiasthmatic effect of olopatadine was also evident in the conscious guinea-pig models of asthma at 0.05 mg/kg/oral.

The effect of olopatadine *in vitro* anaphylactic response was investigated in the guinea-pig tracheal smooth muscle preparations. The IC_{50} of olopatadine was 5.84×10^{-5} M.

The passive cutaneous anaphylaxis reactions in rats *in vivo* were inhibited at 0.11 mg/kg/oral doses. Passively sensitized rats were pretreated with olopatadine two hours before the challenge. The antigen induced pleural exudation in sensitized rats was also inhibited by olopatadine at 0.3 mg/kg doses. Olopatadine prevented the antigen induced deaths in sensitized rats at 24.4 mg/kg/oral doses when administered one hour before the challenge. Olopatadine pretreatment (i.p route) for 15 minutes prevented the migration of leukocytes in the bronchoalveolar lavage fluid when sensitized guinea-pigs were challenged with an aerosolized antigen. 0.1 mg/kg doses of olopatadine inhibited the cell migration in the BAL (broncho-alveolar lavage) fluid.

In vitro affinity of olopatadine for the H_1 receptor was determined by ligand binding experiments using 3H -pyrilamine. Olopatadine displaced pyrilamine binding competitively. The K_i values for inhibition of the binding were 16 nM, 16 nM, 45 nM and 9.5 nM for guinea-pig cerebellum, lung and trachea and mouse cerebral cortex, respectively. Data presented in page 5-0309 of vol. 3 for the radio ligand binding experiment showed that the K_i values for N-mono-demethyl olopatadine in guinea-pig cerebellum and lung were 22 and 33 nM, respectively. The K_i values for N-di-demethyl olopatadine in guinea-pig cerebellum and lung were 33 and 30 nM, respectively. These metabolites of olopatadine showed antihistaminic properties at H_1 receptors. The mono desmethyl olopatadine represents about 1% of the urinary metabolites. Therefore, it does not contribute towards the efficacy substantially.

The *in vitro* H₁ receptor antagonism was investigated using a functional parameter, i.e., PI turnover induced by histamine. The IC₅₀ values for olopatadine were 9.5 nM, 19 nM and 39.9 nM for cultured human conjunctival epithelial, human corneal fibroblast and human trabecular cells, respectively.

Antihistaminic activity of olopatadine in eyes was investigated following topical applications of the drug in guinea-pigs *in vivo*. Guinea-pigs received Evans blue dye intravenously about 45 minutes before a pretreatment with 20 μL of the drug or saline directly onto one eye of each experimental animal. Thirty minutes following the application, animals were anesthetized and injected with 300 ng of histamine subconjunctivally. Thirty minute later animals were sacrificed and the area of blue extravasation was measured. Olopatadine inhibited the histamine induced capillary permeability in the conjunctiva dose dependently. The IC₅₀ for inhibition of the capillary vascular permeability was 0.002%. By modifying the pretreatment time with olopatadine, the onset of inhibition was found to be 15 minutes and the duration was 4-8 hours. The IC₅₀ was 0.465%, 0.0195%, 0.004%, 0.0014%, 0.0056%, 0.035%, 0.114% at 1, 5, 15 minute, 2, 4, 8 and 24 hour pretreatment, respectively.

The above mentioned *in vivo* experiment was replicated in guinea-pigs using clinical formulations of 0.1 and 0.2% olopatadine. The data showed that the pretreatment with olopatadine for 1, 6 and 24 hours at 0.1 and 0.2% formulations inhibited the histamine induced conjunctival vasopermeability by 55 and 82%, respectively. The inhibition was sustained for almost 24 hours.

In an attempt to demonstrate that the antihistaminic effect of olopatadine was topical rather than systemic, an *in vivo* experiment was designed. The ipsilateral eye was pretreated with olopatadine or saline. The contralateral eye was treated with histamine. The permeability scores of the contralateral eyes were 197 and 199 for olopatadine and saline, respectively. The data suggest that pretreatment to the ipsilateral eye did not change the histamine-induced permeability to the contralateral eye. Therefore, olopatadine did not accumulate in the systemic circulation to exert topical antihistaminic activity.

Several *in vivo* experiments were conducted to demonstrate anti histaminic activity of olopatadine given parenterally or orally. Olopatadine pretreatment inhibited the histamine induced bronchoconstriction in guinea-pigs at 3-100 μg/kg, i.v. doses. The histamine induced cutaneous permeability in rats was inhibited at 0.014 mg/kg/oral doses. The histamine induced paw edema in rats was also inhibited at 0.014 mg/kg/oral doses. These

data support the antihistaminic activity of olopatadine in the non-ocular tissues. Overall the sponsor provided data that support the antihistaminic and **antiallergic** responses to olopatadine given orally or topically. The pharmacological effect of olopatadine was seen within 15 to 30 minutes after the topical applications and lasted for several hours. **The pharmacological effect of olopatadine following the topical administration was not mediated through systemic exposures from the bioavailable olopatadine.**

The effects of olopatadine in other receptors were investigated using radio ligand binding experiments. The IC_{50} for displacement of ligands from the specific binding sites was 10-50 nM for H_1 receptor, 1 μM for serotonin₂ receptor and 10 μM for serotonin uptake. Olopatadine has marginal activity toward α_1 adrenergic, dopamine₂, serotonin₁, muscarinic₁, and IL-1 receptor activity. **It has weak effect on the arachidonic acid metabolism and has no effect on the LTD₄ receptor.** The inhibition of prostaglandin cyclooxygenase (PGCO) from sheep vesicular microsomes was investigated. Olopatadine at 0.93-93 μM concentrations did not show PGCO inhibition. At 100 μM concentration the inhibition of PGCO was 26%. Similarly, olopatadine did not inhibit 5-lipoxygenase in the ionophore stimulated rabbit neutrophils up to 100 μM concentration. On the basis of the sensitivity differences for the pharmacological effect, olopatadine may be considered to be a selective H_1 antagonist with an antiallergic response.

Neuropharmacological effects:

Page 5-0039, vol. 2:

At 300 mg/kg/oral doses in mice olopatadine showed an increase in the respiratory rate. It also showed a sedative effect within 30 minutes of dosing at 300 mg/kg/oral doses in mice that disappeared after 2-3 hours. At 300 mg/kg/oral doses in mice, olopatadine did not show anticonvulsant effect. Olopatadine did not change phenyl quinone induced-writhing, reserpine-induced blepharoptosis, physostigmine-induced lethality and the normal body temperature at 300 mg/kg/oral doses in mice. The EEG activity for an arousal response to the auditory stimulation was not altered at 4 mg/kg/i.v. doses of olopatadine in rabbits.

Male cats treated with olopatadine at 3 and 30 mg/kg/oral doses did not change the EEG pattern during the sleep activity. At 30 mg/kg/oral doses vomiting was observed in the male cat. Olopatadine did not show local anesthetic activity on the corneal reflex at 2.0%. At 2% concentration, one out of 8 guinea-pigs

showed skin constriction to olopatadine.

Cardiovascular effect of olopatadine:

The cardiovascular effect of i.v. injections of olopatadine was investigated at 20, 50 and 100 mg/kg doses in the anesthetized dogs. Olopatadine showed a dose dependant hypotensive effect and a decrease in the peripheral resistance. The decrease in the blood pressure was 59% at 100 mg/kg doses. The heart rate was increased. However, hypotension and reflex tachycardia are normally seen with most of the antihistamines on i.v. dosing. The i.v. dose of 0.1 to 5 mg/kg of olopatadine was also investigated in mongrel dogs. These doses also showed pharmacodynamic responses as mentioned above. The guinea-pig right atrium preparations *in vitro* did not show any changes in the rate and contractility at 10^{-6} to 10^{-4} M. The data suggest that the effect of olopatadine on the heart was due to the reflex changes. An i.v. dose of 0.1 mg/kg inhibited the cardiovascular response of histamine in dogs. At 30-100 mg/kg oral doses olopatadine increased the urine volume, levels of sodium and chloride in rats.

A one day ocular irritation/comfort evaluation of olopatadine was examined in New Zealand rabbits. Two drops of 0.1% solution were instilled every 30-minute interval for a total of 10 drops. Comfort to the eye for irritation was examined bio-microscopically. Eyes were examined immediately after the first, second doses and one hour after the last dose. Fluorescein intensity score was zero out of a scale of 4. Data suggest that the topical applications of olopatadine did not show local irritancy and discomfort.

Toxicity studies:

Six-month topical ocular irritation and systemic toxicity of olopatadine ophthalmic solution in rabbits: Interim sacrifice at the end of three months.

Page 5-0868, vol. 5, report TR 030:38520:0395:

The study was conducted at Alcon Laboratories according to the GLP. Male and female rabbits weighing between 2.4 and 3.1 kg were used in the experiment. The animals were about 3 and half months of age at the initiation of the experiment. An biomicroscopic eye examination was conducted in rabbits before enrollment in the study on day zero for both eyes. A score of zero for all parameters and a score of 0-1 for the conjunctival congestion was considered to be normal for the purpose of the study. The

treatment groups are shown in the following table.

| Group | Treatment | Animals (number/sex) | Interim Obs. at 3 month (number/sex) | Final obs. at 6 months (number/sex) |
|-------|----------------------|-------------------------|--|---|
| 1 | untreated control | 10 | 4 | 6 |
| 2 | vehicle | 10 | 4 | 6 |
| 3 | 0.15% | 10 | 4 | 6 |
| 4 | 0.5% | 10 | 4 | 6 |
| 5 | 1.0% | 10 | 4 | 6 |

Among ten rabbits/group/sex, four were assigned for interim evaluation at the end of three months. The remaining six rabbits/group/sex were allowed to complete the six-month treatment period. Each rabbit was treated four times a day. Two drops of the test article or the vehicle were instilled into the right eye at each dosing interval. The left eye served as the contralateral untreated control. The first dose on day 1 and the fourth dose on day 91 were not administered. Considering each drop is equal to 32 μL , each treatment was equal to 64 μL of the drug solution.

Animals in the second group were treated with the vehicle that consist of 0.01% benzalkonium chloride, dibasic sodium phosphate 0.5%, sodium chloride 0.65%, pH of the solution was adjusted to 7.0.

Animals were observed twice daily for the clinical signs and toxicity. Each animal was examined twice per week in details for the toxic signs. Animals were weighed prior to the dosing and on each week up to week 13. Thereafter, body weights were recorded on weeks 16, 21 and on 24.

Both right and left eyes were examined on day 0, 7, 14, 21, 35, 56, 84, 91, 112, 147, 168, and 189. The biomicroscopic examinations included conjunctiva, cornea, anterior chamber, light reflex, lens and iris. In addition to above examinations, indirect ophthalmic examinations were conducted on days 0, 91 and 189. The parameters examined in the indirect ophthalmoscopic examinations included fundus, optic head nerve characteristics, fundic vascular pattern and pigmentation. The pupil of each eye was dilated with mydryacil. Ultrasound pachymetry measurements were obtained on days 1, 90 and 188.

Blood samples were collected on days 91 (interim) and days 188-189 at scheduled necropsy for serum chemistry and hematology. Pharmacokinetic parameters of olopatadine were determined on days 1, 90 and 181. These results have been discussed in the PK section. Rabbits were sacrificed on days 92 (for interim) and 190 for gross changes, organ weight determinations. All tissues and organs were fixed for necessary histological examinations. Eye tissues from all animals for the three-month interim sacrifice were evaluated. Eye tissues from the untreated control and high dose groups were submitted for the histological examinations. Tissues with any gross lesions were also examined for histological changes.

Results:

Chemical potency of the test article and the preservative showed 95-101% of that claimed in the label. There was no treatment related mortality observed in these animals. However, several rabbits were sacrificed as shown in the following table.

| Group | Sex | Remarks |
|-------|--------|---------------------|
| 2 | Female | Day 23, skin wound |
| 3 | Male | Day 88, Broken neck |
| 4 | Female | Day 119, Abscess |

Ocular discharges were seen on days 126, 161 in the right eyes of a male rabbit from the vehicle control group, the left eye on days 115 and 185 from a male rabbit at 0.5% olopatadine, from the right eye of a male rabbit on days 35, 87, 122 and 129 at 0.5% olopatadine, from the right eye of a female rabbit at 0.15% olopatadine on day 80, from the right eye of a female rabbit on day 87 at 0.5% and from unspecified eyes in two female rabbits on days 101, 119 and 168 at 1.0% olopatadine. The data suggest that there was no treatment related trend for the ocular discharge in these rabbits. Some of the animals from the untreated control and treated groups showed loose stool. However, it was considered to be incidental due to a lack of treatment related trend in its occurrence. The body weight changes during the first 91 days of the treatment are shown in the following table.

| Group | Male, Day 0, Kg | Male, Day 91, kg | Female, Day 0, kg | Female, Day 91, kg |
|-------------|--------------------|---------------------|----------------------|--------------------------|
| 1, N=10/sex | 2.9 | 3.5 | 2.7 | 3.6 |
| 2, N=10/sex | 2.9 | 3.6 | 2.7 | 3.4 |
| 3, N=10/sex | 2.9 | 3.5 | 2.7 | 3.5 |
| 4, N=10/sex | 2.9 | 3.5 | 2.7 | 3.5 |
| 5, N=10/sex | 2.9 | 3.5 | 2.6 | 3.4 |

Above data do not show treatment related changes in the weight gain in the male and female rabbits. At the end of the interim sacrifice, remaining animals were observed for the changes in the weight gain. Both male and female rabbits did not show treatment related changes in the body weight gain. However, female rabbits showed a higher body weight gain than the male rabbits for the six-month study.

The slit-lamp examinations conducted on prescreen, days 7, 14, 21, 35, 56, 84, 91, 112, 147, 168 and 189 did not reveal any treatment related conjunctival congestion except isolated incidences of severe congestion in three female rabbits in group 3 and one female rabbit in group 5. The drug treated, untreated and vehicle treatment also showed conjunctival congestion in the left eyes. The right eye did not show any congestion. There was no conjunctival swelling in the treated and untreated control eyes during six months of the treatment. There was no ocular discharge, change in the light reflex from the left and right eyes during 189 days of observations. There was no incidences of aqueous flare in the left and right eyes during 189 days of observations except a single incidence in the left eye of the untreated control rabbit on day 189. **Therefore, olopatadine treatment for 3 and 6 months did not show treatment related congestion, changes in the ocular reflex and flare.**

For the three-month interim observation, cataract was observed in one untreated female rabbit and one female rabbit treated with 0.5% olopatadine. Both eyes of these animals were affected. For the six-month observation period, cataract was observed in one female each from the vehicle and 0.15% olopatadine group, one male from the 1.0% olopatadine group. **Therefore, olopatadine treatment did not induce opacity to lens in the rabbits during 3-6 months of the treatment.**

Indirect ophthalmoscopic examinations did not reveal any treatment related changes in the left and right eyes of rabbits on days 1, 91 and 189.

Male and female rabbits did not show differences in the pachymetry readings for the 91-day observation period. The results for the six-month observation period was also similar to that for the three-month period. It is concluded that olopatadine did not affect the viscosity of the blood in the ocular microcirculation based on the pachymetry data.

Serum chemistry data did not show any treatment related abnormality at the end of three months. However, serum cholesterol and TG levels were increased in male rabbits at 1.0% olopatadine compared to the untreated control. The BUN/creatinine ratios were also decreased at 1.0% in female rabbits compared to the control. The mean albumin values at 0.1% olopatadine in female rabbits were increased. These changes were statistically significant although data were within the normal range of the laboratories.

At the six-month evaluation period, uric acid levels were reduced at 0.15% in male rabbits, GGT activity was reduced at 0.15% in female rabbits.

None of the changes in the serum chemistry parameters at the end of three and six months were considered to be treatment related.

Hematology parameters showed a decrease in the basophil counts in male rabbits at 1.0% at the end of six months. The hematology data at the end of three months of the treatment was unremarkable. Therefore, olopatadine treatment did not affect the blood counts except basophils. The change could have been related to the H₁ antagonism.

There was no organ weight changes in male and female rabbits at the end of six months of the treatment. Gross pathological changes were incidental and there was no treatment related trend.

Histopathology data at the end of three months suggest that there was lymphocyte infiltration in most of the treated and untreated animals. Other than retinal degeneration in one female rabbit at 0.15% olopatadine, there was no treatment related pathological changes observed in eyes at the end of 3 months.

Histology data at the end of six months showed cyst in thyroid in four out of six male rabbits at 1.0%. The untreated control showed cyst in thyroid in one out of six male rabbits. Congestion

and inflammatory changes in the lung were observed at 1.0% olopatadine in male and female rabbits. Other than lymphocyte infiltrations in the untreated and treated rabbits, there was no histological abnormalities in eyes.

To conclude, it can be stated that olopatadine at 0.15, 0.5 and 1.0% ophthalmic solution did not show ocular or systemic toxicity within three to six months.

Six-month topical ocular toxicity test of olopatadine in primates.

Page 5-1443, vol. 7, project 22-5129

The study was conducted according to the GLP. Cynomolgus monkeys approximately 4-15 months of age were used in the study. The condition of the eyes were examined by biomicroscope before the study. Animals that showed conjunctival congestion scores between 0 to 1 were used in the study. The vehicle or the ophthalmic solutions were administered four times daily to the right eye. The left eye of each animal served as the contralateral untreated control. Each animal received two drops of the test article or the vehicle. The study design is shown in the following table.

| Group | Treatment | Dose/day | Vol/dose |
|----------|-----------|----------|------------|
| 1, 4/sex | untreated | 4 | |
| 2, 4/sex | vehicle | 4 | 80 μ L |
| 3, 4/sex | 0.1% | 4 | 80 μ L |
| 4, 4/sex | 0.2% | 4 | 80 μ L |
| 5, 4/sex | 0.5% | 4 | 80 μ L |

All animals were observed daily for clinical signs. Body weights were recorded periodically. Biomicroscopic examinations (slit-lamp) were done on days 0, 7, 14, 28, 42, 56, 84, 112, 140, 168 and 182. Indirect ophthalmoscopic examinations were conducted on days 0, 84 and 182 following dilatation of the pupil by mydriacil. Pachymetry measurements were also taken on days 0, 84 and 182. The protocol of the study is similar to that of the six-month rabbit study described above. Therefore, the protocol for the rabbit study should be referred.

Serum chemistry and hematology were conducted on days -5,-4; days 92-93; and on days 177-178. Blood samples were collected on days 1, 45, 90 and 181 for plasma levels of olopatadine from groups 3,

4 and 5. These data have been presented in the PK section. Tissue samples were fixed and preserved following necropsy on the day 182-183 for histological examinations. Gross changes in the organs were also examined during the necropsy.

Results:

The chemical stability of the drug was determined and found to be satisfactory. Treatment related clinical signs were not observed in the treated animals. However, diarrhea and injuries were observed in some animals. These findings are not unusual for caged primates. There was no statistically significant changes in the body weight gain in male monkeys although animals treated at 0.1 and 0.5% olopatadine showed higher initial body weight on day 0. Female animals did not show significant changes in the body weight gain.

Slit-lamp biomicroscopic examinations did not show treatment related conjunctival congestion and conjunctival swelling. **Corneal cloudiness was observed in one male (OD) animal at 0.2% solution of olopatadine (X1522) from day 7 to the end of the study.** Similar change was also observed in a untreated control animal in the left eye (male X1531). Therefore, the observation was an idiosyncratic response rather than a dose related toxicity. Similarly, fluorescein staining did not show compound related changes in the cornea.

Biomicroscopic examinations of the lens showed superficial and focal **corneal opacity in the nasal cornea** and that was present in both eyes during days 84 to 168 in a male (X-1531) at 0.2% olopatadine. The changes may be due to the natural aging process and not treatment related. No other animals at 0.5% olopatadine showed similar changes in the cornea. Indirect ophthalmoscopic examinations did not reveal any treatment related changes.

There was no treatment related effect in the right eye of animals for the pachymetry data although differences were seen between the initial readings, readings on days 82 and 182. These differences have large standard errors. The untreated left eyes also showed the same trend. Corneal endothelial cell counts in the right and left eyes did not show differences in the cell density. The endothelial cells in the inner surface of the cornea is responsible for the transport of water. The normal process ensures prevention of accumulation of excess water in the cornea. The data signify that olopatadine did not affect the water transport mechanism of the cornea.

Blood chemistry data did not show any treatment related changes in the male and female animals. However, there were intermittent changes in the calcium, glucose and phosphorus levels in some treated animals at the end of three months and recovered at the end of six months.

For hematology parameters, hematocrit values and RBC counts were reduced in all treated females after the end of 3 and 6 months of the treatment. However, there was no changes in the hemoglobin levels. White cell counts were not affected by the treatment.

Plasma levels of olopatadine:

The mean peak plasma levels of olopatadine were 1.72, 2.89, 7.60 ng/ml at 0.1, 0.2 and 0.5% doses, respectively. The trough levels at 0.5% dose was 0.93 ng/ml. The trough levels at other doses were below the limit of detection at 0.5 ng/ml. The data suggest that olopatadine is bioavailable following topical applications in a dose dependent manner.

Organ weight data showed an increase in the percent weight of adrenals in female monkeys. Female monkeys showed a decrease in the weight of the kidney at all doses compared to the vehicle control (13.1, 10.5, 10.4, 11.2 g at control, 0.1, 0.2 and 0.5%, respectively). Gross examination showed that a male monkey (X-1523) had a smaller adrenal on the left side compared to the contralateral side.

Histology data at the end of six months showed periportal hepatocellular vacuolization of livers in one male each from 0.2 and 0.5% doses, and two females from 0.5% dose. The male monkey (X-1523) had hypoplasia of adrenal that was otherwise remarkable. Nematode parasites were present in some animals that was responsible for the granulomatous changes in the GI tract. Protozoan parasitic infections were also noted in some monkeys in the skeletal muscle, tongue and ocular muscle. These protozoans have been reported to be common in monkeys. Inflammatory changes in the lungs were present, however, it was not treatment related. Immature spermatid and spermatozoa were seen in some control and treated animals that represented sexual immaturity. Perioviductal cysts were seen in some animals that was unrelated to the treatment. There was no treatment related changes in the kidney in male and female animals.

There was no treatment related histological changes in the eyes. Mononuclear cell infiltrations were noted in the eyelids of treated animals. However, it was not dose dependent.

To conclude, the findings of the six-month chronic toxicity study of olopatadine ophthalmic solution suggest that there was no treatment related ocular and systemic toxicity in monkeys up to 0.5% solutions given four times a day.

Acute oral toxicity of olopatadine in mice:

Page 5-1900 vol. 8:

(b) (4)

Male and female slc:ICR mice weighing 17-19.6 g of body weight and approximately 3 weeks of age were used in the study. The drug solution was administered orally at 0.25, 0.5, 1.0 and 2.0 g/kg in groups of 5 mice per sex. Animals were observed for 6 hours for the clinical signs and thereafter up to 14 days. Necropsy was performed at the end of 14 days or after the unscheduled death. Histology was performed on selected organs that showed gross changes. The LD₅₀ of olopatadine was 1.15 g/kg for males and 1.83 g/kg for females. Mortality was observed within one hour to day 4 of the study. Reduction in the spontaneous activity, blepharoptosis (inflamed eyelids) and mydriasis were seen as clinical signs. Abnormal gait, tremors, convulsions, hypothermia and dyspnea were seen for animals that died at high doses. Animals that died several days after dosing showed pyelectasis (dilatation of renal pelvis). Hydronephrosis was present among surviving animals. Therefore, the target organ of toxicity in the acute toxicity test was kidney.

Oral and i.v. toxicity of olopatadine in rats:

Page 5-1957 vol 8:

The experiment was conducted according to the GLP. Male and female Slc: Wister rats weighing 101-145 g were used in the study. Rats were approximately 5 weeks old at the time of the drug administration. Each group had 10 rats/sex. Animals were fasted for 17 hours before administration of the drug substance. The test substance was suspended in distilled water for oral and i.v. dosing. It is not clear why the test substance had to be suspended in water when it is soluble in water. The pH of the solution was between 2.07-2.32. Oral doses tested were 0.8, 3.0 and 5.0 g/kg. The i.v. doses were 110, 125 and 130 mg/kg. Animals were observed for mortality and clinical signs initially at 1, 2, 4, 6 and 24 hours after dosing. Following the first day of dosing, the animals were observed twice daily for 14 days. Animals that survived were sacrificed at the end of day 14.

Histology was performed for tissues that showed gross changes. The drug substance in the solution was stable for six hours.

Results:

The male rats showed 40% mortality at 3 and 5 g/kg/oral doses. The oral LD₅₀ of female rats was 3.87 g/kg. The i.v. LD₅₀ of male rats was 127.5 mg/kg and female rats were 142.8 mg/kg. Most of the acute death occurred within 24 hours after oral doses and within few minutes after i.v. doses. Mydriasis, vasodilatation of iris, localized hemorrhage or hyperemia at the rim of iris, lacrimation, writhing movement, atony of skeletal muscle, dyspnea, reduced respiratory rate, convulsions, hypothermia and relaxation of scrotum were seen as toxic signs after oral dosing.

The toxic signs immediately after i.v. administration were relaxation of the scrotum, reduced spontaneous activity and Straub tail response. Dead animals showed dyspnea before the death. Inflammation at the site of injections were also seen in these animals.

Histological examinations of animals that received oral doses showed hydronephrosis and renal papillary necrosis. Dilated renal pelvis was observed for animals that survived after the treatment. Edema in irises was observed among dead animals.

Histology examinations of animals that received i.v treatment showed dilated renal pelvis.

Kidney was the target organ of toxicity at acutely toxic doses. The acute signs of toxicity in eyes include mydriasis, vasodilatation and hemorrhage of the iris and lacrimation.

CNS effects and peripheral irritation are expected from olopatadine at toxic doses that precipitated Straub's tail response, writhing movement and inflammation at the site of injections. However, there was no vehicle control group in the study for comparing the inflammation at the site of injection. Straub's tail response normally seen with opioids, it is interesting to note this type of response in an antihistaminic drug.

Acute oral and i.v. toxicity of olopatadine in dogs:

Page 5-2032, vol. 8, study A-89-62:

The study was conducted according to the GLP. Beagle dogs at 5-6 months of age at the initiation of the study were used. Animals

weighed 7.1-10 kg at the initiation of the experiment. In the preliminary experiment one dog/sex/group was given olopatadine at 0.5, 1.0 and 5.0 g/kg/oral doses. Vomiting was observed at all doses in these dogs. One dog that received the vomited gastric content orally died due to difficulty of swallowing. Rest of the dogs survived during 14 day observation period. On the basis of the preliminary study, 5 g/kg/oral was the dose chosen for the definitive study. The group size was 2/sex. There was no control group in the study.

The preliminary study for the i.v. route was conducted at 150 and 300 mg/kg doses using one dog/sex/group. Convulsions were noted in dogs that received 300 mg/kg dose of olopatadine. All animals survived during the 14-day observation period. The final experiment was conducted at 150 and 300 mg/kg i.v doses in male dogs. The group size was 2 dogs/dose.

Intravenous injections were prepared in distilled water for injection and oral doses were given in capsules. The pH of the solutions were between 1.85-2.32

The animals were observed for the first 6 hours and daily for 14 days. The clinical signs were observed during this period. The body weight, food consumption, blood chemistry, EKG and eye examinations were included in the protocol. At the end of 14 days, animals that survived during the observation period were sacrificed for the determination of organ weights, gross and histological examinations.

Results:

Intravenous doses:

One dog died following an i.v. dose of olopatadine at 300 mg/kg within five minutes. Rest of the animals survived during the 14-day observation period. Clinical signs following the i.v. doses were mydriasis, protrusion of the nictitating membrane, vomiting, tonic and clonic convulsions, irregular breathing and dryness of the nose. However, convulsions were not observed at 150 mg/kg dose. Hypothermia and hyperthermia were noted at 150 and 300 mg/kg doses, respectively. Body weight and food consumption were not affected remarkably except one animal in the 300 mg/kg group had a slight reduction of the body weight gain. Tachycardia was observed at 150 and 300 mg/kg i.v. doses. The sponsor stated that the height of P and T waves were increased on the day of injection for both doses that might resulted from the drug induced hyperirritability of the resting membrane potential. About 50% of the dogs showed normal EKG from day 2 onwards. Transient increase

in the CPK, LDH and HBDH(alpha-hydroxybutyrate dehydrogenase) and transient decrease in the potassium and calcium levels were observed. The funduscopic examinations did not reveal any treatment related changes. Histological examinations showed sign of inflammation at the site of injections. The cause of death was not clear from the histological examination of the dog that died within five minutes after the injection. One animal at 150 mg/kg dose showed inflammatory changes in the heart.

The single dose data suggest that up to 150 mg/kg i.v was tolerated in dogs. Vomiting, mydriasis and protrusion of nictitating membranes were the clinical signs at this dose. Cardiac abnormality, e.g., tachycardia and increase in the P and T waves are expected following olopatadine treatment.

Oral dose:

There was no mortality observed in the treated dogs. Clinical signs were vomiting within an hour after dosing, dryness of nose, mydriasis, salivation, nasal discharge, protrusion of nictitating membrane, conjunctival hyperemia and soft stool. Hypothermia was observed at about 2 hours after the dosing that became normal after about 6 hours. The decrease in the temperature was about 1.5°C. There was no changes in the body weight gain and food consumption compared to the pretest level.

Dogs showed a depression of S-T segment of the EKG that persisted on the next day. However, one of the four dogs showed normal EKG on days 3 and 14. It is possible that olopatadine upon high oral dose induces transient cardiac ischemia. Funduscopic examination in eyes did not reveal any treatment related changes. For the clinical chemistry parameters, there was an increase in the LDH and CPK that might come from the ischemic cardiac tissues. Potassium and calcium levels in the serum were reduced also. However, histology reports did not show any cardiac abnormalities following oral administration of olopatadine.

Oral dose of 5 g/kg was tolerated in male and female dogs. Mydriasis, protrusion of the nictitating membrane, conjunctival hyperemia, hypothermia and EKG changes were clinical signs of toxicity.

A four-week oral toxicity of olopatadine in rats:

Page 5-2081, vol. 8:

This is a GLP study conducted at (b) (4)
(b) (4) The study was conducted in male and female Wister rats.

There were 10 rats/sex/group. Rats were treated with the vehicle (distilled water for injection) or either of the 20, 60, 200 and 600 mg/kg oral doses. Animals were 5 weeks of age and weighed 86-121 g at the initiation of the study. It appears that the rats were very young for a toxicity study point of view. Clinical signs, ophthalmological examinations, body weight food consumption, blood chemistry, hematology, urine analysis, gross and microscopic examinations were conducted during the study.

Results:

There was no treatment related mortality reported in the study. Clinical signs were mydriasis at 600 mg/kg, abnormal respiration at 60 mg/kg and higher doses. Body weight gain was reduced at 600 mg/kg dose by 19% for the male and 27% for the female rats compared to the untreated control. The changes in the body weight gain were due to a reduction in the food consumption. Ophthalmoscopic and hematological examinations did not reveal any treatment related changes. Blood chemistry data showed that serum protein levels were increased, cholesterol and TG levels were decreased at 600 mg/kg dose in male rats. The changes in the serum parameters suggest that the drug might have toxicity in the liver. Urine analysis showed evidence of protein and blood at 200-600 mg/kg doses.

Gross changes were noted at 600 mg/kg dose group. These were atrophy of seminal vesicles and prostate, hemorrhage in the mucosa of the bladder, distended cecum, hemorrhage and opacity of the eye balls, pulmonary hemorrhage and necrosis of the liver.

The histology data suggest that there was inflammatory changes in the liver, urinary bladder, parasternal lymph nodes and hyperplasia of pancreatic ducts. It can be concluded that 600 mg/kg/oral dose was toxic to the liver and urinary bladder. It is possible that the compound or its metabolites might have been concentrated in the urine, induced inflammation in the kidney and the urinary bladder.

A thirteen-week oral toxicity study in rats:

(Page 5-2150, vol. 8):

The experiment was conducted at (b) (4) (b) (4) according to the GLP. Wister rats weighing 87-126 g of body weight and 5 weeks of age were used in the experiment. Experimental design is shown in the following table.

| Dose (mg/kg/oral) | Male | Female |
|-------------------|------|--------|
| Control vehicle | 15 | 15 |
| 6 | 15 | 15 |
| 25 | 15 | 15 |
| 100 | 15 | 15 |
| 400 | 15 | 15 |

The drug solution was prepared in water for injection and administered by oral gavage. During the study mortality, clinical signs, body weight, food consumption, ophthalmoscopic examinations before and after dilatation of the pupil, auditory examination, hematology, blood chemistry, urine analysis, gross and histological examinations were conducted. At the end of 13 weeks, 5 rats/sex/group were allowed to recover for 4 weeks.

Results:

Four female rats at 400 mg/kg dose died, most of the death occurred during 6-8 weeks of the study.

Clinical signs observed at 400 and 100 mg/kg doses were abnormal breathing, relaxation of scrotum, lacrimation and blepharoptosis (drooping of the upper eyelid). Some of the animals showed blood stain around the corneal rim, mydriasis, exophthalmos (abnormal protrusion of the eyeball), alopecia, testicular atrophy and diarrhea. However, these incidences were not dose related.

The body weight (gm) of animals during the days 0, 91 and 119 are shown in the following table.

| Days | control | | 6 mg/kg | | 25 mg/kg | | 100 mg/kg | | 400 mg/kg | |
|-----------|---------|-----|---------|-----|----------|-----|-----------|-----|-----------|-----|
| | M | F | M | F | M | F | M | F | M | F |
| 0 (n=15) | 116 | 93 | 115 | 93 | 115 | 93 | 115 | 93 | 115 | 92 |
| 91 (n=15) | 348 | 190 | 342 | 197 | 330 | 190 | 322 | 190 | 286 | 180 |
| 119 (n=5) | 381 | 211 | 376 | 211 | 373 | 206 | 365 | 212 | 340 | 189 |

Male rats at 100 mg/kg dose showed a decrease in 10.78% of the body weight gain compared to the control. Female rats did not show any change in the body weight gain at 100 mg/kg dose. However, male rats showed a loss of 26.3% body weight gain and female rats showed 9.3% loss of body weight gain at the end of 91

days at 400 mg/kg dose compared to the control. Therefore, olopatadine treatment affected the body weight gain in male and female rats at 100-400 mg/kg doses. The loss of weight gain was partially recovered during the four-week withdrawal period.

The food consumption in male rats varied from 16-18 g/week in the control and that for 400 mg/kg dose group varied from 14-16 g/week during the treatment period. The data suggest that the loss of body weight gain was not related to the food intake.

The sponsor stated that there was no drug related changes observed following the ophthalmological examinations. Similarly there was no change in the auditory reflex.

Hematological examinations showed that RBC counts were decreased in male rats at 400 mg/kg dose on day 91 with a concomitant increase in the MCV. The blood Hb levels did not change. Above-mentioned changes in the RBC parameters were reversible. Female rats did not show any treatment related changes.

Blood chemistry data showed an increase in the serum choline esterase activity at 25 mg/kg and higher doses in male rats. The total cholesterol levels were also increased at 25 mg/kg and higher doses in male rats. Serum alkaline phosphatase activity was also increased in male rats at 100 and 400 mg/kg doses. Although female rats did not show above changes in a dose related manner, serum alkaline phosphatase, GOT and GPT activities were increased in female rats at 400 mg/kg dose. Male rats showed statistically significant increase in the A/G ratios and alkaline phosphatase activity at the end of 119 days recovery. These chemistry data suggest that there might have been treatment related changes in the liver in the male rats specially at 400 mg/kg dose.

Urine analysis data did not show remarkable treatment related changes except a decrease in the pH in female rats at 400 mg/kg dose.

Gross pathological examinations showed atrophy of testes, congestion of kidney and lungs mostly at 400 mg/kg dose. Female rats that died during the dosing period showed blot of eyelids, blood clot in the esophagus and congestion in the liver and lungs.

Relative organ weight data showed an increase in the weight of the lung, kidney, adrenals, testes, thymus and brain at 400 mg/kg dose in male rats. Female rats also showed a decrease in the weight of heart, spleen, liver, adrenals and pituitary at 400

mg/kg dose. A decrease in the weight of the heart was seen in female rats at 100 and 400 mg/kg doses.

Histological changes in the male rats at the end of 91 days were myocardial degeneration, hydronephrosis of the kidney, swelling in the adrenal cortex, inflammation in the prostate, seminal vesicle and lacrimal glands at 400 mg/kg dose.

Female rats also showed myocardial degeneration, hemorrhage in thymus, hypoplastic bone marrow, a decrease in the zymogen granules in the pancreatic acinar cells, inflamed adrenal cortex, atrophy of uterus at 400 mg/kg dose.

Animals (females) that died during the experiment showed degenerative changes in the alveolar wall in the lung and congestion of the kidney.

To summarize the data it may be concluded that olopatadine at 100 mg/kg/oral was tolerated for three months. Female rats that died during the experiment probably died due to degenerative changes and congestion in the lungs. It appears that administration of olopatadine at 400 mg/kg dose showed myocardial degeneration that might be treatment related. The changes in the transaminase and alkaline phosphatase activity would also explain the finding. On the basis of the data, doses close to 25 mg/kg may be chosen for the chronic toxicity study.

A 52-week oral toxicity of olopatadine in rats:

(Page 5-2294, vol. 9):

The study was conducted at (b) (4) according to the GLP. Male and female Wister rats were used in the study. Rats were about 5 weeks of age and weighed 90-120 g at the initiation of the study. The experimental conditions and study design were essentially same as that of the three-month study. The study design is shown below:

| Drug | Dose (mg/kg/oral) | Number of male | Number of female |
|-----------------|-------------------|----------------|------------------|
| vehicle control | distilled water | 30 | 30 |
| Olopatadine | 1 | 30 | 30 |
| Olopatadine | 10 | 30 | 30 |
| Olopatadine | 100 | 30 | 30 |

Ten animals/group/sex were used for an interim evaluation at the end of six months of dosing.

Results:

Mortality data are shown in the following table.

| Dose (mg/kg) | Male/Day | Female/Day |
|--------------|-----------|------------|
| Control | 1/237 | 1/325 |
| 1 | 2/253,355 | 1/327 |
| 10 | | 1/312 |
| 100 | 2/28,97 | |

Labored breathing was observed in some of the animals at death including the control. Clinical signs were abnormal respiratory sound at 100 mg/kg, mydriasis at 10 (female only) and 100 mg/kg doses.

Body weights (gm) of the animals are shown in the following table.

| Days | control | | 1 mg/kg | | 10 mg/kg | | 100 mg/kg | |
|---------------|---------|------|---------|------|----------|------|-----------|------|
| | M | F | M | F | M | F | M | F |
| 0 (n=30) | 119 | 99.5 | 119 | 98.9 | 119 | 99.4 | 119 | 98.9 |
| 182 (n=28-30) | 420 | 225 | 422 | 225 | 429 | 220 | 401 | 219 |
| 364 (n=18-20) | 462 | 285 | 473 | 274 | 476 | 281 | 440 | 262 |

Data in the table showed that at the end of six months, male and female rats had 4-6.4% loss of the body weight gain. At the end of one year, male rats had 6.4% and female rats had 12% loss of body weight gain. The loss in body weight gain was not related to the food consumption.

There was no dose related ophthalmic changes observed in the study. However, opacity of cornea, hyperemia, hemorrhage at the corneal margin, narrowing of fundic blood vessels were observed during the study. The sponsor has not provided the data table on the ophthalmic changes.

Hematology data did not show test substance related changes except transient decrease in the Hb levels in the serum at 100 mg/kg dose at the end of one year. Blood chemistry data at the end of six months showed an increase in the alkaline phosphatase activity at 100 mg/kg dose in female animals. At the end of one year, alkaline phosphatase (male and female) and GOT activities were increased in male rats at 100 mg/kg dose. The pH of the urine was decreased during the study period.

Normalized organ weight data showed a decrease in the weight of pituitary in female rats at 100 mg/kg at the end of six months. Male rats showed a decrease in the weight of spleen, prostate and increase in the weight of testes at 100 mg/kg at the end of one year. Female rats showed an increase in the weights of the heart, uterus and adrenals at 100 mg/kg at the end of one year.

Histological changes at the end of six months of the treatment are shown below

Myocardial fibrosis at 100 mg/kg in male rats, dilatation of uterus at 100 mg/kg.

Histological changes at the end of one year are shown below:

Regeneration of tubular epithelia of kidney in male and female rats dose dependently, micro granuloma of liver at 100 mg/kg in female rats, hypocellular marrow in female rats at 100 mg/kg, interstitial myocarditis in male and female rats dose dependently.

Histological findings among dead animals are given below.

Regeneration of tubular epithelia, thickening of capillary wall in glomerulus, congestion in the lung, myocardial fibrosis, degeneration of myocardium and myocarditis and focal necrosis of hepatocytes. Most of these changes occurred in male rats at 100 mg/kg dose.

It can be concluded from the study that olopatadine was tolerated up to 100 mg/kg dose in rats for one year. Although mortality was observed at 100 mg/kg dose, it could have been due to dosing errors. Regeneration of tubular epithelia of kidney and interstitial myocarditis were noted in male and female rats across the doses, the biological significance of the change is not understood. If there would have been a trend of mortality in the presence of histological changes in the heart and kidney in excess of the control rats, these changes could be a major safety

concern. The incidences are shown in the following table:

| Lesions | control | 1 mg/kg | 10 mg/kg | 100 mg/kg |
|---|--------------|---------------|----------------|---------------|
| regeneration of tubular epithelia in kidney | 8 (M), 4 (F) | 17 (M), 7 (F) | 15 (M), 11 (F) | 14 (M), 5 (F) |
| Interstitial myocarditis | 6 (M), 1 (F) | 6 (M), 5 (F) | 12 (M), 2 (F) | 9 (M), 3 (F) |

A thirteen-week oral toxicity study in dogs:

Page 5-2427, vol. 9:

The study was conducted at (b) (4) according to the GLP. Beagle dogs aged 5-6 months weighing 7.5-11.6 kg were used in the study. The doses were chosen on the basis of the preliminary study. The experimental groups are shown below.

| Treatment | Dose (mg/kg/oral) | No. Of Male | No. Of Female |
|-------------|-------------------|-------------|---------------|
| Control | gelatin capsule | 5 | 5 |
| Olopatadine | 0.6 | 3 | 3 |
| Olopatadine | 10 | 5 | 5 |
| Olopatadine | 40 | 3 | 3 |
| Olopatadine | 160 | 5 | 5 |

Three dogs in each group were sacrificed at the end of the dosing period, two more animals in selected groups were observed for another 4 weeks after completion of dosing.

The drug substance was filled in the gelatin capsules for administration. Mortality, clinical signs, body weight, ophthalmological examinations, EKG, respiratory frequency, hematology, blood chemistry, urine analysis, gross and histopathology were conducted during the study.

Results:

One female at 160 mg/kg dose died on day 82. The clinical signs

at death were vomiting (tainted with blood), conjunctival hyperemia and anorexia.

Clinical signs for animals that survived were mydriasis at 10 mg/kg dose, however, it was not dose related.

Body weight (kg) are shown in the following table.

| Days | Control | | 0.6 | | 10 | | 40 | | 160 mg/kg | |
|------|---------|------|------|-----|------|------|------|------|-----------|------|
| | M | F | M | F | M | F | M | F | M | F |
| 0 | 10.5 | 8.8 | 10.5 | 8.8 | 10.5 | 8.8 | 10.4 | 8.7 | 10.4 | 8.7 |
| 91 | 12.2 | 10.3 | 12.0 | 9.9 | 12 | 10.2 | 12.3 | 10.4 | 12.4 | 10.6 |

The data suggest that there was no treatment related trend in the loss of body weight gain. Although anorexia was noted for the dead animal, the sponsor stated that body weight change was not found in the dead animal. Mean food consumption was not affected by the treatment. On an average male dogs consumed food about 300 g/day and female dogs consumed food about 280 g/day.

Ophthalmological examinations showed dilated state of the pupil in one of 5 female dog at 160 mg/kg dose. The sponsor has not provided EKG data. However, single primary A-V block was observed in one female at 10 mg/kg dose. Besides, abnormality in the ST wave was also observed in one out of 2 male at 160 mg/kg dose. Significance of the finding needs to be correlated to the other studies in dogs. Respiratory rates were increased in control and treated dogs. This was caused probably due to the dosing procedures. Hematological changes were unremarkable except an increase in the fibrinogen levels at 160 mg/kg in male and female dogs. Blood chemistry data in male did not show treatment related changes at the end of 91 days except an decrease in the A/G ratios at 40 and 160 mg/kg doses. Female dogs showed an increase in alkaline phosphatase and HDL cholesterol levels at 40 and 160 mg/kg on day 91. Urine analysis showed a decrease in the sodium excretion in male at 40, 160 mg/kg and female dogs at 160 mg/kg at the end of 91 days. The urine volume was increased in male dogs at 40 and 160 mg/kg doses. The gamma glutamyl transpeptidase activity was increased at 160 mg/kg in male and female dogs at the end of 91 days. This may reflect changes in the glutathione breakdown.

Male dogs at 40 and 160 mg/kg doses showed increase in the weight of the kidney. Similar changes were not observed in female dogs. However, weight of ovaries in female dogs were increased at 160 mg/kg dose. Gross pathology also showed kidney changes and

hematoma of the right A-V valves. Histology data showed clarified cytoplasm of tubular epithelia of kidneys in female dogs at 160 mg/kg dose.

Above data suggest that the dose tolerated was 40 mg/kg in dogs. Kidney toxicity and EKG changes are expected at the dose.

A 52- week oral toxicity of olopatadine in beagle dogs:

Page 5-2588, vol. 9:

This is a GPL study. Dogs weighed 8-11.2 kg and aged 5-6 months at the beginning of the experiment. The drug substance was delivered orally in capsules. Control dogs were treated with empty capsules. Experimental design is shown in the following table.

| Treatment | Dose (mg/kg) | No. Of Male | No. Of Female |
|-------------|----------------|-------------|---------------|
| Control | empty capsules | 4 | 4 |
| Olopatadine | 0.6 | 4 | 4 |
| Olopatadine | 5 | 4 | 4 |
| Olopatadine | 40 | 4 | 4 |

During the study period following parameters were observed. Mortality, clinical signs, body weight changes, food consumption, water intake, ophthalmic changes, EKG, hematology, blood chemistry, urine analysis, gross and histological changes were monitored.

Results:

There was no mortality observed during the treatment period. Clinical signs were dryness of mucosa of the oral cavity in the control and treated groups. It was considered to be unrelated to the treatment. Diarrhea, vomiting, salivation and hyperemia in conjunctiva were also observed in the treated and control groups.

Body weight gain in male dogs at the end of experiment was 3.38, 3.80, 1.73, 1.33 kg at 0, 0.6, 5 and 40 mg/kg doses, respectively. Similarly, the body weight gain in female animals were 3.25, 2.98, 2.45 and 3.33 kg at 0, 0.6, 5 and 40 mg/kg doses, respectively. Therefore, male dogs at 5 and 40 mg/kg doses showed 49 and 60% reduction in the body weight gain compared to the control. The changes in body weight gain was not related to the food consumption.

EKG changes at 5 and 40 mg/kg were noticed in male and female dogs during the treatment period and that comprised of an elevated R-wave, decrease in the Q-wave and prolongation of the P-wave. Some of the changes were present (R wave changes) 24 hours after the drug administration. Although the PK data at 5 mg/kg dose is not available, the C_{max} at 3 mg/kg oral dose was about 2212 ng/ml (page 5-7536, vol 23). The limited systemic exposure from the topical treatment does not suggest any risk to the patients on the basis of the preclinical data.

At the end of the study, treated animals did not show any drug related changes in the hematology parameters. Male dogs showed a significant reduction in the nonesterified free fatty acid and female dogs showed a decrease in the albumin, A/G ratios at 40 mg/kg dose at the end of the dosing period. There was no dose related trend for the change in the urine. Urine volumes were changed in some animals. However, it was considered to be incidental. Except for the reduction in weight of ovaries at 40 mg/kg dose, there was no treatment related trend in the organ weight changes.

Histological examinations showed an increase in the pigmentation and inflammation of submandibular lymph nodes at 40 mg/kg in male and female dogs. A decrease in colloid in the thyroid at 40 mg/kg dose in one out of four dogs was noticed. Interestingly one female dog at 40 mg/kg showed focal fibrosis and deposition of brown pigment in the heart.

The data suggest that olopatadine was tolerated at 40 mg/kg. Clinical signs of vomiting, diarrhea, conjunctival hyperemia and dryness of the of the mouth were observed. However, these changes were observed in control dogs also. Male dogs showed loss of body weight gain at 40 mg/kg dose. There was no treatment related ocular changes following oral administration of the drug. Abnormalities in the EKG and histological changes in the heart were seen at 5-40 mg/kg doses.

Fertility study in rats (segment I):

Page 5-2741, vol. 9:

The experiment was conducted according to the GLP at (b) (4) (b) (4) Male and female Wister rats, 7-8 weeks of age and 148-185 g body weight were used in the study (at the initiation of the experiment). The drug substance was dissolved in distilled water or in the case of a higher concentration, it was suspended in distilled water. Each group had 22 rats/sex. Doses were 0, 6, 50 and 400 mg/kg/oral. Male

rats were treated for 9 weeks before the mating through the mating period. Female rats were dosed 14 days before mating, during the mating through day 7 of the gestation. The clinical signs, mortality, body weight and food consumption were observed. The pregnant dams were sacrificed on gestation day 20 for examinations of the live and dead fetuses, pre and post implantation loss, weight of pups, corpora lutea and number of implantation. External examinations of the live fetuses were conducted and sex ratios were determined. Pups were sacrificed and histological examinations were conducted for abnormalities and variations. The male rats were necropsied after confirmation of gestation of the corresponding female with whom it was mated. Male rats that failed to fertilize female were investigated for the sperm activity.

Results:

Three males at 400 mg/kg dose died on days 17, 26 and 90 of dosing. Males showed mydriasis after the dosage at 6, 50 and 400 mg/kg immediately after the dosing and lasted for almost two hours. Relaxation of scrotum was also noted in some males after the second and third doses. Abnormal respiratory sounds were noted in male and female rats following the drug administration.

Body weight gain of male rats was reduced at 400 mg/kg and that of female was affected at 400 mg/kg dose before gestation and during the gestation period. These data would suggest that 400 mg/kg had adverse effect to the dams. Food consumption at 400 mg/kg dose was also decreased in male and female rats. Necropsy data showed that male rats developed dilatation of renal pelvis at 400 mg/kg dose.

Number of fertile male and female rats was 15 out of 20 (75%) at 400 mg/kg dose. The data on fetuses obtained after the caesarean sections are shown in the following table.

| Dose | Control | 6 | 50 | 400 mg/kg |
|------------------------------|----------|----------|----------|-----------|
| # of dams | 20 | 17 | 21 | 15 |
| Mean Implantation | 16.1 | 14.9 | 15.0 | 13.7 |
| % of corp. Lutea | 96.4 | 91.3 | 90.5 | 87.3 |
| Live fetuses (m, f) | 157, 156 | 131, 107 | 152, 148 | 99, 89 |
| Total live fetuses | 312 | 238 | 300 | 188 |
| Early death | 10 | 15 | 11 | 16 |
| % death to total | 45% | 64.7% | 38.1% | 53.3% |
| Late death | 0 | 0 | 3 | 2 |
| Corpora lutea | 334 | 278 | 360 | 234 |
| Body wt. Live fetuses (m, f) | 2.9, 2.7 | 3.0, 2.7 | 2.9, 2.7 | 3.0, 2.8 |

Above data suggest that corpora lutea and implantation were affected in rats following treatment with olopatadine at 400 mg/kg dose. However, the dose is considered to have maternal toxicity. The 400 mg/kg dose also showed a decrease in male fertility and a decrease in the body weight gain. On the basis of the data it is suggested that at 50 mg/kg dose, a dose at which toxicity to dams and male rats was not observed, did not affect fertility.

Teratogenicity study of olopatadine in rats:

Page 5-2788, vol. 10:

The study was conducted according to the GLP at (b) (4)
 (b) (4) Male and female Jcl:Wister rats 12 weeks of age weighing 173-350 g at the initiation of the experiment was used in the study for copulation. Rats with the presence of sperm cells in the vaginal smears were considered to be pregnant and used for the teratogenicity assessment. Olopatadine was dissolved in the distilled water, the higher concentration of the drug was prepared in a suspension form. An active control Oxatomide was included in the study as a comparator. Oxatomide was suspended in 5% arabic acid. All treatments were given by oral gavage during the day 7 to day 17 of the gestation period. The control rats received distilled water as the vehicle for olopatadine. The study design is shown in the following table.

| Control | 60 mg/kg | 200 mg/kg | 600 mg/kg | 70 mg/kg |
|------------------------|-------------|-------------|-------------|-----------|
| Distilled water | Olopatadine | Olopatadine | Olopatadine | Oxatomide |
| # rats for Caesarean | 25 | 25 | 25 | 25 |
| # of rats for Delivery | 14 | 14 | 14 | 0 |

The standard procedure for a segment II teratogenicity study was followed and therefore it is not described in the review. However, twenty-five rats were sacrificed on day 20 of the gestation for the teratogenicity assessment. Fourteen rats were allowed to deliver. These dams were sacrificed on day 22 after weaning of pups.

Mydriasis was observed at 600 mg/kg dose. One rat at 600 mg/kg dose group died during the day 8 of the study. Hyperemia and congestion of ocular fundus were noticed in one animal each in olopatadine treated groups. Abnormal respiratory sound was noticed in rats treated at 200 and 600 mg/kg doses.

The body weight and food consumption of dams during the gestation and lactation periods did not change significantly from the untreated controls. Food consumption of oxatomide treated animals was reduced during day 9-15 of the gestation compared to the control. There were several incidental gross changes observed in the dams that sacrificed at the end of gestation and lactation. However, the changes appeared to be unrelated to the treatment. Observations following the caesarean section are presented in the following table.

| Dose (mg/kg) | Control | 60 | 200 | 600 | Oxatomide |
|------------------------|----------|----------|----------|-----------------|-----------------|
| # of Dams | 24 | 24 | 22 | 22 | 24 |
| Implantations | 314 | 340 | 308 | 324 | 354 |
| Alive (% of Imp) | 97 | 92.8* | 93.5* | 92.8 | 81.6** |
| Early death (% of Imp) | 2.9 | 7.2* | 6.3* | 7.2 | 15.1** |
| Late death (% of Imp) | 0 | 0 | 0.3 | 0 | 3.3** |
| B.W., M, F (gm) | 3.1, 2.9 | 3.1, 2.9 | 3.0, 2.8 | 2.8**, 2.6** | 2.8**, 2.6** |

* $p < 0.05$, ** $p < 0.01$

Above data suggest that olopatadine at all doses and Oxatomide sustained an increase in the post implantation loss based on the data on the early death. The sponsor suggested that the number of deaths was within the acceptable range. However, consistency of the finding across the group suggest that it was treatment related. The body weight of the live fetuses was also decreased at 600 mg/kg dose of olopatadine and for Oxatomide.

The external anomalies of fetuses showed that cleft palate was observed in two pups from two different litters at 60 mg/kg dose. In the absence of similar findings at 200 and 600 mg/kg doses, it appears that the finding was incidental. Oxatomide treated rats showed cleft palate in two pups, syndactyly (webbing between adjacent digit) in two pups and edema in 3 pups. The data do not suggest that olopatadine treatment induced any external anomalies.

Skeletal anomalies were fusion of ribs in two pups from two different litters at 600 mg/kg dose. Brachydactyly was observed in one pup from rats treated with Oxatomide. The skeletal anomaly in olopatadine treated rats did not show any statistical significance. However, among the skeletal variation, delayed ossification of metacarpus was noted in 11 pups from 2 litters at 600 mg/kg dose. A similar finding was noted in the oxatomide treated group.

Ossification of coccygeal vertebra and sternabrae were also observed at 600 mg/kg dose of olopatadine and in oxatomide treated rats. Visceral anomalies were observed at 200 and 600 mg/kg doses of olopatadine and oxatomide and shown in the following table.

| | Control | 60 | 200 | 600 mg/kg | Oxatomide |
|----------------------------------|------------|----------|---------|-----------|-----------|
| #fetuses | 102 (24) * | 106 (24) | 97 (22) | 98 (21) | 95 (23) |
| Left umbilical artery | 0 | 0 | 2 (2) | 1 (1) | 0 |
| Bilateral azygos vein | 1 (1) | 0 | 3 (3) | 4 (4) | 3 (3) |
| Abnormal origin artery from arch | | | | | 14 (5) |
| Right azygos vein | | | | 1 (1) | 2 (2) |

* number of litters

These data suggest that some visceral anomalies were observed at 200 and 600 mg/kg doses of olopatadine.

For animals that were allowed to deliver, there was no effect on the duration of the pregnancy. Viability, survival and development of F₁ generation were not affected by the treatment. The body weight gain in male and female pups in the F₁ generation was not affected by the treatment. Male rats in the F₁ generation showed shortening the learning behavior (swimming time) after weaning in a dose related manner. However, the reproductive performance of F₁ male and female rats was not affected by the treatment.

It is concluded that the highest dose tolerated in the study was 200 mg/kg. At this dose abnormal respiratory sound was noticed. The 600 mg/kg dose is considered to be a maternal toxic dose since one rat died during the dosing period.

At 60 and 200 mg/kg doses, early fetal deaths were noticed that was significantly higher than the control group. Although the sponsor suggested that the incidences were within the acceptable range, the reviewer suggested that olopatadine contributed to the post implantation losses. The comparator drug also showed similar findings at a higher proportion. Olopatadine treatment did not show any external anomalies. A delayed ossification of coccygeal vertebra was observed at 600 mg/kg dose. The 600 mg/kg dose was considered to have maternal toxicity and thus does not contribute in the determination of the teratogenic potential of olopatadine.

Some visceral anomalies in umbilical artery and azygos vein at 200 mg/kg dose were observed for pups delivered at caesarean section. The F₁ generation of rats did not show any serious physical abnormalities and mating performance. On the basis of the post implantation deaths and visceral abnormalities, olopatadine should be considered for pregnancy category C.

Segment II oral teratogenicity study of olopatadine in rabbits:

Page 5-2865, vol. 10:

The study was conducted at [REDACTED] (b) (4) according to the GLP. Female rabbits (KBL:JW) were mated with the male rabbits from similar strain. At the initiation of pregnancy the rabbits weighed 3.77 to 4.55 kg. Based on the preliminary experiment, doses chosen were 25, 100 and 400 mg/kg/oral. The drug substance was dissolved in distilled water except the high dose which was administered as suspensions. All doses were given from gestation days 6-18. The control rabbits received distilled water. Each group had 17 animals with positive sign of copulation. The procedures of the experiment is standard. Clinical signs, mortality body weight gain and food consumption were monitored during the study. At the end of day 29 of gestation, animals were sacrificed to evaluate the uterine content, viability of fetuses. External, visceral and skeletal examinations of the fetuses were carried out.

Results:

At caesarean section, descending of a part of the fetus into the vagina was observed at 100 mg/kg dose in one animal. Polypoid process of the endometrium was also noticed at this dose. The body weight gain and food consumption were not affected by the treatment. The litter data following the caesarean section are shown in the following table.

| Dose mg/kg | Control | 25 | 100 | 400 |
|------------------------|---------|---------|---------|---------|
| #of Dams | 16 | 13 | 15 | 15 |
| Mean Impl. | 9.7 | 9.4 | 7.4 | 7.9 |
| Total fetuses(alive) | 137 | 99 | 99 | 98 |
| Early Death % Impl. | 10.6 | 16.9 | 8.5 | 13.2 |
| Late Death %Impl. | 2.3 | 5.5 | 2.3 | 7.4 |
| Total corpora lutea | 206 | 172 | 170 | 168 |
| Body wt. fetuses | 43.3 g | 44.47 g | 45.81 g | 47.19 g |
| Placental wt. | 5.02 g | 5.12 g | 5.44 g | 5.77 g |

The data showed that number of fetuses alive per litter or as % of implantation was reduced in all treatment groups compared to the control. However, it was not statistically significant. There was an increase in the late death at 400 mg/kg dose compared to the control. Body weight of live fetuses were not changed. External examinations also did not show any abnormality compared to the control. Skeletal abnormality data showed abnormal alignment of lumbar vertebra one each at 100 and 400 mg/kg dose. However, above changes in the skeleton did not show any statistical significance. There was no visceral anomalies observed in the F₁ pups. On the basis of the data, it is concluded that olopatadine did not show teratogenicity up to 400 mg/kg dose in rabbits.

Peri- and postnatal development of fetuses in rats treated with olopatadine (segment III):

Page 5-2905, vol. 10:

The study was conducted at (b) (4) according to the GLP. Male and female rats of SPF jcl:wister strain were mated under the laboratory condition. The pregnant rats were 11 weeks old at the start of mating and weighed 177-214 g on the gestation day 0. The test substance was dissolved in distilled water for injection. In the case of a concentrated preparation, i.e., 25 mg/ml or more, the test substance was suspended in water. Animals were dosed by oral gavage from gestation day 17 through post partum day 21. The design of the experiment is shown below.

| Group | Dose mg/kg/day | Number of animals |
|-------------|-----------------|-------------------|
| Vehicle | Distilled water | 26 |
| Olopatadine | 60 | 26 |
| Olopatadine | 200 | 26 |
| Olopatadine | 600 | 26 |

Animals were observed for mortality, body weight gain, food consumption, clinical signs, delivery and lactation. The gestation period of the F₀ animals was noted. At the end of lactation day 22, F₀ dams were sacrificed, corpora lutea and number of implantation sites were counted. Number of still births, the sex ratio and body weights of F₁ pups were noted following the delivery. On the day 4 after birth, litters were culled to 4 males and 4 females. Where number of pups were less than 8, all pups were reared. If the number of male or female were less than 4, number of animal was adjusted to 8. Among these F₁ pups, one male and one female from each litter were grown and allowed to mate around week 10. Remaining animals were used for skeletal examinations, reflex development and for organ weight determinations. The copulation and fertility index of F₁ generation were determined. Females were necropsied on day 20 of the gestation for the examination of corpora lutea, number of implantations, fetal death, number of live fetuses, body weight of fetuses and external examination. About half of the fetuses were preserved for visceral and skeletal examinations.

Results:

Number of pregnant animals in each group were 25 in the control, 24 in the 60 mg/kg, 24 in 200 mg/kg and 25 in 600 mg/kg dose. Mortality was observed at 600 mg/kg dose as shown in the table.

| Number of animals died | Time of death |
|------------------------|-------------------|
| 3 | Gestation day 18 |
| 1 | Gestation day 19 |
| 1 | Postpartum day 9 |
| 1 | Postpartum day 11 |

Clinical signs:

Reddish tear in 2 animals at 200 mg/kg dose observed on days 19 and 20 of gestation and from days 14-22 postpartum. Blood stained urine was observed in one animal on day 8 post partum at 600 mg/kg dose. Animals that survived at 600 mg/kg dose showed poor lactation condition.

Body weight:

The body weight gain was reduced compared to the control for rats in the treatment group at 600 mg/kg dose from post partum day 4. This loss of weight gain was observed during the administration of olopatadine. Body weight gain at 60 mg/kg doses was comparable to the control throughout the study. The body weight gain at 200 mg/kg was reduced slightly from the control from post partum day 14.

Food consumption:

During the gestation period, food consumption was reduced at 600 mg/kg dose. The food consumption of F₀ rats at 60, 200 and 600 mg/kg doses were reduced during the lactation period. The following table shows the data.

Mean food consumption (g) during nursing period

| Dose (mg/kg) | Day 1 | Day 5 | Day 8 | Day 15 | Day 22 |
|--------------|-------|--------|--------|--------|--------|
| Control | 13.0 | 32.5 | 35.2 | 48.3 | 54.7 |
| 60 | 14.0 | 30.6 | 31.6 | 42.0 | 45.8* |
| 200 | 13.9 | 28.5 | 29.2 | 37.4** | 40.1** |
| 600 | 12.4 | 20.8** | 21.7** | 29.4** | 29.0** |

** p<0.01

Data suggest that the low food consumption impaired the body weight gain at 600 mg/kg dose during the nursing period. Similar effect on the food consumption was seen at 200 mg/kg dose from post parturition day 15. However, the magnitude of changes in the body weight gain at 200 mg/kg dose was smaller than that for 600 mg/kg. The data clearly suggest that 600 mg/kg dose had maternal toxicity and 200 mg/kg dose was tolerated.

Necropsy findings for F₀ females:

Rats treated with 600 mg/kg dose of olopatadine showed atrophy in thymus and spleen, hemorrhage of thymus, lungs, stomach and uterus, hydronephrosis, distension of ureter and bladder, lymph node swelling. The result included findings from the dead animals also. Caesarean findings:

| Observations | Control | 60 | 200 | 600 mg/kg |
|------------------------------|-----------|-----------|-----------|-----------|
| # of Dams | 25 | 24 | 24 | 21 |
| Duration of Pregnancy (days) | 21.9 | 21.9 | 22 | 22 |
| Birth to Day 4 | | | | |
| # of New Births | 330 | 322 | 314 | 261 |
| # of still births (%) | 9/2.6% | 5/1.4% | 6/1.8% | 3/1.1% |
| # of live births (%) | 321/91.2% | 317/91.9% | 308/91.4% | 258/93.1% |
| Number of dead | 22/6.9% | 57/18% | 88/28.6% | 133/51.6% |
| # Live on day 4 | 93.2% | 82% | 71.4% | 48.4% |
| Days 4-22 | | | | |
| #New births (culling) | 193 | 172 | 145 | 93 |
| # Dead | 2 | 3 | 5 | 11 |
| # Weaning | 191 | 169 | 140 | 82 |
| After weaning | | | | |
| # Breeding | 144 | 129 | 105 | 63 |
| # of Dead animals | 0 | 0 | 0 | 5 |
| # of live animals | 144 | 129 | 105 | 58 |

Above data clearly suggest that 600 mg/kg dose was toxic for delivery and nursing performance of the F₀ dams, pre and post weaning survival of F₁ pups. However, At 60 and 200 mg/kg doses, increased death among the weaning pups was also noticed. Based on

the data 60 and 200 mg/kg doses should be considered as the toxic dose for postnatal development of pups.

There was no external anomalies observed in the F₁ generation. Among the surviving pups, delayed opening of vagina was observed at 600 mg/kg. Body weight gain of F₁ pups at 60 and 200 mg/kg doses were reduced from day 14 onwards although it was statistically not significant compared to the control group up to weaning day 21. Thereafter, a statistically significant decrease in the body weight gain was also observed at 60 and 200 mg/kg doses. Changes in the body weight gain of F₁ pups at 600 mg/kg were more severe than the control from postnatal day 4 and statistically significant compared to the control. The F₁ Pups did not show developmental anomalies in series of tests on reflex and swimming activities. There was no treatment related gross pathological changes observed in the F₁ pups. Relative organ weights of spleen, lung, thymus and brain were increased for male F₁ rats at 600 mg/kg. The relative organ weights of brain, and ovary were increased in female F₁ rats at 600 mg/kg doses. There was no skeletal abnormality observed in the treated F₁ rats. The reproductive performance of F₁ generation of rats was not affected by the treatment. The F₂ pups were normal.

Above experiment was repeated at 6 and 20 mg/kg doses in rats (page 5-3227, vol. 11). The food consumption of F₀ dams was decreased from post partum day 11 at 20 mg/kg dose. Body weight gain of F₁ pups was also reduced at 6 and 20 mg/kg doses during weaning period. Number of variation in the cervical ribs was noted at 20 mg/kg dose.

In the third study (page 5-3522, vol. 12), body weight gain was suppressed in the F₁ generation at 4 mg/kg dose. When dams of the 60 mg/kg dose group nursed F₁ pups from the control group, a similar effect on the body weight gain was observed. It was concluded that olopatadine was excreted in the breast milk and affected the weight gain of F₁ pups. At 2 mg/kg dose, toxicity to F₁ pups was not evident.

Ames test for mutagenicity of olopatadine:

Page 5-3724, vol. 12:

The experiment was conducted at [REDACTED] (b) (4) according to the GLP. The strains of S. Typhimurium used in the study were TA 98, TA 100, TA 1535 and TA 1537. The strain of E. Coli used was WP2. The experiment was conducted in the presence and absence of S-9 liver homogenate. Concentrations used were 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in the presence

and absence of the S-9 fraction. Positive controls were 2-nitrofluorene, 9-aminoacridine, N-ethyl-N'-nitro-N-nitrosoguanidine and 2-aminoanthracene. Data showed that olopatadine did not increase the number of revertant colonies in the presence and absence of S-9 up to 5000 ug/plate concentration. Therefore, olopatadine is considered to be non-mutagenic in the Ames assay.

Chromosomal aberration test in Chinese hamster lung cell line:

Page 5-3747, vol. 12:

The experiment was conducted at the (b) (4) according to the GLP. Cytotoxicity to the drug was determined prior to the selection of doses. The result suggest that olopatadine at 1.5, 2.0, 2.5 and 3.0 mM did not show increased aberrations at metaphase up to 48 hours of incubation in the absence of S-9 homogenate. The positive control, MNNG at 2 µg/ml concentration showed an increase in the total aberrations when a total of 200 cells were scored. Olopatadine also did not show chromosomal aberrations at 5, 6, 7 and 8 mM concentrations in the presence of the S-9 homogenate. In the similar experiment, benzopyrene at 20 µg/ml showed considerable increase in the aberration. These date suggest that olopatadine is not mutagenic in the chromosomal aberration assay.

Micronucleus test in mice in vivo:

Page 5-3774, vol. 5:

The experiment was conducted at (b) (4) according to the GLP. Male mice six week of age at the time of the procurement were used in the study. The body weight of mice was 34.1 to 40.6 g. Each group had 5 mice. Doses were selected on the basis of acute toxicity data. The drug substance was given as oral suspensions at 10 ml/kg dose. The experimental design is shown in the following table.

| Compound | Dose (mg/kg/day) | No. Of injection | No. Of mice |
|-------------|------------------|------------------|-------------|
| Water | 0 | 1 | p.o |
| Olopatadine | 100 | 1 | p.o |
| Olopatadine | 200 | 1 | p.o |
| Olopatadine | 400 | 1 | p.o |
| Mitomycin C | 3 | 1 | i.p |
| Water | 0 | 4 | p.o |
| Olopatadine | 100 | 4 | p.o |

The drug substance was administered once a day except one group of animals received 4 doses of 100 mg/kg at 24 hour intervals to a total dose of 400 mg/kg/day.

Animals were sacrificed 30 hours after the single dose or 24 hours after the repeat doses of the drug or vehicle. Bone marrow cells from the femur were collected and stained. Two thousand polychromatic and 2000 normochromatic erythrocytes were prepared per animal for the determination of the increase in micro nucleated cells. Olopatadine did not increase percent of polychromatic erythrocytes (PCE), micro nucleated polychromatic erythrocytes (MNPCE) and micro nucleated normochromatic erythrocytes (MNNCE) compared to the control. However, mitomycin treatment increased percent of PCE and MNPCE that was statistically significant. The data suggest that olopatadine did not induce chromosome damage or changes in the spindle function in vivo. Therefore, it is not mutagenic in the micronucleus test in mice.

78-week carcinogenicity of olopatadine in mice:

Page 5-3794, vol. 12:

The study was conducted at [REDACTED] (b) (4) according to the GLP. Male and female CD-1 mice were procured from Charles River, England for the study. Animals weighed 18-27 g within a week after the arrival and were 29-36 days of age at the initiation of the treatment. The experimental design is shown in the following table.

| Group | Treatment | Dosage mg/kg/day | No. Of Male | No. Of Female |
|-------|-------------|---------------------|-------------|---------------|
| 1 | Control | 0 | 52 | 52 |
| 2 | Olopatadine | 50 | 52 | 52 |
| 3 | Olopatadine | 160 | 52 | 52 |
| 4 | Olopatadine | 500 | 52 | 52 |

In addition to above groups, two satellite groups comprising of 8 animals/sex were maintained as veterinary control and for the purpose of the health monitoring process. The control animals received powdered rodent diet. The treated animals received diet and drug mixtures ad lib. Water was available ad lib. The stability and homogeneity of the test material in the diet was analyzed in weeks 1, 13, 26, 52, 65 and 78 at (b) (4). The food consumption was determined on weekly basis. The dietary concentrations of olopatadine were adjusted weekly for the first 14 weeks of the treatment and biweekly thereafter so as to provide appropriate dosage.

The dosages of the study were selected on the basis of a preliminary study #91/KKY007/0120 that showed loss of the body weight gain at 600 and 2000 mg/kg doses.

Animals were inspected twice daily for ill health, mortality and weekly for palpable masses. Animals were weighed weekly up to 14 weeks and biweekly thereafter. The oral dose of the drug was calculated weekly from the nominal dietary concentration, food consumption and body weight data.

Differential blood counts were taken from the surviving animals at the end of 50 and 77 weeks. Blood samples were also collected at the end of week 77 for the RBC, WBC and platelet counts, Hb levels, mean cell hemoglobin concentrations and mean red cell volume determinations. Bone marrow smears were also prepared at the end of necropsy for determining myeloid and erythroid ratios. Gross changes, organ weights and histological examinations of the organs were conducted following necropsy at the end of 78 weeks.

Results:

The number of animals with palpable swelling was comparable between the control and treated mice. Male mice in the third and fourth groups showed skin aberration, swollen and reddening of pinna, right forelimb and fast respiration.

Mortality of the mice is shown in the following table.

| Week | control ,M | 50 mg/k g,M | 160 mg/kg ,M | 500 mg/kg ,M | Control ,F | 50 mg/k g,F | 160 mg/kg ,F | 500 mg/kg ,F |
|------|---------------|-------------------|--------------------|--------------------|---------------|-------------------|--------------------|--------------------|
| 78 | 28 | 24 | 27 | 28 | 16 | 22 | 9 | 15 |

Percent of survival is shown in the following table.

| Week | control ,M | 50 mg/k g,M | 160 mg/kg ,M | 500 mg/kg ,M | Control ,F | 50 mg/k g,F | 160 mg/kg ,F | 500 mg/kg ,F |
|------|---------------|-------------------|--------------------|--------------------|---------------|-------------------|--------------------|--------------------|
| 78 | 46% | 52% | 48% | 46% | 69% | 58% | 83% | 65% |

Two male mice in the control and one male at 500 mg/kg dose died by accident during the routine animal husbandry procedures. Above data suggest that there was no trend in the treatment related mortality. However, a detail statistical analysis of the intercurrent mortality will be provided in the statistical review.

The body weight (gm) of mice are shown in the following table.

| Weeks | Control | | 50 mg/kg | | 160 mg/kg | | 500 mg/kg | |
|-------|---------|------|----------|------|-----------|------|-----------|------|
| | M | F | M | F | M | F | M | F |
| 0 | 25.7 | 21.3 | 25.5 | 21.5 | 25.7 | 21.4 | 25.7 | 21.3 |
| N= | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 |
| 78 | 52 | 41.7 | 50.6 | 38.2 | 51 | 39 | 53.8 | 38.9 |
| N= | 22 | 36 | 27 | 30 | 25 | 43 | 23 | 37 |

The weight gain in male mice was comparable for the control and treated groups. The weight gain in the female mice was 20.4 g in the control and 17.6 g in the high dose group i.e. about 14% less than the control.

Food consumption:

The food intake in the treated male mice was 7-9% less than the control. However, the body weight gain was not affected by the food intake in male mice. For female mice, the food intake in the group 3 and 4 was 6% less than the control. The data suggest that

the body weight changes were independent of the food consumption.

The dosage achieved in the experiment was 50.6, 161.5 and 500.9 for the male mice; 50.9, 161.4 and 509.1 for the female mice. The data suggest that animals were exposed to the target dose.

The hematology data at the end of 50 weeks of the treatment showed a statistically significant increase in the lymphocyte counts in female mice at 500 mg/kg dose. Hematology data at the end of week 78 did not show any dose related change in the total and differential cell counts in male mice. Changes in the white cell and platelet counts in female mice at the end of 77 weeks are shown in the following table.

| Groups | Total WBC1000/cmm | Lymphocytes 1000/cmm | Platelets 1000/cmm |
|--------|-------------------|----------------------|--------------------|
| 1. | 5.4 | 3.7 | 1083 |
| 2. | 5.1 | 3.2 | 1038 |
| 3. | 4.5 | 3.0 | 1336* *p<0.05 |
| 4. | 4.6 | 3.3 | 1271* *p<0.05 |

The data suggest that female rats had higher platelet counts than the control at 160 and 500 mg/kg doses.

None of the organ weight data (% of body weight) showed statistically significant changes in male and female mice.

The macroscopic data in mice that died or sacrificed before the scheduled necropsy are shown in the following table.

| Organs | gr 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
|------------------------|------|------|------|------|--------|----|---|----|
| | Male | Male | Male | Male | Female | F | F | F |
| #Animals | 30 | 25 | 27 | 29 | 16 | 22 | 9 | 18 |
| Thickened uterus | | | | | 5 | 1 | 1 | 0 |
| thickened stomach wall | 6 | 6 | 4 | 11 | | | | |
| excoriation in skin | 6 | 1 | 13 | 9 | | | | |
| mandibular lymph node | 4 | 1 | 11 | 4 | | | | |
| masses in the heart | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 2 |

Macroscopic findings at the end of 78 weeks are shown below:

| Organs | gr 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
|-----------------------------|------|------|------|------|--------|----|----|----|
| | Male | Male | Male | Male | Female | F | F | F |
| #Animals | 22 | 27 | 25 | 23 | 36 | 30 | 43 | 34 |
| dark mesenteric lymph nodes | | | | | 1 | 2 | 6 | 4 |
| masses in the liver | 6 | 7 | 4 | 10 | | | | |
| hair loss from the skin | 20 | 24 | 17 | 12* | | | | |
| thickened stomach wall | 2 | 6 | 8 | 9* | | | | |
| thickened uterus | | | | | 10 | 7 | 3* | 2* |

* P<0.05

Above data suggest that male mice at 500 mg/kg dose showed most of the treatment related macroscopic changes.

The non-neoplastic histological changes are shown in the following table.

| Gr 1 | Gr 2 | Gr 3 | Gr 4 | Gr 1 | Gr 2 | Gr 3 | Gr 4 |
|--------------------------------------|------|------|------|------|------|------|------|
| M | M | M | M | F | F | F | F |
| n=52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 |
| Amyloidosis in cardiac ventricles, 7 | 9 | 8 | 11 | 6 | 2 | 1 | 8 |
| Arteritis, 1 | 0 | 2 | 2 | 1 | 1 | 0 | 2 |
| Mes.lymph node hyperpl., 0 | 0 | 0 | 3 | 0 | 1 | 0 | 0 |
| Liver Amyloidosis, 7 | 12 | 5 | 6 | 5 | 5 | 1 | 8 |
| Liver leuko. foci, 5 | 0 | 2 | 6 | 8 | 2 | 0 | 15 |
| Lung alveolar hemor., 4 | 0 | 2 | 7 | 3 | 0 | 0 | 3 |
| Ovary Folli. cyst, | | | | 1 | 2 | 0 | 4 |
| Ovary Amyloidosis | | | | 3 | 3 | 1 | 5 |
| Prostate Inflamm, 0 | 1 | 1 | 4 | | | | |
| Sem. Ves. Inflamm, 0 | 0 | 1 | 3 | | | | |
| Stomach glandular hyper, 11 | 10 | 13 | 16 | | | | |

Above data suggest that nonneoplastic histological changes in the heart, liver, lung, ovary and prostate are expected at 500 mg/kg dose following chronic treatment with olopatadine in mice.

The neoplastic findings are shown in the following table.

| Lesions | Gr 1, M | Gr 2, M | Gr 3, M | Gr 4, M | Gr 1, F | Gr 2, F | Gr 3, F | Gr 4, F |
|------------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| N= | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 52 |
| Liver adenoma | 8 | 6 | 4 | 10 | 0 | 0 | 0 | 0 |
| Liver carcinoma | 2 | 4 | 1 | 2 | 0 | 1 | 0 | 0 |
| Liver carc and adenoma | 10 | 10 | 5 | 12 | 0 | 1 | 0 | 0 |

The above table shows the neoplastic changes that occurred with a high prevalence in the control and treated male mice during the treatment period. Incidentally none of the above lesions show a significant treatment related trend. The biostatistics review would elaborate more on the findings.

It is concluded from the report that olopatadine treatment for 78 weeks did not show higher mortality due to the treatment. Male mice did not show an increase in the loss of weight gain compared to the control. However, female mice showed about 14% loss of body weight gain. One of the criteria for a valid carcinogenicity study is a reduction of the body weight gain by about 10% compared to the control. Accordingly, the highest dose used in the study could be considered as the MTD. Olopatadine did not show neoplastic changes up to 500 mg/kg (1500 mg/sqm) dose given in the diet. It should be noted that the sponsor did not conduct histology of all animals at low and mid doses following the terminal sacrifice.

A 104-week carcinogenicity study of olopatadine in F-344 rats:

Page 5-5193, vol. 16:

The study was conducted at (b) (4) according to the GLP. The dosage of the study was selected from a preliminary study # 91/KKY006/0112 at 100, 300 and 1000 mg/kg/day. Male and

female F-344 rats procured from the (b) (4) (b) (4) were used in the study. Animals were 28-35 days old at the time of the procurement and weighed 56-90 g. Animals were acclimatized for another 5 days before the experiment. Rats were given powdered rodent diet and water ad lib. The experimental design is shown below.

| Group | Dosage (mg/kg/day) | Male | Female |
|-------|--------------------|------|--------|
| 1 | control | 50 | 50 |
| 2 | 20 | 50 | 50 |
| 3 | 65 | 50 | 50 |
| 4 | 200 | 50 | 50 |

In addition to above groups, a group of 10 rats/sex were selected as the veterinary control and another batch of 10/sex were selected for monitoring health of the animals. The diet and drug mixtures were prepared each week and quality control of the drug was monitored. The mixing equipment used for the preparation of the diet was changed from week 34 due to a low dosage level achieved in the group 2 rats. The new equipment corrected the problem of homogeneity of the drug in the diet mixtures. The changes were required on the basis of the observation on week 26 that the achieved doses in male and female rats were 83 and 87% of the intended doses, respectively, for the low dose group.

The dietary concentration of the drug was adjusted weekly up to 14 weeks and biweekly thereafter. Animals were inspected twice daily for clinical signs. A weekly examination was conducted for palpable masses. Body weights of the animals were recorded weekly for the first 14 weeks and biweekly thereafter. The food consumption was recorded every week throughout the study. The dosage achieved was calculated every week on the basis of the food consumption and expressed as mg/kg/day.

Differential white cell counts were taken after 50 and 76 weeks of the treatment. Finally, a complete hematological examination was done on week 103 for the surviving animals. At necropsy, macroscopical examinations were conducted and organ weights were recorded. Tissues were fixed for histopathological examinations. Following tissues were preserved but not processed for histological examinations:

Eye and optic nerve, right

Harderian glands
 Mammary gland-cranial
 Salivary gland-submandibular, right
 sciatic nerve, right

Results:

Clinical signs: High incidences of ungroomed coat was observed in the dorsal surface of females at 65 mg/kg dose from the week 13 and at 200 mg/kg dose from week 62 in male rats. A high incidences of hair loss was also observed in female rats at 200 mg/kg during the first 30 weeks of the treatment. Besides the changes in the fur, 73 male and 43 female rats from the control and the treated groups showed palpable swellings. However, number of animals with swellings were almost similar in the control and treated groups. The cumulative mortality up to 105-108 weeks during the necropsy is shown in the following table.

| Groups | 1M | 2M | 3M | 4M | 1F | 2F | 3F | 4F |
|-----------|----|----|----|----|----|----|----|----|
| Total | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Mortality | 25 | 27 | 23 | 13 | 19 | 13 | 9 | 9 |
| %survival | 50 | 46 | 54 | 74 | 62 | 74 | 82 | 82 |

Above data showed that there was no treatment related mortality. However, from the survival data it appears that there were enough animals left at the end of the treatment period who had exposure to the drug. The mortality rate will be discussed in the statistical report.

The body weight gain in male and female rats was reduced at 200 mg/kg dose from week 4 of the treatment. The loss of body weight gain at the end of the treatment period was statistically significant. Following table shows the change in the body weight (gm) between weeks 0 and 104 in male and female rats

| Group | 1M | 2M | 3M | 4M | 1F | 2F | 3F | 4F |
|------------------------|-------|-----|-----|-----|-----|-----|-----|-----|
| Week 0 | 90 g | 91 | 89 | 91 | 80 | 82 | 81 | 81 |
| Week 104 | 468 g | 442 | 438 | 410 | 337 | 336 | 324 | 306 |
| Gain 1-104 weeks | 377 g | 352 | 352 | 319 | 258 | 255 | 242 | 225 |
| % of control | | 93 | 93 | 85 | | 99 | 94 | 87 |

One of the criteria of a valid carcinogenicity study is the loss of weight gain by about 10% in the high dose animals (MTD). The data suggest that the high dose fulfilled the criteria. There was no differences in the food consumption at low and mid doses when compared to the control. The food consumption at the high dose was 95 and 94% for male and female rats, respectively. The decrease in the food consumption at the high dose was consistent. It may be possible that the low food consumption contributed to the loss of body weight gain at the high dose. The achieved dosage was calculated from the food consumption. Data showed the achieved dose for male was 19.9, 64.7, 199.7 mg/kg/day and that for female rats was 20, 65.2 and 198.3 mg/kg.

The differential white cell counts did not show any change during weeks 50 and 76. However, RBC counts and hemoglobin levels were reduced at the end of 103 weeks in all treated male rats, the change was not statistically significant. The female rats showed an increase in the platelet counts that was statistically significant.

The organ weight relative to the body weight was increased for kidneys. The weight of kidney was 0.837, 0.944, 0.939 and 0.936 gm% at control, low, mid and high doses for male rats, respectively. Data for mid and high doses were statistically significant. Similarly the kidney weight (gm/100 gm body weight) was 0.766, 0.816, 0.803 and 0.827 in control, low, mid and high doses in female rats, respectively. No other changes were statistically significant.

Macroscopic examinations for animals that died or killed before the termination of the experiment showed prominent tubules in the testes at mid and high doses (1,0,3,4 at control, low, mid and high doses). The incidence at the high dose was statistically significant.

Opaque eyes were observed in male and female rats that were sacrificed at the end of the experiment. The incidences were increased dose dependently in left and right eyes at low (7M, 12F), mid (8M, 13F) and high (10M, 20F) doses compared to the control (2M,9F). Prostate at the high dose group appeared small. An increase in the staining of the fur was observed in male and female rats at the high dose.

Histological examinations for non-neoplastic lesions in all animals showed the presence of keratitis in male and female rats. An increased incidences of corneal mineralization was also present in the high dose female rats. Male rats showed an increased incidences of dilated glands in the stomach and interstitial hyperplasia of testes at high dose. The following table summarizes the non-neoplastic incidences for all animals.

| Observations | 1M | 2M | 3M | 4M | 1F | 2F | 3F | 4F |
|---------------------------------|----|----|----|----|----|----|----|----|
| Keratitis, left eye | 2 | 5 | 6 | 7 | 1 | 5 | 3 | 3 |
| Right eye | 1 | 4 | 6 | 4 | 7 | 4 | 4 | 6 |
| Cornel mineralization, left eye | 9 | 8 | 8 | 7 | 10 | 7 | 9 | 15 |
| Right eye | 1 | 10 | 8 | 5 | 6 | 9 | 8 | 12 |
| Dilated glands, stomach | 5 | 3 | 6 | 10 | 4 | 0 | 2 | 2 |
| Testes, inter. Hyperplasia | 6 | 4 | 4 | 11 | | | | |

The neoplastic observations for all animals are shown in the following table.

| Observations | 1M | 2M | 3M | 4M | 1F | 2F | 3F | 4F |
|--|----|----|----|----|----|----|----|----|
| Benign islet cell adenoma of pancreas | 3 | 0 | 2 | 4 | 0 | 1 | 0 | 1 |
| Malignant islet cell carcinoma of pancreas | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 |

Conclusion:

A 104-week carcinogenicity study was conducted in F-344 rats at 20, 65 and 200 mg/kg/day doses given in the diet. Doses were selected on the basis of a preliminary study #91/KKY006/0112. A thirteen-week oral gavage toxicity study was conducted in Wister rats at 6, 25, 100 and 400 mg/kg/day doses. Mortality was observed at 400 mg/kg dose. The 100 mg/kg dose was tolerated. However, the loss of body weight gain was observed at this dose. No remarkable histopathological change was noted at 100 mg/kg dose. Considering these data and results of the loss of body weight gain in this study, the doses selected for the 104-week carcinogenicity study appeared to be adequate to be considered as the MTD. The food consumption was reduced by about 5% at the high dose in male and female rats. It appears that the drug-diet mixtures might have some palatability problem that confounded the food intake. If it is so, the maximum dose used in the study can also be considered as the MTD since a higher dose than that used in the study would not be achievable for practical reasons.

There was no treatment related mortality observed in the study. Increased incidences of opaque eyes were reported in the macroscopic findings. Keratitis in left and right eyes were observed in male and female rats.

There was an increase in the incidences of benign and malignant adenoma and carcinoma, respectively, in the islet cells of the pancreas. The statistical trend will be determined in the biostatistical review. However, clinical significance of islet cell neoplasm cannot be determined in the absence of blood chemistry data and the impact on carbohydrate metabolism.

It should be mentioned in this context that the sponsor did not conduct histological examinations of tissues of all animals from the low and mid dose groups that were sacrificed at the end of the experiment. The islet cell carcinoma was coded as mineralization of pancreas in the statistical review.

Preclinical Pharmacokinetics and disposition:

Ocular tissue distribution of radioactivity following single ocular dose of 0.15% ¹⁴C-Olopatadine solution in male N-Z rabbits: Page 5-6979, vol. 21, Report # N-94-113

The radiolabelled drug substance was prepared in an ophthalmic vehicle so as to obtain 1.5 mg/ml (0.15%) concentration of the free base. Male New Zealand white rabbits were used in the study.

The animals weighed about 2.2 kg and were 70-90 days old at the time of dosing. The experimental design is shown below.

| Sample groups | Number of animals | Sampling Time (hours) |
|---------------|-------------------|-----------------------|
| 1 | 1 | control |
| 2 | 4 | 0.5 |
| 3 | 4 | 1 |
| 4 | 4 | 2 |
| 5 | 4 | 4 |
| 6 | 4 | 6 |
| 7 | 4 | 8 |
| 8 | 4 | 10 |
| 9 | 4 | 24 |

Each animal in the treated group received 30 μ L of the test solution in the right eye which was equivalent to 45 μ g base of olopatadine. Animals were sacrificed at the appropriate time point. Blood samples were collected from each animal including the control prior to the sacrifice. Blood and plasma levels of the radioactivity was determined. Following tissue samples were collected from the dosed and control eyes:

Aqueous humor, choroid, conjunctiva, cornea, iris-ciliary body, lens, vitreous humor and retina.

All ocular tissues were weighed, radioactivity of the tissue samples and fluids were determined by liquid scintillation spectrometry. Results were expressed as the μ g equivalent of free base/gram of the tissue. Following table shows the kinetics of distribution of the drug within the ocular tissues and blood.

| Samples | C _{max} (µg eq/g) | T _{max} (hr) | T _{1/2} (hr) |
|-------------------------------|----------------------------|-----------------------|-----------------------|
| Blood | 0.0026 | 0.5 | NA |
| Plasma | 0.0033 | 0.5 | 1.3 |
| Aqueous Humor, dosed eyes | 0.155 | 1 | 1.4 |
| Conjunctiva, dosed eyes | 0.398 | 0.5 | 1.9 |
| Cornea, dosed eyes | 1.85 | 0.5 | 1.8 |
| Iris-ciliary body, dosed eyes | 0.108 | 1 | 1.4 |
| Lens, dosed eyes | 0.0026 | 2 | 9.0 |
| Choroid, dosed eyes | 0.0219 | 0.5 | 0.9 |
| Conjunctiva, control eyes | 0.0014 | 0.5 | NA |
| Cornea, control eyes | 0.0026 | 0.5 | NA |

Above data suggest that olopatadine mostly distributed to the anterior ocular tissues. Most of the drug was concentrated in conjunctival tissues. The distribution in the posterior ocular tissues of the doses eye was minimal. Also, the contralateral eye showed less than 0.0026 µg eq/g of the radio labeled olopatadine in conjunctiva and cornea. The amount in the conjunctiva and cornea in the control eye was almost similar to that in the blood.

To conclude, the data showed that topical application of 45 µg of olopatadine in the right eye showed detectable level of the radio labeled compound in blood within half hour. Radioactivity in the blood and plasma were below the limit of detection after 2 hours of dosing. The systemic exposure was limited. Most of the drug is distributed in the anterior ocular tissue. The clearance of the drug from the lens was slow as evident from the longer half life. Otherwise, most of the drug/ radioactivity was disappeared from the tissues after the single dose without any accumulation.

Ocular tissue distribution of ¹⁴C-olopatadine following single topical ocular dose of 0.15% ophthalmic solution to male Dutch rabbits.

Page 5-7051, vol. 21, Protocol N-95-48 (PKDM285):

Male Dutch rabbits weighing 1.6 kg and 90-120 days of age used in

the study. A single dose of 30 μ L of 0.15% solution containing 45 μ g of the drug with 7.2 μ Ci was instilled into the right eye. Four rabbits were sacrificed per time point at 0.5, 1, 3, 5, 10 and 24 hours. Blood, aqueous humor, iris ciliary body, choroid, and retina were collected for determining the radioactivity. Data were expressed as the μ g equivalent of olopatadine per gm of tissues. The distribution of radioactivity is shown in the following table.

| Tissue | C _{max} (μ g eq./g) | T _{max} (hrs) | T _{1/2} (hrs) |
|-------------------|-----------------------------------|------------------------|------------------------|
| Aqueous humor | 0.129 | 1 | 1.6 |
| Iris-ciliary body | 0.238 | 5 | 12.8 |
| Retina | 0.008 | 0.5 | BLQ |
| Chroid | 0.055 | 1 | 15.6 |
| Plasma | 0.008 | 1 | BLQ |

Above data suggest that the radioactivity was rapidly absorbed in the ocular tissue. The radioactivity in the plasma, retina and ocular tissues of the anterior chamber of Dutch rabbits was higher than the N-Z rabbits. The T_{1/2} in the pigmented tissues, i.e., ICB and choroid were longer. The data suggest that the binding of olopatadine in the pigmented rabbit eyes was greater and resulted in 10-20 fold increase in the T_{1/2} in pigmented tissues. The comparative study suggest that olopatadine binds with melanin pigment. Therefore, the ocular toxicity of olopatadine should have been conducted in the Dutch rabbits.

Plasma PK of olopatadine following single topical dose to the eye or a single iv dose in rabbits:

Page 5-7018, vol. 21, Protocol: N-94-121.

Male N-Z rabbits weighing 2.8 kg and 90-120 days of age were used in the study. A single dose of olopatadine ophthalmic solution (0.15%) were applied to both eyes at 30 μ L volume. The ophthalmic solution was also injected intravenously to two separate groups of rabbits at 0.1 and 1.0 mg/kg doses. Blood samples were drawn at several time points up to 48 hours for the pharmacokinetic determinations. The assay of olopatadine was done by GC-Mass spect analysis. The limit of detection was 0.5 ng/ml. The design of the experiment is shown below.

| Group | Dose | Animals |
|-------|----------------------|---------|
| 1 | Control | 2 |
| 2 | 0.032 mg/kg, topical | 4 |
| 3 | 0.1 mg/kg, iv | 4 |
| 4 | 1.0 mg/kg, iv | 4 |

The pharmacokinetic data are presented in the following table.

| Dose | AUC _{0-alpha} (ng.hr/ml) | T _{1/2} (hr) | BF |
|---------------|-----------------------------------|-----------------------|----|
| 0.1 mg/kg, iv | 48.5 | 0.67 | |
| 1.0 mg/kg, iv | 538 | 0.66 | |
| 0.032 mg/kg | 12.9 | 0.81 | 83 |

The clearance, volume of distribution and mean residence time at 0.1 and 1.0 mg/kg doses were 1.89, 2.10 L/hr/kg; 1.79, 2.06 L/kg and 0.57, 0.66 hr, respectively.

Above data suggest that olopatadine is highly bioavailable (83%) following topical application in eyes in rabbits. However, the drug is rapidly cleared from the systemic circulation.

Plasma concentrations of olopatadine during one month ocular dosing in rabbits:

Page 5-7082, vol. 21, Protocol: N-93-156:

This is an extension of the study report on the one month ocular safety of olopatadine in rabbits presented in page 5-0576 vol 4 of the NDA.

N-Z white rabbits were used in the study. Ophthalmic solutions of olopatadine at 0.1, 0.5 and 1.0% strength were applied topically to eyes. In the experimental design for the report it is also stated that 0.1% and 0.2% ophthalmic solutions were used. Following table shows the experimental design.

| Test solution | Treatment Group | # of Animal | Treatment Regimen | Plasma sampling days |
|---------------|-----------------|-------------|-------------------|----------------------|
| 0.1% | 3 | 4M/4F | QID | days 1, 16, 27 |
| 0.2% | 4 | 4M/4F | QID | days 1, 16, 27 |
| 0.2% | 5 | 4M/4F | HID (six times) | days 1, 16, 27 |

Animals in the q.i.d groups received two drops of the drug solution in the right eye at 8 AM, 11 AM, 1 PM and 4 PM. Animals in the HID group two more doses were given at 9:30 and 2:30 PM. The objective of two additional doses was to determine dose proportionality of the PK parameters. Blood samples were taken at several time points on days 1, 16 and 27. Plasma levels of olopatadine was determined by GC/Mass spect analysis with the limit of detection of 0.5 ng/ml. Plasma trough levels were determined from the sample that was collected ten minutes before 8 AM. The samples for the peak plasma levels were collected 30 minutes after the 4 PM dose. Following table shows the PK data.

| Treatment | 18 hr trough, day 1 | 30 min peak, day 1 | 18 hr trough, day 16 | 30 min peak, day 16 | 18 hr trough, day 27 | 30 min peak, day 27 |
|-----------|---------------------|--------------------|----------------------|---------------------|----------------------|---------------------|
| 0.1%qid | below Limit (BLQ) | 1.75 | BLQ | 1.00 | BLQ | 0.97 ng/ml |
| 0.2%qid | BLQ | 3.71 | 0.37 | 2.14 | 0.48 | 1.87 ng/ml |
| 0.2%hid | BLQ | 3.37 | 0.36 | 2.22 | 0.75 | 1.90 ng/ml |

The data suggest that following multiple topical dosing, olopatadine was detected in the plasma at 0.1% concentration 2 drops 4 times a day schedule. The peak levels were increased with doses. However, any increase in the doses from the q.i.d. dose of 8 drops a day of 0.2% ophthalmic solution did not increase the peak plasma levels. Therefore, a steady state systemic exposure to olopatadine was achieved following multiple dosing for one month in rabbits. The steady state was observed on the day 16 data as shown above.

Plasma olopatadine levels in rabbits following six month O.I.D. topical doses in rabbit eyes.

Page 5-7100, vol. 21, protocol N-94-06:

This report is an extension of the six-month ocular toxicity report described in page 5-0872, vol 5. The report has been reviewed under the toxicity section and details of the experimental design has been discussed above. Following table shows the experimental design for the PK portion of the study. Two drops were instilled into the right eyes four times a day for each animal as shown in the following table.

| Test article | Treatment group | Treatment Regimen | Number of animals | Plasma sampling |
|--------------|-----------------|-------------------|-------------------|-----------------|
| 0.1% | 3 | q.i.d. | 4M, 4F | days 1, 45, 181 |
| 0.5% | 4 | q.i.d. | 4M, 4F | days 1, 45, 181 |
| 1.0% | 5 | q.i.d. | 4M, 4F | days 1, 45, 181 |

Plasma samples were analyzed by GC/Mass spect with a limit of detection at 0.5 ng/ml. Following table provides the data.

| Treatment group | 18 hr trough, day 1 | 30 min peak, day 1 | 18 hr trough, day 90 | 30 min peak, day 90 | 18 hr trough, day 181 | 30 min peak, day 181 |
|-----------------|---------------------|--------------------|----------------------|---------------------|-----------------------|----------------------|
| 0.1% q.i.d. | BLQ | 2.05 | BLQ | 1.53 | BLQ | 1.35 ng/ml |
| 0.5% q.i.d. | BLQ | 4.67 | 1.42 | 6.99 | 1.63 | 3.68 |
| 1.0% q.i.d. | BLQ | 11.6 | 3.25 | 10.0 | 3.70 | 9.48 |

BLQ - below limit of detection

The above data show that repeated topical dosing of olopatadine in rabbit eyes provide a systemic exposure dose dependently. However, 0.1% q.i.d. dose cleared faster than the high dose that resulted a insignificant trough concentration in the plasma.

Plasma concentrations of olopatadine following topical dosing in the monkey eyes for six month.

Page 5-7118, vol. 21, Protocol N-94-172

The toxicity of olopatadine following ocular administration has been reviewed above. The study design is shown below.

| Concentration | Group | Animal # | Treatment | Plasma sampling days |
|------------------|-------|----------|-----------|----------------------|
| 0.1% ophtl. Soln | 3 | 4M/4F | q.i.d. | 1, 45, 90 and 181 |
| 0.2% | 4 | 4M/4F | q.i.d. | 1, 45, 90 and 181 |
| 0.5% | 5 | 4M/4F | q.i.d. | 1, 45, 90 and 181 |

Two drops were given into the right eyes. Blood samples were drawn before the 8 A.M. dose and 30 minutes after the 3 PM dose for the trough and peak levels, respectively. Plasma assay of olopatadine was conducted by GC/MS at the quantitation limit of 0.5 ng/ml.

The plasma half-life of olopatadine in monkey has been reported to be about 10 hours.

The 18 hour trough and the 30 min peak levels (ng/ml) are shown in the table below.

| Group | 18 hour trough, day 1 | Day 45 | Day 90 | Day 181 |
|--------------|-----------------------|--------|--------|---------|
| 0.1%, q.i.d. | BLQ | BLQ | BLQ | BLQ |
| 0.2%, q.i.d. | BLQ | BLQ | BLQ | 0.584 |
| 0.5%, q.i.d. | BLQ | 1.04 | 1.23 | 1.46 |
| | 30 min peak levels | | | |
| 0.1% | 1.64 | 1.53 | 1.91 | 1.79 |
| 0.2% | 2.48 | 3.17 | 3.12 | 2.81 |
| 0.5% | 6.75 | 7.80 | 8.64 | 7.21 |

BLQ = below the level of detection.

Above data showed that up to 0.2% q.i.d. dose (two drops in the right eye), there was no systemic accumulation of olopatadine in monkeys. The peak levels were consistent and were dose proportionate. However, the clearance rate was possibly high, that resulted in an undetectable trough level. It can be concluded that olopatadine ophthalmic solution (0.1-0.5%) is bioavailable in monkeys on repeated dosing.

Disposition of olopatadine in monkeys:

Page 5-7564, vol. 23, report 052385700995:

Male crab eating monkeys (macaques) were given single 1 mg/kg iv or oral doses. Plasma and urine levels of olopatadine were determined. After the oral dose, absorption of olopatadine was rapid and within 1.67 hours. The elimination took place in two phases. Half-life was 12.4 hours and bioavailability was 102%. About 40% of the drug was eliminated unchanged in the urine. About 50% of the drug was eliminated unchanged in the urine after the iv dose.

Absorption, distribution and excretion of ¹⁴C-olopatadine in rats:

Page 5-7136, vol. 22, Report # 043:38570:0995:

In male Wistar rats, 7 weeks old were given oral or iv injection of ¹⁴C-olopatadine at 1 mg/kg dose. Blood, tissues, urine and feces were collected for the determination of radioactivity.

The radio tracer was absorbed rapidly (92%) after oral dosing. The plasma C_{max} of radioactivity was 209 ng.eq./ml at 0.5 hr. The $AUC_{0-\infty}$ was 1216 ng.eq.hr/ml. The elimination half life was 16.8 hr and biphasic.

The plasma level of radioactivity after iv dose of 1 mg/kg was 994 ng.eq/ml within 3 mins. The $AUC_{0-\infty}$ was 2475 ngeq.hr/ml, elimination was triphasic, $T_{1/2}$ was 34.2 hr.

Plasma and whole blood radioactivity was almost similar. Most of the radioactivity from tissues were eliminated within 8-24 hour of dosing. Mass balance study showed 97 and 95% excretion of the radioactivity. Most of the radioactivity was eliminated in the urine and feces. The radioactivity excreted in the feces was about 10% higher than the urine samples. The radio labeled Olopatadine showed 30% enterohepatic circulation.

The above study was repeated in male and female Wister rats (see page 5-7199, vol. 22). The data suggest that plasma $AUC_{0-\infty}$ of radioactivity in male rats was 1215.9 ng.eq.hr/ml vrs 1437.5 in the female rats at 1 mg/kg oral dose. The differences were statistically significant. Based on the data, one can anticipate greater toxicity in female rats compared to the male rats at equal dose.

The site of absorption of ^{14}C -olopatadine was investigated in the male Wister rats (page 5-7222, vol. 22). The data suggest that the absorption of radioactivity at 1 mg/kg dose of olopatadine was 88% from the jejunum, 57% from duodenum, 49% from the ileum and 6% from the stomach. The data suggest that most of the absorption took place from the small intestine after an oral dose.

Comparison of PK parameters at 1 and 25 mg/kg/oral dose of ^{14}C -olopatadine in male Wister rats:

Page 5-7247, vol. 22, Report # 054-385700995:

The plasma data normalized to the dose presented in the following table.

| Dose | C _{max} /dose | Plasma | Blood |
|---------------|------------------------|--------|----------------|
| 1 mg/kg/oral | ng.eq/ml/mg/kg | 208 | 187.9 |
| 25 mg/kg/oral | | 257.6 | 263.3 |
| | AUC/dose | | |
| 1 mg/kg/oral | ng.eq.hr/ml/mg/kg | 1077.8 | 843.1 |
| 25 mg/kg/oral | | 961.0 | Not calculated |

The serum unbound fraction was 30-39% at 1 mg/kg dose between 0.5-4 hours. The unbound fraction was 28-38% between 0.5-4 hours at 25 mg/kg dose. The data suggest that olopatadine had linear kinetics within the dose range studied.

Urinary and biliary metabolites of olopatadine in Wister rats:

Page 5-2402, 5-6920, vol. 22; Report# 044385700995 and 045385700995: ?

Male Wister rats were given a oral dose of olopatadine at 50 mg/kg dose in bile cannulated rats. Bile and urine samples were collected over 24 hours for the identification of metabolites.

The data suggest that M₄ = sulphate conjugate of 8 hydroxy olopatadine; M₅ = hydroxy derivative (minor), M₆ = oxidized metabolite (minor); M₁ = mono des methyl olopatadine; UD = parent drug; M₃ = olopatadine N oxide were detected in the bile.

The 0-24 hour urine samples showed unchanged drug and a small amount of M₁ metabolite.

Above data suggest that unchanged drug and metabolites were excreted in the bile and urine in rats.

Disposition of ¹⁴C-olopatadine in rats and dogs:

Page 5-7456, vol 22, Report: 066385700995:

Plasma, bile and urine samples of rats (strain not mentioned), plasma and urine samples of dogs (strain not mentioned) were collected after 1 mg/kg/oral dose of olopatadine. The percent of metabolites are shown below.

| Species | sample | monodesmethyl (M ₁) | olopatadine | N-oxide (M ₃) |
|---------|----------------|------------------------------------|-------------|------------------------------|
| Rat | plasma | 5.9% | 79.9% | ND |
| Dog | Plasma | 3.5% | 58.4% | 4.1% |
| Rat | Urine (0-24hr) | 9.7% | 67% | ND |
| Dog | Urine (0-24hr) | 5.8% | 68% | 2.2% |
| Rat | Bile | 8.4% | 12.2% | ND |

ND=not detected

Above data suggest that most of the olopatadine was excreted unchanged in the urine and two minor metabolites M₁ and M₃ were excreted in the urine and feces after 1 mg/kg dose.

Disposition of olopatadine in male Wister rats:

Page 5-7481, vol. 22, Report 046385700995:

A comparative PK study was conducted at 1 mg/kg iv dose and 0.3, 1 and 3 mg/kg oral doses of olopatadine (all single doses). The data suggest that olopatadine was rapidly absorbed after oral dosing. The elimination half life was between 6-7 hours. The pharmacokinetics data at 0.3 - 3 mg/kg was linear. The oral bioavailability was about 61 -74%.

Gender differences in the kinetics of olopatadine in Wister rats:

Page 5-7510, vol. 23 and Report 055385700995:

Male and female rats were given single dose of 1mg/kg either by oral or iv route. PK parameters were analysed. AUC 0-∞ in male rats was 431 ng.hr/ml and that of female was 542 ng.hr/ml. The bioavailability was 60 and 76.3%, in male and female respectively. The data were statistically significant. It can be concluded that olopatadine was more bioavailable in female rats compared to the male rats.

A radio-tracer study was conducted in lactating Wister rats (Page 5-7282, vol. 22, Report 062385700995). Results of the study showed that most of the parent drug is excreted in the milk from the lactating rats at 1 mg/kg oral dose. The C_{max} of olopatadine in the plasma and milk were 339 and 285 ng/ml, respectively. Suckling rats from the dosed lactating females also showed radioactivity in the plasma. The data suggest that olopatadine is

excreted in the milk and transferred to the nursing pups.

Effect of olopatadine on hepatic metabolizing enzymes in female rats.

Page 5-7647, vol. 23, report#059385700995:

The effect of olopatadine in hepatic enzymes was investigated in female Sprague-Dawley rats. Rats were treated orally at 0.1, 1.0 and 25 mg/kg for 7 days. Olopatadine did not change the weight of liver, microsomal protein, CYP-450 and several drug metabolizing enzymes. It was concluded that olopatadine does not affect hepatic drug metabolizing enzymes up to 25 mg/kg oral dose for 7 days.

In a separate study (page 5-7675, vol. 23) in male Wister rats renal clearance of olopatadine was investigated. The data suggest that olopatadine was filtered in the glomerular bed and also involve in the tubular secretion. The effect supports higher excretion of olopatadine in the urine.

Hepatic clearance of olopatadine was 11.1 ml/min/kg compared to the total clearance of 17.9 ml/min/kg after 0.2 mg/kg iv injection in male Wister rats (page 5-7694, vol. 23).

Transfer of olopatadine to fetuses in the pregnant rats:

Page 5-7318, vol. 22, Report: 061385700995:

Pregnant Wister rats were given oral doses of ¹⁴C-olopatadine on gestation day 12 and 19th. Blood and plasma levels of radioactivity were determined at several time points on gestation days 12 and 19. Two fetuses were removed from each animal on day 12 and 19 of gestation. Fetal blood and tissue samples were collected for determining the radioactivity.

There was no statistically significant differences in the PK parameters in the blood and plasma from pregnant rats on days 12 and 19 of gestation. However, plasma exposures (AUC 0-∞) of radioactivity of the pregnant rats at 1 mg/kg dose levels were higher compared to that of previously conducted male rats (page 5-7171 vol 22) as shown in the following table.

| Rats | Plasma AUC | Blood AUC, ng.eq.hr/ml |
|------------------|------------|------------------------|
| Male | 1215 | 936 |
| Pregnant, day 12 | 1792 | 1450 |
| Pregnant, day 19 | 2219 | 2069 |

The data suggest that the clearance of the radioactivity in pregnant animals was slow. Most of the radioactivity in the maternal tissues were detected in the liver and kidney up to 4 hours post dose and reduced considerably at 24 hours.

Following table shows the distribution of radioactivity in the maternal blood, amniotic fluid and fetus on days 12 and 19 of the gestation.

| Days | Maternal blood | Amniotic fluid | Fetus |
|----------------|--------------------|----------------|-------|
| day 12, 0.5 hr | 284 ng.eq/ml or gm | 40.3 | 61.2 |
| Day 12, 4 hr | 85.5 | 16.8 | 23.2 |
| Day 19, 0.5 hr | 402.2 | 7.5 | 107 |
| Day 19, 4 hr | 127.2 | 46.3 | 64.8 |

The data suggest that olopatadine or its metabolites crossed the placental barrier in pregnant rats. The data was confirmed by an imaging technique in a separate study as presented in page 5-7351, vol. 22.

Disposition of olopatadine: absorption and excretion of ¹⁴C-radioactive olopatadine after oral dosing in dogs.

Page 5-7378, vol. 22, 053385700995:

Male beagle dogs weighing 10-11 kg and 10 months of age were used. Animals were given ¹⁴C-olopatadine at 1 mg/kg/oral dose. Blood samples were collected at several time points for PK analysis. In vivo protein binding and excretion of radioactivity were determined. Pharmacokinetic parameters in the blood and plasma are shown below.

| Parameter | Units | Plasma | Blood |
|--------------------|-------------|--------|-------|
| T _{max} | hr | 1.13 | 1.13 |
| C _{max} | ng.eq/ml | 723 | 661 |
| T _{1/2} | hr | NC | NC |
| AUC _{0-t} | ng.eq.hr/ml | 3483 | 2837 |

The percent of unbound radioactivity varied from 43-46%. Radioactivity was also distributed in the red cell. About 96% of the radioactivity was excreted within 168 hours. About 74% of the radioactivity was excreted in the urine and 23% in the feces.

It can be concluded that olopatadine was rapidly absorbed after oral dosing in dogs and did not accumulate following administration of single dose of 1 mg/kg/oral.

In a separate experiment (page 5-7536, vol. 23), pharmacokinetic parameters of olopatadine were determined in male beagle dogs at 1 mg/kg iv dose and 0.3, 1, and 3 mg/kg oral doses (all doses were single-dose). Data suggest that olopatadine was rapidly absorbed after oral administration within one hour. The C_{max} at 0.3, 1.0 and 3.0 mg/kg doses were 291, 808 and 2212 ng/ml of plasma, respectively. The plasma exposure was dose proportionate. The elimination was biphasic. The elimination half life was 6-8 hours. Bioavailability was 83-87%.

Comparison of olopatadine metabolites in rats, dogs, monkeys and humans.

Page 5-6940, vol 21:

Percent of metabolites and olopatadine in several species

| Species | Route | Dose | Samples | % of (M ₁) | % of Olopata dine | % of N-Oxide (M ₂) |
|---------|-------|---------|---------|------------------------|-------------------|--------------------------------|
| Rat | Oral | 1 mg/kg | Plasma | 5.9 | 79.9 | ND |
| Dog | Oral | 1 mg/kg | Plasma | 3.5 | 58.4 | 4.1 |
| Rat | Oral | 1 mg/kg | Urine | 9.7 | 67 | ND |
| Dog | Oral | 1 mg/kg | urine | 5.8 | 68 | 2.2 |
| Monkey | oral | 1 mg/kg | Urine | NA | 39.6 | NA |
| Monkey | IV | 1 mg/kg | Urine | NA | 50.1 | NA |
| Human | Oral | 5 mg | Urine | 0.6 | 68.4 | 4.1 |
| Human | Oral | 10 mg | Urine | 1.1 | 71.7 | 2.3 |
| Human | Oral | 10 mg | Urine | 0.2 | 62.9 | 2.1 |
| Human | Oral | 20 mg | Urine | 1.3 | 73.4 | 2.1 |
| Human | Oral | 40 mg | Urine | 0.9 | 62.2 | 3.2 |
| Human | Oral | 80 mg | Urine | 0.8 | 58.7 | 3.1 |
| Rat | Oral | 1 mg/kg | Bile | 8.4 | 12.2 | ND |

ND = Not detected, NA = Not analyzed.

These data suggest that olopatadine is excreted unchanged in urine. Two minor metabolites were also detected i.e. Monodesmethyl (M₁) and N-Oxide (M₂). Metabolic profiles in the species studied were same.

Protein binding in human, rat, guinea-pig and dog sera in vitro:

Page 5-7596, vol. 23, 069385700995:

At 10 $\mu\text{g/ml}$ protein binding was 54-61% in all species. The binding was determined by equilibrium dialysis of ^{14}C -olopatadine. Binding to human albumin was 36-44% at 0.1-1000 $\mu\text{g/ml}$ concentration. The binding of olopatadine to 4% human serum albumin was unaffected by warferin, diazepam or digitoxin.

Effect of pH for in vitro protein binding of olopatadine in human, rat and bovine serum albumin:

Page 5-7613, vol 23, report# 070385700995:

At pH 6, 7 and 8 the in vitro protein binding of ^{14}C -olopatadine was investigated. The unbound fraction of olopatadine was increased from 49-59% with the increase in pH in human serum albumin. The amount of unbound fraction in bovine serum albumin was decreased with the increase in pH. Therefore, high affinity binding was decreased in human serum albumin with the increase in the pH and that for bovine serum albumin was increased. Free fatty acid interfered with the binding of rat serum albumin.

Summary and Discussion :

Olopatadine is a non-sedative antihistamine that blocks H_1 receptors competitively and possesses anti-allergic activities. It also inhibits the release of histamine, PGD_2 and tryptase from mast cells. Several *in vivo* and *in vitro* experiments were conducted to demonstrate its pharmacodynamic properties. Olopatadine inhibited the allergic response to guinea-pig eyes *in vivo*. The ED_{50} for inhibition of the conjunctival permeability changes was 0.017 and 0.0067% for topical and i.v. routes, respectively. The anti-allergic response was observed within 30 minutes and sustained for 4-8 hours. The anti-allergic response was also demonstrated by the inhibition of allergic bronchospasm in passively sensitized guinea-pigs at 0.011 to 0.05 mg/kg doses. However, a dose of 12.1 mg/kg/oral was necessary for preventing the bronchospastic effect in actively sensitized guinea-pigs. Olopatadine inhibited passive cutaneous anaphylaxis at 0.11 mg/kg/oral doses in rats.

Olopatadine showed *in vivo* antihistaminic activity in guinea-pig eyes at 0.002% concentration when applied topically. The 0.1 and 0.2% clinical formulations also showed antihistaminic effect in guinea-pigs when applied topically. Histamine-induced bronchoconstriction in guinea-pigs, capillary permeability and

paw edema in rats were inhibited at 3-100 $\mu\text{g/ml/i.v}$ and 0.014 mg/kg/oral doses, respectively. The effect of olopatadine in LTD₄-induced smooth muscle contractions was not inhibited at concentrations between 10^{-6} and 1.4×10^{-4} M *in vitro*. It did not show lipoxygenase inhibitory activity up to 100 μM concentrations. Olopatadine showed about 26% inhibition of PG cyclooxygenase (PGCO) at 100 μM concentration. Considering the single clinical dose to be 2 drops of 0.1% ophthalmic solution in each eye, it is equivalent to about 100 μg of olopatadine. Therefore, it is expected that olopatadine would inhibit PGCO at the clinical dose. Considering the fact that olopatadine does not inhibit the release of other known mediators from the mast cells, it is better to define its action as an antiallergic rather than that of a mast cell stabilizer.

In an *in vivo* experiment, olopatadine did not inhibit PAF or serotonin induced vascular permeability in rat conjunctiva at 0.01% concentration (20 μL) applied topically.

The anti-allergic and antihistaminic responses were validated in several *in vitro* experiments. Olopatadine inhibited the histamine and PGD₂ release in passively sensitized rat basophil cells at 559 to 736 μM concentrations. The antigen induced smooth muscle contractions were inhibited at 5.84×10^{-5} M. The K_i for inhibition of ³H-pyrimidine binding in the guinea-pig lung and tracheal smooth muscle was between 16-45 nM. Olopatadine also inhibited H₁ receptor transduction at 9.5 to 39.5 nM in human conjunctival epithelial, human corneal fibroblast and in human trabecular cells.

The *in vitro* binding studies suggest that olopatadine possesses some affinity to 5-HT₂ receptors. The ratio of IC₅₀ for the 5-HT₂ and H₁ receptor will be about 20-100 folds. On the basis of the selectivity it is concluded that the pharmacodynamic response of olopatadine will be primarily due to the antihistaminic and anti allergic responses. Olopatadine did not show local anesthetic activity in the guinea-pig corneal reflex experiments. It should be mentioned in this context that several antihistaminic shares local anesthetic and anti muscarinic properties. The metabolites of olopatadine N-mono-demethyl and N-di-demethyl olopatadine also showed a competitive displacement of radioligand in guinea-pig cerebellum and lung tissues. The K_i values were approximately half or less than that for olopatadine. However, these metabolites do not constitute as the major metabolites and their contributions to the efficacy is considered to be minimal.

Its CNS effect was not evident at doses lower than 300 mg/kg/oral in mice. Acute clinical sign of the increase in the respiratory

rate and occasional vomiting was observed at 300 and 30 mg/kg/oral doses in mice and cats, respectively. Olopatadine showed antihypertensive effect in dogs. However, several antihistaminic showed hypotensive response upon i.v. injections. Topical application of olopatadine in eyes did not show ocular discomfort and irritation immediately after the dosing in rabbits.

Above data suggest that olopatadine is a potent antihistaminic and antiallergic drug without sedative effect. The mode of action is through the end organ blockade on H₁ receptor and inhibition of histamine release from the inflammatory cells. Olopatadine showed weak inhibition of cyclooxygenase and without 5-lipoxygenase inhibitory activity in vitro at concentrations that demonstrated antihistaminic and antiallergic responses. Based on these facts the product should be considered as an antiallergic compound rather than a mast cell stabilizer.

In vivo ocular toxicity study in N-Z rabbits at 0.15, 0.5 and 1.0% olopatadine did not show ocular and systemic toxicity at the end of six months.

Olopatadine was given to male and female monkeys at 0.1, 0.2 and 0.5% ophthalmic solutions q.i.d. for six months. The treatment did not show sign of toxicity to eyes and other organs. The mean plasma concentrations of olopatadine was 7.60 ng/ml at 0.5% and it was bioavailable in a dose dependent manner. However, the trough levels were not high enough for systemic toxicities.

Acute toxicity of olopatadine was investigated in mice. The LD₅₀ was 1.15 g/kg/oral in male and 1.83 g/kg/oral in female mice. Clinical signs were reduction in the spontaneous activity, abnormal gait, tremors, convulsions, hypothermia and dyspnea. Ocular toxicity was mydriasis and inflamed eyelids. The target organ of toxicity was kidney.

The data on acute toxicity of rats following oral and i.v doses showed kidney toxicity associated with hydronephrosis and papillary necrosis, mydriasis, hemorrhage and vasodilatation in the iris and lacrimation in eyes. Peripheral irritation is expected due to the fact that some of the animals showed writhing movement after oral dosing. The LD₅₀ for rats was more than 5 g/kg/oral for male rats, 3.87 g/kg/oral for female rats, 127.5 mg/kg/i.v for male rats and 142.8 mg/kg/i.v for female rats.

Data for the one month toxicity study in rats showed enlargement of liver, hemorrhage in the kidney and eyeballs at 600 mg/kg/oral dose. There was no mortality reported at this dose.

A three month oral gavage study was undertaken in Wister rats at 6, 25, 100 and 400 mg/kg doses. The result of the study showed that 4 out of 15 female rats died during the study at 400 mg/kg dose possibly from the congestion in lungs and degeneration of the alveolar wall. The loss of body weight gain was observed at 400 mg/kg dose in male and female rats. Mydriasis was observed as the sign of toxicity at 400 mg/kg. Histology data showed degenerative changes in the myocardium at 400 mg/kg dose in male and female rats. On the basis of the data a dose of 100 mg/kg was chosen for the one year study. Report of the one year study up to 100 mg/kg dose suggest that the dose was tolerated. However, regeneration of tubular epithelia of kidney and interstitial myocarditis were noted in male and female rats when treated for one year. Biological significance of the finding is not understood. Mydriasis was also noted in rats treated at 10-100 mg/kg for one year. Labored breathing and congestion of lungs were noted in several studies in rats. The sponsor indicated that the changes could have been due to irritation of laryngeal mucosa induced by the drug substance.

Olopatadine was tolerated up to 150 mg/kg i.v. and 5 g/kg per oral in beagle dogs in the acute toxicity study. Clinical signs were vomiting, hypothermia, convulsions, mydriasis, protrusion of the nictitating membrane and abnormal EKG. The activity of CPK and LDH was increased. This may reflect ischemia of the heart. However, there was no histological changes in the heart in animals sacrificed on day 14 after the single dose. It is interesting to note that kidney was the target organ of toxicity in rodents, however, in dogs heart may be the target organ of toxicity following acute overdoses.

Olopatadine was tolerated at 40 mg/kg/oral dose in beagle dogs for three months. At 160 mg/kg dose mortality was observed. Clinical signs of toxicity were vomiting, conjunctival hyperemia, anorexia and mydriasis. EKG abnormality at 160 mg/kg dose and a single incidence of primary A-V block was noted at 10 mg/kg. Histology data showed clarified cytoplasm of tubular epithelia of kidney at 160 mg/kg in female dogs. Based on these data, 10-40 mg/kg/oral dose seems to be chosen as the highest dose for a chronic toxicity study. When the treatment was extended to one year at 0.6, 5 and 40 mg/kg doses, abnormality in the EKG (male and female) and fibrosis of the heart (one out of 4 female at 40 mg/kg) were observed at 5-40 mg/kg doses. Male dogs showed loss of body weight gain at 40 mg/kg dose. However, there was no treatment related ocular changes observed following the systemic delivery of olopatadine.

In male and female rats, olopatadine did not affect fertility at 50 mg/kg/oral dose (seg I study). At higher dose maternal toxicity and a decrease in the body weight gain was observed in male and female animals. Fertility to male and female rats was also affected at 400 mg/kg dose. The segment II teratogenicity data in rats showed that olopatadine treated rats had higher incidences of post implantation loss and visceral anomalies at 200 mg/kg dose. A segment (III) reproductive toxicity was conducted in rats at 60, 200 and 600 mg/kg/oral doses. The 600 mg/kg dose appeared to be toxic to rats and an increase in mortality was observed. Results of the study suggest that olopatadine showed postnatal toxicity to F₁ rats at 60 mg/kg and higher doses. Viability of F₁ pups was reduced at these doses. The experiment was repeated at 4, 6 and 20 mg/kg doses. However, body weight gain of the F₁ pups was reduced at these doses even though F₀ rats appeared to be normal. It was concluded that olopatadine was excreted in the breast milk and affected the body weight gain of the F₁ pups. If the data is extrapolated to the human safety, olopatadine treated patients should not nurse babies. **On the basis of the decrease in the number of live fetuses and a decrease in the viability of fetuses after delivery (within 4 days), olopatadine should be considered for pregnancy category C.**

Olopatadine is excreted in the milk of nursing mothers in rats. It also crosses the placental barrier and distributed to the fetuses in rats.

Olopatadine did not show mutagenicity in the Ames test, chromosomal aberration assay and in the in vivo micronucleus test.

A 78-week carcinogenicity study of olopatadine was conducted in CD-1 mice. The doses were 50, 160 and 500 mg/kg/day given in the diet mixtures. The doses were selected on the basis of a preliminary study #91/KKY/007/0120. At the highest dose, female mice showed a 14% decrease in the body weight gain. Treated mice also showed a reduction of food consumption specially at the mid and high doses. Considering these data, the maximum dose used in the study could be considered as the MTD. The target dose was also achieved in the study. The histopathology data suggest that nonneoplastic changes were present in the heart, liver, lung, ovary and prostate at 500 mg/kg dose. However, survival of mice was not affected in the presence of above mentioned nonneoplastic changes. For the neoplastic changes, an increase incidences of adenoma and carcinoma of liver were observed in male mice at 500 mg/kg dose. Biostatistics review did not show statistical significance of the findings. Considering the hepatic adenoma and

carcinoma as common tumors, it is unlikely that the finding would be of any significance. Overall, it appears that mice did not show any carcinogenic potential to olopatadine. Olopatadine did not show carcinogenic lesions at 50 mg/kg (150 mg/msq), 160 mg/kg (480 mg/msq) and 500 mg/kg (1500 mg/msq) doses in mice treated for 78 weeks when the drug was administered orally in the diet.

A 104 week carcinogenicity study was conducted in F-344 rats at 20, 65 and 200 mg/kg doses given in the diet mixture. More than 10% loss of body weight gain was observed at the highest dose in male and female rats. Based on the toxicity and the data from the 3 month oral gavage study, it appears that the MTD has been reached. Long term oral dosing of olopatadine in rats showed increased incidences of keratitis at the low dose in male and female rats in the left eye. The right eye showed more susceptibility to keratitis for male rats. Pancreatic islet cells showed an increase in the incidences of adenoma and carcinoma. The statistical review of the data did not confirm that the findings had statistical significance. Based on the findings it can be concluded that olopatadine given in the diet did not show carcinogenic potential at 20 mg/kg (118 mg/msq), 65 mg/kg (383.5 mg/msq), 200 mg/kg (1180 mg/msq).

The pharmacokinetic and the ocular tissue distribution study of topical olopatadine was conducted in N-Z rabbits. A single topical dose of radio labeled olopatadine (45 μ g) in the eye in N-Z rabbits showed distribution of the drug was mostly in the anterior chamber. The bioavailability of olopatadine after a single dose was 83% at 0.032 mg/kg topical dose when compared to the i.v. dose. Multi-dose study in N-Z rabbits showed no accumulation of olopatadine in the systemic circulation at 0.1% q.i.d dose (2 drops/dose) for up to six months. However, a steady state level (trough) of 0.48 and 0.75 ng/ml was obtained at 0.2% q.i.d and 0.2% H.I.D (2 drops/dose), respectively, on day 27. In the Dutch rabbit model, binding in pigmented eye tissues were higher than that observed in the N-Z rabbits.

Chronic topical dosing of olopatadine at 0.1 and 0.2% q.i.d (2 drops/dose) for six months in cynomolgus monkeys did not show systemic accumulation of olopatadine. However, it was bioavailable dose proportionately within 30 min after the last dose.

Olopatadine is rapidly absorbed after oral administration and bioavailability was 61-71% in rats, 83-87% in dogs and 102% in monkeys. The major site of the drug absorption is small intestine in rats after oral doses. Olopatadine is excreted unchanged in urine and feces in rats, dogs, monkeys and humans. Two

metabolites were identified as M₁ (monodesmethyl) and M₂ (N-oxide) in rats, dogs and humans. The plasma half-life of olopatadine is 6-8 hrs in dogs, 5.5-7.45 hrs in rats and about 12 hours in monkeys. It has linear plasma kinetics up to 25 mg/kg oral dose in rats. Female rats showed higher exposures than the male rats at equal oral doses. However, it would not show similar differences for a topical product. Olopatadine does not have any effect on hepatic P-450 drug metabolizing systems in rats. Its binding to plasma protein is about 54-61% in rat, guinea-pig, dog and human plasma proteins. It is not displaced by digitoxin, diazepam and warferin from the plasma protein binding sites.

Conclusion:

Olopatadine is an antagonist to the H₁ receptor, inhibits the release of PGD₂ and tryptase from the activated mast cells. The product should be considered as an antiallergic rather than mast cell stabilizer because there is no evidence to support that it inhibits all known mediators of anaphylaxis by interfering with the antigen-IgE interactions in the mast cell. In the preclinical models, the antiallergic effect of olopatadine was topical when the drug was instilled into eyes. Olopatadine ophthalmic solutions at 0.1%-0.2% concentration did not show any ocular toxicity when applied directly to the N-Z rabbit and monkey eyes. The sponsor should have conducted the experiments in Dutch rabbits since binding of olopatadine in the pigmented ocular tissues in Dutch rabbits was higher than that of N-Z rabbits. Chronic oral administration of the drug in rats and mice (carcinogenicity studies) did not show any statistically significant neoplastic changes at 200 and 500 mg/kg/oral doses, respectively. However, fetotoxicity was observed in several reproductive toxicity experiments in rats. It is also excreted in the milk in rats. Based on the data, pregnancy category C is recommended for the label. An warning for the nursing mother needs to be incorporated in the label. In the chronic toxicity study in rats, keratitis was observed in some rats. However, the medical team leader suggested that the vehicle could contribute to the inflammatory changes in the cornea. In several oral toxicity studies, mydriasis was also observed in rats, mice and dogs at a high oral dose. However, considering the differences in the topical and oral doses, the findings may not be relevant to the product.

The recommended dose is one to two drops of 0.1% in each eye twice daily which is equivalent to about 400 µg/day. The ophthalmic doses are much lower than the oral doses used in the toxicity study in rats and mice. At above prescribed doses, the systemic exposures to olopatadine will be very low compared to

that necessary for the antiallergic response following oral dosing. On the basis of the preclinical data on the chronic toxicity studies, the chronic use of olopatadine ophthalmic solution (0.1%) would be safe. The product is approvable. However, CAC committee needs to be consulted for a discussion and recommendation.

Recommendation:

The NDA is approvable on the basis of the preclinical data. The proposed package insert needs to be modified in accordance to the review of the reproductive toxicity data. It is recommended that the pregnancy category C should be stated in the label.

Recommendation for the label:

1. Product description and clinical pharmacology:

Olopatadine should be considered as a H₁ antagonist and antiallergic rather than a mast cell stabilizer.

2. Carcinogenesis, mutagenesis, impairment of fertility:

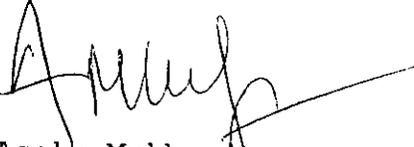
Olopatadine was not carcinogenic in mice and rats up to 500 mg/kg/day (1500 mg/m²) and 200 mg/kg/day (1180 mg/m²) doses, respectively. These doses were 83,333 and 33,333 times higher than the maximum recommended ocular human dose (MRHOD). No mutagenic potential was observed when olopatadine was tested in the Ames Assay, in vitro chromosomal aberration assay in the Chinese Hamster lung cell line and the in vivo micronucleus assay in mice. Olopatadine did not show impairment of fertility in male and female rats up to 50 mg/kg (295 mg/m²) which was 8,333 times MRHOD.

3. Pregnancy:

Category C: Olopatadine was found not to be teratogenic in rats and rabbits. However, rats and rabbits treated at 60 mg/kg (354 mg/m²), 10,000 MRHOD and 400 mg/kg (4400 mg/m²), 66,666 MRHOD, respectively, during organogenesis showed a decrease in live fetuses. There are, however, no adequate and well controlled studies in pregnant women. Because animal studies are not always predictive of human responses, this drug should be used in pregnant women only if the potential benefit to the mother justifies the potential risk to the embryo or fetus.

4. Nursing mothers:

Olopatadine has been identified in the milk of nursing rats following oral dosing. It is not known whether topical ocular administration could result in sufficient systemic absorption to produce detectable quantities in the human breast milk. Nevertheless, caution should be exercised when OPATANOL 0.1% (olopatadine ophthalmic solution) is administered to a nursing mother.



Asoke Mukherjee
Pharmacologist

*Concurred by Conrad H. Chen
Aug. 7, 1996*

cc:
Orig. NDA 20-688
HFD-550/Div. File
HFD-550/AMukherjee
HFD-550/JHolmes
HFD-345
Doc#nda 20-688

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT
AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

NDA:20-688 IND:
DRUG:Olopatadine 1% Ophthalmic Solution CODE#:
CAS#: 140462-76-6 DATE: Aug 7, 1996
DIVISION(s): HFD-550
DRUG NAME(s):Opatanol 0.1%

SPONSOR: Alcon Lab, Texas
LABORATORY:Pharmaco LSR LTD, England
P/T REVIEWER(s):Asoke Mukherjee
P/T REVIEW DATE:July 16, 1996
CARCINOGENICITY STUDY REPORT DATE: Mar 3, 1994 (rat); Oct 28,
1993 (mouse)
THERAPEUTIC CATEGORY: Antihistaminic and antiallergic
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION:H₁ antagonist and
inhibitor of histamine release from mast cells.

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date):No, as far as
I know

MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay):No

RAT CARCINOGENICITY STUDY (multiple studies? Std1;Std2 etc.):
One study

RAT STUDY DURATION (weeks):104 weeks
STUDY STARTING DATE: Feb 11, 1991
STUDY ENDING DATE:Mar 1, 1993
RAT STRAIN: F344
ROUTE: Oral, Diet
DOSING COMMENTS:The diet mixing equipment changed from week 34.

No. Rats in Control1 (C1):50/sex Control2 (C2):
Low Dose (LD):50/sex
Middle Dose (MD):50/sex
High Dose (HD):50/sex High Dose2 (HD2):

RAT DOSE LEVELS (mg/kg/day)

Rat Low Dose:20 Rat Middle Dose:65
Rat High Dose:200 Rat High Dose2:

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum
feasible):MTD, three month oral gavage and diet studies

RAT CARCINOGENICITY (negative;positive;MF;M;F):

positive male rats

RAT TUMOR FINDINGS:

Malignant islet cell carcinoma of pancreas in male rats:

| Control | Low | Mid | High |
|---------|------|------|------|
| 0/50 | 0/28 | 1/25 | 2/49 |

RAT STUDY COMMENTS:

Statistical review did not show statistically significant finding. However, the sponsor examined tissue histology for all rats in the control and high doses. For low and mid doses, histology was done for moribund animals, those died during the study and those showed gross changes in the organ. The total number examined was that attempted to be microscopically examined. However, Due to autolysis and other factors all tissues were not examined.

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1;Std2 etc.):one

MOUSE STUDY DURATION (weeks): 78 weeks
STUDY STARTING DATE:Feb 7, 1991
STUDY ENDING DATE:Aug 19, 1992
MOUSE STRAIN: CD-1 strain
ROUTE:Oral,diet
DOSING COMMENTS:

| | |
|------------------------------|---------------------|
| No. Mice in Control1 (C1):52 | Control2 (C2) |
| Low Dose (LD):52 | Middle Dose (MD):52 |
| High Dose (HD):52 | High Dose2 (HD2): |

MOUSE DOSE LEVELS (mg/kg/day)

| | |
|--------------|--------------|
| Mouse LD:50 | Mouse MD:160 |
| Mouse HD:500 | Mouse HD2: |

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible):MTD, preliminary dose range finding study

Prior FDA Concurrence (Div/CAC)? (y/n;Date):no, as far as I know

MOUSE CARCINOGENICITY (negative;positive;MF;M;F):

Negative in male and female

MOUSE TUMOR FINDINGS: Male mice

| | Control | Low | Mid | High |
|-----------------|---------|------|------|-------|
| Liver adenoma | 8/52 | 6/33 | 4/32 | 10/52 |
| Liver carcinoma | 2/52 | 4/33 | 1/32 | 2/52 |

Statistical review did not show statistically significant finding. However, the sponsor examined tissue histology for all mice in the control and high doses. For low and mid doses, histology was done for moribund animals, those died during the study and those showed gross changes in the organ. The total number examined was that attempted to be microscopically examined. However, Due to autolysis and other factors all tissues were not examined.

ABBREVIATIONS FOR TUMOR SITE IDENTIFICATION*

Aden=adenoma
AG = adrenal gland
B = brain
BD = bile duct
Carc=carcinoma
CG = clitoral gland
CS = circulatory system (e.g. hemangioepithelioma/sarcoma)
HG = harderian gland
HS = hematopoietic system (e.g. leukemia;lymphoma)
I = intestine
IS = integumentary system (e.g. connective tissue)
K = kidney
L = liver
LU = lung
MG = mammary gland
MT = mesothelial tissue
N = nose
O = ovary
OC = oral cavity
OST= osteosarcoma in bone
PG = preputial gland
PTG= pituitary gland
S = stomach
SB = subcutaneous tissue
SK = skin
SP = spleen
TG = thyroid gland
ZG = zymbals gland
U = uterus
TV = tunica vaginalis

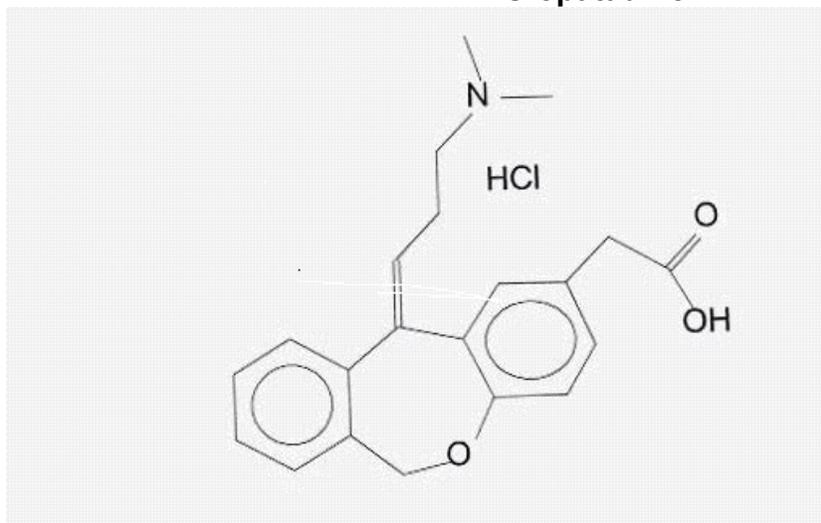
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

IND number: 60,116
Review number: 3
Sequence number/date/type of submission: N007 PN/3-12-01
N011 IT/4-27-01
Information to sponsor: No
Sponsor and/or agent: Alcon Universal, Ltd.
Manufacturer for drug substance: (b) (4)

Reviewer name: Jui R. Shah, Ph.D.
Division Name: Division of Pulmonary and Allergy Drug Products
HFD#: 570
Review Completion Date: September, 2001

Drug:
Code name: AL-4943A, (b) (4)-4679
Generic Name: Olopatadine HCl
Trade Name: N/A
Chemical Name: (Z)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrobenz[b,e]-oxepine-2-acetic acid hydrochloride
Molecular Formula/ Molecular Weight: $C_{21}H_{23}NO_3 \cdot HCl$, 373.88
Structure:

Olopatadine HCl



Relevant INDs/NDAs/DMFs: NDA 20-688

Drug class: Olopatadine HCl is an antihistamine as well as a conjunctival mast cell stabilizer.

Indication: For the treatment of allergic rhinoconjunctivitis.

Clinical Formulation: Topical aqueous nasal solution with benzalkonium chloride (0.01%), dibasic sodium phosphate, sodium chloride, and hydrochloric acid/sodium hydroxide (to adjust pH)

Route of Administration: Intranasal

Previous clinical experience: Olopatadine is currently marketed for ophthalmic treatment of allergic conjunctivitis.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

OVERALL SUMMARY AND EVALUATION:

Introduction:

Olopatadine is currently marketed as an ophthalmic solution for treatment of allergic conjunctivitis. Olopatadine HCl is an antihistamine as well as a conjunctival mast cell stabilizer and is currently in trials for the treatment of allergic rhinoconjunctivitis. The clinical formulation is a topical aqueous nasal solution with benzalkonium chloride (0.01%), dibasic sodium phosphate, sodium chloride, and hydrochloric acid/sodium hydroxide (to adjust pH).

Safety evaluation:

In the intranasal rat studies reviewed here (13-week & 8 week interim sacrifice from a 6-month toxicity study), olopatadine was well-tolerated up to 0.2 mg/d ($40 \mu\text{g}/\text{cm}^2/\text{day}$) for 8 weeks. Nasal cavity inflammation observed at $80 \mu\text{g}/\text{cm}^2/\text{day}$. No NOAEL was identified in the 13-week study due to incomplete histopathology assessment although there were no indications of nasal or pulmonary toxicity at doses up to 0.16 mg/day ($0.32 \mu\text{g}/\text{cm}^2/\text{day}$). In addition, no effects of BAC (benzalkonium chloride) were evident in these studies. Although the vehicle in these studies induced adverse findings in the nasal cavity & lung, the clinical formulation is different from that used in these studies. In an earlier 14-day intranasal toxicity study in rats, doses of 0.2 and 0.4 mg/day were administered; these gave local exposures of $57 \mu\text{g}/\text{cm}^2$ ($400 \mu\text{g}/7 \text{ cm}^2$) per nostril at the highest dose of 0.2% calculated based on surface area, while the highest human dose gives an exposure of $10 \mu\text{g}/\text{cm}^2$ ($1600 \mu\text{g}/160 \text{ cm}^2$). In the current submission, the nasal surface area being used for a single nare is 5 cm^2 (a more conservative estimate); based on that surface area, the high dose used in the 14-day study gives an exposure of $80 \mu\text{g}/\text{cm}^2$ ($400 \mu\text{g}/5 \text{ cm}^2$) per nostril.

In addition, although a CV safety study had been conducted earlier, the possible QT prolonging effects of olopatadine were tested in males beagles treated with itraconazole (to block CYP3A4, the extent to which CYP3A4 was blocked at the dose used is not stated). The co-administration of olopatadine and itraconazole did not prolong QT in male beagles (please note females were not tested). Furthermore, the effect of olopatadine on cloned hERG channels was also evaluated. Olopatadine blocks hERG with an IC_{50} of 1.1mM and shows no temperature or use-dependence.

Conclusions:

(b) (4) nasal spray was well tolerated up to doses of 0.2 mg/day ($40 \mu\text{g}/\text{cm}^2/\text{day}$) intranasally in rats for up to 8 weeks. The excipient benzalkonium chloride appeared to have no adverse effects. Evaluation of a non-rodent species has not been provided.

RECOMMENDATIONS:

Internal comments:

In these studies, the animals were dosed only in a single naris and the contralateral naris was used as a control. This is not a good experimental design since a perforation exists between the two nares, which would allow the drug to also reach the contralateral naris. However, the use of adequate control groups resolves this issue. A similar design (dosing in a single naris and using the contralateral naris as a control) was used for the acute study submitted with the original IND.

External comments:

We remind you (see previous letter dated May 24, 2000) that the species selection for a 6-month nasal toxicity study should be based upon the results of 2-week studies in two species (one of which is a non-rodent). The most appropriate specie should be selected for long term assessment. Based on the submitted data it is unclear that the rat is the most appropriate specie since data in a non-rodent species have not been submitted.

Reviewer signature:

Team leader signature [concurrence/non-concurrence]:

cc:

HFD-570/Division file
HFD-570/T. McGovern
HFD-570/J. Shah
HFD-570/D. Hilfiker
HFD-570/C. Lee

Studies reviewed within this submission:

Effect of Combination of (b) (4)-4679 and Itraconazole on the ECG in Conscious Dogs (N007, Vol. 4.3, p. 1027).

Effects of Olopatadine HCl on Cloned hERG Channels (N007, Vol. 4.3, p. 1044).

Repeated Dose Toxicity Study of (b) (4)-4679 NS Administered Intranasally to Rats for 13-Weeks. (A-95-132, vol. 4.3, p.0759).

6-Month Intranasal Toxicity Study of (b) (4) Nasal Spray (0.1 & 0.2%) in Rats. 8-Week Interim Report. (b) (4) Study #: 298-051, vol. 1)

Studies not reviewed within this submission:

14-day Intranasal Toxicity Study of (b) (4) Nasal Spray (0.1 & 0.2%) in Rats. (b) (4)
Study #: 298-050, vol. 4.2, p.14). This study was already submitted as N004 (June 16, 2000) and has already been reviewed (July 20, 2000).

Introduction and drug history:

Olopatadine is currently marketed as an ophthalmic solution for treatment of allergic conjunctivitis. Olopatadine HCl is an antihistamine as well as a conjunctival mast cell stabilizer and is in clinical trials for the treatment of allergic rhinoconjunctivitis. The clinical formulation is a topical aqueous nasal solution with benzalkonium chloride (0.01%), dibasic sodium phosphate, sodium chloride, and hydrochloric acid/sodium hydroxide (to adjust pH).

TABLE OF CONTENTS

| | |
|--|----|
| SAFETY PHARMACOLOGY:..... | 1 |
| TOXICOLOGY:..... | 3 |
| Histopathology Inventory for IND # | 10 |

SAFETY PHARMACOLOGY:**Cardiovascular effects:****Effect of Combination of (b)(4)-4679 and Itraconazole on the ECG in Conscious Dogs.** (N007, Vol. 4.3, p. 1027).

Earlier cardiovascular safety studies showed that (b)(4)-4679 (20, 50 & 100 mg/kg i.v.) elicited a dose dependent hypotension and decreased peripheral resistance followed by (reflex) tachycardia but no prolongation of the QT interval. This study was undertaken to study the effects of (b)(4)-4679 following itraconazole mediated inhibition of CYP3A4 in conscious dogs. Reviewer note: (b)(4)-4679 is assumed to be olopatadine.

Male beagles (7-9 months old, n=6/group) were instrumented to allow for the measurement of blood pressure, heart rate and ECG. Cardiovascular parameters in response to a single dose of (b)(4)-4679 (30 mg/kg) were determined, followed by a 7-day washout. Animals were then dosed with 100 mg/kg itraconazole p.o. followed 1 hr later by the same dose of (b)(4)-4679. The data are tabulated below. It should be noted that there is no justification for the dose of itraconazole used (Does this dose adequately block CYP3A4?).

| Time (h) | Vehicle | | | (b)(4)-4679 | | | Itr+ (b)(4)-4679 | | |
|----------|----------|------------|------------|-------------|------------|------------|------------------|------------|------------|
| | HR (bpm) | ΔQT (ms.h) | MBP (mmHg) | HR (bpm) | ΔQT (ms.h) | MBP (mmHg) | HR (bpm) | ΔQT (ms.h) | MBP (mmHg) |
| 0 | 71.5 | - | 95.6 | 71.0 | - | 93.5 | 70.8 | - | 91.2 |
| 3 | 70.9 | 5.2 | 88.3 | 85.0* | 3.0 | 103.0* | 77.6 | 3.9 | 102.7 |
| 6 | 72.5 | 7.9 | 86.1 | 74.5 | 3.1 | 99.7* | 69.3 | 12.8 | 93.9 |
| 8 | 67.0 | 11.9 | 93.0 | 74.7* | 4.7 | 96.1 | 72.9 | 7.8 | 93.1 |
| 12 | 72.7 | 11.3 | 90.7 | 71.6 | 2.8* | 91.1 | 76.9** | 4.9 | 92.8 |

* indicates statistically significant ($p < 0.05$) compared to control, ** indicates statistically significant ($p < 0.05$) compared to (b)(4)-4679 alone.

(b)(4)-4679 alone causes a greater increase in HR and mBP (in contrast to the earlier experiment where (b)(4)-4679 caused hypotension) than when administered along with itraconazole, while ΔQT tended to be less affected. These data suggest that (b)(4)-4679 may not elicit QT prolongation even when co-administered with the CYP 3A4-inhibitor itraconazole.

Effects of Olopatadine HCl on Cloned hERG Channels (N007, Vol.4.3, p. 1044)

This study was conducted to measure the effects of olopatadine on cloned hERG channels. Concentration, temperature and use-dependence were also studied. Olopatadine (0.3, 1.0 & 7.0 mM) was administered and gave an IC₅₀ of 1.1 mM; terfenadine (500 nM) was used as a positive control. Olopatadine (1 mM) block of hERG currents was independent of depolarization frequency (0.3-3 Hz). To evaluate temperature dependence the temperature of the bath was raised from 22-25 °C to 32.4

°C. Raising the temperature of the bath did not significantly change the amount of inhibition elicited by 1-mM olopatadine.

Thus, olopatadine blocked hERG channels with an IC_{50} of 1.1 mM. This block showed no use or time dependence.

Safety pharmacology summary:

In male beagles, olopatadine alone elicited slight increases in HR and slight decreases in ΔQT ; olopatadine increased HR when administered in combination with itraconazole, but there was a smaller decrease in ΔQT (at 12 hr: vehicle: 11.3, olo: 2.8, itr+olo: 4.9 ms.h). These data suggest that olopatadine may not elicit QT prolongation even when CYP3A4 is blocked (Note: the extent of CYP3A4 block achieved by the administered dose of itraconazole is not stated). Olopatadine appears to block hERG channels with an IC_{50} of 1.1 mM. The block shows no use or time dependence.

Safety pharmacology conclusions:

The data show that olopatadine has little potential to prolong the QT interval and thus to induce polymorphic ventricular tachycardias (PVMTs) such as torsades de pointes.

TOXICOLOGY:

Study title: Repeated Dose Toxicity Study of (b) (4)-4679NS Administered Intranasally to Rats for 13-weeks.

Key Study findings: In this 13-week intranasal toxicology study in rats, olopatadine was well tolerated. No definitive drug-related effects were identified. The NOAEL for nasal and pulmonary toxicities was the high dose since no significant effects were seen at doses of 0.04, 0.08 and 0.16 mg/day (8, 16 & 32 $\mu\text{g}/\text{cm}^2/\text{day}$). However, an overall NOAEL could not be determined since a complete histopathological assessment was not performed. In addition, the vehicle produced adverse effects such as decreased goblet cells.

Study no: A-95-132/ Ref. Doc. 94-305

Volume #, and page #: Vol. 4.3, p. 0759, N007IT

Conducting laboratory and location: (b) (4)

No Address Provided

Date of study initiation: Sept. 1993

GLP compliance: No

QA report: No

Drug, lot #, radiolabel, and % purity: No radiolabel, % purity not provided

| Label | Batch |
|-------------------------------|----------|
| Olopatadine Nasal Spray 0.05% | 4679-8L4 |
| Olopatadine Nasal Spray 0.1% | 4679-8M4 |
| Olopatadine Nasal Spray 0.2% | 4679-8N4 |
| Base Agent | 4679-8P4 |

Formulation/vehicle: not stated

Methods (unique aspects): In this intranasal study, animals were dosed 4 times daily in the left naris only at approximately 2 hr intervals. Contralateral naris was used as a control.

Dosing:

Species/strain: Wistar rats.

#/sex/group or time point (main study): Not stated, appears to be 15/sex/group.

Satellite groups used for toxicokinetics or recovery: None.

Age: ~ 7-weeks

Weight: M: 185-204 g, F: 135-152 g

Doses in administered units: 0.04, 0.08 & 0.16 mg/day (8, 16 & 32 $\mu\text{g}/\text{cm}^2/\text{day}$ calculated based on single naris surface area).

Route, form, volume, and infusion rate: Intranasal, spray, 20 μl /instillation

Observations and times:

| | |
|---------------------|---|
| Clinical signs: | Twice daily (predosing and after final dose) |
| Body weights: | Weekly |
| Food consumption: | Weekly. |
| Ophthalmoscopy: | Pretest & week 12. |
| EKG: | Not done |
| Hematology: | Terminal. |
| Clinical chemistry: | Terminal. |
| Urinalysis: | Week 9. Tested for Na, K, protein, sp. gravity, and urinary sediment at a later unspecified time point. |
| Gross pathology: | Terminal |
| Organs weighed: | Terminal |
| Histopathology: | Terminal. Assessment was limited; see histopathology table (p. 10). |
| Toxicokinetics: | Not done |

Results:

| | |
|-------------------|---|
| Mortality: | No treatment related mortalities. |
| Clinical signs: | A few clinical signs were seen in both control and treated groups. These included animals with cut or broken teeth (vehicle: 2M, 1F, LD: 3M, 2F, MD: 2M, 1F, HD: 4M), urine leakage (LD M and treated females), and soft stools (males of vehicle, LD and HD groups). |
| Body weights: | No notable changes. |
| Food consumption: | The mean food consumption was slightly reduced in vehicle, LD and HD males and LD and MD females. These values were less than 10% and thus of questionable significance. |
| Ophthalmoscopy: | Minor changes such as corneal clouding, indentation of the optic disk, abnormal blood vessel patterns and dilation of the optic disk were seen in treated and control animals, however, the time (whether before or during treatment) at which these observations were made is not stated. Furthermore, since these |

were seen in both control and treated animals, they may not be treatment-related.

Hematology:

No notable changes were seen.

Clinical chemistry:

Minor changes (<10%) were seen in total protein and ALP, which are of doubtful toxicological significance.

Urinalysis:

No notable changes were seen.

Organ weights:

Small changes in organ weights were noted in absolute ovary weights and relative adrenal weights. These changes show no relation to treatment.

Gross pathology:

No notable changes.

Histopathology:

The nasal cavity was divided into 3 levels (1-3) and the histopathological changes observed were listed according to the level at which they were observed and are as tabulated below.

| Treat | Nasal Cavity Level 1 | | | Nasal Cavity Level 2 | | | Nasal Cavity Level 3 | |
|---------|------------------------------|----------|--------|------------------------------|---------------------------------|-----------------------------------|-----------------------------------|----------------------------|
| | ↓ goblet cells - left septum | | | ↓ goblet cells - left septum | ↑ resp epithelium - left septum | Inflam. Infiltratn-lamina propria | Edema - ethmoturbinal lamina prop | Inflam. Infit. Lamina prop |
| | slight | moderate | marked | | | | | |
| Saline | - | - | - | - | - | 2M, 1F | - | - |
| Vehicle | 6M, 9F | 3M, 2F | 1F | 1M, 2F | - | 4M | - | - |
| LD | 5M, 3F | 1M, 4F | 2F | 2M | - | 2M, 1F | - | 1F |
| MD | 7M, 7F | 4M | - | - | 1M | 4M, 6F | - | - |
| HD | 9M, 8F | 2M, 2F | 2F | 4M, 2F | - | 2M | 1M | 1M |

- indicates not seen

Summary of individual study findings:

No drug-related effects were seen in this 13-week intranasal study in rats using doses of 0.04, 0.08 and 0.16 mg/day (8, 16 & 32 µg/cm²/day); however, the vehicle produced adverse effects in the nasal cavity. Histopathologic alterations in control, vehicle and treated animals included decreased goblet cells in the left nasal septum, infiltration of the nasal cavity and lamina propria, etc. The sponsor suggests that the goblet cell changes occurred in response to the viscosity of the vehicle and therefore are not of concern; however, this may become an issue with prolonged use. The clinical formulation is different from that used in this 13-week study. The NOAEL for nasal and

pulmonary findings is the high dose of 0.16 mg/day. An overall NOAEL cannot be determined since full histopathological assessment was not performed.

Study title: 6-Month Intranasal Toxicity Study of (b) (4) Nasal Spray (0.1 & 0.2%) in Rats. 8-Week Interim Report.

Key study findings: In this interim sacrifice data from the 6-month intranasal toxicology study in rats, olopatadine was well tolerated. The NOAEL was the low dose of 0.4 mg/day (40 µg/cm²/day calculated based on single naris surface area of 5 cm²) based on nasal cavity infiltration. In addition, no effects of BAC (benzalkonium chloride) were evident in this study; however, the vehicle did cause adverse effects such as laryngeal infiltration as well as the appearance of hemoglobin crystals in the lungs with associated inflammation.

Study no: (b) (4) Study #: 298-051
Volume #, and page #: Vol. 1, N011IT
Conducting laboratory and location: (b) (4)
Date of study initiation: Jan. 3, 2001
GLP compliance: No, This is a draft report
QA report: No, This is a draft report
Drug, lot #, radiolabel, and % purity: No radiolabel, % purity not provided

| Label | Batch |
|---|----------|
| Olopatadine Nasal Spray 0.1% with PEG 400 and PVP | 01-28064 |
| 0.2% with PEG 400 and PVP | 01-28065 |
| Olopatadine Nasal Spray Vehicle without BAC | 01-28062 |
| Olopatadine Nasal Spray Vehicle with BAC | 01-28063 |

Methods (unique aspects): In this intranasal study, animals were dosed 4 times daily in the right naris only at approximately 2 hr intervals, with the contralateral naris used as a control.

Dosing:
 Species/strain: Crl:CD rats.
 #/sex/group or time point (main study): 30/sex/group, with 10 sacrificed at the 8-week time point.
 Satellite groups used for toxicokinetics or recovery: 10/sex/group – no interim sacrifice.
 Age: ~ 4-weeks

Weight: M: 266-274 g, F: 182-185 g
Doses in administered units: 0.2 & 0.4 mg/day (40 & 80 $\mu\text{g}/\text{cm}^2$ /day based on single naris surface area of 5 cm^2).
Route, form, volume, and infusion rate: Intranasal, spray, 50 μl /instillation

Observations and times:

| | |
|---------------------|---|
| Clinical signs: | Twice daily |
| Body weights: | Pretest and weekly |
| Food consumption: | Weekly. |
| Ophthalmoscopy: | Pretest & immediately prior to termination. |
| EKG: | Not done |
| Hematology: | Time point not stated. |
| Clinical chemistry: | Time point not stated. |
| Urinalysis: | Time point not stated. |
| Gross pathology: | Terminal |
| Organs weighed: | Terminal |
| Histopathology: | Terminal |
| Toxicokinetics: | Not done |

Results:

| | |
|-------------------|--|
| Mortality: | No treatment related mortalities. One HD male died following blood collection. |
| Clinical signs: | A few clinical signs were seen in both control and treated groups. These included animals with cut or broken teeth, malocclusion, and alopecia. The most outstanding observation however, was the absence of hair (Vehicle w/o BAC: 1F, Vehicle w/ BAC: 2M, 4F, LD: 7M, 3F, HD: 1M). This does not appear to be treatment related. |
| Body weights: | Statistically significant but only slightly higher (<10%) body weights were seen in HD males compared to the vehicle treated groups from weeks 6-8. |
| Food consumption: | The mean food consumption for treated males was higher than that for vehicle treated animals during weeks 2, 3, 6, 7, & 8. These values were less than 10% and thus of questionable significance. |
| Ophthalmoscopy: | Minor changes such as conjunctivitis and keratitis were seen, which do not appear to be treatment related. |

| | |
|----------------------|---|
| Electrocardiography: | Not done. |
| Hematology: | No notable changes were seen. |
| Clinical chemistry: | Minor changes were seen which are of doubtful toxicologic significance. |
| Urinalysis: | No notable changes were seen. |
| Organ weights: | Small changes in organ weights were noted with several organs. These changes show no relation to treatment. |
| Gross pathology: | No notable changes. |
| Histopathology: | Inflammatory cell infiltration of the anterior nasal cavity was seen in 3/sex HD animals and appears to be treatment related. Infiltration was also seen in the larynx as well as subacute inflammation of the trachea. The lungs showed the presence of hemoglobin crystals with associated inflammation. These changes were seen in both vehicle and drug treated animals and may not be due to the drug but caused by the vehicle. |

| Organ | Vehicle w/o BAC | | Vehicle w/ BAC | | LD | | HD | |
|-------------------------|-----------------|---|----------------|---|----|---|----|---|
| | M | F | M | F | M | F | M | F |
| Nasal Cavity | | | | | | | | |
| Infiltration | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| Larynx | | | | | | | | |
| Infiltration | 3 | 4 | 2 | 1 | 1 | 2 | 4 | 4 |
| Lungs | | | | | | | | |
| Hemoglobin crystals | 7 | 8 | 6 | 6 | 5 | 7 | 6 | 5 |
| Associated inflammation | 8 | 2 | 7 | 0 | 8 | 0 | 7 | 1 |

Summary of individual study findings:

In all, 8-week administration of (b) (4) nasal spray vehicle (with or without BAC) and (b) (4) nasal spray (0.1 & 0.2% with PEG 400 and PVP) was well tolerated. It appears that the vehicle of PEG 400 and PVP induced inflammation in the larynx and lung at the concentrations tested. The NOAEL for the 8-week interim sacrifice animals is the low

dose of 40 $\mu\text{g}/\text{cm}^2/\text{day}$ calculated based on single naris surface area of 5 cm^2 based on nasal infiltration.

Toxicology summary:

No drug-related effects were seen in this 13-week intranasal study in rats using doses of 0.04, 0.08 and 0.16 mg/day (8, 16 & 32 $\mu\text{g}/\text{cm}^2/\text{day}$); however, the vehicle produced adverse effects in the nasal cavity. Histopathologic alterations in control, vehicle and treated animals included decreased goblet cells in the left nasal septum, infiltration of the nasal cavity and lamina propria, etc. The sponsor suggests that the goblet cell changes occurred in response to the viscosity of the vehicle and therefore are not of concern; however, this may become an issue with prolonged use however. The clinical formulation is different from that used in this 13-week study. The NOAEL for nasal and pulmonary findings is the high dose of 0.16 mg/day. An overall NOAEL cannot be determined since full histopathological assessment was not performed.

In the interim (8-week) sacrifice data from the 6-month intranasal toxicology study in rats, (b) (4) was well tolerated at doses of 0.2 and 0.4 mg (40 & 80 $\mu\text{g}/\text{cm}^2/\text{day}$). Nasal inflammation was seen at the high dose. The vehicle of PEG 400 and PVP induced inflammation in the larynx and lung at the concentrations tested. In addition, no effects of BAC (benzalkonium chloride) were evident in this study. The NOAEL for 8 weeks dosing was the low dose of 0.2 mg/day (40 $\mu\text{g}/\text{cm}^2/\text{day}$).

Toxicology conclusions:

(b) (4) nasal spray was well tolerated at doses of up to 0.2 mg/day (40 $\mu\text{g}/\text{cm}^2/\text{day}$) intranasally in rats for up to 8 weeks. The excipient benzalkonium chloride appeared to have no adverse effects. Nasal cavity inflammation was observed at 0.4 mg/day (80 $\mu\text{g}/\text{cm}^2/\text{day}$) after 8 weeks of dosing. Although the vehicle used in the studies reviewed induced adverse effects in the nasal cavity and lungs, the clinical formulation appears to be different from the formulations used in these studies. A 13-week study with only selective histopathologic assessment in rats resulted in no nasal or pulmonary toxicity at doses up to 0.16 mg/day (32 $\mu\text{g}/\text{cm}^2/\text{day}$).

Histopathology Inventory for IND # 60,116

| Study | 13-Week Intranasal | 8-wk interim sacrifice from 6-month study |
|-------------------------|--------------------|---|
| Species | Rat | Rat |
| Adrenals | X* | X* |
| Aorta | | X |
| Bone Marrow smear | | X |
| Bone (femur) | | X |
| Brain | X* | X |
| Cecum | | X |
| Cervix | | X* |
| Colon | | X |
| Duodenum | | X |
| Epididymis | | X |
| Esophagus | | X |
| Eye | X | X |
| Fallopian tube | | |
| Gall bladder | | |
| Gross lesions | | |
| Harderian gland | | X |
| Heart | X* | X* |
| Ileum | | X |
| Injection site | | |
| Jejunum | | X |
| Kidneys | X* | X* |
| Lachrymal gland | | X |
| Larynx | | X |
| Liver | X* | X* |
| Lungs | X* | X* |
| Lymph nodes, cervical | | |
| Lymph nodes mandibular | | X |
| Lymph nodes, mesenteric | | X |
| Mammary Gland | | X |
| Nasal cavity | X | X |
| Optic nerves | | |
| Ovaries | X* | X |
| Pancreas | | X |
| Parathyroid | | X* |

| | | |
|------------------|----|----|
| Peripheral nerve | | |
| Pharynx | | X |
| Pituitary | | X* |
| Prostate | X* | X* |
| Rectum | | X |
| Salivary gland | | X* |
| Sciatic nerve | | X |
| Seminal vesicles | X* | X |
| Skeletal muscle | | X |
| Skin | | X |
| Spinal cord | | X |
| Spleen | X* | X* |
| Sternum | | X |
| Stomach | | X |
| Testes | X* | X* |
| Thymus | | X* |
| Thyroid | | X* |
| Tongue | | X |
| Trachea | | X |
| Urinary bladder | | X |
| Uterus | X* | X |
| Vagina | | X |
| Zymbal gland | | |
| Standard List | | |
| | | |

X, histopathology performed

*, organ weight obtained

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

/s/

Jui Shah
9/26/01 10:52:36 AM
PHARMACOLOGIST

Timothy McGovern
10/2/01 10:58:45 AM
PHARMACOLOGIST
I concur.

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 60,116
Review number: 6
Sequence number/date/type of submission: N031 IT/1-20-03
N035/4-4-2003
N036 IT/4-21-03
N037 IT/5-13-03
N038 IT/5-30-03

Information to sponsor: Yes
Sponsor and/or agent: Alcon Universal, Ltd.

Manufacturer for drug substance: [REDACTED] (b) (4)

Reviewer name: Jui R. Shah, Ph.D.
Division Name: Division of Pulmonary and Allergy Drug Products
HFD#: 570
Review Completion Date: June 2003

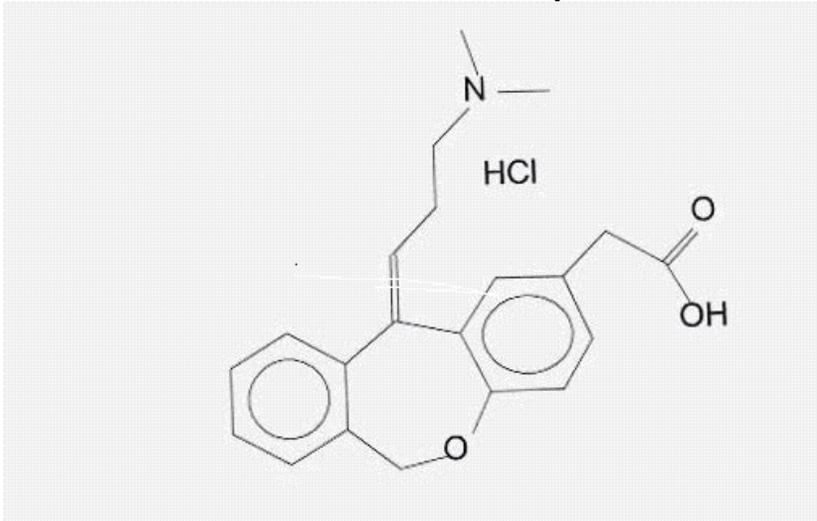
Drug:

Code name: AL-4943A, (b) (4)-4679
Generic Name: Olopatadine HCl
Trade Name: N/A
Chemical Name: (Z)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrobenz[b,e]-oxepine-2-acetic acid hydrochloride

Molecular Formula/ Molecular Weight: C₂₁H₂₃NO₃.HCl, 373.88

Structure:

Olopatadine HCl



| | |
|--------------------------------------|---|
| Relevant INDs/NDAs/DMFs: | NDA 20-688 |
| Drug class: | Olopatadine HCl is an antihistamine as well as a conjunctival mast cell stabilizer. |
| Indication: | For the treatment of allergic rhinoconjunctivitis. |
| Clinical Formulation: | Topical aqueous nasal solution with benzalkonium chloride, Povidone (b) (4) disodium EDTA, dibasic sodium phosphate, sodium chloride, and hydrochloric acid/sodium hydroxide (to adjust pH) |
| Route of Administration: | Intranasal |
| Previous clinical experience: | Olopatadine is currently marketed for ophthalmic treatment of allergic conjunctivitis. |

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history:

Olopatadine is currently marketed as an ophthalmic solution for treatment of allergic conjunctivitis. Olopatadine HCl is an antihistamine as well as a conjunctival mast cell stabilizer and is currently in trials for the treatment of allergic rhinoconjunctivitis.

In intranasal rat studies (13-week & 8 week interim sacrifice from a 6-month toxicity study) reviewed in review #3 (September 2001), olopatadine was well tolerated up to 0.2 mg/d (40 $\mu\text{g}/\text{cm}^2/\text{day}$) for 8 weeks. Nasal cavity inflammation observed at 80 $\mu\text{g}/\text{cm}^2/\text{day}$. No NOAEL was identified in the 13-week study due to incomplete histopathology assessment, although there were no indications of nasal or pulmonary toxicity at doses up to 0.16 mg/day (0.32 $\mu\text{g}/\text{cm}^2/\text{day}$). In addition, no effects of BAC (benzalkonium chloride) were evident in these studies. Although the vehicle in these studies induced adverse findings in the nasal cavity & lung, the clinical formulation is different from that used in these studies. In an earlier 14-day intranasal toxicity study in rats, doses of 0.2 and 0.4 mg/day were administered; these gave local exposures of 57 $\mu\text{g}/\text{cm}^2$ (400 $\mu\text{g}/7 \text{ cm}^2$) per nostril at the highest dose of 0.2% calculated based on surface area, while the highest human dose gives an exposure of 10 $\mu\text{g}/\text{cm}^2$ (1600 $\mu\text{g}/160 \text{ cm}^2$).

In addition, although a CV safety study had been conducted earlier, the possible QT prolonging effects of olopatadine were tested in males beagles treated with itraconazole (to block CYP3A4, the extent to which CYP3A4 was blocked at the dose used is not stated). The co-administration of olopatadine and itraconazole did not prolong QT in

male beagles (please note, females were not tested). Furthermore, the effect of olopatadine on cloned hERG channels was also evaluated. Olopatadine blocks hERG with an IC₅₀ of 1.1mM and shows no temperature or use-dependence.

Olopatadine has been studied in 102 subjects at doses of 5 mg PO (study # C-00-23), which gave C_{max} of 76.7±19.2 ng/ml (range: 34-127 ng/ml) and shown to be safe and well tolerated without any QTc prolongation. In a separate intranasal PK study using 0.1% QD, 0.1% BID and 0.2% BID, C_{max} after the first dose was 3.46 & 8.6 ng/ml for the 0.1% QD and 0.2% dose, respectively. In an intranasal PK study (C-00-21) male and female volunteers received either placebo (n=4) or 0.6% olopatadine (n=8). C_{max} was 29.3 ng/ml (M: 13.6-21.4 and F: 30.8-58.4) observed at a t_{max} of 0.5-2.0 (M: 0.5-2.0 and F: 0.5-1.0) hr and AUC was 75.0 ng/ml (M: 31.84-71.43 and F: 70.28-126.3). Comparing data from studies conducted with the 0.1 & 0.2% doses and the study using the 0.6% dose, it appears that the systemic exposure from 0.6% is proportional to the exposures at 0.1% and 0.2%.

The sponsor states that Olopatadine has been reformulated such that the current product contains 0.6% olopatadine as well as (b) (4) Povidone (PVP K- (b) (4)) and the pH of the product has been adjusted to (b) (4) (same as for Nasonex[®]). The sponsor has conducted a 2-week intranasal bridging study in rats with concentrations up to 1.2% formulated to include Povidone (b) (4) adjusted to a pH of (b) (4). In addition, the same concentration of Povidone was present in the formulation during the first 8 weeks of the 6-month intranasal rat toxicity study.

Submission N031 contains a draft report of the 6-month rat study, a 14-day rat study using 0.6 & 1.2% olopatadine with povidone and a pH of (b) (4). In addition, the final report of the 8-week interim sacrifice for the 6-month rat study is included. We reminded the sponsor that the final complete report is to be accompanied by a detailed summary of any and all changes made to the previously submitted draft report. The sponsor has not submitted such a list. Therefore, the final report will be reviewed once such a list is submitted. Submission N035 only contains resubmitted Appendices 21 & 22 from the 14-day rat study. They were resubmitted because they were illegible (6 point font) in the original submission. Submission N036 contains a preclinical development plan update. In this submission, Alcon refers to recent data where it was discovered that for the 9-month dog study, the animals were administered a dose of 0.16% olopatadine rather than the target dose of 0.2%. Alcon informed us of this in N025 (7-16-02). In the current submission Alcon states that 'Because no specific feedback was received, Alcon has presumed that the margin of safety provided by this study is acceptable to the agency for the support of the planned NDA.' We will inform the sponsor that we decline to comment on this with reference to acceptability, safety margins, etc. until the completed study report is submitted. Submission N037 contains the final study report for the 6-month rat study (a detailed summary of changes made to the draft report is not included). Submission N038 contains a brief update only (not a draft or final report) on the 14-day dog PVP study in which the sponsor states that no notable changes were seen. Therefore, the 6-month PVP bridging study will be carried out in rats. This submission contains a protocol outline for such a study. In addition, N038 also contains a protocol (C-02-54) for a double-masked, multiple dose, 2-way crossover study of cardiovascular safety and pharmacokinetics of Olopatadine 20 mg oral solution versus

placebo administered twice daily (BID) for fourteen days in healthy subjects, to obtain longer term QT interval safety data.

GENERAL TOXICOLOGY: **1**

DETAILED CONCLUSIONS AND RECOMMENDATIONS: **14**

PHARMACOLOGY/TOXICOLOGY REVIEW**GENERAL TOXICOLOGY:**

Study title: **6-Month Intranasal Toxicity Study of**
(b) (4) Nasal Spray (0.1 & 0.2%) in Rats

Key study findings: Rats (20/sex/group) were treated 4 times daily for 6 months with either vehicle with BAC, vehicle w/o BAC, 0.2 mg/d or 0.4 mg/d of olopatadine. Treated males had decreased relative thyroid/parathyroid weights while HDF had increased absolute and relative uterus/cervix and heart weights. These changes were minor. Histopathologic changes included testicular hypertrophy and aspermatogenesis in HDM and increased mammary lobular hyperplasia in HDF. Low dose animals were not examined for these changes. In addition hepatic inflammation and lung histiocytosis were seen in animals from all groups. No NOAEL can be determined for this study based on histopathology since LD animals were not examined. Toxicokinetic data showed that animals from both treated groups were exposed to olopatadine on all days tested. Exposure was greater in F compared to M and increased with dose. No accumulation was seen with time.

Study no: 298-051 and technical report #: 015:33:0402
Volume #, and page #: 2, p. 1
Conducting laboratory and location: (b) (4)
Date of study initiation: January 3, 2001
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity: No % purity available, no radiolabel.

| Label | Batch # | TMC # |
|--|----------|-------|
| Olopatadine Nasal Spray 0.1% w/ PEG 400 & PVP | 01-28064 | 14294 |
| Olopatadine Nasal Spray 0.2% w/ PEG 400 & PVP | 01-28065 | 14295 |
| Olopatadine Nasal Spray 0.1% | 01-28070 | 14481 |
| Olopatadine Nasal Spray 0.2% | 01-28471 | 14482 |
| Olopatadine Nasal Spray Vehicle w/o BAC, PEG 400 & PVP | 01-28062 | 14292 |
| Olopatadine Nasal Spray Vehicle w/ BAC, PEG 400 & PVP | 01-28063 | 14293 |
| Olopatadine Nasal Spray Vehicle w/o BAC | 01-28516 | 14479 |
| Olopatadine Nasal Spray Vehicle w/ BAC | 01-28469 | 14480 |

Formulation/vehicle: Olopatadine nasal spray vehicles with and without benzalkonium chloride were used. For the first 8 weeks of the study, vehicles also contained PEG 400 and PVP and were at a pH of (b) (4). For the remainder of the study, no PEG 400 or PVP were present, pH of the vehicle used for the latter part of the study are not stated.

Methods (unique aspects): Animals were dosed in the right naris only with the left naris as the contralateral control.

Dosing:

Species/strain: Crl:CD(SD)IGS rats

#/sex/group or time point: Main study: 20/sex/group, 8-week sacrifice: 10/sex/group

Satellite groups used for toxicokinetics: 10/sex/group

Age: 4 weeks

Weight: Main study: M: 202-236g, F: 165-204g
Replacement TK: M: 219-245g, F: 178-201g

Doses in administered units: Vehicle without BAC: 0, Vehicle with BAC: 0, (b) (4) nasal spray (0.1%): 0.2 mg/day, (b) (4) nasal spray (0.2%): 0.4 mg/day.

Route, form, volume, and infusion rate: Intranasal, Spray, 50µl x 4 approximately 2 hr apart.

Observations and times:

Clinical signs: Twice daily.

Body weights: Prerandomization, day -1, weekly thereafter.

Food consumption: Weekly.

Ophthalmoscopy: Prerandomization, and prior to interim and terminal euthanasia.

Hematology: At interim and terminal euthanasia.

Clinical chemistry: At interim and terminal euthanasia.

Urinalysis: At interim and terminal euthanasia.

Gross pathology: Terminal.

Organs weighed: Terminal, see histopathology inventory p. 11.

Histopathology: Terminal, see histopathology inventory p. 11.
Toxicokinetics: Day 1, weeks 13 & 26 at predose, 0.5, 1, 2 & 4 hr after the last dose, n=5/sex/timepoint.

Results:

Mortality: One male (#1474) and one female (#1506) from the vehicle w/ BAC group and one HD F (#1566) died on day 183 after blood collection but prior to euthanasia. On day 117 one LD M (#1535) and on day 57 one HD M (#1589) were found dead. No cause of death was determined for the latter two animals.

Clinical signs: Numerous signs were noted such as malocclusion, material (red or brown) around nose, hair loss and skin nodules, none of the changes showed any relationship to treatment or dose.

Body weights: High dose males had significantly increased body weights during weeks 6, 7, 8, 11 & 12. Treated males had higher body weights (not statistically significant) than vehicle treated males starting week 8 and lasting for the duration of the study, HD M had slightly (not statistically significant) higher body weights than LD M. No differences were seen in body weights of female animals.

Food consumption: High dose males had significantly increased food consumption during weeks 2, 3, 6-14, 17-21, 23 & 25. No differences were seen for female animals.

Ophthalmoscopy: No notable changes.

Hematology: A significant decrease was seen in prothrombin time in HDM compared to vehicle w/o BAC group (V w/o BAC: 16.17 s, V w/ BAC: 14.79* s, HD: 14.97* s) during week 8; however, this value appears to be within the 'normal range', was not seen at the terminal analysis nor in females at any time point and may not be biologically relevant. Low dose F showed a slight but statistically significant increase in MCH, which is not biologically relevant.

| | |
|---------------------|--|
| Clinical chemistry: | Minor, statistically significant changes were seen which showed no relationship to treatment and were not toxicologically relevant. |
| Urinalysis: | All males had lower urine volumes during week 26 compared to those seen during week 8. Low dose males showed a significant decrease in volume during week 26. Vehicle w/o BAC, LD & HD females had lower urine volumes during week 26 compared to those seen during week 8. Due to the large variability seen with the values, the toxicological relevance of this is not clear. |
| Organ weights: | Minor changes were seen in organ weights as tabulated below. Treated males had statistically significant decreases in relative (to body weight) thyroid/parathyroid weights. HDF had significantly increased absolute and relative (body and brain) uterus/cervix weights as well as absolute and relative (to body weight) heart weights. |

| Organ | 0 (w/o BAC) | | 0 (w/ BAC) | | 0.2 mg/d | | 0.4 mg.d | |
|--------------------------------------|-------------|-------|------------|--------|----------|--------|----------|-------|
| | M | F | M | F | M | F | M | F |
| Heart | | | | | | | | |
| Absolute (g) | 1.76 | 1.14 | 1.79 | 1.12 | 1.77 | 1.15 | 1.85 | 1.2* |
| Rel. to BW (%x10) | 3.26 | 3.78 | 3.32 | 3.7 | 3.21 | 3.85 | 3.25 | 4.06* |
| Rel. to BrW (%x10 ⁻¹) | 8.81 | 6.18 | 8.76 | 6.09 | 8.77 | 6.26 | 9.02 | 6.42 |
| Pituitary | | | | | | | | |
| Absolute (mg) | 15 | 32 | 15 | 23 | 13 | 24 | 14 | 29 |
| Rel. to BW (%x10 ³) | 2.73 | 10.52 | 2.79 | 7.62* | 2.39* | 7.99* | 2.52 | 9.68 |
| Rel. to BrW (%x10) | 7.29 | 17.39 | 7.38 | 12.54* | 6.5* | 13.01* | 6.96 | 15.26 |
| Thyroid/Parathyroid | | | | | | | | |
| Absolute (mg) | 32 | 32 | 35 | 29 | 33 | 28* | 33 | 30 |
| Rel. to BW (%x10 ³) | 6.08 | 10.53 | 6.59 | 9.61 | 5.91* | 9.49 | 5.82* | 10.06 |
| Rel. to BrW (%x10) | 16.2 | 17.2 | 17.3 | 15.8 | 16.1 | 15.5 | 16.1 | 15.9 |
| Uterus/Cervix | | | | | | | | |
| Absolute (g) | | 0.71 | | 0.78 | | 0.87 | | 0.91* |
| Rel. to BW (%x10 ²) | | 23.5 | | 25.9 | | 29.4 | | 31.0* |
| Rel. to BrW (%x10 ⁻¹) | | 3.86 | | 4.26 | | 4.73 | | 4.86* |

BW: body weight, BrW: brain weight, * indicates significantly different (p<0.05) from vehicle w/ BAC

Gross pathology:

No notable changes.

Histopathology:

Testicular tubular atrophy and aspermatogenesis was seen in HDM and mammary lobular hyperplasia in HDF; since the LD animals were not examined, no dose relationship for these can be determined. Other histopathologic changes were minor and included inflammation of the liver and histiocytosis of the lung in animals from all groups including control.

| Organ | 0 (w/o BAC) | | 0 (w/ BAC) | | 0.2 mg/d | | 0.4 mg.d | |
|---|-------------|---|------------|---|----------|----|----------|---|
| | M | F | M | F | M | F | M | F |
| Testis | | | | | | | | |
| <i>tubular atrophy & aspermatogenesis</i> | 0 | - | 0 | - | ND | - | 4 | - |
| Mammary Gland | | | | | | | | |
| <i>lobular hyperplasia</i> | - | 4 | - | 4 | - | ND | - | 8 |
| Liver | | | | | | | | |
| <i>inflammation</i> | 17 | 4 | 15 | 5 | 18 | 13 | 18 | 3 |
| Lung | | | | | | | | |
| <i>histiocytosis</i> | 15 | 5 | 12 | 4 | 13 | 7 | 11 | 6 |

Toxicokinetics:

Blood was collected from the orbital sinus on day 1, and during weeks 13 & 26 from TK animals treated with 0.1 and 0.2% olopatadine. Five animals/sex were evaluated per time point. The data are presented in technical report # 015:33:0402 (p.1, v. 5), the analyses were conducted by (b) (4)

All treated animals were exposed to drug, F had greater exposures than males. Exposure increased with dose. No accumulation was apparent with time.

| Parameter | Time | 0.2 mg/d | | | 0.4 mg/d | | |
|--|------------|----------|------|------|----------|-------|------|
| | | M | F | M+F | M | F | M+F |
| C_{max} (ng/ml) | <i>D1</i> | 23.2 | 37.3 | 30.2 | 63.6 | 74.3 | 69.0 |
| | <i>W13</i> | 24.3 | 44.7 | 34.5 | 41.7 | 85.5 | 63.6 |
| | <i>W26</i> | 19.7 | 43.3 | 32.8 | 44.5 | 71.9 | 58.1 |
| AUC₀₋₂₄ (ng.h/ml) | <i>D1</i> | 30.8 | 42.7 | 36.8 | 66.3 | 90.5 | 78.4 |
| | <i>W13</i> | 25.8 | 52.5 | 39.9 | 51.9 | 102.0 | 77.5 |
| | <i>W26</i> | 29.6 | 43.7 | 37.4 | 52.0 | 86.2 | 69.1 |

Summary of individual study findings:

Rats (20/sex/group) were treated 4 times daily for 6 months with either vehicle with BAC, vehicle w/o BAC, 0.2 mg/d or 0.4 mg/d of olopatadine. The initial 8 weeks of the study were conducted using a vehicle containing PEG 400, PVP and with a pH of (b) (4) the remainder of the study was conducted using a vehicle with no PVP or PEG 400 and of unknown pH. Five animals died during the study, 3 following blood collection on the final day, no cause of death was determined for the latter two animals. Treated males had higher body weights than controls from week 8 onwards and high dose males had higher body weights than LDM. HD M had increased food consumption during weeks 2, 3, 6-14, 17-21, 23 & 25. No differences were seen amongst F for either body weights or

food consumption. Minor, statistically significant changes were seen in hematological and clinical chemistry parameters, which are of doubtful toxicological relevance. Treated males had decreased relative thyroid/parathyroid weights while HDF had increased absolute and relative uterus/cervix and heart weights. These changes were minor. Histopathologic changes included testicular hypertrophy and aspermatogenesis in HDM and increased mammary lobular hyperplasia in HDF. Low dose animals were not examined for these changes. In addition hepatic inflammation and lung histiocytosis were seen in animals from all groups. No NOAEL can be determined for this study based on histopathology since LD animals were not examined.

Toxicokinetic data showed that animals from both treated groups were exposed to olopatadine on all days tested. Exposure was greater in F compared to M and increased with dose. No accumulation was seen with time.

Study title: **14-Day Intra-Nasal Toxicity Study of 0.6% and 1.2% Olopatadine Hydrochloride Nasal Spray Solution in Rats.**

Key study findings: In this 14-day toxicity study, rats were dosed with 0.9 & 1.8 mg/d (0.6% and 1.2%) olopatadine hydrochloride intranasally. Body weight gains were slightly lower in HDM compared to controls. Changes in organ weights included decreased absolute spleen weights in HDM only, while absolute kidney weights were increased in HDF only. Both absolute & relative liver weights were decreased in LDM and HD M and increased in HD F. One HD M & F had pigmented mandibular lymph nodes while 2 HD F had red/mottled thymus. Since the liver weight changes were not accompanied by any clinical chemistry or histopathological changes, they may not be toxicologically relevant. Therefore, the NOAEL for this study is the HD based on lack of notable changes.

Study no: TR 055:30:0602
Volume #, and page #: V. 1, p. 1
Conducting laboratory and location: [REDACTED] (b) (4)
Date of study initiation: March 7, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity: No radiolabel, % purity unavailable.

| Solution | Lot # |
|---------------------------------------|------------|
| Vehicle | 02-30721 |
| Olopatadine nasal spray solution 0.6% | 02-30719-1 |
| Olopatadine nasal spray solution 1.2% | 02-30720 |

| | |
|---|---|
| Formulation/vehicle: | Olopatadine nasal spray containing povidone and PEG 400 at a pH of (b) (4) |
| Methods (unique aspects): | Animals were dosed in the right naris only, the contralateral naris served as a control. |
| Dosing: | |
| Species/strain: | CrI:CD(SD)IGS BR rats |
| #/sex/group or time point (main study): | n10/sex/group |
| Satellite groups: | None |
| Age: | ~5 weeks |
| Weight: | M: 101-128g, F: 73.5-108 g |
| Doses in administered units: | 0, 0.9 mg/d, 1.8 mg/d |
| Route, form, volume, and infusion rate: | Intranasal, spray solution, 50 µl 3 times/day, ~2 hr apart. |
| Observations and times: | |
| Clinical signs: | Daily. |
| Body weights: | At randomization, then weekly and at termination. |
| Food consumption: | N/A |
| Hematology: | Rats were fasted overnight after the last dose and blood collected prior to termination. |
| Clinical chemistry: | Rats were fasted overnight after the last dose and blood collected prior to termination. |
| Urinalysis: | Rats were fasted overnight after the last dose and blood collected prior to termination. |
| Gross pathology: | Terminal. |
| Organs weighed: | See histopathology inventory p. 11. |
| Histopathology: | See histopathology inventory p. 11. |
| Toxicokinetics: | Not done. |
| Results: | |
| Mortality: | No animals died. |
| Clinical signs: | No test material related effects were seen. |
| Body weights: | There were no significant differences in the body weights of treated animals vs. control animals; however, body weight gains of HDM was lower than control. |

| Parameter | Vehicle control | | 0.6% (0.9 mg/d) | | 1.2% (1.8 mg/d) | |
|------------------------|-----------------|------|-----------------|------|-----------------|------|
| | M | F | M | F | M | F |
| Body weight (g) | | | | | | |
| Week 0 | 176 | 129 | 178 | 132 | 174 | 134 |
| Week 2 | 291 | 187 | 292 | 194 | 278 | 193 |
| Weight gain (g) | | | | | | |
| Total | 115 | 57.8 | 114 | 62.2 | 103.7* | 59.3 |

* indicates significantly ($p < 0.05$) different from control.

Food consumption: A statistically significant increase in food consumption was seen in HDF during week 1 (C: 127 g, LD: 132, HD: 136*) but not 2 (C: 141 g, LD: 147, HD: 151). The toxicological significance of this is unclear.

Hematology: No notable changes.

Clinical chemistry: No notable changes.

Urinalysis: No notable changes.

Organ weights: Changes in organ weights are noted below. Absolute spleen weights were decreased in HDM only, while absolute kidney weights were increased in HDF only. Both absolute & relative liver weights were decreased in LDM and HD M & F.

| Organ | | Control | | 0.9 mg/d (0.6%) | | 1.8 mg/d (1.2%) | |
|---------|-----------------|---------|-------|-----------------|-------|-----------------|-------|
| | | M | F | M | F | M | F |
| Kidneys | <i>absolute</i> | 2.44 | 1.5 | 2.61 | 1.6 | 2.35 | 1.66* |
| | <i>relative</i> | 0.945 | 0.904 | 0.989 | 0.940 | 0.947 | 0.967 |
| Liver | <i>absolute</i> | 9.13 | 5.87 | 8.45* | 6.3 | 8.21* | 6.49* |
| | <i>relative</i> | 3.54 | 3.53 | 3.21* | 3.69 | 3.31* | 3.79* |
| Spleen | <i>absolute</i> | 0.724 | 0.461 | 0.67 | 0.482 | 0.607* | 0.452 |
| | <i>relative</i> | 0.28 | 0.277 | 0.255 | 0.284 | 0.243 | 0.264 |

* indicates significantly different ($p < 0.05$) from respective control.

Gross pathology: One HD M & F had pigmented mandibular lymph nodes while 2 HD F had red/mottled thymus. These changes do not appear toxicologically relevant.

Histopathology: No notable findings.

Summary of individual study findings:

In this 14-day toxicity study, rats were dosed with 0.9 & 1.8 mg/d (0.6% and 1.2%) olopatadine hydrochloride intranasally. No notable clinical signs were seen, nor were there any consistent changes in food consumption. Body weight gains were slightly lower in HDM compared to controls. Changes in organ weights included decreased absolute spleen weights in HDM only, while absolute kidney weights were increased in HDF only. Both absolute & relative liver weights were decreased in LDM and HD M and increased in HD F. One HD M & F had pigmented mandibular lymph nodes while 2 HD F had red/mottled thymus. Since the liver weight changes were not accompanied by any clinical chemistry or histopathological changes, they may not be toxicologically relevant. Therefore, the NOAEL for this study is the HD based on lack of notable changes.

Toxicology summary:

Rats (20/sex/group) were treated 4 times daily for 6 months with either vehicle with BAC, vehicle w/o BAC, 0.2 mg/d or 0.4 mg/d of olopatadine. The initial 8 weeks of the study were conducted using a vehicle containing PEG 400, PVP and with a pH of (b) (4) the last 4 months of the study were conducted using a vehicle with no PVP or PEG 400 of unknown pH. Five animals died during the study, 3 following blood collection on the final day, no cause of death was determined for the other two animals. Treated males had higher body weights than controls from week 8 onwards and high dose males had higher body weights than LDM. HD M had increased food consumption during weeks 2, 3, 6-14, 17-21, 23 & 25. No differences were seen amongst F for either body weights or food consumption. Minor, statistically significant changes were seen in hematological and clinical chemistry parameters, which are of doubtful toxicological relevance. Treated males had decreased relative thyroid/parathyroid weights while HDF had increased absolute and relative uterus/cervix and heart weights. These changes were minor. Histopathologic changes included testicular hypertrophy and aspermatogenesis in HDM and increased mammary lobular hyperplasia in HDF. Low dose animals were not examined for these changes. In addition hepatic inflammation and lung histiocytosis were seen in animals from all groups. No NOAEL can be determined for this study based on histopathology since LD animals were not examined. Toxicokinetic data showed that animals from both treated groups were exposed to olopatadine on all days tested. Exposure was greater in F compared to M and increased with dose. No accumulation was seen with time.

In this 14-day toxicity study, rats were dosed with 0.9 & 1.8 mg/d (0.6% and 1.2%) olopatadine hydrochloride intranasally. No notable clinical signs were seen, nor were there any consistent changes in food consumption. Body weight gains were slightly lower in HDM compared to controls. Changes in organ weights included decreased absolute spleen weights in HDM only, while absolute kidney weights were increased in HDF only. Both absolute & relative liver weights were decreased in LDM and HD M and increased in HD F. One HD M & F had pigmented mandibular lymph nodes while 2 HD F had red/mottled thymus. Since the liver weight changes were not accompanied by any clinical chemistry or histopathological changes, they may not be toxicologically relevant. Therefore, the NOAEL for this study is the HD based on lack of notable changes.

Toxicology conclusions:

Intranasal olopatadine was administered to rats at doses of up to 1.8 mg/day for 14 days (in a vehicle containing povidone, PEG 400 and pH ^(b)₍₄₎) and at doses of 0.4 mg/day for 6 months. The study report for the interim (8-week) sacrifice from the 6-month rat study was submitted in submission N07IT, dated 3-12-01 and reviewed in the September 2001 review. Treatment-induced changes were minor and included organ weight changes of the thyroid/parathyroid, uterus/cervix, heart, spleen, kidney and liver. Histopathologic changes included testicular hypertrophy and aspermatogenesis, increased mammary lobular hyperplasia, pigmented mandibular lymph nodes and red/mottled thymus. No effect of povidone, PEG 400 and pH ^(b)₍₄₎ were noted in these studies for up to 8 weeks.

Histopathology Inventory for IND # 60116

| Study Species | 6-month Rat | 14-day Rat |
|-------------------------|--------------------|-------------------|
| Adrenals | X* | X* |
| Aorta | X | X |
| Bone Marrow smear | X | X |
| Bone (femur) | X | X |
| Brain | X* | X* |
| Cecum | X | X |
| Cervix | X* | X* |
| Colon | X | X |
| Duodenum | X | X |
| Epididymis | X* | X* |
| Esophagus | X | X |
| Eye | X | X |
| Fallopian tube | | |
| Gall bladder | | |
| Gross lesions | | |
| Harderian gland | X | X |
| Heart | X* | X* |
| Ileum | X | X |
| Injection site | | |
| Jejunum | X | X |
| Kidneys | X* | X* |
| Lachrymal gland | X | X |
| Larynx | X | X |
| Liver | X* | X* |
| Lungs | X* | X* |
| Lymph nodes, cervical | | |
| Lymph nodes mandibular | X | X |
| Lymph nodes, mesenteric | X | X |
| Mammary Gland | X | X |
| Nasal cavity | X | X |
| Optic nerves | X | X |
| Ovaries | X* | X* |
| Pancreas | X | X |
| Parathyroid | X* | X* |
| Peripheral nerve | X | X |
| Pharynx | X | X |
| Pituitary | X* | X* |
| Prostate | X* | X* |
| Rectum | X | X |
| Salivary gland | X* | X* |

| | | |
|------------------|----|----|
| Sciatic nerve | X | X |
| Seminal vesicles | | |
| Skeletal muscle | X | X |
| Skin | X | X |
| Spinal cord | X | X |
| Spleen | X* | X* |
| Sternum | X | X |
| Stomach | X | X |
| Testes | X* | X* |
| Thymus | X* | X* |
| Thyroid | X* | X* |
| Tongue | X | X |
| Trachea | X | X |
| Urinary bladder | X | X |
| Uterus | X* | X* |
| Vagina | X | X |
| Zymbal gland | | |

X, histopathology performed

*, organ weight obtained

DETAILED CONCLUSIONS AND RECOMMENDATIONS:**Conclusions:**

Intranasal olopatadine was administered to rats at doses of up to 1.8 mg/day for 14 days (in a vehicle containing povidone, PEG 400 and pH (b) (4)) and at doses of 0.4 mg/day for 6 months. The study report for the interim (8-week) sacrifice from the 6-month rat study was submitted in submission N07IT, dated 3-12-01 and reviewed in the September 2001 review. Treatment-induced changes were minor and included organ weight changes of the thyroid/parathyroid, uterus/cervix, heart, spleen, kidney and liver. Histopathologic changes included testicular hypertrophy and aspermatogenesis, increased mammary lobular hyperplasia, pigmented mandibular lymph nodes and red/mottled thymus. No effect of povidone, PEG 400 and pH (b) (4) were noted in these studies for up to 8 weeks.

Povidone is used in a variety of products; in cosmetics, in food (clarifying agent, stabilizer and dispersant), in OTC drugs (demulcent) and in betadine, an antifungal and first-aid antiseptic. PVP was used during WWII as a plasma extender but this was stopped when it was found to deposit within the monocyte-macrophage system. In this submission, the sponsor has also provided the DMF (b) (4) for Povidone as well as a final report on the safety assessment of polyvinylpyrrolidone (PVP) from the International Journal of Toxicology 17(suppl. 1):95-130; 1998. In the DMF for Povidone, data from acute, subchronic and chronic toxicity studies as well as carcinogenicity studies are presented and briefly summarized below. The acute toxicity of PVP in rats, mice, rabbits, dogs and rhesus monkeys show no histopathological changes.

| Species | Route & Dose (mg/kg) | Changes |
|-------------|----------------------|---|
| Rats & mice | IP & PO | LD ₅₀ >12 g/kg Osmotic diarrhea @ doses > 500 mg/kg |
| Rabbits | 300-2700 | Slight inhibition of BW gain was seen @ 2700 mg/kg |
| Dogs | IV | Shock due to viscosity @ >10 g/kg – recovered |
| Monkeys | IV | Shock due to viscosity @ >10 g/kg – monkeys died |

In the chronic dietary toxicity studies in rats & dogs, no toxicity was seen although dogs appeared to assimilate PVP from the GI tract into surrounding lymph glands at doses >5%. In the four reproductive toxicity studies in rats and rabbits, there was no evidence of embryotoxicity or teratogenicity of PVP. Similarly in six genotoxicity studies (Ames, mouse lymphoma, Balb/c cell transformation, in vivo dominant lethal test in mice and a chromosomal aberration assay) no evidence of genotoxic potential was detected. Carcinogenicity studies showed varying results. Of 11 studies in rats, 6 showed no difference from controls. A 73 week SC study showed tumors at the injection site only

(43% each in PVP & CMC groups and 17% in controls). A 32 month IV study (200 mg/d) showed spontaneous uterine carcinoma. A lifetime study with weekly IP injections of 25% solution showed 3 sarcomas in the K25 group, 1 in the K17 group and none in controls. In two 2-year studies utilizing a variety of routes rats were positive, while in another similar study they were negative. Of the 3 studies in mice, two were border line while the third showed no increase in carcinomas. PVP was negative in the 2 rabbit carcinogenicity studies.

The sponsor was informed in a teleconference dated February 14, 2003 that since two months of intranasal animal data in a single species (namely, the rat) were available with Povidone, and Povidone had been used via other routes, it would be acceptable for them to proceed with their long term clinical trial (1 year) provided they initiated the requested preclinical studies with the Povidone containing formulation. Theses studies would involve initiation of the 2-week non-rodent toxicology study to determine the most appropriate species (prior to initiation of the clinical trial) and within a reasonable time following completion of this 2-week study, Alcon would conduct a 6-month preclinical study in the most appropriate species.

Submission N036 IT (dated 4/21/03), contains protocol outlines for a 6 month bridging study to qualify Povidone as an excipient. This outline has proposed doses for both rats as well as dogs as the species to be used has not been selected yet. The sponsor proposes to use two different concentrations of Povidone, namely, (b) (4) (the concentration present in the clinical formulation) and (b) (4), which the sponsor states were selected based on the limited pump characteristics and stability data available. The species for this study will be selected pending data from a 2-week dog study; the 2-week rat study is complete. The proposed dose in rats would be (b) (4) in the right naris only ((b) (4), given 2 hr apart) while dogs would receive (b) (4) 4 doses of (b) (4), given 2 hr apart) in both nares. Below is a table with the calculated exposures and safety margins for both species. Based on the exposure calculated on nasal surface area, the HD provides a ~29 fold safety margin in rats and a ~11 fold safety margin in dogs compared to the maximum human exposure.

| Species | Povidone (mg/day) | | Exposure to Povidone (mg/cm ²) | | Safety Margin | |
|---------|-------------------|---------|--|---------|---------------|---------|
| | (b) (4) | (b) (4) | (b) (4) | (b) (4) | (b) (4) | (b) (4) |
| Rat | (b) (4) | | 0.51 | 1.29 | 11.33 | 28.67 |
| Dog | (b) (4) | | 0.2 | 0.49 | 4.0 | 10.87 |
| Man | (b) (4) | | 0.045 | | | |

These calculations assume a surface area of 7 cm² for the rat (right naris only – total volume: (b) (4)), (b) (4) for the dog (dosed in both nares– total volume: (b) (4) ml/d), and (b) (4) for man (dosed in both nares – total volume: (b) (4) containing (b) (4) Povidone).

Although, the doses selected are appropriate, the appropriateness of the species could not be determined for lack of a 2-week study in a second species.

In addition, N038 also contains a protocol (C-02-54) for a double-masked, multiple dose, 2-way crossover study of cardiovascular safety and pharmacokinetics of

Olopatadine 20 mg oral solution versus placebo administered twice daily (BID) for fourteen days in healthy subjects, to obtain longer term QT interval safety data. The safety calculations are based on 4-week rat and 13-week dog oral studies conducted to support NDA 20-688 (Ophthalmic drug products) reviewed by Dr. Asoke Mukherjee (July 16, 1996). The rat NOAEL is based on histopathology changes (inflammation of the liver, bladder, parasternal lymph nodes and hyperplasia of the pancreatic ducts), while the dog NOAEL is based on organ weight (kidneys, ovaries), EKG (ST wave) and histopathology (kidneys, heart valves) changes. The safety calculations for proposed clinical trial are shown below.

| Parameter | Proposed Oral human dose | Rat NOAEL | Dog NOAEL |
|---------------------------|--------------------------|------------|-------------|
| Study/duration/species | 2 weeks | 4-weeks PO | 13-weeks PO |
| Dose (mg/kg) | 0.8 | 200 | 40 |
| Dose (mg/m ²) | 29.6 | 1200 | 800 |
| Safety Factor | | 40 | 26.67 |

Alcon informed us (N022IT, dated 3/13/02) that they had initiated a 9-month dog intranasal toxicity study in June 2001. A summary draft report was (single page) was submitted as appendix 2 of N025 IT, dated 7/18/02. The sponsor states that no effects were seen and concludes that the intranasal instillation of 1.6 & 3.2 mg/day (0.1 & 0.2%) resulted in no apparent toxicity to dogs. The final study report has not yet been submitted. In submission N036, Alcon refers to recent data where it was discovered that for the 9-month dog study, the animals were administered a dose of 0.16% olopatadine rather than the target dose of 0.2%. Alcon states that 'Because no specific feedback was received, Alcon has presumed that the margin of safety provided by this study is acceptable to the agency for the support of the planned NDA.' We will inform the sponsor that we decline to comment on this with reference to acceptability, safety margins, etc. until the completed study report is submitted.

Recommendations (to be conveyed to sponsor):

We acknowledge receipt of your submission N036 and inform you that the proposed 6-month bridging studies for PVP are acceptable. However, the most appropriate species has not yet been determined as the 14-day study in a second (non-rodent) species is not yet available for review.

The Agency cannot comment on the 9-month toxicity study in dogs with reference to acceptability, exposure margins, etc. since the complete report for the 9-month dog study is not available for review.

Submit the complete study reports from both studies for review.

Evaluate and submit terminal histopathology data for the low dose group for the 6-month rat intranasal toxicology study.

A. Reviewer signature: Jui Shah, Ph.D.

B. Supervisor signature: Concurrence – Joseph C. Sun, Ph.D.

Non-Concurrence - _____
(see memo attached)

C.
cc:
HFD-570/Division file
HFD-570/C. Sun
HFD-570/J. Shah
HFD-570/A. Zeccola
HFD-570/C. Lee

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

/s/

Jui Shah
8/8/03 11:28:51 AM
PHARMACOLOGIST

Joseph Sun
8/8/03 01:09:32 PM
PHARMACOLOGIST
I concur.

Drug: **Olopatadine**

| | age | mg/dose | # daily doses | mg/day | kg | mg/kg | factor | mg/m ² |
|-----------|-----|---------|---------------------|--------|----|-------|--------|-------------------|
| Pediatric | | | | 0 | 3 | 0 | 25 | 0 |
| Adult | >12 | 2.4 | 2 | 4.8 | 50 | 0.096 | 37 | 3.552 |

| | route | mg/kg/d | conv. factor | mg/m ² | Dose Ratio | | Rounded Dose Ratio | |
|--------------------------------|-------------|---------|-----------------|-------------------|------------|----------|--------------------|----------|
| | | | | | Adults | Children | Adults | Children |
| <u>Carcinogenicity:</u> | | | | | | | | |
| rat | oral | 200 | 6 | 1200 | 337.84 | --- | 340 | --- |
| mouse | oral | 500 | 3 | 1500 | 422.3 | --- | 420 | --- |
| extra | | | --- | --- | --- | --- | --- | --- |
| extra | | | --- | --- | --- | --- | --- | --- |
| extra | | | --- | --- | --- | --- | --- | --- |
| <u>Repro/Fertility:</u> | | | | | | | | |
| rat | oral | 50 | 6 | 300 | 84.459 | N/A | 85 | N/A |
| rat | oral | 400 | 6 | 2400 | 675.68 | N/A | 680 | N/A |
| extra | | | --- | --- | --- | N/A | --- | N/A |
| extra | | | --- | --- | --- | N/A | --- | N/A |
| <u>Teratogenicity:</u> | | | | | | | | |
| rat | oral | 20 | 6 | 120 | 33.784 | N/A | 35 | N/A |
| rat | oral | 60 | 6 | 360 | 101.35 | N/A | 100 | N/A |
| rat | oral | 600 | 6 | 3600 | 1013.5 | N/A | 1,000 | N/A |
| rabbit | oral | 400 | 12 | 4800 | 1351.4 | N/A | 1,400 | N/A |
| extra | | | --- | --- | --- | N/A | --- | N/A |
| <u>Overdosage:</u> | | | | | | | | |
| rat | intranasal | 3.6 | 6 | 21.6 | 6.0811 | --- | 6 | --- |
| mouse | oral male | 1150 | 3 | 3450 | 971.28 | --- | 970 | --- |
| mouse | oral female | 1830 | 3 | 5490 | 1545.6 | --- | 1,500 | --- |
| dog | oral | 5000 | 20 | 1E+05 | 28153 | --- | 28,000 | --- |
| <u>Overdosage:</u> | | | | | | | | |
| rat | oral | 3870 | 6 | 23220 | 6537.2 | --- | 6,500 | --- |
| extra | | | --- | --- | --- | --- | --- | --- |
| extra | | | --- | --- | --- | --- | --- | --- |
| extra | | | --- | --- | --- | --- | --- | --- |
| extra | | | --- | --- | --- | --- | --- | --- |

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

/s/

Gary Bond
8/26/2005 03:05:23 PM
PHARMACOLOGIST

Joseph Sun
8/26/2005 03:11:20 PM
PHARMACOLOGIST
I concur.

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

/s/

Jean Wu
3/4/2008 05:12:19 PM
PHARMACOLOGIST

Joseph Sun
3/4/2008 05:18:29 PM
PHARMACOLOGIST
I concur.

Executive CAC

Date of Meeting: February 12, 2008

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair
Paul Brown, Ph.D., OND IO, Member
John Leighton, Ph.D., DDOP, Alternate Member
Bill Taylor, Ph.D., DSPTP, Alternate Member
C. Joseph Sun, Ph.D., DPAP, Team Leader:
Jean Q. Wu, MD, Ph.D., DPAP, Presenting Reviewer

Author of Minutes: Jean Q. Wu

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-861

Drug Name: Patanase® Nasal Spray

Sponsor: Alcon, Inc.

Mouse Carcinogenicity Studies

The carcinogenicity potential of two degradants of the drug product, (b) (4) and (b) (4) was tested in the following two 26-week mouse carcinogenicity studies. Both degradants showed positive results in mouse lymphoma assay and Syrian hamster embryo assay, and the intake of both degradants at the proposed acceptance criteria (b) (4) would exceed 1.5 µg/day. The protocols of these two studies were not submitted for Executive CAC concurrence before the studies were conducted.

Study 1:

(b) (4) in vehicle (b) (4) povidone, (b) (4) dibasic sodium phosphate, (b) (4) NaCl, 0.01% benzalkonium chloride, (b) (4) disodium EDTA, pH (b) (4), was administered subcutaneously at doses of 3, 10 and 30 mg/kg/day to groups of 25 male and 25 female C57BL/6TacrBR-P53^{+/-} mice. Untreated sham control, vehicle control and positive control groups (400 mg/kg *p*-cresidine) were included. Dose selection was based on a 28-day repeated dose study in C57BL/6NTacr BR mice at dose levels of 1, 3, 10, 30 and 100 mg/kg/day, in which significant test article-related mortality was observed at 100 mg/kg/day. The incidences of sarcomas observed at injection site skin in all treated and vehicle control groups of males and females were higher than those in the sham controls. The incidence in the male mid-dose group was notably higher than that in male vehicle group, but the increase in incidences in the treated male mice was not dose-dependent. There were no other test-article related neoplastic findings in this study.

Study 2:

(b) (4), in vehicle (b) (4) povidone, (b) (4) dibasic sodium phosphate, (b) (4) NaCl, 0.01% benzalkonium chloride, (b) (4) disodium EDTA, pH (b) (4), was administered subcutaneously at doses of 1, 5 and 12.5 mg/kg/day to groups of 25 male and 25 female C57BL/6TacrBR-P53^{+/-} mice. The initial high dose, 12.5 mg/kg/day, was lowered to 8

mg/kg/day on Day 62 (males) and Day 56 (females) due to the early deaths. Untreated sham control, vehicle control and positive control (400 mg/kg *p*-cresidine) groups were included. Dose selection was based on a 28-day repeated dose study in C57BL/6 NTac^f BR mice at dose levels of 5, 12.5, and 20.0 mg/kg/day, in which significant test article-related toxicity and slight mortality were observed at 12.5 mg/kg/day and 20 mg/kg/day. The incidences of sarcomas observed at injection site skin in all treated and vehicle control groups were significantly higher than those in the sham control. The incidence in high dose females was higher than that in the female vehicle-control group. The survival-unadjusted trend test on the incidence of sarcomas showed that the trend was statistically significant at the 0.05 level in females but not in males. However, the further pair-wise comparison between female high dose and vehicle-control groups was not statistically significant at the 0.05 level. There were no other test-article related neoplastic findings in this study.

Executive CAC Recommendations and Conclusions:

1. The high-dose selection in the second study exceeded the MTD. The SC route is generally not recommended for the P53^{+/-} mouse studies. The inclusion of povidone (known to cause sc sarcomas in rodents) in the vehicle was considered inappropriate for evaluation of the local effects of the two genotoxic impurities.
2. The interpretation of sarcomas at injection sites of skin in both vehicle and test article-treated groups was confounded by the subcutaneous route of administration, the vehicle effects, and the lack of a proper control group for the vehicle. Therefore, the local effects of the test articles in both studies can not be determined.
3. Since no other tumor findings were observed in either study, the Committee concluded that the two test articles have no systemic carcinogenic potential related to p53.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\

/NDA 21-861 Division File, DPAP
/CSun, DPAP
/JWu, DPAP
/MRaggio/PM, DPAP
/CBertha, OND/QA/DPMA
/PPeri, OND/QA/DPMA
/ASeifried, OND IO

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

/s/

Abby Jacobs
2/21/2008 07:10:56 AM

**PHARMACOLOGY/TOXICOLOGY REVIEW
FOR CONSULTATION REQUEST**

NDA number: 22-861

Request date:/Type of Request: October 16, 2007/Review-General

Requested by: ONDQA/DPA1/Branch 2

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Alcon Inc.

Reviewer name: Jean Q. Wu

Division name: Division of Pulmonary and Allergy Products

HFD #: 570

Review completion date: February 4, 2008

Drug:

Trade name: Patanase® Nasal Spray

Active Drug: olapatadine HCl

Relevant INDs/NDAs/DMFs: IND 60,116, IND (b)(4) & NDA20-688, IND 60,991

Drug class: histamine H₁ antagonist and inhibitor of pro-inflammatory cytokine secretion

Intended clinical population: Patients with seasonal allergic rhinitis at age of 12 years and above

Clinical formulation: The current proposed formulation and the formulation in the original NDA are listed in the table below (excerpted from Module 2, Vol. 2. Section 2.4, Page 2).

Table 2.4.1-1
Compositions of PATANASE NDA Formula (FID^a: (b) (4)) and
Proposed Reformulation (FID: 109941)

| Components | NDA Formulation (1.8% PVP) FID (b) (4) | Proposed Reformulation (PVP-free) FID 109941 |
|---|--|--|
| | % w/v | % w/v |
| Olopatadine Hydrochloride | 0.665 ^b | Same |
| Benzalkonium Chloride ^c | 0.01 | Same |
| Edetate Disodium ^d | (b) (4) | Same |
| Povidone (PVP) | | None |
| Sodium Chloride | | (b) (4) |
| Dibasic Sodium Phosphate ^e | | Same |
| Hydrochloric Acid and/or Sodium Hydroxide | | Adjust pH to 3.7 |
| Purified Water | | Same |

^aFormulation identification number.

^b0.665% w/v olopatadine hydrochloride (665 mcg/spray) is equivalent to 0.6% w/v olopatadine as base (600 mcg/spray).

^cAn equivalent amount of benzalkonium chloride solution may be used in the manufacture of the drug product.

^dEdetate disodium, (b) (4) is used.

(b) (4)

Route of administration: Intranasal

Intended Dosage: 2 sprays (100 µL/spray, 0.6% olopatadine)/nostril BID = 4.8 mg olopatadine/day

Consultation requested:

This chemistry consult was requested by the review chemist, Craig M. Bertha, PhD to evaluate the applicant's response to comment 37 (see below) of the October 27, 2005, NA letter that resulted from the original NDA 21-861 consult review (Gary Bond, PhD) dated June 15, 2005. Dr. Bertha indicated that the proposed acceptance criteria for (b) (4) (b) (4) and (b) (4) are (b) (4) respectively.

Issue 37:

Tighten the acceptance criteria for the (b) (4) and (b) (4) degradants in the drug product to less than (<) 0.1% relative to the olopatadine, or conduct a carcinogenicity assay with the isolated impurities. This is based on the positive genotoxicity results of (b) (4) and (b) (4) (Mouse Lymphoma Assays and Syrian Hamster Embryo Assays).

Response:

Technical reports for the p53^{+/+} transgenic mouse carcinogenicity studies conducted with (b) (4) have been filed to IND 60,116 and will be included in the planned amendment to NDA 21-861. As the results of these studies demonstrate that both the (b) (4) and (b) degradation products of olopatadine are non-carcinogenic in the p53^{+/+} transgenic mouse model, despite demonstrated bioavailability, no changes in the acceptance criteria for these agents have been made at this time. However, the Sponsor acknowledges that a final agreement regarding the acceptance criteria for the (b) (4) degradation products may not be feasible until the pharmacology/toxicology review of NDA 21-861 is completed. (b) (4) has not been observed in PATANASE PVP-free product to date. Through 26 weeks, no degradation products have been observed at any of the storage conditions or orientations (upright or horizontal) for the PATANASE PVP-free lots. This represents an improvement over the original NDA formula for which greater degradation was observed in the horizontal versus upright orientation.

Due to the limited stability data for the PATANASE PVP-free lots, the specifications for degradation products are being established based upon the stability data for the upright orientation of the original NDA formulation.

Review and Evaluation**2.6.6.5 Carcinogenicity**

Study title: 26-Week Repeated Subcutaneous Dose Carcinogenicity Study In p53^{+/+} Mice with A Toxicokinetic Study in C57BL/6 Mice with (b) (4)

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:
The study protocol was not evaluated by ECAC prior to the study initiation.

The dose selection was based on the 28-day repeated dose toxicity study of (b) (4) in C57BL/6 mice at dose levels of 3, 10, 30 and 100 mg/kg/day. Mortality was observed at high dose. The high mid-dose, 30 mg/kg/day, was selected as the MTD for 26-week carcinogenicity study, which is considered acceptable.

The positive control, p-cresidine in corn oil, at dose of 400 mg/kg, produced expected neoplastic and hyperplastic lesions in the urinary bladder and degenerative lesions in the kidneys.

The genotoxic potential of (b) (4) was evaluated in several in vitro and in vivo assays including Ames bacterial mutation assay, mouse lymphoma assay (MLA), Syrian hamster embryo assay (SHE) and in vivo mouse micronucleus assay (inadequate dose tested). The positive results were observed in SHE and MLA assays.

Evaluation of tumor findings:

The incidence of sarcoma observed at injection site skin in the treated and vehicle control groups was significant higher than the sham control. Although the incidence in male mid-dose group was higher than that in male vehicle group, the increase in incidence was

not dose-dependent, not observed in females groups. There are no significant differences in the incidence rate of sarcoma of skin between treated and vehicle control groups. The sarcomas in this study appeared to be a vehicle-related, repeated trauma from subcutaneous injection and unlikely to be test article-related.

The test article, as a degradant of olopatadine which is intended to be intranasally administered to human, is not considered to be carcinogenic.

Study no.: AA68DK.7S8P.BTL (Alcon Document No. TDOC-0002519, version 3.0)

Volume #, and page #: Module 4, Vol. 18, Page 1 to Vol. 20, Page 1011

Conducting laboratory and location: (b) (4)

Date of study initiation: January 20, 2005

GLP compliance: GLP compliant

QA report: yes (X) no ()

Drug, lot #, and % purity: (b) (4), Lot No. 11421:007, purity: (b) (4) dry basis

CAC concurrence: Study protocol and dose selection were not submitted for ECAC evaluation prior to study initiation. The study report evaluation by ECAC will be added as an addendum to this review.

Methods

Doses: (b) (4) was administered at doses of 3, 10 and 30 mg/kg/day for 26 weeks.

Basis of dose selection (MTD, MFD, AUC etc.): MTD.

The dose selection was based on a 28-day repeated dose study titled "28-Day Repeated Dose Mechanistic Subcutaneous Toxicity Study in C57BL/6 Mice of (b) (4)" (Document No. TDOC-0002104). Male and female mice (n=12/sex/group) were administered subcutaneously with vehicle or (b) (4) in the vehicle at dose levels of 1, 3, 10, 30 and 100.0 mg/kg/day. Mortality was observed in high dose main animals (12/12 males, 6/12 females) and TK animals (9/16 males, 2/16 females). Clinical signs of toxicity observed in the high dose groups included lethargy, ruffled fur at the site of injection, coma, rapid and shallow breathing and hyperactivity. The decrease in body weight gain was observed in high dose males and females. The hyperplastic lesions and inflammation (involving the subcutis and ulcerative and pustular changes) at site of injection (SOI), and chronic inflammation in the non-SOI skin were observed in vehicle and test article-treated groups. The incidence and severity of the findings at SOI in vehicle and test article-treated groups were comparable, hence the findings were considered vehicle-related. The hyperplasia of non-glandular stomach in the high dose groups was considered test article-related. The high mid-dose of 30 mg/kg/day was selected as the MTD for 26-week carcinogenicity study, which was considered acceptable when the lethal dose was 100 mg/kg/day.

Study design is shown in the table below.

| Treatment Group Number | Treatment Group | Number of Animals | | | | | |
|------------------------|---|---------------------------------|--------|----------------------|--------|--------------------|--------|
| | | p53 ^{+/-} (Main Study) | | C57BL/6 (Main Study) | | C57BL/6 (TK Study) | |
| | | Male | Female | Male | Female | Male | Female |
| 1 | Sham Control | 25 | 25 | 25 | 25 | - | - |
| 2 | Vehicle Control | 25 | 25 | 25 | 25 | 12 | 12 |
| 3 | Positive Control, p-cresidine 400 mg/kg | 25 | 25 | - | - | - | - |
| 4 | Low Dose, 3 mg/kg/day | 25 | 25 | - | - | 26 | 26 |
| 5 | Mid-Dose, 10 mg/kg/day | 25 | 25 | - | - | 26 | 26 |
| 6 | High Dose, 30 mg/kg/day | 25 | 25 | 25 | 25 | 26 | 26 |
| Total mice | | 150 | 150 | 75 | 75 | 90 | 90 |

Species/strain: mouse/ C57BL/6TacfBR-[KO]p53(p53^{+/-}) (heterozygous knockout mouse) and C57BL/6NTacfBR (conventional inbred mouse). Both p53^{+/-} and C57BL/6 mice were obtained from (b) (4)

Number/sex/group (main study): n=25/sex/group

Route, formulation, volume: Vehicle (b) (4) Povidone (b) (4) Diabasic sodium phosphate, (b) (4) NaCl, 0.01% Benzalkonium chloride (b) (4) Disodium EDTA, pH (b) (4) and the test article in vehicle at 0.3, 1 and 3 mg/mL were administered subcutaneously at dose volume of 10 mL/kg. Sham control animals were not dosed with any materials. The positive control animals were administered by oral gavage at dose volume of 10 mL/kg with p-cresidine in corn oil at dose of 400 mg/kg.

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: n=12/sex for vehicle control group, n=26/sex/group for (b) (4) treated groups

Age: Both p53^{+/-} and C57BL/6 mice were 9-11 weeks at initiation of dosing. Body weight for p53^{+/-} mice ranged 20.2 g to 28.8 g in males and 15.9 g to 22.3 g in females. Body weight for C57BL/6 mice ranged 17.7 g to 25.6 g in males and 16.1 g to 21.3 g in females.

Animal housing: The animals were individually housed during the treatment of the study in polycarbonate cages containing (b) (4) Hardwood bedding. The

animals were provided Harlan TEDLAD Global Diet #2018C in meal form and *ad libitum* access to drinking water.

Restriction paradigm for dietary restriction studies: No.

Drug stability/homogeneity: The analysis of the benzalkonium chloride (BAC) content of the vehicle and the analysis of the test article, (b) (4) were performed by HPLC. The stability of (b) (4) in Olopatadine Hydrochloride Nasal Spray Vehicle was established at a concentration of 0.3 and 3 mg/mL when stored at 2-8 °C. The result showed (b) (4) in Olopatadine Hydrochloride Nasal Spray Vehicle was stable for 19 days when stored at 2-8 °C. The samples were taken from the top, middle and bottom of the first, last and four interim dose preparations for homogeneity analysis. The result indicated that all the formulations met the acceptance criterion of $\leq 10\%$ of the target concentration. Content of (b) (4) was verified by analysis of the samples taken from the middle of each formulation. The concentrations found for all formulations met the acceptance criterion of 80-120% of the test article target concentrations.

Dual controls employed: No

Interim sacrifices: No

Deviations from original study protocol: There were no significant deviations from the study protocol that had significant impact on the outcome of the study.

Observation times

Mortality: All animals were observed twice daily for moribundity and mortality.

Clinical signs: Main study animals were observed for clinical signs of toxicity immediately after the last animal was dosed once weekly. Detailed hands-on examinations were performed for main study animals from Day 1 and weekly thereafter, in addition to the cageside observations.

Body weights: All animal body weights were measured once weekly from Day 1 through Week 13 and biweekly thereafter, with a pre-fasting body weight taken on Day 182 and a terminal fasting body weight taken at the day of scheduled sacrifice (for main study animal only).

Food consumption: Food consumption of main study animals was recorded weekly through the duration of the study with the exception of the final week when food consumption was recorded on Day 182. No food consumption was recorded for TK animals.

Clinical pathology: Samples were taken from all survival main animals at the termination.

Histopathology: All main study animals that died during study were necropsied as soon as possible after being found. A scheduled necropsy was performed on all survived main study animals. The specified organs as listed in the Histopathology Table below were weighed at the scheduled necropsy. The tissues/organs from all necropsied main study animals and 5/sex positive control animals, as listed in the Histopathology Table below, were preserved in 10% neutral-buffered formalin unless specified otherwise. The urinary bladder and kidneys were collected from all necropsied positive control animals. All

collected tissues/organs from the necropsied animals were stained with hematoxylin and eosin and examined for histopathological effects.

Peer review: yes (), no (x)

Toxicokinetics: Blood samples from TK animals were taken at the following timepoints:

For vehicle group only, pre-dose on Day 1 and at pre-dose and 0.5 hour post dose on Days 91 and 181;

For other treated groups: pre-dose, and at 0.5, 3 and 6 hours post dose on Days 91 and 181.

Results

Mortality: The incidence of the mortality in main study p53^{+/-} animals and C57BL/6 animals is listed in the table below (excerpted from Tables 1A and 1B of Vol. 18, page 59-61).

TABLE 1A - SUMMARY OF MORTALITY (Main Study p53^{+/-} Animals)

| MALES | | | | | | |
|----------------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Group: | 1 | 2 | 3 | 4 | 5 | 6 |
| Possible Gavage Accident | - | - | 4/25 | - | - | - |
| Found Dead | 1/25 | 2/25 | 1/25 | 1/25 | - | 4/25 |
| Moribund Sacrifice | - | - | - | - | - | 1/25 |
| Day 185 Terminal Sacrifice | 24/25 | 23/25 | 20/25 | 24/25 | 25/25 | 20/25 |
| TOTAL | 25/25 | 25/25 | 25/25 | 25/25 | 25/25 | 25/25 |

| FEMALES | | | | | | |
|----------------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Group: | 1 | 2 | 3** | 4 | 5 | 6 |
| Found Dead | - | - | 5/25 | - | - | 1/25 |
| Moribund Sacrifice | - | 1/25 | 4/25 | - | 1/25 | 1/25 |
| Day 185 Terminal Sacrifice | 25/25 | 24/25 | 16/25 | 25/25 | 24/25 | 23/25 |
| TOTAL | 25/25 | 25/25 | 25/25 | 25/25 | 25/25 | 25/25 |

** The number of animals found dead or sacrificed in a moribund condition within this Group was significantly (p ≤ 0.05; Fisher's Exact test) increased when compared to the sham control group (Group 1) and to the vehicle control group (Group 2).

Note: Represents the number of animals affected / the number of animals started on test.

- = Not applicable.

Nominal Dose: Group 1 - Sham Control Group 2 - Vehicle Control
 Group 3 - 400 mg/kg Positive Control Group 4 - 3 mg/kg/day (b) (4)
 Group 5 - 10 mg/kg/day (b) (4) Group 6 - 30 mg/kg/day

TABLE 1B - SUMMARY OF MORTALITY (C57BL/6 Main and TK Study Animals)

| MALES | | | | | | |
|---------|---------------------|-------|-------|-------|-------|-------|
| Group: | | 1 | 2 | 4 | 5 | 6 |
| Day 1 | Scheduled Sacrifice | - | 3/3 | - | - | - |
| | Found Dead | - | - | - | - | 3/12 |
| | Moribund Sacrifice | - | - | - | - | - |
| Day 91 | Scheduled Sacrifice | - | 3/3 | 12/12 | 12/12 | 9/12 |
| | Accidentally Killed | - | - | 1/14 | - | - |
| | Found Dead | - | - | - | - | - |
| | Moribund Sacrifice | - | - | - | - | - |
| Day 181 | Scheduled Sacrifice | - | 6/6 | 13/14 | 14/14 | 14/14 |
| | Accidentally Killed | - | 1/25 | - | - | - |
| | Found Dead | - | 1/25 | - | - | - |
| | Moribund Sacrifice | - | - | - | - | - |
| Day 188 | Terminal Sacrifice | 25/25 | 23/25 | - | - | 25/25 |
| | TOTAL: | 25/25 | 37/37 | 26/26 | 26/26 | 51/51 |

There were no statistically significant differences found when number of animals that died early within Groups 2-6 were compared to Group 1 or when Groups 4-6 were compared to Group 2 (Fisher's Exact test).

Note: Represents the number of animals affected / the number of animals started on test within the study cohort (C57BL/6 Main Study or Day 1, Day 91 or End-of-Study TK).

- = Not applicable.

Nominal Dose: Group 1 - Sham Control Group 2 - Vehicle Control
 Group 3 - 400 mg/kg Positive Control Group 4 - 3 mg/kg/day (b) (4)
 Group 5 - 10 mg/kg/day (b) (4) Group 6 - 30 mg/kg/day

TABLE 1B - SUMMARY OF MORTALITY (C57BL/6 Main and TK Study Animals CONTINUED)

| FEMALES | | | | | | |
|---------|---------------------|-------|-------|-------|-------|-------|
| Group: | | 1 | 2 | 4 | 5 | 6 |
| Day 1 | Scheduled Sacrifice | - | 3/3 | - | - | - |
| | Found Dead | - | - | - | - | 1/12 |
| | Moribund Sacrifice | - | - | - | - | - |
| Day 91 | Scheduled Sacrifice | - | 3/3 | 12/12 | 12/12 | 11/12 |
| | Found Dead | - | - | - | 1/14 | - |
| | Moribund Sacrifice | - | - | - | 1/14 | - |
| Day 181 | Scheduled Sacrifice | - | 6/6 | 14/14 | 12/14 | 14/14 |
| | Found Dead | - | - | - | - | 1/25 |
| | Moribund Sacrifice | - | - | - | - | - |
| Day 186 | Terminal Sacrifice | 25/25 | 25/25 | - | - | 24/25 |
| | TOTAL: | 25/25 | 37/37 | 26/26 | 26/26 | 51/51 |

There were no statistically significant differences found when number of animals that died early within Groups 2-6 were compared to Group 1 or when Groups 4-6 were compared to Group 2 (Fisher's Exact test).

Note: Represents the number of animals affected / the number of animals started on test within the study cohort (C57BL/6 Main Study or Day 1, Day 91 or End-of-Study TK).

- = Not applicable.

Nominal Dose: Group 1 - Sham Control Group 2 - Vehicle Control
 Group 3 - 400 mg/kg Positive Control Group 4 - 3 mg/kg/day (b) (4)
 Group 5 - 10 mg/kg/day (b) (4) Group 6 - 30 mg/kg/day (b) (4)

As summarized in the table below, for p53^{+/+} mice, 5 of 25 positive control males and 9 of 25 positive females died early, in which 4 of 5 positive control male early deaths may be related to the gavage errors. For both p53^{+/+} and C57BL/6 mice, there were no significant differences in mortality (including found dead and moribund killed) in the test article-treated groups when compared to the vehicle control group or in the test article-treated groups when compared to the sham-control group. The cause of deaths is unclear. There were no related histopathologic findings reported.

| Mortality | Males | | | | | | Females | | | | | |
|--------------------|-------|------|-------|------|------|-------|---------|------|------|------|------|------|
| | Sham | Veh | 3 | 10 | 30 | PC | Sham | Veh | 3 | 10 | 30 | PC |
| p53 mice Total | 1/25 | 2/25 | 1/25 | 0/25 | 5/25 | #1/25 | 0/25 | 1/25 | 0/25 | 1/25 | 2/25 | 9/25 |
| Days 43-91 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Days 91-184 | 1 | 1 | 1 | 0 | 5 | 1 | 0 | 1 | 0 | 1 | 2 | 7 |
| C57BL/6 mice Total | 0/25 | 1/37 | *0/26 | 0/26 | 3/51 | NA | 0/25 | 0/37 | 0/26 | 2/26 | 2/51 | NA |
| Days 39-83 | 0 | 1 | 0 | 0 | 3 | NA | 0 | 0 | 0 | 2 | 2 | NA |

Sham—Sham control group; Veh---Vehicle control group; PC---positive control group *one male accidentally killed was not included; #4 males died of dosing accident are not included.

Clinical signs: For p53^{+/-} mice, alopecia (ventral surface in most sham control animals, dorsal surface in most vehicle control and treated animals, foreleg, body urogenital area and site of injection) was observed in 3/25 sham control males, 24/25 vehicle control males, and 24/25, 25/25 and 24/25 in the low, mid, and high dose test article treated males, respectively. Alopecia was also observed in 6/25 sham control females, and all females in the vehicle control, low, mid and high dose test article-treated groups. For C57BL/6 mice, alopecia was observed in 1/25, 24/25 and 20/25 of sham control, vehicle control and high dose test article-treated males, and in 1/25, 25/25, and 24/25 of sham control, vehicle control and high dose test article-treated females. Alopecia was considered to be related to the vehicle treatment.

For p53^{+/-} mice, mass was observed in vehicle control male (4/25), low (6/25), mid (8/25) and high dose (7/25) test article-treated males, and observed in vehicle control female (6/25), low (0/25), mid (5/25) and high (1/25) dose test article-treated females. The incidence of the mass was significantly higher in vehicle and test article-treated groups than sham-control groups but comparable between vehicle control and test article-treated groups, hence, was considered vehicle related. For C57BL/6 mice, masses were only observed in two high dose males. However, no mass was reported in the gross pathology evaluation.

In positive control p53^{+/-} mice, the clinical signs of toxicity in one or both sexes included thin appearance, alopecia, ruffled fur, hunched posture and labored breathing. A mass was observed in one female.

There were no other significant test article or vehicle related clinical observations.

Body weights:

The significant decrease in body weight (about 20% in males and females) was observed in the positive control group. The body weights on Day 183 are listed below. The body weight was decreased up to about 10%-12% in both strains male and female mice treated with vehicle and test article, when compared to the sham control. There were no significant changes in body weight in test article-treated groups when compared to the vehicle control group in both strains.

| Body Weight (BW) | Males | | | | | | Females | | | | | | |
|------------------------|------------------|------|------|------|------|------|---------|------|------|------|------|------|------|
| | Dose (mg/kg/day) | Sham | Veh | 3 | 10 | 30 | PC | Sham | Veh | 3 | 10 | 30 | PC |
| BW (g) in p53 mice | | 33.1 | 29.9 | 30.8 | 29.9 | 30.2 | 26.3 | 28.5 | 26.6 | 26.5 | 26.6 | 26.0 | 22.8 |
| BW (g) in C57BL/6 mice | | 34.1 | 30.7 | NA | NA | 30.9 | NA | 31.1 | 28.2 | NA | NA | 27.3 | NA |

Sham—Sham control group; Veh---Vehicle control group; PC---positive control group

Food consumption: There was a significant decrease (~25%) in total food consumptions of positive control mice. The decreases in total food consumption of vehicle control groups and test article-treated groups were less than 5 % in males from both of strains, less than 10 % in female p53^{+/-} mice, and less than 12% in female C57BL/6 mice, when compared to the sham control groups. Therefore, there was no significant test article-related effect on food consumption.