

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPROVAL PACKAGE FOR:**

**APPLICATION NUMBER**

**50-804 (formerly 21-675)**

**Pharmacology Review(s)**



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: 21-675  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: Sept 10, 2003  
DRUG NAME: Zylet  
INDICATION: Steroid responsive inflammation in the eye with the risk of superficial ocular infection.

SPONSOR: Bausch & Lomb, Tampa, FL 33637  
DOCUMENTS REVIEWED: Volume 1.13-1.17  
REVIEW DIVISION: Anti-inflammatory, Analgesic and Ophthalmic Drug Products, HFD-550.

PHARM/TOX REVIEWER: Asoke Mukherjee, Ph.D.  
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PROJECT MANAGER: Rapheal Rodriguez

Date of review submission to Division File System (DFS): Dec 19, 2003

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## ***EXECUTIVE SUMMARY***

### **1. Recommendations**

1.1 Recommendation on approvability: The sponsor provided evidence that up to 6 times higher than daily clinical doses on the basis of mg/kg into the rabbit eyes, no untoward events were noted in the eye. Chronic administration of Zylet may induce systemic corticosteroid-like toxicity, e.g., adrenal atrophy. Based on the preclinical data, the NDA is approvable for the clinical uses as indicated. However, package insert should provide adequate warning for the possibility of systemic glucocorticoid-like effect following chronic ophthalmic administration.

1.2 Recommendation for nonclinical studies: Nil

1.3 Recommendations on labeling:

Carcinogenicity and mutagenicity section of the label is acceptable as stated by the sponsor.

Impairment of fertility:

Treatment of male and female rats at 50 mg/kg/day and 25 mg/kg/day of loteprednol etabonate, respectively, (500 and 250 times the maximum daily clinical dose, respectively) prior to and during mating did not impair fertility in either gender. No impairment of fertility was noted in studies of subcutaneous tobramycin in rats and rabbits at 100 mg/kg/day (1666 times the maximum daily clinical dose).

Pregnancy:

Teratogenic effects: Pregnancy Category C:

Loteprednol etabonate was shown to be teratogenic when administered orally to rats and rabbits during organogenesis at 5 and 3 mg/kg/day, respectively (50 and 30 times maximum daily clinical dose in rats and rabbits, respectively). An oral dose of loteprednol in rats at 50 mg/kg/day (500 times maximum daily clinical dose) during late pregnancy through weaning period showed a decrease in the growth and survival of pups without dystocia. However, no adverse effect in the pups was observed at 5 mg/kg/day (50 times maximum daily clinical dose).

Parenteral doses of tobramycin did not show any harm to fetuses up to 100 mg/kg/day (1666 times the maximum daily clinical dose) in rats and rabbits.

There are no adequate and well controlled studies in pregnant women. Zylet should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing mothers: Same as proposed by the sponsor.

## 2. Summary of nonclinical findings

2.1 Brief overview of nonclinical findings: Repeated administration of loteprednol etabonate/tobramycin drop at 6 times /day in Dutch-belted rabbits did not show any ocular toxicity. A slight elevation of IOP was noted in a 14-day study. However, it was not evident in the chronic study in rabbits. Atrophy of adrenal glands was noted in the six month ocular toxicity study. The sponsor conducted an acute study on the comparison of loteprednol alone with loteprednol and tobramycin ophthalmic suspensions for ocular bioavailability. The presence of tobramycin did not affect the distribution of loteprednol into aqueous humor. However, concomitant administration of local anesthetics reduced the level of loteprednol in the aqueous humor. Data suggest that local anesthetics might reduce the penetrability of the drug into aqueous humor.

The sponsor did not conduct any new mutagenicity studies for the NDA since these data are available in the approved package insert for loteprednol. No mutagenicity data for tobramycin is provided in the NDA. The issue was discussed with the ophthalmic team leader. However, need for the data as a Phase IV commitment was not felt necessary due to wide marketing experience of the product as an antibiotic.

The reproductive safety data indicated teratogenic effect of the drug in rats and rabbits. The sponsor stated that loteprednol is susceptible to esterase hydrolysis. The hydrolyzed product (PJ-91) is devoid of glucocorticoid activity. However, reproductive safety data showed glucocorticoid-like toxicity. Considering the reproductive and ocular safety data, it is unlikely that the chronic doses of the drug will be free from systemic glucocorticoid-like effect as claimed by the sponsor. The six-month ophthalmic safety study did not show any cataract formation in the eye. In the absence of any positive control in the experiment, absence of cataractogenicity of loteprednol is not resolved on the basis of the preclinical study.

### 2.2 Pharmacologic activity:

The sponsor conducted endotoxin-induced ocular inflammation study in the New Zealand rabbit. Data showed that tobramycin did not affect the anti-inflammatory effect of loteprednol.

2.3 Nonclinical safety issues relevant to clinical use: Systemic glucocorticoid like effect may be observed following chronic administration of the ophthalmic suspension.

**PHARMACOLOGY/TOXICOLOGY REVIEW**

**3.1 INTRODUCTION AND DRUG HISTORY**

**NDA number:** 21-675

**Review number:** One

**Sequence number/date/type of submission:** 000, Sept 8, 2003, 4P

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

**Sponsor and/or agent:** Bausch & Lomb, Tampa, FL 33637

**Manufacturer for drug substance:** For loteprednol:

For tobramycin

**Reviewer name:** Asoke Mukherjee, Ph.D.

**Division name:** Anti-inflammatory, Analgesic and Ophthalmic Drug Products.

**HFD #:** 550

**Review completion date:** Nov 19, 2003

**Drug:**

Trade name: Zylet

Generic name: Loteprednol etabonate 0.5% and tobramycin 0.3% ophthalmic suspension

Code name: Nil

Chemical name: For loteprednol: Chloromethyl 17 $\alpha$ -[(ethoxycarbonyl)oxy]-11 $\beta$ -hydroxy-3-oxoandrosta-1,4-diene-17 $\beta$  carboxylate.

For tobramycin: O-3-Amino-3-deoxy-a-D-glucopyranosyl-(1 $\rightarrow$ 4)-O-[2,6-diamino-2,3,6-trideoxy-a-D-ribo-hexopyranosyl-1(1 $\rightarrow$ 6)]-2-deoxy-Lstreptamine.

CAS registry number: For loteprednol, 82034-46-6. For tobramycin, 32986-56-4

Molecular formula/molecular weight: For loteprednol, C<sub>24</sub>H<sub>31</sub>ClO<sub>7</sub>, MW 466.96. For tobramycin, C<sub>18</sub>H<sub>37</sub>N<sub>5</sub>O<sub>9</sub>. MW 467.52.

Structure:

Appears This Way  
On Original

**Relevant INDs/NDAs/DMFs:** NDA 20-583, NDA 20-803 and, DMF [redacted] (for loteprednol). NDA 50-541 ANDA 64-052, DMF [redacted], DMF [redacted] (for tobramycin).

**Drug class:** Corticosteroid and antibiotic in combination.

**Indication:** Steroid responsive inflammation in the eye with the risk of superficial ocular infection.

**Clinical formulation:** Quantitative formulation per ml of the suspension (page 114, vol 2.01).

Quantity (mg)	Component
5.00	Loteprednol etabonate, micronized
3	Tobramycin
	Benzalkonium chloride solution
	Edetate disodium dehydrate
	Glycerine
	Povidone
	Tyloxapol
Qs	
Adjust pH	Sulfuric acid,
Adjust pH	Sodium hydroxide,

**Route of administration:** Ophthalmic drops

**Proposed use:** Ocular inflammation associated with infection or risk of infection.

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

**Studies reviewed within this submission:**

1. The anti-inflammatory activity of loteprednol etabonate-tobramycin combination, page 089, vol 1.13.
2. Determination of test article levels in aqueous humor, cornea, and iris-ciliary body of male Dutch-belted rabbits following ocular administration of test article with and without concomitant administration of various medications. Pages 190 and 210, vol 1.13.
3. Fourteen-day ocular toxicity study in Dutch belted rabbits, page 1, vol 1.14.
4. Six-month ocular toxicity in Dutch belted rabbits. Page 1, vol 15.

**Studies not reviewed within this submission:** Nil

### 3.2 PHARMACOLOGY

**3.2.1 Brief summary:** Tobramycin did not affect the bioavailability of loteprednol in the cornea, aqueous humor and iris-ciliary body in rabbits. The anti-inflammatory activity of loteprednol was not affected by the presence of tobramycin.

#### 3.2.2 Primary pharmacodynamics:

Mechanism of action: The effect of the drug is mediated by the anti-inflammatory and anti-infective activities of the drugs in Zylet.

#### Drug activity related to proposed indication:

The sponsor submitted a study titled "The anti-inflammatory activity of loteprednol etabonate-tobramycin combination", study # PHA-39, page 089 vol 1.13.

The effect of loteprednol was compared to the combination in endotoxin induced intraocular inflammation in the rabbit eye. Male NZ white rabbits weighed 4 kg were used in the study. LPS (endotoxin) was dissolved in 45% hydroxypropyl cyclodextrin so that each ml contained 50 µg of LPS. Rabbits were injected with 40 µL of the solution into mid-vitreous of each eye. Animals were anesthetized with ketamine at 35 mg/kg before the injection.

Eyes were treated five times for 2 days with one drop (50 µL) of saline, LE or LET immediately after the LPS injections. The study design is shown in the table below.

Group	Treatment	# animal
1	Saline	5
2	Loteprednol (0.5%) (LE)	5
3	LET (0.5% loteprednol/0.3% tobramycin)	5

Forty eight hours after the injection, eyes were examined with slit lamp for conjunctiva, iris and fibrin in the anterior chamber of the eye. Inflammatory conditions were graded using a 0-4 scale. Aqueous humor samples were collected for cell counts and protein levels. The sponsor did not indicate that the vitreous samples were collected for cell counts and protein contents. However, data for these parameters were presented.

Data show that a statistically significant decrease in the iridal hyperemia and fibrin content in the anterior chamber in rabbits treated with LET compared to loteprednol alone. Aqueous humor protein content was lower in the loteprednol and LET treated rabbit eyes compared to the saline treated animals. The sponsor stated that due to a large variability, no conclusion could be made for the aqueous humor cell count.

It is concluded that presence of tobramycin did not reduce the anti-inflammatory activity of loteprednol.

The data are shown in the table below.

Mean score and data	LET	LE	Saline
Conjunctival inflammation	1.6*	1.9	2.1
Iridal hyperemia	1.1*	1.5*	1.9
Anterior chamber fibrin	0.9*	1.5*	2.5
Total score	3.5*	4.8*	6.4
Aqueous humor cell (x 1000/ml)	145*	109	71
Aqueous humor proteins, mg/ml	50.1	48.8*	61.9
Vitreous humor cell (x1000/ml)	453	336	
Vitreous humor proteins, mg/ml	163	127	

\* Statistically significant from saline control.

**3.2.3 Secondary pharmacodynamics:** No study report was submitted in the NDA.

**3.2.4 Safety pharmacology:** No new data were submitted in the NDA.

**3.2.5 Pharmacodynamic drug interactions:** See reports for the ocular bioavailability of loteprednol and tobramycin study and drug distribution study below.

### 3.3 PHARMACOKINETICS/TOXICOKINETICS

#### 3.3.1 Brief summary:

The sponsor submitted a report on the distribution of loteprednol in the presence of tobramycin in Dutch belted rabbit eyes. Data suggest that bioavailability of the drug in aqueous humor was not affected by tobramycin following ophthalmic doses. Also, penetration of loteprednol across the cornea was reduced in the presence of local anesthetics applied topically into the eye.

**3.3.3 Absorption:** No new data or report was submitted.

#### 3.3.4 Distribution:

Determination of test article levels in aqueous humor, cornea, and iris-ciliary body of male Dutch belted rabbits following ocular administration of test article with and without concomitant administration of various medications.

Study # 0852LB16.002 and BLP-358-P002, pages 210 and 190, vol 1.13, June 15, 2000.

The sponsor determined the concentration of the drug substance in aqueous humor, cornea, and iris-ciliary body following ophthalmic drops of 0.5% loteprednol or the combination of loteprednol etabonate and tobramycin (LET) ophthalmic suspensions with or without the presence of other drugs. The sponsor did not mention the

concentration of each component in LET. However, 0.5% is mentioned which does not specify whether 0.5% of loteprednol or tobramycin was used in the combinations.

Twenty four male rabbits, 3-4 months of age were used in the experiment. The rabbit weighed 1.5-1.9 kg. Rabbits were divided into six groups of 4 animals. Concomitant medications were shown below.

1. Anesthetic-Alcaine (proparacaine 0.5%)
2. Anesthetic #2-4% Xylocaine (lidocaine)
3. Antibiotic-Ocuflex (ofloxacin 0.3%)
4. Dilator-Neosynephrine 2.5%
5. Dilator- Mydriacyl (tropicamide 0.5%)
6. IOP lowering agent Iopidine 0.5% (apraclonidine)
7. NSAID-Acular (ketorolac tromethamine 0.5%)

The sponsor stated that either loteprednol (LE 0.5%) or loteprednol and tobramycin (0.5/0.3%, LET) was applied twice into each eye with or without above drugs. Each application of loteprednol (0.5%) LE or LET was one drop. Page 190, vol 1.13 stated that the drop size for the experiment was about 32  $\mu$ L. Treatment with co-medication was given as one drop for all medications except xylocaine that was given in 35  $\mu$ L volume using a pipette. Four animals from each group was euthanized using barbiturate anesthesia, aqueous humor (AH), cornea and iris-ciliary body (ICB) were collected for the determination of loteprednol and its major metabolites (PJ-90 and PJ-91). However, data for LE and PJ-91 were provided only. The following table shows the experimental design.

Minute	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 6
0	Alcaine	Alcaine	Alcaine	LE	LET	LE
5	Neosynephrine	Neosynephrine		LE	LET	LE
10	Mydriacyl	Mydriacyl				
15	Iopidine	Iopidine				
20	Ocuflox					Euthanasia
25	-					
30	LE	LE	LE			
35	LE	LE	LE	Euthanasia	Euthanasia	
40						
45						
50						
55	Acular	Acular	Lidocaine			
60	Lidocaine	Lidocaine	Euthanasia			
65	Euthanasia	Euthanasia				

The sponsor provided tables for the body weight and weight of tissue samples. However, no data for the loteprednol or tobramycin have been provided in the report.

Data for loteprednol and its metabolite (PJ-91) are shown in the table below. PJ-91 is formed by the hydrolysis of the ester at 17 position to form carboxylic acid derivative. The sponsor stated that PJ-91 does not have glucocorticoid activity.

Intraocular tissue (nmoles/g or ml)	Aq. Humor, LE	Cornea, LE	Cornea, PJ-91	ICB, LE	ICB, PJ-91
Gr 1, All medicines	0.0623	0.9466	0.1998	0.8399	0.0567
Gr 2, All medicines, no antibiotics	0.0727	0.8526	0.2404	0.1303	0.0263
Gr 3, Anesthetics only	0.0390	0.9518	0.1548	0.3485	0.0187
Gr 4, LE only	0.0638	0.7205	0.1442	0.2911	0.0167
Gr 5, LET only	0.0649	Not tested	Not tested	Not tested	Not tested
Gr 6, LET only	0.0592	0.03752	0.0683	0.3155	0.0269

Above data suggest that,

1. Loteprednol level in the anterior chamber was not affected by the presence of tobramycin.
2. Local anesthetics reduced the penetration of the drug into iris and aqueous humor.
3. Loteprednol levels in cornea were higher at 35 min post dose compared to 25 min postdose.
4. Loteprednol concentrations in cornea were not affected by medications.
5. Traces of PJ-91 were noted in the cornea indicating slower metabolism of loteprednol in the cornea.
6. Tobramycin did not affect the distribution of the drug in aqueous humor.

**3.3.5 Metabolism:** No new data were submitted.

**3.3.6 Excretion:** No new data were submitted.

**3.3.7 Pharmacokinetic drug interactions:** The study report discussed under distribution indicates that the bioavailability of loteprednol in the aqueous humor was reduced in the presence of ophthalmic doses of local anesthetics.

**3.3.10 Tables and figures to include comparative TK summary:** Nil

## 3.4 TOXICOLOGY

### 3.4.1 Overall toxicology summary

General toxicology: No general toxicity data were provided.

Genetic toxicology: The approved package insert for loteprednol etabonate stated that the drug substance was not mutagenic in several in vitro assays i.e., Ames test, mouse lymphoma assay in TK locus, chromosomal aberration in human lymphocytes. Loteprednol was not genotoxic in mouse micronucleus assay in vivo.

The sponsor submitted a study report on page 102, vol. 1.13 for the degraded product of loteprednol in Ames assay. Data showed no mutagenic effect in the Ames assay. The degradation was due to irradiation of the drug substance for sterility purpose.

However, approved package insert for tobramycin indicated that no mutagenicity study was conducted. Since it is an antibiotic and the drug has been used for eye infections for a long time, the ophthalmic medical team leader suggested that there is no need to conduct a battery of mutagenicity studies as a Phase IV commitment.

Carcinogenicity: The sponsor stated that long term carcinogenicity studies for loteprednol and tobramycin were not conducted. Loteprednol ophthalmic drop was approved under NDA 20-583 and 20-803. Tobramycin was approved under NDA 50-541 and ANDA 64-052.

Reproductive toxicology:

Loteprednol etabonate: The approved package insert of the product shows that male and female rats up to 50 and 25 mg/kg/day doses, respectively did not impair fertility. However, at 3 mg/kg oral dose, a segment II study in rabbits showed teratogenicity. The no effect dose was 0.5 mg/kg/day. Teratogenicity was also observed at 5 mg/kg/day/oral and above doses in rats. The no effect dose for teratogenicity was 0.5 mg/kg/day/oral in rats.

Segment III reproductive safety study was conducted in pregnant rats during fetal period to the end of lactation at 50 mg/kg/day/oral dose. Reduced growth and compromised survival of pups were observed. The sponsor stated that body weight gain of the dam was also reduced at 50 mg/kg without any adverse effect on the gestation and parturition. However, no adverse effect in the dam and off spring was noted at 5 mg/kg/day/oral dose when given during the late stage of pregnancy through the end of lactation in rats.

Tobramycin:

The approved package insert stated that tobramycin did not show impairment of fertility and harm to fetuses up to 100 mg/kg/day subcutaneous dose in rats.

Special toxicology: Nil

**3.4.2 Single-dose toxicity:** The sponsor did not provide single dose toxicity data for loteprednol and tobramycin ophthalmic suspensions.

**3.4.3 Repeat-dose toxicity**

**Study title:** 14-day ocular toxicity study in Dutch Belted rabbits

**Key study findings:** Ophthalmic drops of 0.5% Loteprednol and 0.3% tobramycin four times a day for 14 days did not show any ocular and systemic toxicity.

**Study no.:** 0437LP27.001

**Volume #1.14, and page #:** 1

**Conducting laboratory and location:** C

3

**Date of study initiation:** May 14, 1997

**GLP compliance:** Yes

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

**Drug, lot #, and % purity:** Lot # for loteprednol and tobramycin suspension, 0.5%/0.3%, 884001; Lot # for placebo 873581. The sponsor did not provide the purity of the drug substance in the report.

### Methods

Doses: The study design is shown in the table below.

Group	Treatment	Dose, Concentration	Drops/day	Male	female
1	placebo	0	4	10	10
2	LET (loteprednol/tobramycin)	0.5% loteprednol/0.3% tobramycin	4	10	10

One drop was instilled/dose into the right eye. The doses were given 4 hours interval.

Species/strain: Dutch belted, HAZ(DB) SPF

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

Route, formulation, volume, and infusion rate: Ophthalmic drop. The sponsor did not provide the drop size.

Satellite groups used for toxicokinetics or recovery: Nil

Age: 11-13 weeks old

Weight (nonrodents only): 1.2-1.6 kg

Unique study design or methodology (if any): Nil

### Observation times and results

Mortality: Mortality was checked twice daily.

No mortality was reported in the study.

Clinical signs: Clinical signs were recorded once prior to the first dose every day and prior to the terminal sacrifice.

No adverse clinical sign was reported in the study.

Body weights: Body weights were recorded on the day of randomization, days 1, 8 and 14.

There was no treatment related change in the body weight of rabbits.

Food consumption: Food consumption was recorded daily during the treatment.

Food consumption was not affected by the treatment.

Ophthalmoscopy: Ocular changes were observed daily and scored according to the Draize's scale shown below.

Tissue	Description	Score
1. Cornea	No ulcer or opacity	0
	Scattered or diffused opacity	1
	Translucent area	2
	Translucent area and no details of iris visible	3
	Opaque, iris invisible	4
2. Area of cornea involved	1/4 or less	1
	Between 1/4 and 1/2	2
	Between 1/2 and 3/4	3
	Between 3/4 and 1	4
3. Iris	Normal	0
	Markedly deepened congestion, still reacting to light	1
	No reaction to light	2
4. Conjunctiva redness	Vessels normal	0
	Above normal	1
	Diffuse, deeper crimson red	2
	Diffuse beefy red	3
5. Chemosis	No swelling	0
	Any swelling above normal	1
	Swelling and partial aversion of lids	2
	Swelling with lids half closed	3
	Swelling with lids completely closed	4
6. Discharge	No discharge	0
	Slight discharge without moistening eyelids and hair	1
	Discharge with moistening eyelids and hair	2
	Discharge with moistening eyelids and hair and considerable area around eyes	3

Intraocular pressure (IOP) was measured by a pneumatonometer on the day before treatment and on days 8 and 14. Each eye was anesthetized with drops of tetracaine hydrochloride and measurements were taken between first and second doses. Ophthalmological examination was conducted using an ophthalmoscope and a slit lamp before randomization, and on days 8 and 14 following dilatation of the pupil with 0.5% tropicamide.

Group 2 males showed slight incidences of macroscopical changes on days 7 and 14 with average Draize's score of 0.2 and 0.4, respectively. However, the score was 0 during the rest of the treatment period. Female rabbits did not show any macroscopical changes in the eye.

The IOP (mm of Hg) data are shown in the table below.

Group, sex	Predose		Day 8		Day 14	
	OD	OS	OD	OS	OD	OS
1, Male	27	28.1	27.7	27.6	27.7	28.6
2, Male	27.8	27.4	29.4*	27.2	29.8	27.5
1, Female	28.9	28.3	28.7	27.7	29.0	27.6
2, Female	29.8	28.1	29.4	28.3	31.2*	28.8

\* Statistically significant

Above data show that male and female rabbits had a slight increase in the IOP on day 14. However, clinical significance of the small change is not known. Ophthalmoscopic and slit lamp examinations did not show any treatment related lesion.

EKG: Not recorded

Hematology: Blood samples were collected from the central ear artery before the initiation of treatment and before terminal sacrifice on day 15 for hematology and coagulation parameters. Animals were fasted overnight before blood collection.

There were no treatment related changes in the hematological parameters except a small decrease in the platelet counts ( $10^3/\text{mm}^3$ ) in group 2 male rabbits (550 in the control, 466 in the treated rabbits). Female rabbits did not show clinically important changes in the hematological parameters. The coagulation parameter showed a slight decrease in the APTT from 11.4 sec in the control to 9.5 sec in group 2 male rabbits at terminal sacrifice. Female rabbits did not show any treatment related changes.

Clinical chemistry: Standard parameters of blood chemistry were assayed for the blood samples collected for hematology.

Clinical chemistry parameters did not show any treatment related changes.

Urinalysis: Not conducted

Gross pathology: All animals were euthanized on day 15 by barbiturate overdose. Gross examinations were conducted for body surface, orifice, cranial, thoracic and abdominal cavities. Organs indicated in the table below were preserved in 10% formalin. Eye tissues were preserved in Davidson’s solution.

Male and female rabbits did not show any treatment related gross lesions.

Organ weights (specify organs weighed if not in histopathology table): The following organs were weighed at necropsy.

Liver, adrenal glands, brain, kidneys, ovaries, testes and eyes.

Organ weight data did not show any treatment related change.

Histopathology:

Eyes and gross lesions were evaluated after staining the paraffin embedded tissues with hematoxylin and eosin.

Histopathology data did not show any treatment related changes in the treated eye except focal mononuclear cells in the cornea in one treated male rabbit. It could be incidental in nature. Focal mononuclear cell infiltration in the cornea was also noted in the placebo and untreated eye of female rabbits. Female rabbits did not show any treatment related changes.

Adequate Battery:    yes ( ), no ( x )—explain, only eye and macroscopic changes were examined.

Peer review:            yes ( ), no ( x )

Toxicokinetics: Nil

**Histopathology inventory (optional)**

Study	14-day Ophthalmic safety			
Species	Dutch belted Rabbit			
Adrenals	x			
Aorta	x			
Bone Marrow smear				
Bone (femur)	x			
Brain	x			
Cecum	x			

Cervix	x			
Colon	x			
Duodenum	x			
Epididymis	X			
Esophagus	x			
Eye	x			
Fallopian tube				
Gall bladder	x			
Gross lesions				
Harderian gland				
Heart	x			
Ileum	x			
Injection site				
Jejunum	x			
Kidneys	x			
Lachrymal gland	x			
Larynx				
Liver	x			
Lungs	x			
Lymph nodes, cervical				
Lymph nodes mandibular				
Lymph nodes, mesenteric	x			
Mammary Gland	x			
Nasal cavity	x			
Nasolarymal duct				
Optic nerves				
Ovaries	x			
Pancreas	x			
Parathyroid				
Peripheral nerve	x			
Pharynx				
Pituitary	x			
Prostate	x			
Rectum	x			
Salivary gland	x			
Sciatic nerve	x			
Seminal vesicles	x			
Skeletal muscle	x			
Skin	x			
Spinal cord	x			
Spleen	x			
Sternum	x			
Stomach	x			
Testes	x			
Thymus	x			

Thyroid	x			
Tongue	x			
Trachea	x			
Urinary bladder	x			
Uterus	x			
Vagina	x			
Zymbal gland				

X indicate tissue samples were collected.

**Study title:** Six-month ocular toxicity in Dutch belted rabbits.

**Key study findings:** Mild redness, discharge in the conjunctiva, and hair loss were noted as side effects. Treatment with the combination drug showed sign of systemic glucocorticoid like activity associated with adrenal atrophy.

**Study no.:** 0460LP27.001

**Volume # 15, and page #:** 1

**Conducting laboratory and location:** C

J

**Date of study initiation:** Jun 11-12, 1997

**GLP compliance:** Yes

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

**Drug, lot #, and % purity:**

1. Loteprednol etabonate 0.5%, lot # 873472.
2. Loteprednol etabonate (0.5%) and Tobramycin (0.3%), lot # 884001.
3. Tobramycin 0.3%, lot # 863231.

The sponsor did not provide the purity of the drug substance.

**Methods**

Doses:

The study design is shown in the table below.

Group/Treatment	Concentration	Number of treatment/day	Dose, drop/dose	Number of rabbits	
				Male	Female
1. Placebo	0	6 times	1	10	10
2. Loteprednol (LE)	0.5%	6 times	1	10	10
3. Tobramycin (T)	0.3%	6 times	1	10	10
4. Loteprednol/Tobramycin (LET)	0.5%/0.3%	4 times	1	10	10

5. Loteprednol/Tobramycin (LET)	0.5%/0.3%	6 times	1	10	10
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Species/strain: Dutch, belted rabbit, HAZ(DB)SPF

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

Route, formulation, volume, and infusion rate: Ophthalmic drops into the right eye, left eye was untreated control.

Satellite groups used for toxicokinetics or recovery: Nil

Age: Age range at the initiation of experiment, 14-18 weeks for males and 13-17 weeks for females.

Weight: 1.3-2.0 kg for males and 1.0-2.0 kg for females.

Unique study design or methodology (if any): Nil

### Observation times and results

**Mortality:** Recorded twice daily throughout the study. The sponsor stated that no mortality was reported in the study. However, page 37, vol 15 showed one group 3 male was found dead on day 120.

**Clinical signs:** Once daily prior to the first dose of the day. Slight redness under the treated eye was noted in all drug treated animals. Hair loss was noted in groups 2, 4 and 5 male and female rabbits from month 3 onwards. It appeared that hair loss was not evident in the tobramycin treated male and female rabbits.

**Body weights:** Body weights were recorded before initiation of the study and once weekly for remainder of the study. No treatment related body weight change was noted at the end of treatment as shown in the table below.

Group	Day 1 (kg)		Day 182 (kg)	
	Male	Female	Male	Female
1	1.7	1.5	2.3	2.4
2	1.7	1.6	2.1	2.5
3	1.6	1.6	2.2	2.5
4	1.6	1.6	2.1	2.4
5	1.7	1.6	2.2	2.4

**Food consumption:** Beginning the treatment initiation food consumption was recorded daily. There was no treatment related change in the food consumption as shown in the table below.

Group	Day 2 (gm)		Day 182 (gm)	
	Male	Female	Male	Female
1	146	129	103	115
2	146	137	100	119

3	147	137	104	107
4	134	148	98	105
5	148	139	106	117

**Ophthalmoscopy:** One hour following the last dose, any changes in the eye were scored twice a week up to day 28 and once a week for rest of the study according to the Draize's scoring system. Eye examinations were also conducted by slit lamp, direct and indirect ophthalmoscope at pretest, and during weeks 2, 4, 8, 13, 20 and on day 180. Pupils were dilated with 0.5% tropicamide. Intraocular pressure was measured by pneumatonometer prior to the treatment, and during weeks 2, 4, 8, 13, 20 and 27.

Macroscopic eye examination showed Draize's score of 0.2 and higher in the treated animals. The control animals also showed an average score of 0.2 at the middle of the experimental period. Based on the data mild redness and discharge of conjunctiva were present in the treated eye with Zylet. The average score, the day when it was observed first and the score on the last day of treatment, is shown in the table below.

Group, sex	Day	Score	Group, sex	Day	Score
1, male	83	0.2	1, female	34	0.2
1, male	183	0.2	1, female	183	0
2, male	6	0.2	2, female	23	0.2
2, male	90	0.4	2, female	183	0.2
2, male	183	0	3, female	9	0.2
3, male	2	0.2	3, female	183	0
3, male	183	0.2	4, female	13	0.2
4, male	16	0.2	4, female	183	1.0
4, male	183	0.4	5, female	16	0.2
5, male	2	0.2	5, female	183	0.6
5, male	183	2.4			

#### Intraocular pressure:

The treatment did not show significant changes in the drug treated eye compared to the placebo or untreated eye. Intraocular pressure (mm of Hg) at predose, and on days 90 and 181 is shown in the table below.

Group, sex	Predose		Day 90		Day 181	
	OD	OS	OD	OS	OD	OS
1, male	28.85	27.65	26.45	25.80	24.15	24.45
2, male	28.10	27.80	25.35	26.35	24.60	24.45
3, male	28.50	27.50	26.85	25.50	23.90	23.80
4, male	29.0	28.70	27.60	27.10	25.30	25.25
5, male	28.60	28.25	26.05	25.95	26.60	26.35
1, female	25.80	25.95	27.15	26.45	25.25	24.80
2, female	27.45	26.75	26.10	26.35	24.40	26.90

3, female	28.30	27.80	27.90	27.05	26.40	27.50
4, female	30.05	29.05	28.45	28.45	27.80	28.35
5, female	27.90	27.10	27.55	27.40	25.70	27.65

Ophthalmoscopic examinations did not show consistent ocular changes attributed by the treatment. The ophthalmologist's report showed changes were incidental in nature.

EKG: Not recorded

Hematology: Blood samples were collected prior to dosing, during weeks 13, 20 and prior to terminal sacrifice. Animals were fasted overnight prior to blood collection. Standard hematology and coagulation parameters were determined. Blood samples were collected from the central ear artery.

Male and female rabbits did not show any treatment related changes in the hematology parameters. Lower WBC counts were noted in group 5 male rabbits at terminal sacrifice and group 5 female rabbits in week 13. However, the data were within the normal range. Coagulation data were also unremarkable in male rabbits. A slight decrease in the prothrombin time (sec) was noted in female animals at terminal sacrifice. However, clinical significance of the finding is unknown. Data are shown in the table below.

Gr 1	Gr 2	Gr 3	Gr 4	Gr 5
7.5	7.0*	7.3	7.2*	7.1*

Clinical chemistry: Serum chemistry was performed from the blood samples collected for hematology.

No clinically significant change in the clinical chemistry parameters was noted. A slight decrease (statistically significant) in the potassium was noted in groups 3 and 5 male rabbits in week 13 and at terminal sacrifice. However, the change was minimal and considered to be insignificant from clinical perspective.

Female rabbits in group 5 showed significant increase in the cholesterol levels (32 mg/dl) from the control (17 mg/dl) at terminal sacrifice. Biological significance of the change is unknown. No other changes were clinically significant.

Urinalysis: Not conducted

Gross pathology: All surviving animals were sacrificed on day 183. External surface, orifice, cranial, thoracic and abdominal cavities and its contents were examined.

Gross changes are shown in the table below.

	Group 1		Group 2		Group 3		Group 4		Group 5	
	Male	Female								
Hair loss, right eyelid	0	2	2	6	2	0	4	9	4	5
Adrenal gland, flabby		0		0		0		0		1

Above data show that hair loss in the right eyelid was the only gross change observed in male and female rabbits.

Organ weights (specify organs weighed if not in histopathology table): Organ weights were recorded for following organs:

Adrenals, brain, eyes, kidneys, liver, ovaries and testes.

Absolute organ weight (g) data showed a decrease in the weight of adrenal in male and female animals as shown in the table below.

	Group 1		Group 2		Group 3		Group 4		Group 5	
	Male	Female								
Adrenal glands	0.36	0.26	0.10	0.12	0.34	0.23	0.15	0.18	0.10	0.12
% change from control			-73%	-54%	-6%	-12%	-64%	-31%	-73%	-54%

Histopathology: Adequate Battery: yes ( ), no (x)—explain, only eye tissues and macroscopic changes were characterized histopathologically.

Peer review: yes ( ), no (x)

Organs listed in the table below were preserved in 10% neutral buffered formalin. Testes were preserved in Bouin's fixative. The sponsor stated that histological examinations were performed on all eye tissues and gross lesions. Remaining tissues were preserved for the future analysis if required.

Some of the histological changes are shown in the table below.

Lesion	Group 1		Group 2		Group 3		Group 4		Group 5	
	M	F	M	F	M	F	M	F	M	F
Adrenals, pigmentation	5/10	7/9	7/10	10/10	5/10	4/10	8/10	7/10	9/10	10/10
Adrenals, congestion	1/10	0/9	2/7	0/10	0/10	0/10	2/9	0/10	2/10	2/10

The histology data did not show any adverse effects of the treatment with loteprednol 0.5%, loteprednol 0.5% and tobramycin 0.3% combination when instilled up to 6 times a

day for 182 days. The histopathology report on page 15 vol 17 stated that atrophy of zona fasciculate of adrenals was noted in male and female rabbits in groups 2, 4 and 5. However, the sponsor did not provide the data on the summary table on pages 21 and 23 of the volume 17.

Based on the histopathology report, loteprednol 0.5%, and the combination of loteprednol 0.5% and tobramycin 0.3% (Zylet) showed adrenal atrophy that could be due to systemic glucocorticoid-like activity of loteprednol following ophthalmic dosing.

It is concluded that loteprednol 0.5% and tobramycin 0.3% combination did not show ocular toxicity except mild redness and discharge in the conjunctiva when applied one drop 4-6 times a day for six months. However, a reduction of the absolute weight and atrophy of adrenal glands were observed in Zylet treated group that could be due to the systemic glucocorticoid effect of loteprednol. Hair loss from the right eyelid and loss of hair from the body surface were also noted in Zylet treated animals. Loss of hair could be due to glucocorticoid activity of the drug also.

Toxicokinetics: No toxicokinetic data were provided in the report.

**Histopathology inventory (optional)**

Study	6-month ocular Tox			
Species	Dutch-belted rabbit			
Adrenals	x			
Aorta	x			
Bone Marrow smear				
Bone (femur)	x			
Brain	x			
Cecum	x			
Cervix	x			
Colon	x			
Duodenum	x			
Epididymis	x			
Esophagus	x			
Eye	x			
Fallopian tube				
Gall bladder	x			
Gross lesions				
Harderian gland				
Heart	x			
Ileum	x			
Injection site				
Jejunum	x			

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

X, tissue samples were collected.

**3.4.4. Genetic toxicology**

No new genotoxicity study report was submitted in the NDA.

**3.4.5. Carcinogenicity**

No carcinogenicity study was submitted in the NDA.

Toxicokinetics: No toxicokinetic data was submitted.

#### **3.4.6. Reproductive and developmental toxicology**

No reproductive safety study was submitted in the NDA.

#### **3.4.7 Local tolerance: Nil**

#### **3.4.8 Special toxicology studies: Nil**

### **3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS**

Conclusions: Zylet, a combination of loteprednol (0.5%) and tobramycin (0.3%), has been developed for treatment of inflammatory conditions of eyes where corticosteroid is indicated and a risk of bacterial infections exists. Both products are available individually for the ophthalmic treatment. Two ocular safety studies in rabbits were conducted with 14 days and 6 months duration. One drop of Zylet given six times a day did not show remarkable ocular toxicity in rabbits in both studies. The maximum dose for an average 2.3 kg rabbit would be 0.652 mg/kg/day of loteprednol and 0.391 mg/kg/day of tobramycin considering 50  $\mu$ L drop size. Although slight increase in the IOP was noted in loteprednol alone or in combination with tobramycin in the treated rabbit eye in the 14-day study, the change was clinically insignificant. There was no elevation of IOP in the rabbit eye following six months of treatment.

Considering 60 kg body weight of a patient and 50  $\mu$ L drop size, maximum human dose for loteprednol and tobramycin would be 0.1 mg/kg/day and 0.06 mg/kg/day, respectively. The calculation was done using 24 drops/day as the maximum dose. The rabbit to human dose ratio (safety factor) was 6.5 times for each of loteprednol and tobramycin.

The sponsor stated that loteprednol hydrolyzed by tissue esterase and forms an inactive carboxylic acid derivative. Therefore, it is a topical steroid and its chance of IOP elevation is lesser than other glucocorticoids. The sponsor stated that the product is not expected to cause cataract (NDA page 108, vol 2.01). A definite conclusion of the claim could not be made on the basis of the preclinical data in the absence of a positive control. On the other hand, atrophy of adrenal glands was noted following six months of treatment with ophthalmic drops. Data provide evidence that the product may show systemic glucocorticoid like effect following chronic ocular administration.

Both the components are approved at concentrations similar to Zylet. The product is designated for pregnancy category C due to teratogenicity of loteprednol in rats and rabbits. No additional toxicity was reported when compared to the individual component in the preclinical studies.

The sponsor calculated the rabbit dose as 0.032 to 0.054 mg/kg/dose. The human dose was calculated to be 0.004-0.006 mg/kg/single dose (page 122 vol 2.01). The sponsor listed a large number of preclinical studies that were submitted in support of the approvals of loteprednol and tobramycin, respectively.

No carcinogenicity, mutagenicity, reproductive safety data other than that available in the approved products have been submitted in the NDA.

From preclinical point of view, the product is safe for an approval for its clinical uses as indicated.

Unresolved toxicology issues (if any): Nil

Recommendations: The product is approvable on the basis of preclinical ocular toxicity. However, systemic glucocorticoid like effect may be expected following chronic ocular administration of Zylet.

Suggested labeling: Carcinogenicity and mutagenicity section of the label is acceptable as stated by the sponsor.

Impairment of fertility:

Sponsor's proposed label:

[ ]

The reviewer's proposed label:

Oral treatment of male and female rats at 50 mg/kg/day and 25 mg/kg/day of loteprednol etabonate, respectively, (500 and 250 times the maximum daily clinical dose, respectively) prior to and during mating did not impair fertility in either gender. No impairment of fertility was noted in studies of subcutaneous tobramycin in rats and rabbits at 100 mg/kg/day (1700 times the maximum daily clinical dose).

Pregnancy:

Sponsor's proposed label:

[ ]

The reviewer's proposed label:

Teratogenic effects: Pregnancy Category C:

Loteprednol etabonate was shown to be teratogenic when administered orally to rats and rabbits during organogenesis at 5 and 3 mg/kg/day, respectively (50 and 30 times maximum daily clinical dose in rats and rabbits, respectively). An oral dose of loteprednol in rats at 50 mg/kg/day (500 times maximum daily clinical dose) during late pregnancy through weaning period showed a decrease in the growth and survival of pups without dystocia. However, no adverse effect in the pups was observed at 5 mg/kg/day (50 times maximum daily clinical dose).

Parenteral doses of tobramycin did not show any harm to fetuses up to 100 mg/kg/day (1700 times the maximum daily clinical dose) in rats and rabbits.

There are no adequate and well controlled studies in pregnant women. Zylet should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing mothers: Same as proposed by the sponsor.

Signatures (optional):

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

Supervisor Signature \_\_\_\_\_

Concurrence Yes \_\_\_ No \_\_\_

IS/IS/

**3.7. APPENDIX/ATTACHMENTS : NIL**

**C.C LIST:**

Orig. NDA 21-675  
HFD-550/Div File  
HFD-550/Reviewer/A. Mukherjee  
HFD-550/Team Leader/ J. Yang  
HFD-550/Chemist/ Su Tso  
HFD-550/MO/L.Lim  
C: NDA21675oct2703.doc

REVISED ON DEC 9, 2003. DEC 16, 2003, DEC 18, 2003.

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/s/

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12/19/03 01:28:53 PM  
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12/19/03 01:36:06 PM  
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