CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-862

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:	**21-862**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**3/2/2005**
DRUG NAME:	**NEVANAC**
INDICATION:	[redacted] .reatment of pain and inflammation associated with cataract surgery
SPONSOR:	**Alcon, Inc., Mail Code R7-18, 6201 South Freeway, Fort Worth, TX 76134-2099** **Tel: 817-551-4399; Fax: 817-551-4630**
DOCUMENTS REVIEWED:	**Module 4**
REVIEW DIVISION:	**Division of Anti-Infective and Ophthalmology Products (HFD-520)**
PHARM/TOX REVIEWER:	**Zhou Chen, MD, PhD**
PHARM/TOX SUPERVISOR:	**Robert Osterberg, PhD**
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PROJECT MANAGER:	**Mike Puglisi**

Date of review submission to Division File System (DFS): July 19, 2005

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This application is approvable from a nonclinical perspective with some modifications of labeling as revised in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

B. Recommendation for nonclinical studies

No recommendation is necessary.

C. Recommendations on labeling

Several modifications of labeling in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section (see Labeling Review) are recommended.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Nepafenac, an NSAID, is being developed for the — treatment of pain and inflammation associated with cataract surgery. Pharmacological studies showed that nepafenac rapidly penetrated the cornea and was converted to the active moiety amfenac by tissue hydrolases. The cyclooxygenase inhibitory activity of nepafenac was weaker than that of amfenac. Topical administration of nepafenac significantly inhibited trauma-induced prostaglandin production and leakage of the ocular vasculature.

AL-6515 was hydrolyzed to amfenac (AL-6295) in all species with all routes tested. Plasma exposures to amfenac were higher than those to AL-6515. The plasma half-life for amfenac amide and amfenac was short. The plasma half-lives of radioactivity were long, suggesting the existence of other uncharacterized metabolites. Following topical ocular administration of ^{14}C-AL-6515 to rabbits, radioactivity was absorbed into the eye with high concentrations of radioactivity in the conjunctiva and cornea. Radioactivity was also distributed to the posterior ocular tissues (retina and choroids). ^{14}C-AL-6515 or its radioactive drug equivalents did not bind to melanin pigmented tissues. Oral administration of ^{14}C-AL-6515 to pregnant rats resulted in placental transfer of radioactivity into the developing fetus. Radioactivity was also found in the milk of lactating rats. ^{14}C-AL-6515 bound moderately to plasma proteins of rat, monkey, and human *in vitro* (73% to 84%) in a concentration-independent manner over the concentration range of 10 to 1000 ng/ml. Incubation of ^{14}C-amfenac amide in precision-cut human liver slices produced 12 metabolites. The major metabolite was amfenac with the remaining metabolites being present in relatively low amounts. Drug-derived radioactivity was rapidly excreted after iv administration to rats. The major route of excretion was via urine. Biliary excretion was also an important elimination pathway.

Several acute and repeated-dose oral systemic toxicity studies were conducted in rats with the duration up to 6 months. In the 2-week study, Jejunal serositis and mesenteric lymphoid hyperplasia were noted in rats of 25 mg/kg/day group. The sponsor indicated that these changes were considered to be secondary to intra-abdominal trauma, possibly associated with gavage procedures. However, distinct gavage trauma was not observed grossly or microscopically in abdominal tissues, and a relationship between drug treatment and these findings could not be entirely ruled out. In the 3-month toxicity study in SD rats, histopathological examination showed renal papillary necrosis in two of ten females at 15 mg/kg only. GI and renal lesions were common findings in animals treated with high doses of NSAIDs. GI abnormalities including stomach or intestine distended with fluid or gas, abnormal mucoid contents in the stomach and small intestine, abnormal fluid, granular or gelatinous material in the abdominal cavity, abdominal adhesions, and perforated or eroded mucosa of the GI tract were noted in rats at $\geq$ 30 mg/kg doses in acute and reproductive studies, indicating that the GI tissues were the target organs of toxicity. TK evaluations showed that at 10 mg/kg/day dose (at which dose no GI toxicity was noted), systemic exposures to AL-6515 and AL-6295 were 500 and 1600 times human exposure under the proposed clinical dosage (see table below). Because of the great safety margin, GI toxicity is not a concern for this drug in this indication.

AUC (ng-hr/ml)	Rats (10 mg/kg)	Human (0.1%, tid x 4 days)	Animal/human
AL-6515	189±22	0.368±0.106	500
AL-6295	1550±106	0.976±0.284	1600
Cmax (ng/ml)			
AL-6515	49.5±21.9	0.310±0.104	160
AL-6295	388±99	0.422±0.121	900

In 6-month toxicity study in F344 rats, higher incidences of corneal mineralization (5 of 25 in males vs. 0 in control animals) and uterus hydrometra (5 of 25 in females vs. control's 1 of 25) were seen at 10 mg/kg/day. Similar changes were not seen in other studies including 6-month ocular toxicity in which 1.0% AL-6515 ophthalmic suspension was used. Corneal and uterus abnormalities were not listed in the common adverse events seen in clinical studies. In addition, the systemic exposure to AL-6515 and AL-6295 at 10 mg/kg/day was much higher than that in humans. These findings might not be toxicologically significant for an ophthalmic drug.

Several repeated dose ocular toxicity studies were conducted with duration up to 3 months in monkeys (concentrations up to 1.0%, qid) and NZW rabbits (concentrations up to 1.0%, qid), and 6 months in pigmented rabbits (concentrations up to 1.5%, tid). The drug was well tolerated. No drug-induced systemic and ocular toxicity was observed. In all studies, minimal to moderate conjunctival congestion and transient and sporadic incidences of minimal conjunctival discharge were seen in the eye treated with vehicle and drugs. Because of the similar incidences and severity between control and treated eyes, these changes were not considered as drug-related. In a rabbit study in which nepafenac ophthalmic suspension (up to 1.0%) was administered prior and subsequent to a corneal incision, no significant ocular and systemic toxicity as well as postoperative ocular complications were noted.

AL-6515 was nonmutagenic in the Ames test and mouse lymphoma TK assay. The drug was also negative in *in vivo* micronucleus assay. AL-6515 was positive for the induction of structural chromosome aberrations in CHO cells.

In a fertility and early embryonic development study conducted in SD rats. Male animals of the 15 mg/kg group showed lower sperm motility and sperm concentrations compared to the control males. Histological examination on the epididymis in the 15 mg/kg group showed slightly decreased spermatozoa

and slightly more intraluminal single necrotic cells in two of three animals tested. In females, there were no toxicologically significant differences in copulation and fertility indices between control and treated groups. However, a decrease in the number of viable fetuses and an increase in the early resorption and post-implantation loss were noted in animals at 10 and 15 mg/kg. Oral administration of AL-6515 in rats at 3.0 mg/kg showed no developmental toxicity in this study.

In the embryo-fetal development study in pregnant rats, a slight decrease in fetal body weight (3.3 ± 0.5 g vs. control's 3.5 ± 0.2 g) was seen in HD (30 mg/kg) group. One HD animal had 9 dead fetuses, 6 resorptions, and no viable fetuses. There were no treatment-related malformations. Regarding developmental variations, the incidences of unossified 5th and 6th sternebrae and 7th cervical ribs were significantly higher in the HD group than in the control group. Based on the study results, the dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

In the embryo-fetal development study in pregnant rabbits, abortion occurred in one MD (10 mg/kg) animal and one HD (30 mg/kg) animal. One HD animal had a premature delivery. HD animals showed an increase in post-implantation loss which was mainly due to an increase in early resorptions. There was a statistically significant increase in the number of litters with skeletal malformations and in the number of litters with total malformations in the 30 mg/kg/day group. Low incidences of malformations were seen in the MD and LD (3 mg/kg) groups and were not considered drug-related because they occurred at a low incidence, were dissimilar in nature, and were not statistically different from the controls. Based on the study results, the dose of 3 mg/kg was considered a NOEL for maternal toxicity and a dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

The dose of 10 mg/kg/day was the NOEL for both rat and rabbit segment 2 studies. The following table compares the plasma exposure to AL-6515 and AL-6295 between animals at 10 mg/kg/day and humans following multiple bilateral dosing of nepafenac ophthalmic suspension 0.1%.

AUC (ng-hr/ml)	Rats (10 mg/kg)	Rabbits (10 mg/kg)	Human (0.1%, tid x 4 days)	Rat/human	Rabbit/human
AL-6515	97 0-207	28.4-62.5	0.368±0.106	260	77
AL-6295	2340-4190	663-3070	0.976±0.284	2400	680
Cmax (ng/ml)					
AL-6515	69 6-242	39 3-70.8	0 310±0.104	225	127
AL-6295	793-1710	666-2100	0 422±0.121	1900	1578

In the perinatal and postnatal study in rats, AL-6515 produced dystocia and associated maternal mortality in F0 females at levels ≥ 3 mg/kg/day, and developmental toxicity in F1 offspring at levels ≥ 10 mg/kg/day. The developmental toxicity was characterized by decreased F1 pup survival and decreased F1 pup body weights during lactation and growth phases. The NOEL for maternal effects in F0 females was not established in this study. The NOEL for developmental toxicity in F1 offspring was determined to be 3 mg/kg/day.

— a degradation product of AL-6515, was negative in the Ames test and *in vivo* mouse bone marrow micronucleus assay. In an *in vitro* mouse lymphoma TK assay, — was negative for inducing forward mutations under nonactivation conditions, but the test article was positive under activation conditions. In an ocular toxicity study conducted in NZW rabbits, AL-6515 ophthalmic suspension (0.1%) with 0.003% and 0.0075% — tid for one month) showed no local and systemic toxicity. The drug was well tolerated. The reviewer has discussed with the chemistry team for this degradation product. Considering the positive finding in one *in vitro* genotoxicity study, and the level of the degradation product

that was below the qualification threshold, the chemistry team will set a low acceptance criterion for this degradation product.

B. Pharmacologic activity

Nepafenac (amfenac amide), a nonsteroidal anti-inflammatory drug (NSAID), is being developed for the treatment of pain and inflammation associated with cataract surgery. As a prodrug, nepafenac rapidly penetrates the cornea and is converted to the active moiety amfenac by intraocular tissue hydrolases. Unlike the prodrug which has very weak cyclooxygenase inhibitory activity, amfenac exhibits potent cyclooxygenase inhibitory activity. Topical ocular dosing of the nepafenac to the rabbit eye inhibited both ocular inflammation and PGE_2 accumulation in ocular tissues. Amfenac sodium has been marketed in Japan since 1986 (as Fenazox) in an oral dosage form (50 mg qid) for the treatment of pain and inflammation associated with rheumatoid and osteoarthritis and low back pain, as well as for the treatment of pain and inflammation following surgery, injury, or tooth extraction.

C. Nonclinical safety issues relevant to clinical use

There are no drug-related safety issues relevant to clinical use.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

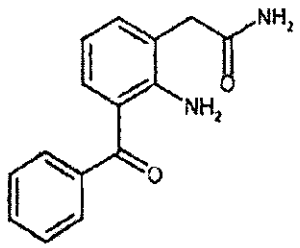
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 21-862
Review number: 000
Sequence number/date/type of submission: 000/February 25, 2005/Commercial
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Alcon Research, Ltd., Mail Code R7-18, 6201 South Freeway, Fort Worth, TX 76134-2099
Manufacturer for drug substance: —

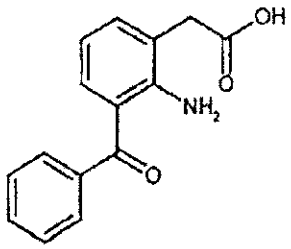
Reviewer name: Zhou Chen
Division name: Division of Anti-Infective and Ophthalmology Products
HFD #: 520
Review completion date: July 19, 2005

Drug:

Trade name: **Nevanac**
Generic name: Nepafenac (nepafenac ophthalmic suspension) 0.1%
Code name: AL-6515
Chemical name: 2-amino-3-benzoylbenzeneacetamide
CAS registry number: 78281-72-8
Molecular formula/molecular weight: $C_{15}H_{14}N_2O_2$, MW: 254.28
Structure:



Amfenac Amide, AL-6515



Amfenac

Relevant INDs/NDAs/DMFs: IND 49,924, DMFs —

Drug class: NSAID

Indication: — treatment of pain and inflammation associated with cataract surgery

Clinical formulation of nepafenac ophthalmic suspension, 0.1%:

Component	% w/v	Function	Compendial status
Nepafenac	0.1	Active ingredient	
Benzalkonium chloride	0.005		NF
Carbomer 974P			NF
Tyloxapol			USP
Edentate disodium		Preservative	USP
Mannitol			USP
Sodium chloride			USP
NaOH or HCl	qs for pH tc	pH adjustment	NF
Purified water	qs 100	Vehicle	USP

Route of administration: Topical ocular

Proposed use: One drop, three times daily (approximately 150 µg/person/day, 2.5 µg/kg/day for a 60 kg person) beginning 1 day prior to cataract surgery, on the day of surgery and through the first 2 weeks of the postoperative period

Studies reviewed within this submission:

Pharmacology:

Primary pharmacodynamics

017:39900:0694: AL-6515 (AHR-9434): Summary of preclinical pharmacology evaluation
008:39900:0495: Inhibition of prostaglandin E_2 synthesis by AL-6515 in the anterior and posterior portions of the eye
008:39900:0396: Preclinical evaluation of proposed clinical formulations of AL-6515
001:43:0100: Efficacy of nepafenac in a model of concanavalin A-mediated pan-retinal inflammation in rabbits
014:39900:0695: Corneal penetration profile of AL-6515
015:39200:1299: *In vitro* bioactivation and permeation of external ocular barriers
001:35:1002: *In vitro* corneal drug penetration of preserved and un-preserved nepafenac formulations

Secondary pharmacodynamics

TDOC-0001020: Effects of anecortave acetate (AL-3789) and amfenac (AL-6295) on BRMEC and HRMEC *in vitro* angiogenesis and retinal VEGF expression in a rat model of oxygen-induced retinopathy
TDOC-0001234: Effect of AL-6515 (nepafenac) on VEGF-induced *in vitro* angiogenesis: Report from Dr. —

TDOC-0001235: Effect of topical nepafenac (AL-6515) on preretinal neovascularization in the rat model of oxygen-induced retinopathy
097:43:1101: Effect of topical AL-6515 on preretinal neovascularization versus marketed topical NSAIDs in a rat model of oxygen-induced retinopathy
TDOC-0001237: Peroxide-induced choroidal neovascularization in adult rabbits: Final report from Dr. — uly 2001
TDOC-0001238: Effect of topical nepafenac (AL-6515) on early nonproliferative diabetic retinopathy in streptozotocin-treated adult rats: Report from —

TDOC-0000815: Effect of topical nepafenac (AL-6515) on VEGF-induced retinal vascular permeability in the adult rabbit

Safety pharmacology

009:39930:1294: *In vitro* receptor binding profile of the non-steroidal anti-inflammatory agent, AL-6515
006:39200:0196: AL-6515: Neuropharmacological profile in mice
017:39200:0196: Evaluation of proconvulsant potential in a submaximal electroshock assay
018:39200:1196: AL-6515: Effect on phenylquinone-induced writhing in mice
010:39200:0196: Acute hemodynamic effects of the subcutaneous administration of AL-6515 in the open-chest anesthetized dog
019:39200:1196: Effect on airway resistance and dynamic lung compliance in the guinea pig
021:39200:1196: Effect on barbiturate-induced sleep time in mice
007:39200:0196: Determination of electrolyte concentration and volume diuresis in rats
009:39200:0196: Gastrointestinal propulsion study in male mice
008:39200:0296: Ulcerogenic study in intact rats
002:39200:1196: Evaluation of antagonism to acetylcholine, histamine and barium chloride using the isolated guinea pig ileum
049:39500:0795: Effect of 50 μg and 500 μg of AL-6515 on corneal reflex in New Zealand albino rabbits following single topical ocular instillation

PK:

Absorption

001:38570:0198: Pharmacokinetics of amfenac and amfenac amide in male rats following 0.5 mg/kg intravenous and 3, 10 and 30 mg/kg oral doses
021:38570:1097: Pharmacokinetics of radioactivity in male Sprague-Dawley rats following administration of a single 0.5 mg/kg intravenous dose or a single 3 mg/kg oral dose of ^{14}C-AL-6515
017:38570:0598: Plasma pharmacokinetics of amfenac and amfenac amide in rabbits following intravenous and topical ocular dosing
018:38570:0797: Pharmacokinetics of radioactivity in male New Zealand white rabbits following administration of a single 1 mg/kg intravenous dose or 0.3% topical ocular dose of ^{14}C-AL-6515
TDOC-0001509: Pharmacokinetics of AL-6515 and amfenac following a 0.5 mg/kg intravenous dose of AL-6515 and pharmacokinetics and metabolism of ^{14}C-AL-6515 following a single 0.5 mg/kg intravenous dose to male cynomolgus monkeys

Distribution

022:38570:1097: Distribution of radioactivity in ocular tissues following a single topical ocular dose 0.3% ^{14}C-AL-6515 ophthalmic suspension to male New Zealand white and Dutch belted rabbits
012:38570:0299: Distribution of radioactivity in tissues of Sprague-Dawley rats following single and multiple oral doses of ^{14}C-AL-6515
014:38570:0299: Distribution of radioactivity in dams and fetal rats following a single oral dose of ^{14}C-AL-6515

049:38570:1298: Secretion of radioactivity in milk of lactating rats following a single oral dose of ^{14}C-AL-6515
TDOC-0002142: The *in vitro* protein binding of ^{14}C-AL-6515 in rat, monkey and human plasma

Metabolism

TDOC-0001077: Metabolism of ^{14}C-amfenac amide (nepafenac, AL-6515) *in vitro* by human precision cut liver slices.
TDOC-0001353: Chromatographic profiles of radioactivity in ocular tissues following a single bilateral topical ocular dose of 0.3% ^{14}C-amfenac amide (nepafenac, AL-6515) ophthalmic suspension to New Zealand white rabbits
TDOC-0001352: Chromatographic profiles of radioactivity in plasma and urine following a 0.5 mg/kg intravenous dose of 0.3% ^{14}C-amfenac amide (nepafenac, AL-6515) in Sprague Dawley rats
TDOC-0001076: Chromatographic profiles of radioactivity in plasma and urine following a 0.5 mg/kg intravenous dose of 0.3% ^{14}C-amfenac amide (nepafenac, AL-6515) in cynomolgus monkeys
TDOC-0002143: Evaluation of the inhibitory potential of AL-6515 towards metabolic activities of cDNA-expressed human cytochrome P450 isozymes

Excretion

011:38570:0299: Excretion and mass balance of radioactivity in male Sprague Dawley rats following a single intravenous dose of ^{14}C-AL-6515

Other pharmacokinetic studies

TDOC-0001327: Toxicokinetics of nepafenac and amfenac in toxicology study N-00-309: Six-month topical ocular irritation and systemic toxicity evaluation of nepafenac ophthalmic suspension in pigmented rabbits
TDOC-0001735: Toxicokinetics of nepafenac and amfenac in toxicology study N-03-090: Three-month topical ocular irritation and systemic toxicity evaluation of AL-6515 ophthalmic suspension in nonhuman primates
TDOC-0001557: Toxicokinetics of nepafenac (AL-6515) and amfenac (AL-6295) in toxicology study N-01-024: Six-month oral (gavage) toxicity study of in rats
TDOC-0001901: Retrospective toxicokinetic study of nepafenac and amfenac in pregnant rats following repeated oral doses of AL-6515 (N-03-161)
TDOC-0002069: Retrospective toxicokinetic study of nepafenac and amfenac in pregnant rabbits following repeated oral doses of AL-6515 (N-03-160)

Toxicology:

Single dose studies

129:38520:0995: Acute toxicity evaluation of AL-6515 in mice (up and down procedure)
130:38520:0995: Acute oral toxicity evaluation of AL-6515 in rats (up and down procedure)

Repeated dose studies

044:30:0501: Topical ocular irritation evaluation of nepafenac ophthalmic suspension used in conjunction with corneal incisions in New Zealand white rabbits
140:38520:1195: Three-month topical ocular irritation and systemic toxicity evaluation of AL-6515 ophthalmic suspension in rabbits (one-month interim)
032:38520:0196: Three-month topical ocular irritation and systemic toxicity evaluation of AL-6515 ophthalmic suspension in rabbits
TDOC-0001960: Six-month topical ocular irritation and systemic toxicity evaluation of nepafenac ophthalmic suspension in pigmented rabbits
TDOC 0001434: Three-month topical ocular irritation and systemic toxicity evaluation of nepafenac ophthalmic suspension in nonhuman primates
131:38520:0995: Two-week oral toxicity evaluation of AL-6515 in rats
025:38520:0196: Three month oral toxicity evaluation of AL-6515 in rats
TDOC-0001935: Six-month oral (gavage) toxicity study of AL-6515 in rats

Genetic toxicology

141:38520:1195: Mutagenicity test with AL-6515 in the *Salmonella—E. coli*/mammalian-microsome reverse mutation assay with AL-6515
007:38520:0298: *In vitro* mammalian cell gene mutation test with an independent repeat assay with AL-6515
008:38520:0298: *In vitro* mammalian cytogenetic test with an independent repeat assay with AL-6515
006:38520:0298: Micronucleus cytogenetic assay in mice with AL-6515

Reproductive and developmental toxicology

155:30:0801: A fertility and general reproduction study in rats with AL-6515
153:30:0801: A range finding development toxicity study in rats with AL-6515
156:30:0801: A development toxicity study in rats with AL-6515
154:30:0801: A range finding development toxicity study in rabbits with AL-6515
157:30:0801: A development toxicity study in rabbits with AL-6515
158:30:0801: A perinatal and postnatal study in rats with AL-6515

Special toxicology

TDOC-0001891: A skin sensitization study of AL-6515 (nepafenac) in guinea pigs using the maximization method
TDOC-0001457: Four-week topical ocular irritation and toxicity evaluation of nepafenac (AL-6515) ophthalmic suspension degradation product [redacted] in New Zealand white rabbits
TDOC-0001324: Bacterial reverse mutation assay with a confirmatory assay using [redacted], a degradation product of AL-6515 (nepafenac)
TDOC-0001325: L5178Y $TK^{+/-}$ mouse lymphoma forward mutation assay with a confirmatory assay using [redacted], a degradation product of AL-6515 (nepafenac)
TDOC-0001890: *In vivo* micronucleus assay in CD-1 mice using [redacted], a degradation product of AL-6515 (nepafenac)

Studies not reviewed within this submission:

Pharmacokinetics

TDOC-0001748: Validation of — method for the determination of nepafenac (AL-6515) and amfenac in rat plasma
TDOC-0001750: Validation of — method for the determination of nepafenac (AL-6515) and amfenac in rabbit plasma
TDOC-0001751: Validation of — method for the determination of nepafenac (AL-6515) and amfenac in cynomolgus monkey plasma

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

As a prodrug, AL-6515 was less potent than amfenac and diclofenac for the *in vitro* inhibition of cyclooxygenase activity. Topical dosing of AL-6515 at 0.1% to the eye of NZW rabbits inhibited both vascular permeability and PGE_2 accumulation induced by trauma with efficacy similar to diclofenac 0.1%, suggesting that AL-6515 is a prodrug requiring *in vivo* conversion to the free acid (AL-6295) for the inhibition of cyclooxygenase activity. The conversion was also supported by *in vitro* studies conducted in human and rabbit iris/ciliary body and retinal/choroids tissues. Pretreatment of AL-6515 to the rabbit eye inhibited PG synthesis in the iris/ciliary body and retina/choroid tissues. Concanavalin-A-induced pan-retinal inflammation was also inhibited by topical ocular administration of AL-6515 in NZW rabbits. *In vitro* corneal penetration test showed that AL-6515 was a more effective corneal penetrating agent than AL-6295.

One *in vitro* study showed that AL-6295 inhibited VEGF-induced cell proliferation and tube formation of BRMECs and HRMECs. However, similar study with AL-6515 did not achieve the same results. The sponsor indicated that this might be related to a lack of metabolism of nepafenac to its active metabolite, amfenac. In studies using rat OIR (oxygen-induced retinopathy) model or rabbit CNV (choroidal neovascularization) model, topical administration of nepafenac significantly inhibited pathological angiogenesis. In a study using diabetic rats, topical ocular treatment with AL-6515 significantly inhibited diabetes-induced retinal PGE_2 production, superoxide production, and leukostasis.

In safety pharmacology studies, AL-6515 showed no significant effects on general behavior and body temperature changes, peristalsis, phenylquinone-induced writhing, and electroshock-induced convulsions in mice, GI ulcer potential, urine volume, pH and urinary electrolyte changes in rats, airway resistance and dynamic lung compliance in guinea pigs, cardiac function in beagle dogs, and corneal reflex in NZW rabbits. In *in vitro* studies, AL-6515 did not demonstrate any interaction with 21 receptor and binding sites examined. The drug had no effects on acetylcholine, histamine, and barium chloride-induced contraction in the isolated guinea pig ileum,

2.6.2.2 Primary pharmacodynamics

017:39900:0694: AL-6515 (AHR-9434): Summary of preclinical pharmacology evaluation. Vol. 1
014:39900:0695: Corneal penetration profile of AL-6515. Vol. 1

Inhibition of cyclooxygenase activity *in vitro*

The effect of AL-6515, AL-6295 (amfenac) and diclofenac on inhibiting prostaglandin synthesis was evaluated in several *in vitro* studies. Test compounds were incubated with PGH synthase from sheep vesicular glands for 2 min before initiating the reaction by addition of ammonium arachidonate (10 mM). The IC_{50} was 64.3 μM for AL-6515, 0.25 μM for AL-6295, and 0.12 μM for diclofenac, respectively. As an NSAID, AL-6515 was about 257-fold less potent than amfenac and 536-fold less potent than diclofenac, respectively.

Trauma-induced breakdown of the blood-aqueous barrier

The effect of AL-6515 on preventing trauma-induced breakdown of the blood-aqueous barrier was measured in NZW rabbits. Following a single 45 min pretreatment (topical, 50 μl) period in NZW rabbits, ocular trauma was induced by removal of aqueous humor. Thirty min later the animals were terminated and aqueous humor was removed for protein analysis. AL-6515, AL-6295 and diclofenac showed a dose-dependent effect (see table below) on inhibiting paracentesis-induced vascular permeability (protein extravasation). Accumulation of PGE_2 in the aqueous humor was inhibited to a similar level (97-98%) by both AL-6515 and diclofenac. In another study, the onset and duration of action of AL-6515 (0.1%) were determined. AL-6515's inhibitory effect was seen at 15 min (the 1st observation time) and continued through 8 hr. Diclofenac inhibited vascular permeability response from 15 min through 4 hr postdose. The results indicated that AL-6515 at 0.1% inhibited both protein influx and PGE_2 accumulation induced by trauma with efficacy similar to that of diclofenac 0.1%.

Inhibition of paracentesis-induced break-down of the blood-aqueous barrier in NZW rabbits (%, mean±SD)

Concentration (%, w/v)	AL-6515	AL-6295	Diclofenac
0.0001	-11.1±16.9	14.9±22.5	3.0±30.3
0.001	9.3±21.7	40.4±20.6	40.3±20.0
0.01	38.2±29.1	58.8±26.8	45.0±20.8
0.1	52.2±26.0	63.8±26.8	49.5±24.3

Ex vivo inhibition of prostaglandin synthesis in rabbit iris/ciliary body (ICB) or retina/choroid homogenates

The effect of topical applied AL-6515 and AL-6295 on *ex vivo* PG synthesis by homogenates of rabbit ICB or retina/choroids was evaluated. AL-6515 was administered to both eyes of NZW rabbits at 50 μl volumes of 0.1% suspension. Sixty min post treatment ICB and retina/choroid tissues were collected and homogenized. Arachidonic acid was added to the homogenate and incubated for 10 min for PG synthesis. PG levels were determined by HPLC.

Results are summarized in the table below. AL-6515 inhibited both individual and total PG production in ICB with a pattern similar to that of AL-6295.

Topical ocular AL-6515 and AL-6295 on ICB PG synthesis in NZW rabbits (% inhibition)

PG	AL-6515		AL-6295A
	Test 1	Test 2	
6-keto-PGF1α	58 1	68 2	68 4
TXB2	65 5	90 8	71 1
PGF2α	93 6	100	86 6
PGE2	81 6	100	100
PGD2	100	100	100
HHT	64 4	60 6	64 3
Total PG	**73.0**	**78.7**	**77.6**

The time course of inhibition of PG synthesis was also investigated following the pretreatment of rabbit eyes for 2 to 80 min with 0.05% or 0.1% of AL-6515 or diclofenac, respectively. Results are summarized in the table below. The delayed inhibitory effects observed in AL-6515 groups might be due to the delay in the conversion to amfenac.

Topical ocular AL-6515 and diclofenac on ICB PG synthesis in NZW rabbits (% inhibition, mean ± SD)

		2 min	10 min	20 min	40 min	80 min
ICB						
AL-6515 (0.05%)	PGE_2	Not detected	40 0± 28 9	75 6±20 9	96 9±6 9	96 7±8 0
	Total PG	Not detected	25 1±16 6	68 1±10 4	89 4±11 8	90 6±5 0
Diclofenac (0 1%)	PGE_2	100	100	100	100	100
	Total PG	41 3±10 0	48 7±7 0	36 9±9 4	54 4±10 6	52 9±9 2
Retina/choroid						
AL-6515 (0 05%)	PGE_2		26 1±38 9	16 1±31 7	22 1±44 6	93 5±16 0
	Total PG		18 6±44 1	3 7±30 5	4 4±38 5	66 1±29 1

Corneal penetration

Freshly excised cornea from NZW rabbits were mounted in a perfusion chamber. The buffer of the donor side (facing the corneal epithelial cell layer) contained either AL-6515 (116.8 μM) or AL-6295 (613.0 μM). Samples were collected concurrently from both donor and receiver chambers at the beginning of the experiment and at 20-30 min intervals thereafter for 6 hr. Samples were examined by HPLC for AL-6515 and AL-6295.

Results (summarized in the table below) showed that the penetration rate of AL-6515 was 25-fold greater than that for its free arylacetic acid counterpart AL-6295. The lag time for the detection of AL-6515 on the endothelial side of the cornea was shorter than that for AL-6295, indicating that AL-6515 was a more effective corneal penetrating agent than AL-6295.

***In vitro* permeability of AL-6515 and AL-6295 with corneal preparations of rabbits**

Compound	Concentration (μM)	Permeability coefficient (cm/sec)	Lag time (min)
AL-6515	116 8 (maximum solubility)	74×10^{-6}	12
AL-6295	613 0	3×10^{-6}	115
Diclofenac (historical)	920 0	$12 1 \times 10^{-6}$	77

008:39900:0495: Inhibition of prostaglandin E_2 synthesis by AL-6515 in the anterior and posterior portions of the eye. Vol. 1

This study was reviewed by Dr. Asoke Mukherjee in 1996. Minor modifications were made by the current reviewer.

AL-6515 solution was administered topically at 50 µl (0.1%) dose to both eyes of three rabbits. Vehicle was given to the control animals. At various time points following the pretreatment, ICB or retina/choroid tissues were excised and homogenized. The homogenates were incubated for 10 min with arachidonic acid for the production of PGE_2. The PGE_2 levels were determined by a RIA kit.

In vitro metabolism of AL-6515 to AL-6295 (amfenac) was investigated using retina/choroid tissues from NZW rabbits and human eye tissues obtained from a cadaver (10 hr post mortem). The conversion of AL-6515 to AL-6295 was determined by HPLC.

Results are summarized in the table below. For retina/choroid tissues, AL-6515 (0.05% and 0.1%) dose-dependently inhibited PGE_2 synthesis (38% and 50% inhibition, respectively) within 80 min. The onset of action was evident within 15 min. Diclofenac only showed marginal inhibition (< 33%). For ICB tissues, the maximum inhibition (83-93%) was reached within about an hr and sustained for almost 8 hr.

Inhibition of *ex vivo* ocular PG synthesis by AL-6515 (%)

Treatment	Concentration (%, w/v)	ICB	Choroid/retina
Vehicle			
AL-6515	0 1	93	50
	0.05	83	38
Diclofenac	0 1	83	27

The hydrolysis rate was evaluated at 140 µM AL-6515 in an *in vitro* study using retina/choroid tissues. Human retina/choroid tissues showed a lower rate (0.13 nmol/min/mg tissue) relative to the rabbit tissues (6.40 nmol/min/mg tissue).

008:39900:0396: Preclinical evaluation of proposed clinical formulations of AL-6515. Vol. 1

The effect of AL-6515 in different formulations on preventing trauma-induced breakdown of the blood-aqueous barrier was measured in NZW rabbits. Following a single 45 min pretreatment (ocular, topical, 50 µl) period in NZW rabbits (4/group), ocular trauma was induced by removal of aqueous humor. Thirty min later the animals were terminated and aqueous humor was collected for PGE_2 and protein analysis. Formulations summarized in the table below are similar to the clinical formulation.

Formulation #	**Clinical formulation**	**90378**	**90377**	**90998**
Component		% w/v		
Nepafenac	0 1	0 3	0 1	0 03
Benzalkonium chloride	0.005			
Carbomer 974P				
Tyloxapol				
Edentate disodium				
Mannitol				
Sodium chloride				
NaOH or HCl	qs for pH to	qs	qs	qs
Purified water	qs 100	qs	qs	qs

Results, summarized in the table below, indicated that AL-6515 at 0.03 to 0.3% inhibited both protein influx and PGE_2 accumulation induced by trauma with efficacy similar to that of diclofenac 0.1%.

Inhibition of paracentesis-induced break-down of the blood-aqueous barrier and PGE_2 synthesis in NZW rabbits (mean±SD)

Treatment	Concentration (%, w/v)	Aqueous protein (mg/ml)	% inhibition	PGE_2 (ng/ml)	% inhibition
Vehicle		39 8+4 4		1 46±1 68	
AL-6515 (90378)	0 3	16 3±5 1	59	0 03±0 02	98
AL-6515 (90377)	0 1	17 7+4 3	55	0 02±0 01	98
AL-6515 (90998)	0 03	15 4±5 0	61	0 03±0 01	98
Diclofenac (Voltaren)	0 1	17 5±6 6	56	0 03±0 01	98

001:43:0100: Efficacy of nepafenac in a model of concanavalin A-mediated pan-retinal inflammation in rabbits. Vol. 1

The purpose of this study was to determine the effect of AL-6515 in a preclinical model of pan-retinal inflammation. Retinal edema was induced by intravitreal injection of concanavalin A (ConA, 30 μg) in Dutch belted rabbits. AL-6515 was administered (by sc route, 10 mg/kg, qd or ocular topical, 0.1-1.0%, 50 μl, 5 times/day) on Days -1, 0, 1, 2, and 3 relative to ConA injection. Changes in retinal thickness were assessed by scanning laser ophthalmoscopy (SLO) image analysis. Blood-retina and blood-aqueous barrier breakdown was evaluated by determining vitreous and aqueous protein concentrations. Vitreous PGE_2 levels were determined by direct enzyme immunoassay (EIA).

Topical ocular administration of AL-6515 suspension (0.1-1.0%) dose-dependently inhibited increases in retinal thickness and suppressed blood-retinal and blood-aqueous barrier breakdown. At the concentration of 0.5%, AL-6515 inhibited increases in retinal thickness by 65% and suppressed blood-retinal and blood-aqueous barrier breakdown by 62% and 78%, respectively. Vitreous PGE_2 synthesis was also inhibited by 96% with 0.5% AL-6515 suspension. Two marketed NSAID products, diclofenac 0.1% and ketorolac 0.5% did not show similar inhibition for blood-retinal barrier breakdown and vitreous PGE_2 synthesis. Subcutaneous injections of AL-6515 also inhibited increases in retinal thickness (88%) and suppressed blood-retinal (92%) and blood-aqueous barrier breakdown (51%). In conclusion, nepafenac showed superior pharmacodynamic properties in the posterior segment following topical ocular dosing.

015:39200:1299: *In vitro* bioactivation and permeation of external ocular barriers. Vol. 1

The purpose of this *in vitro* study was to determine the bioactivation (hydrolysis) of AL-6515 by ocular tissue components and its ability to permeate external ocular barriers. Hydrolysis was assessed in the corneal, ICB, and retina/choroid tissues from the rabbit or human cadaver eyes. Tissues were incubated with AL-6515 (8.9, 111, and 140 μM) for 4 to 6 hr and amfenac formation was determined by HPLC analysis. Drug permeation was evaluated using NZW rabbit corneal, scleral, and bulbar conjunctival tissues mounted in a modified perfusion chamber. AL-6515 (7 to 117 μM) or diclofenac (17 to 1200 μM) was added to the donor side of the chamber. Drug accumulation in the receiver side was determined over 360 min by HPLC analysis.

In vitro hydrolysis data are summarized in the table below. The hydrolytic activity in rabbit retina/choroid tissues was much higher than in ICB tissues. Minimal activity was seen in the corneal tissues. In human tissues, activity in the ICB was greater than in the corneal tissues.

***In vitro* hydrolysis of AL-6515 by ocular tissues (rate of amfenac formation, pM/min/mg wet weight, mean ± range)**

Ocular tissue	Concentrations (μM)	NZW rabbit	Human
Cornea	8 9	74	26±7
	111	131	107±47
	140	290±52	
ICB	8 9	154	39±4
	111	685	454±159
	140	480±94	
Retina/choroid	140	6400±1259	135±6

Regarding drug permeability, the corneal tissue showed a 6-fold greater permeation coefficient for AL-6515 (k_p = 727 x 10^{-6}/min) than diclofenac (k_p = 127 x 10^{-6}/min). Superior permeation in the conjunctival and scleral tissues was also evident when AL-6515 (k_p = 128 x 10^{-6}/min) was compared to diclofenac (k_p = 80 x 10^{-6}/min). Short time perfusion (5 min) of the corneal epithelial surface with 0.1% nepafenac also showed a sustain drug flux across the cornea for 6 hr. Under the same conditions, AL-6515 flux across the cornea resulted in an accumulation of 16.7 μM drug on the receiver side compared to 3.3 μM diclofenac.

001:35:1002: *In vitro* corneal drug penetration of preserved and un-preserved nepafenac formulations. Vol. 1

The purpose of this *in vitro* study was to compare four different formulations of AL-6515 suspension 0.1% for their corneal penetration ability. Different formulations are summarized in the table below.

Formulation #	**Clinical formulation**	**FID 90377**	**FID 103788**	**FID 104629**	**FID 104596**
Component	% w/v				
Nepafenac	0 1	0 1	0 1	0 1	0 1
Benzalkonium chloride	0.005				
Carbomer 974P					
Tyloxapol					
Edentate disodium					
Mannitol					
Sodium chloride					
NaOH or HCl	qs for pH tc				
Purified water	qs 100				

Drug permeation was evaluated using NZW rabbit corneal tissues mounted in a modified perfusion chamber. The corneal epithelium (the donor side of the chamber) was exposed to AL-6515 (0.1%) in different formulations for 5 min. Drug accumulation in the endothelium side (the receiver compartment) was determined by HPLC analysis.

Results, summarized in the table below, showed that lowering carbopol concentration (from 0.5% to 0.35%) reduced corneal drug permeation by 35%. Removal of benzalkonium and EDTA did not affect the drug permeation.

Comparison of the in vitro corneal penetration of 0.1% nepafenac with different formulations (nM/min, mean ± SD)

Formulation	FID 90377	FID 103788	FID 104629	FID 104596
Rate of corneal penetration	17 2±1.2	10 7±0.6	18.3±2 2	12.0±1.9

2.6.2.3 Secondary pharmacodynamics

TDOC-0001020: Effects of anecortave acetate (AL-3789) and amfenac (AL-6295) on BRMEC and HRMEC *in vitro* angiogenesis and retinal VEGF expression in a rat model of oxygen-induced retinopathy. Vol. 1

The purpose of this *in vitro* screening study was to evaluate the inhibitory effect of AL-6295 on VEGF-induced proliferation and tube formation in bovine retinal microvascular endothelial cells (BRMECs) and human retinal microvascular endothelial cells (HRMECs). The concentrations of AL-6295 used in cell cultures were 0.01, 0.1, 1, and 10 μM.

Results showed that AL-6295 dose-dependently inhibited VEGF-induced BRMEC proliferation and tube formation. Inhibition in the cell proliferation and tube formation of HRMECs was also seen. No detailed data were provided by the sponsor.

TDOC-0001234: Effect of AL-6515 (nepafenac) on VEGF-induced *in vitro* angiogenesis: Report from Dr. — Vol. 1

The purpose of this *in vitro* study was to determine the effects of nepafenac on VEGF-induced angiogenesis in bovine retinal endothelial cells. Cell proliferation and tube formation was induced by recombinant human VEGF165 (25 ng/ml). AL-6515 (nepafenac, 0.3-10 μM) was added at the same time as VEGF. Results showed that nepafenac at the concentrations up to 10 μM exhibited no inhibition in VEGF-induced bovine retinal endothelial cell migration and capillary tube formation. The sponsor indicated that the lack of efficacy might be related to a lack of metabolism of nepafenac to its active metabolite, amfenac.

TDOC-0001235: Effect of topical nepafenac (AL-6515) on preretinal neovascularization in the rat model of oxygen-induced retinopathy. Vol. 1

The purpose of this study was to evaluate the inhibitory effect of topical nepafenac on preretinal neovascularization in a rat OIR (oxygen-induced retinopathy) model. Newborn SD rat pups were placed into separate shoebox cages inside oxygen delivery chamber and subjected to the "Double 50" oxygen-exposure profile from postpartum Days 0 to 14. From postpartum Day 14, animals were placed into room air and were treated by bilateral qid topical ocular dosing with AL-6515 (0.1% or 0.5%) or vehicle. All animals were terminated on postpartum Day 20 and retinal samples were collected. Neovascularization scores were determined using computerized image analysis. Topical qid administration of 0.1% nepafenac significantly decreased preretinal neovascularization by 55% (neovascularization score = 1.98). At 0.5%, the median neovascularization score (2.49) was lower than that of control (3.4), but the decrease was not significant.

097:43:1101: Effect of topical AL-6515 on preretinal neovascularization versus marketed topical NSAIDs in a rat model of oxygen-induced retinopathy. Vol. 1

The purpose of this study was to compare the inhibitory effect of topical ocular delivery of 0.1% AL-6515, 0.5% ketorolac tromethamine, and 0.1 diclofenac (qid, both eyes, from post partum Days 14 to 20) on preretinal neovascularization in a rat OIR (oxygen-induced retinopathy) model. SD neonatal rat pups were placed into an oxygen incubator immediately after birth. At the end of postpartum Day 14, pups were placed into room air and were treated by bilateral qid topical ocular dosing with 0.1% AL-6515, 0.5% ketorolac tromethamine, 0.1 diclofenac, or AL-6515 vehicle (n = 6 or 7/group). All animals were terminated

on postpartum Day 20 and retinal samples were collected. Neovascularization scores were determined using computerized image analysis. Topical qid administration of 0.1% nepafenac significantly decreased preretinal neovascularization by 57%. The degree of preretinal neovascularization in ketorolac and diclofenac groups did not differ significantly from the vehicle-treated group. In conclusion, topical nepafenac inhibited posterior segment inflammation in rats, but ketorolac and diclofenac did not.

TDOC-0001237: Peroxide-induced choroidal neovascularization in adult rabbits: Final report from Dr. — July 2001. Vol. 1

The purpose of this study was to determine the effect of topical nepafenac on choroidal neovascularization (CNV) induced by subretinal injection of the lipid peroxide (LPO, 50 µg in 50 µl). NZW rabbits were treated (by topical ocular administration) with 0.5% AL-6515 five times per day beginning at 24 hr prior to subretinal injection and continued for 4 weeks. Animals were sacrificed after the 4-week treatment and histopathology examination on retina-RPE-choroid tissues was conducted.

Subretinal LPO injections induced CNV as observed through fluorescein angiography and histopathology. Topical 0.5% AL-6515 inhibited choroidal endothelial cell proliferation by 32% relative to the vehicle evidenced by the number of free vascular endothelial cells in an expanded space between the RPE and Bruch's membrane. The study result suggested that AL-6515 inhibit new vessel formation in the rabbit CNV model.

TDOC-0001238: Effect of topical nepafenac (AL-6515) on early nonproliferative diabetic retinopathy in streptozotocin-treated adult rats: Report from Dr. — Vol. 1

The purpose of this study was to evaluate the effects of AL-6515 on nonproliferative diabetic retinopathy in adult diabetic rats induced by streptozotocin. Animals were treated topically with 0.3% AL-6515 in one or both eyes qid for two months. After two months treatment, animals were sacrificed, retinal samples were collected, and several parameters were examined including PGE_2 accumulation and leukostasis (the number of leukocytes adherent to the wall of the retinal vasculature, and possibly occluding the vessel).

Diabetic rats had significant increases in production of retinal PGE_2 and superoxide, and in leukostasis within retinal vessels. Topical ocular treatment with AL-6515 significantly inhibited all three of these retinal abnormalities. AL-6515 showed no effects on diabetes-induced increases in nitric oxide production and VEGF expression in the retina. The drug showed no effects on glycemia, demonstrating that the effects were not due to reducing the severity of hyperglycemia.

TDOC-0000815: Effect of topical nepafenac (AL-6515) on VEGF-induced retinal vascular permeability in the adult rabbit. Vol. 1

The purpose of this study was to evaluate the potential ability of topical nepafenac to prevent VEGF-induced retinal vascular permeability in a rabbit model. Dutch belted rabbits (4/group) were treated topically with 0.3% AL-6515 qid for 6 days. On Day 4, animals were treated with $hrVEGF_{165}$ (2 µg/20 µl) by intravitreal injection. On Day 6, animals were injected (iv) with Evans blue dye and terminated. Retinal samples were collected and processed. Retinal vascular permeability was evaluated using a spectrophotometry method.

Results showed that VEGF induced a typical increased permeability pattern at 48 hr after intravitreal injection. In the AL-6515 treated group, VEGF-induced retinal vascular permeability was not suppressed. Topical ocular administration of 0.3% AL-6515 did not demonstrate efficacy.

2.6.2.4 Safety pharmacology

009:39930:1294: *In vitro* receptor binding profile of the non-steroidal anti-inflammatory agent, AL-6515. Vol. 1

Al-6515 (1 μM, 10 μM, and 100 μM) was tested for its ability to interact with 21 different receptors and binding sites to evaluate the drug's specificity and side effect potential. AL-6515 did not demonstrate any interaction with any of the receptor binding sites examined including various neurotransmitter receptors, opioids, peptides, growth factors, PGs, second messenger system, steroid receptors, and immunological factor receptors.

006:39200:0196: AL-6515: Neuropharmacological profile in mice. Vol. 1

The purpose of this study was to evaluate the potential neuropharmacological effects of AL-6515 in male CD-1 mice. Five groups of animals (10/group) were treated (by gavage) with AL-6515 at 0 (vehicle, 0.25% methylcellulose, 20 ml/kg), 0.1, 0.3, 1.0, or 3.0 mg/kg and were observed for apparent neuropharmacological or toxicological activity for 24 hr. Body temperature was measured at 60 min after dosing.

Results showed no apparent neuropharmacological signs, toxicity, and abnormal body temperature changes in any mice in any groups.

017:39200:0196: Evaluation of proconvulsant potential in a submaximal electroshock assay. Vol. 1

The purpose of this study was to evaluate potential convulsant activity of AL-6515 in mice. Male CD-1 mice (10/group) were treated with AL-6515 (3 mg/kg) or vehicle (0.25% methylcellulose, 20 ml/kg), respectively, by ip injection. One hr after dosing, animals received a submaximal electroshock (10 mA) administered transcorneally and were observed for tonic extension of the hind limbs for a one-min period post shock. The electroshock dose of 10 mA was chosen based on data from a preliminary study. AL-6515 (3 mg/kg, ip) neither potentiated nor inhibited the effect of the submaximal electroshock.

018:39200:1196: AL-6515: Effect on phenylquinone-induced writhing in mice. Vol. 1

The purpose of this study was to evaluate potential analgesic activity of AL-6515 in mice. Male CD-1 mice (10/group) were treated with AL-6515 (3 mg/kg) or vehicle (0.25% methylcellulose, 20 ml/kg), respectively, by ip injection. One hr after dosing, animals were challenged with an ip injection of 0.02% phenylquinone at 0.25 ml/kg and were observed for writhing for 10 min.

Intraperitoneal administration of AL-6515 at 3 mg/kg produced a 36% decrease in the mean number of writhes when compared to the vehicle control group (8.7 ± 2.5 vs. control's 13.6 ± 1.9). However, the effect was not statistically significant.

010:39200:0196: Acute hemodynamic effects of the subcutaneous administration of AL-6515 in the open-chest anesthetized dog. Vol. 1

The purpose of this study was to determine the potential acute effects of a subcutaneous dose of AL-6515 upon cardiac and circulatory functions in acutely prepared, open-chest anesthetized dogs. Four male beagle dogs were used in this study. Effects on the cardiovascular system, as assessed by changes in arterial pressure (systolic, diastolic, and mean), heart rate, left ventricular pressure, left ventricular end diastolic pressure, +dP/dt (at 40 mmHg intraventricular pressure), cardiac output, and lead II ECG were evaluated. Baseline values for each parameter were established over a 10-min pretreatment period. The dogs were then treated with AL-6515 at 1 mg/kg by sc injection. Cardiovascular parameters were evaluated at 5-min intervals for 60 min, and then at 15-min intervals for another hr.

Subcutaneous injection of AL-6515 (1 mg/kg) produced no biologically relevant changes in blood pressure, heart rate, cardiac output, left ventricular pressure, and ECG.

019:39200:1196: Effect on airway resistance and dynamic lung compliance in the guinea pig. Vol. 1

The purpose of this study was to determine the potential effects of the intravenous administration of AL-6515 upon pulmonary mechanics in guinea pigs. Male guinea pigs (4/group) were anesthetized. The trachea and esophagus were cannulated to facilitate pulmonary mechanic measurements. Baseline determinations of airway resistance and dynamic lung compliance, as calculated from intrapleural pressure, tidal volume, and airflow rate, were performed. Following AL-6515 (1 mg/kg, iv) or vehicle administration, pulmonary parameters were evaluated every min for the first 5 min, and every 5 min thereafter for a total of 30 min.

Intravenous administration of AL-6515 (1 mg/kg) produced no biologically relevant, statistically significant changes in airway resistance and dynamic lung compliance compared to vehicle control or to baseline measurements.

021:39200:1196: Effect on barbiturate-induced sleep time in mice. Vol. 1

The purpose of this study was to evaluate potential ability of AL-6515 to affect sleep time induced by barbiturate in mice. Male CD-1 mice (10/group) were treated with AL-6515 (3 mg/kg) or vehicle (0.25% methylcellulose, 20 ml/kg), respectively, by ip injection. One hr after dosing, animals were treated with an ip injection of sodium pentobarbital at 50 mg/kg and were observed for sleep time (the difference between the time of loss of the righting reflex and the time at which righting reflex was regained).

Intraperitoneal administration of AL-6515 at 3 mg/kg produced a 70% increase in the mean sleep time when compared to the vehicle control group (115.3 ± 12.4 vs. control's 68.0 ± 5.4). However, the sponsor indicated that the effect was not considered clinically meaningful.

007:39200:0196: Determination of electrolyte concentration and volume diuresis in rats. Vol. 1

The purpose of this study was to evaluate the potential effect of AL-6515 on electrolyte concentrations and volume diuresis in rats. Following 17 hr fast, male SD rats (10/group) were hydrated

with 0.9% saline (25 ml/kg, po) and then administered (by gavage) AL-6515 at 0 (vehicle, 0.25% methylcellulose, 10 ml/kg), 0.1, 0.3, 1.0, or 3.0 mg/kg, or hydrochlorothiazide (HCTZ) at 10 mg/kg. Urine was collected for 4 hr after dosing. Urine volume, pH and urinary electrolytes were analyzed.

Oral administration of AL-6515 at doses up to 3.0 mg/kg showed no statistically significant effects on urine volume, pH and Na^+, K^+, or Cl^- concentrations when compared to the vehicle control group. On the other hand, the reference compound, HCTZ (10 mg/kg, po), produced statistically significant increases in urine volume and Na^+, K^+, or Cl^- concentrations. pH was not affected.

009:39200:0196: Gastrointestinal propulsion study in male mice. Vol. 1

The purpose of this study was to evaluate the potential effect of AL-6515 on peristalsis in mice. Following 17 hr fast, Male CD-1 mice (10/group) were administered (by gavage) AL-6515 at 0 (vehicle, 0.25% methylcellulose, 20 ml/kg), 0.1, 0.3, 1.0, or 3.0 mg/kg, or atropine sulfate (reference standard) at 10 mg/kg. Thirty min after dosing, a 10% suspension of activated charcoal in 0.25% methylcellulose was given orally to the mice at 10 ml/kg. The animals were terminated 30 min after receiving charcoal and the intestines were removed. The length of the intestine (from pyloric sphincter to cecum) and the distance traveled by the charcoal as a fraction of that length were evaluated as mean motility ratio for each mouse.

Oral administration of AL-6515 at doses up to 3.0 mg/kg showed no statistically significant effects on gastric motility when compared to the vehicle control group. On the other hand, the reference compound, atropine sulfate (10 mg/kg, po), produced statistically significant inhibition (-30%) in gastric motility.

008:39200:0296: Ulcerogenic study in intact rats. Vol. 1

The purpose of this study was to evaluate the potential ulcerogenic effect of AL-6515 on the GI tract of the rat. Following 18-24 hr fast, male SD rats (10/group) were administered (by gavage) AL-6515 at 0 (vehicle, 0.25% methylcellulose, 10 ml/kg), 0.1, 0.3, 1.0, or 3.0 mg/kg, or indomethacin (reference standard) at 30 mg/kg. Four hr after dosing the animals were sacrificed and the stomach and intestines were examined for ulcerogenic effects.

Oral administration of AL-6515 at doses up to 3.0 mg/kg showed no statistically significant incidence of stomach ulceration when compared to the vehicle control group. On the other hand, the reference compound, indomethacin (30 mg/kg, po), produced 100% incidence of grade 1 level gastric ulceration. The mean percent ulcerogenicity for indomethacin (17%) was statistically significant when compared to the vehicle control group. No ulceration was seen in the intestine of the animals.

020:39200:1196: Evaluation of antagonism to acetylcholine, histamine and barium chloride using the isolated guinea pig ileum, Vol. 1

The purpose of this study was to evaluate the effect of AL-6515 on responses to acetylcholine (0.01 μg/ml), histamine (0.1 μg/ml), and barium chloride (0.1 mg/ml) in the isolated guinea pig ileum. Ileal tissue segments (4/group) were exposed to AL-6515 at concentrations of 0.25 and 2.5 μg/ml, or to DMSO at 0.3 ml/30 ml of bath volume for two min. Each agonist was then added to the tissue bath and contractile responses were evaluated and compared to the reference contraction values recorded prior to exposure to AL-6515.

Results, summarized in the table below, showed that AL-6515 at 0.25 or 2.5 μg/ml had no statistically significant effects on responses to acetylcholine, histamine, and barium chloride in the isolated guinea pig ileum.

Inhibitory effects of AL-6515 on the contraction induced by acetylcholine, histamine, and barium chloride in guinea pig ileum (% inhibition)

Treatment	Acetylcholine (0 01 μg/ml)	Histamine (0 1 μg/ml)	Barium chloride (0 1 mg/ml)
DMSO	2	2	0
AL-6515 (0 25 μg/ml)	2	0	0
AL-6515 (2 5 μg/ml)	7	7	0

049:39500:0795: Effect of 50 μg and 500 μg of AL-6515 on corneal reflex in New Zealand albino rabbits following single topical ocular instillation. Vol. 1

The purpose of these studies was to determine the effect of AL-6515 on corneal reflex (anesthesia) in New Zealand white (albino) rabbits following ocular topical instillation. Animals (3/group) were treated with AL-6515 (50 or 500 μg) and vehicle for AL-6515 (50 μl) topically to both eyes. Blink responses to corneal touch (3 touches per eye per time point) were measured at baseline and 0.25, 0.5, 0.75, 1, 1.5, and 2 hr. Number of blinks at each time point were recorded.

Results are summarized in the table below. In Study 15431, lowered blink responses were seen with AL-6515. The sponsor indicated that considerable tearing interfered with the blink reflex. In the other two studies (Studies 15442 and 15458), AL-6515 did not show any effects on the corneal reflex. In conclusion, topical ocular instillation of AL-6515 (0.1%) did not affect blink reflex in NZW rabbits.

Effect of AL-6515 on blink reflex in NZW rabbits

	Score time	0.25 hr	0 5 hr	0 75 hr	1 hr	1 5 hr	2 0 hr
Vehicle in all studies		18	18	18	18	18	18
Study 15431	AL-6515 (50 μg)	3	4	16	18	17	18
	AL-6515 (500 μg)	6	8	12	14	15	18
Study 15442	AL-6515 (50 μg)	18	18	18	18	18	18
Study 15458	AL-6515 (50 μg)	18	18	18	18	18	18

2.6.2.5 Pharmacodynamic drug interactions

No drug interaction studies were conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Please see Module 2, Vol. 4.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

AL-6515 was hydrolyzed to amfenac in all species with all routes tested. Plasma exposures to amfenac were higher than those to AL-6515.

Following oral administration in rats, the bioavailbility of AL-6515 was low. Following topical ocular administration in rabbits, the bioavailbility of AL-6515 was high. The plasma half-life for amfenac amide and amfenac was short in both studies. In studies with ^{14}C-AL-6515 in rats, rabbits, and monkeys, systemic absorption of radioactivity was high, and the plasma half-lives of radioactivity were long, suggesting other uncharacterized metabolites.

Following topical ocular administration of ^{14}C-AL-6515 to both NZW and Dutch belted rabbits, high concentrations of radioactivity were seen in the conjunctiva and cornea. Radioactivity was also distributed to the posterior ocular tissues (retina and choroid). The radioactivity concentrations of the retinal and choroid tissues in the untreated eye were very low. ^{14}C-AL-6515 or its radioactive drug equivalents did not bind to melanin pigmented tissues.

High radioactivity concentrations were seen in the GI, liver and kidney tissues after oral dosing in rats. The tissues with the lowest Cmax values were bone, eyes, testes, and brain. The long half-lives might be related to the formation of relatively small amounts of metabolites that were well distributed to tissue compartment.

Oral administration of ^{14}C-AL-6515 to pregnant rats resulted in distribution of radioactivity to maternal tissues and placental transfer of radioactivity into the developing fetus. Radioactivity was also found in the milk of lactating rats. ^{14}C-AL-6515 bound moderately to plasma proteins of rat, monkey, and human *in vitro* (73% to 84%) in a concentration-independent manner over the concentration range of 10 to 1000 ng/ml.

The metabolism profiles were similar in rats, monkeys, and humans. Amfenac was the major metabolite. There was a mixture of conjugated (glucuronide or sulfate) and non-conjugated metabolites. However, both amfenac amide and amfenac were not found to be conjugated. AL-6515 at plasma concentrations up to 1000 ng/ml did not inhibit cytochrome P450 metabolism.

Drug-derived radioactivity was rapidly excreted after iv administration to rats. The major route of excretion was via urine. A substantial fraction was excreted from feces, suggesting that biliary excretion was an important elimination pathway.

In TK evaluations in different animal species, systemic exposure to both AL-6515 and AL-6295 following ocular topical dosing was found in all dose levels and increased with doses. Exposure to AL-6295 was higher than that to AL-6515. No accumulation and gender-related differences were noted.

2.6.4.2 Methods of Analysis

See descriptions under individual study reviews.

2.6.4.3 Absorption

001:38570:0198: Pharmacokinetics of amfenac and amfenac amide in male rats following 0.5 mg/kg intravenous and 3, 10 and 30 mg/kg oral doses. Vol. 2

Report Nº: 001:38570:0198

Compound: Amfenac amide (AL-6515, Lot#: 6869:017)
Route: Intravenous or oral
Dose Level: 0.5 mg/kg for iv and 3, 10 or 30 mg/kg for po
Dosing Regimen: Single dose
Animal: Sprague-Dawley rats, 278.6 ± 15.6 g for iv group and 204.4 ± 21.7 g for po group
Testing Facility: Alcon Research, Ltd., 6201 S. Freeway, Fort Worth, TX 76134
GLP: No

The purpose of this study was to determine the pharmacokinetics of amfenac and amfenac amide following iv and oral doses of amfenac amide to male rats. Male SD rats (6/group) were treated with amfenac amide (AL-6515) at 0.5 mg/kg by intravenous injection or at 3, 10 and 30 mg/kg by oral administration. Plasma samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 hr after dosing. Plasma concentrations of amfenac amide and amfenac were determined using a , method with a quantitation limit of — ng/ml.

Results:

Results are summarized in the table below. Both amfenac and amfenac amide had short plasma half-lives. Bioavailbility after oral dosing was low.

Plasma PK parameters of amfenac and amfenac amide in rats treated with amfenac

	Oral						Intravenous	
Dose (mg/kg)	3		10		30		0 5	
	Amfenac							
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cmax* (ng/ml)	288	86	1350	546	6380	2490	206	38
Tmax (hr)	0 650	0 762	0 417	0 303	0 938	0 774	0 5	
T1/2 (hr)	0 871	0 344	0 73	0 14	1 67	0 67	0 546	0 066
$AUC_{1-4\ hr}$ (ng-hr/ml)	519	250	2000	985	17200	8970	267	78
$AUC_{0-\infty}$ (ng-hr/ml)	523	250	2010	982	17300	9080	269	79
	Amfenac amide							
Cmax* (ng/ml)	29 2	12 6	156	70	358	114	232	65
Tmax (hr)	0.217	0 075	0 375	0 306	0 362	0 314		
T1/2 (hr)			0 493	0 066	1 06	0 19	0 21	0 02
$AUC_{1-4\ hr}$ (ng-hr/ml)	46 1	10 3	155	64	792	362	84 9**	14 7
$AUC_{0-\infty}$ (ng-hr/ml)	85 9	4 9	156	64	797	365	87 5	15 0
%F	4 6	2 0	7 3	3 7	6 1	1 9		

*C0 for AL-6515 in iv group
**AUC was determined between 0.083 to 1 hr.

021:38570:1097: Pharmacokinetics of radioactivity in male Sprague-Dawley rats following administration of a single 0.5 mg/kg intravenous dose or a single 3 mg/kg oral dose of ^{14}C-AL-6515. Vol. 2

Report Nº: 021:38570:1097
Compound: ^{14}C-Amfenac amide (AL-6515, Lot#: CFQ9917, 63 mCi/mmol)
Route: Intravenous or oral
Dose Level: 0.5 mg/0.8 ml/kg (11.5 μCi) for iv and 3 mg/3 ml/kg (29.4 μCi) for po
Dosing Regimen: Single dose
Animal: Sprague-Dawley rats, 0.28 ± 0.005 kg
Testing Facility: Alcon Research, Ltd., 6201 S. Freeway, Fort Worth, TX 76134

GLP: No

The purpose of this study was to determine the plasma pharmacokinetics of total radioactivity following iv and oral doses of ^{14}C-AL-6515 to male rats. Male SD rats (4 for iv group and 5 for po group) were treated with ^{14}C-amfenac amide (AL-6515) at 0.5 mg/kg by intravenous injection or at 3 mg/kg by oral administration. Plasma samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 30, 48, 54, and 72 hr after dosing. Radioactivity was determined by liquid scintillation counting (LSC).

Results:

Results are summarized in the table below. The absorption of radioactivity was high (85.4%) following oral administration. The disappearance of radioactivity was biphasic for both iv and po routes. The radioactivity concentrations declined rapidly for the initial phase. The plasma half-lives of radioactivity in the terminal phase were similar for both iv (20.4 ± 1.3 hr) and po (26.7 ± 2.2 hr) routes.

Plasma PK parameters of radioactivity in rats treated with ^{14}C-AL-6515 (mean ± SD)

	Dose (μg)	Tmax (hr)	Cmax* (μg-eq/ml)	AUC (μg-eq-hr/ml)	T1/2 (hr)
Intravenous injection	134.1±6.0		0.453±0.033	1.01±0.08	20.4±1.3
Oral administration	839.3±21.2	2.5±1.5	0.486±0.125	5.35±0.94	26.7±2.2

*C0 for iv group

017:38570:0598: Plasma pharmacokinetics of amfenac and amfenac amide in rabbits following intravenous and topical ocular dosing. Vol. 2

Report Nº: 017:38570:0598
Compound: Amfenac amide ophthalmic suspension 0.3% (Lot#: 3132-01) for topical route and amfenac amide solution 0.4% (Lot#: 6869-014) for iv route
Route: Intravenous or ocular topical
Dose Level: 0.98 ± 0.01 mg/kg for iv and 30 μl (0.18 mg)/animal for ocular dosing
Dosing Regimen: Single dose
Animal: New Zealand white rabbits, 2.5 ± 0.07 kg for iv and 2.6 ± 0.08 kg for ocular route
Testing Facility: Alcon Research, Ltd., 6201 S. Freeway, Fort Worth, TX 76134
GLP: No

The purpose of this study was to determine the pharmacokinetics of amfenac and amfenac amide following iv and topical ocular doses of amfenac amide to male NZW rabbits. Plasma samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hr after dosing. Plasma concentrations of amfenac amide and amfenac were determined using a validated [redacted] method with a quantitation limit of [redacted] ng/ml.

Results:

Results are summarized in the table below. Systemic bioavailability of amfenac amide was high after topical ocular dosing. Amfenac amide was hydrolyzed to amfenac. In both routes, the Cmax and AUC levels for amfenac were much higher than those for amfenac amide. The elimination of both amfenac and amfenac amide was rapid.

Plasma PK parameters of amfenac and amfenac amide in rabbits treated with amfenac amide

	Amfenac		Amfenac amide	
	Topical ocular	intravenous	Topical ocular	intravenous
Cmax* (ng/ml)	45 5±20 5	1500±329	8 8±2 4	749±117
Tmax (hr)	0 6±0 2	0 5	0.15±0 09	
T1/2 (hr)	0 58±0 10	0 66±0 03	0.41±0 01	0 27±0 04
AUC $_{0\text{-}0.083\text{-}2\,hr}$ (ng-hr/ml)	63 9±26 0	1680±368	8.07±3 30	218±35
AUC $_{0\text{-}\infty}$ (ng-hr/ml)	83 4±27 9	2065±440	8.63±3 08	253±35
%F			101±41	

*C0 for AL-6515 in iv group

018:38570:0797: Pharmacokinetics of radioactivity in male New Zealand white rabbits following administration of a single 1 mg/kg intravenous dose or 0.3% topical ocular dose of ^{14}C-AL-6515. Vol. 2

Report N°: 018:38570:0797
Compound: ^{14}C-Amfenac amide (AL-6515, Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity =
Route: Intravenous or topical ocular
Dose Level: 1 mg/0.25 ml/kg (11.5 μCi) for iv and 30 μl of 0.3% ^{14}C-AL-6515 ophthalmic suspension (0.18 mg)
Dosing Regimen: Single dose
Animal: Male New Zealand white rabbits, 2.40 ± 0.12 kg for the initial iv group, 2.36 ± 0.11 kg for ocular dose group, and 2.16 ± 0.05 kg for the amended iv dose group
Testing Facility: Alcon Research, Ltd., 6201 S. Freeway, Fort Worth, TX 76134
GLP: No

The purpose of this study was to determine the plasma pharmacokinetics of total radioactivity following iv and topical ocular doses of ^{14}C-AL-6515 to male NZW rabbits. Animals (5/group) were treated with ^{14}C-amfenac amide (AL-6515) at 1 mg/kg by intravenous injection (12.7 μCi and 89.2 μCi for initial and amended iv groups, respectively) or at 30 μl bilateral topical ocular doses of 0.3% ^{14}C-AL-6515 (0.18 mg, 21.2 μCi). Plasma samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 hr after dosing for ocular and initial iv groups, and at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hr after dosing for the amended iv group. The amended iv group was added to this study to allow for a longer collection period after iv dosing. Radioactivity was determined by liquid scintillation counting.

Results:

Results are summarized in the table below. The absorption of radioactivity was relatively high (56%) following topical ocular administration. The disappearance of radioactivity was biphasic for both the iv and topical ocular routes. The radioactivity concentrations declined rapidly for the first 8 hr.

Plasma PK parameters of radioactivity in rabbits treated with ^{14}C-AL-6515 (mean ± SD)

	Dose (μg)	Tmax (hr)	Cmax *(μg-eq/ml)	AUC $_{0\text{-}8\,hr}$ (μg-eq-hr/ml)	AUC$_{0\text{-}\infty}$ (μg-eq-hr/ml)	T1/2 (β) (hr)	T1/2 (α) (hr)
Initial iv group	2376±136		2.25±0.43	3 29±0 58		Insufficient values available	
Amended iv group	2103±74		1 96±0 33		3 84±0 59	32.1±2.5	0.89±0 07
Topical ocular group	185±0	0 5±0	0 0697±0 0229	0 141±0.037		Insufficient values available	

* C0 for iv groups

TDOC-0001509: Pharmacokinetics of AL-6515 and amfenac following a 0.5 mg/kg intravenous dose of AL-6515 and pharmacokinetics and metabolism of ^{14}C-AL-6515 following a single 0.5 mg/kg intravenous dose to male cynomolgus monkeys. Vol. 2

Report N^{o}: TDOC-0001509
Compound: 0.2% AL-6515 solution (Lot #: 10147:031), 0.2% ^{14}C- AL-6515 solution (Lot#: 10147:040, 297.6 μCi/g, radiochemical purity =
Route: Intravenous
Dose Level: 0.5 mg/kg AL-6515 (Treatment 1) and 0.5 mg/kg ^{14}C-AL-6515 (Treatment 2)
Dosing Regimen: Single dose
Animal: Male cynomolgus monkeys, 7.17 ± 0.67 kg for Treatment 1, 7.11 ± 0.54 kg for Treatment 2
Testing Facility: Alcon Research, Ltd., 6201 S. Freeway, Fort Worth, TX 76134
GLP: No

The purpose of this study was to determine the plasma pharmacokinetics of AL-6515, AL-6295 and total radioactivity following a single 0.5 mg/kg iv dose of AL-6515 or ^{14}C-AL-6515 to male cynomolgus monkeys using a crossover design. Three animals were treated with AL-6515 and ^{14}C-amfenac amide (AL-6515) at 0.5 mg/kg by intravenous injection. There was a 12-day wash-out period between the two treatments. Blood and urine samples were collected as shown in the table below. Concentrations of amfenac amide and amfenac in plasma were determined using a validated method. Radioactivity concentrations were determined by liquid scintillation counting. The lower limits of quantitation (LOQ) were ng eq/g for amfenac amide, amfenac, and radioactivity, respectively.

Sample collection (hr after dosing)

Treatment	N	Blood	Urine
1	3	0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24	N/A
2	3	0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96	0-6 and 6-24 hr intervals

Results:

Results are summarized in the table below. Amfenac amide was cleared from systemic circulation with a half-life less than one hr. Amfenac concentrations reached Cmax levels within approximately 20 min following administration of amfenac amide. After reaching C_{max}, plasma amfenac concentrations declined in a biphasic manner with a log-linear distribution phase half-life of 63 ± 6 min and a terminal elimination half-life of 5.51 ± 0.22 hr. The $AUC_{0\text{-}inf}$ of amfenac was approximately twice that of amfenac amide. The high radioactivity exposure and radioactivity half-lives suggested other uncharacterized metabolites.

Plasma PK parameters of AL-6515, AL-6295, and radioactivity in monkeys treated with AL-6515 and ^{14}C-AL-6515

(mean ± SD)	AL-6515	AL-6295	Radioactivity
C0 or Cmax (ng/ml or ng-eq/ml))	587±183	489±140	989±123
Tmax (min)		20±8	15
T1/2$_z$ (hr)	0 856±0 188	5.51±0 22	52 8±7 7
AUC$_{0-\infty}$ (ng-hr/ml or ng-eq-hr/ml)	445±47	801±289	2906±728
T1/2 α (min)		63±6	
AUC$_{0-8\ hr}$ (ng-hr/ml)	444±47		

2.6.4.4 Distribution

022:38570:1097: Distribution of radioactivity in ocular tissues following a single topical ocular dose 0.3% ^{14}C-AL-6515 ophthalmic suspension to male New Zealand white and Dutch belted rabbits. Vol. 3

Report N^{o}: 022:38570:1097
Compound: 0.3% ^{14}C-AL-6515 ophthalmic suspension (Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity = —)
Route: Topical ocular, right eye only for NZW rabbits and both eyes for Dutch belted rabbits
Dosing Regimen: Single dose
Animal: New Zealand white rabbits (males, 2.2 ± 0.1 kg) and Dutch belted rabbits (1.8 ± 0.1 kg)
GLP: No

The purpose of this study was to determine the pharmacokinetics and distribution of radioactivity in NZW rabbits and Dutch belted rabbits following topical ocular doses of a 0.3% ^{14}C-AL-6515 ophthalmic suspension. For NZW rabbits, blood and ocular samples (aqueous humor, inferior bulbar conjunctiva, cornea, lens, ICB, vitreous humor, retina and choroid tissues from the right eye and aqueous humor, retina and choroids from the left eye) were collected from 4 animals per time point at 0.5, 1, 2, 4, 8, 10, 24, 48, and 72 hr after dosing. For Dutch belted rabbits, blood and ocular samples (aqueous, ICB, retina and choroids) were collected from both eyes of one animal per time point at 0, 0.5, 8, 24, and 72 hr after dosing. Radioactivity was determined by LSC.

Results:

Results are summarized in the table below. Radioactivity was absorbed into the eye following topical ocular administration in both NZW and Dutch belted rabbits. High concentrations of radioactivity were seen in the conjunctiva and cornea. The results also showed that radioactivity distributed to the posterior ocular tissues by ocular distribution of the drug. Plasma exposure to the radioactivity was low and was below the quantitation limit (— μg-eq/ml) at 8 hr after dosing. The radioactivity concentrations of the retinal and choroid tissues in the untreated eye were very low (Cmax was 0.0040 μg-eq/g for the retina and 0.0168 μg-eq/g for the choroid) and were only seen in the first one and 4 time points, respectively. The radioactive concentrations in the ICB (iris and ciliary body), retinal, and choroids tissues were similar, suggesting that ^{14}C-AL-6515 or its radioactive drug equivalents did not bind to melanin pigmented tissues.

PK parameters of radioactivity in ocular tissues and plasma of NZW rabbits and Dutch belted rabbits (mean ± SD)

Parameter	Aqueous humor	Conjunctiva	Cornea	ICB	Lens	Choroids	Retina	Vitreous humor	Plasma
NZW rabbits, right eyes									
Tmax (hr)	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5
Cmax (µg-eq/ml)	0.577±0 228	4 62±2 78	3 90±0 87	0 639±0 186	0 043±0 014	0 115±0 066	0 051±0 024	0 002±0 002	0 022±0 009
T1/2 (hr)	2 14	14 3	14	20	22 9	2 59	1 99	2 65	1 56
AUC_{0-inf} (µg-eq-hr/ml)	0 765	47 0	14 4	1 86	0 676	0 232	0 066	0 0053	0 0325
Dutch belted rabbits, both eyes									
Tmax (hr)	0 5			0 5		0 5	0 5		0 5
Cmax (µg-eq/ml)	0 632			1 18		0 0862	0 0168		0 0271
T1/2 (hr)	2.46			29 2		ND (not determined)			ND
AUC_{0-inf} (µg-eq-hr/ml)	2 73			6 54					ND

012:38570:0299: Distribution of radioactivity in tissues of Sprague-Dawley rats following single and multiple oral doses of ^{14}C-AL-6515. Vol. 3

Report Nº: 012:38570:0299
Compound: ^{14}C-AL-6515 (Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity =) and AL-6515 (Lot#: 6558:041, purity =
Route: Oral by gavage
Dose Level: 3 mg/kg
Dosing Regimen: Single dose and qd x 14 days
Animal: Sprague Dawley rats (0.268 ± 0.010 kg for single dose group and 0.310 ± 0.012 for multiple dose group)
Study Facility:

GLP: No

The purpose of this study was to determine the distribution of radioactivity in SD rats following single and multiple (qd x 14 days) 3 mg/kg oral doses of ^{14}C-AL-6515. Three animals per time-point were terminated and blood and tissue samples (see table below) were collected at 0.5, 2, 4, 12, 48, 72, 96, 120, and 168 hr after dosing. Radioactivity was determined by LSC.

Adrenal	Bone	Brain	Small intestine	Cecum/colon	Stomach
Heart	Lungs	Eyes	Pancreas	Liver skeletal muscle	Kidney
Spleen	Lymph nodes (mesenteric)	Thyroid	Sciatic nerve	Trachea	Skin
Fat	Testes	Salivary glands	Thymus	Blood	Urinary bladder

Results:

Results are summarized in the table below. High radioactivity concentrations were seen in the GI, liver and kidney tissues. The tissues with the lowest Cmax values were bone, eyes, testes, and brain. Radioactivity was rapidly absorbed with Tmax of 0.5 hr for most tissues after single or multiple dosing. Radioactivity accumulation was noted in most tissues. Elimination half-lives after multiple dosing were generally longer than after single dose. The long half-lives might be related to the formation of relatively small amounts of metabolites that were well distributed to tissue compartment.

Summary of PK data for radioactivity following a single or 14 daily oral doses of ^{14}C-AL-6515 (3 mg/kg) to SD rats

Tissue	Single Oral Dose			14 Daily Oral Doses		
	C_{max} (ug eq/g)	T_{max} (hr)	$t_{1/2}$ (hr)	C_{max} (ug eq/g)	T_{max} (hr)	$t_{1/2}$ (hr)
Adrenals	2.47	0.5	46.7	3.23	0.5	125
Blood	1.66	0.5	64.9	1.57	0.5	172
Bone (both femurs, including marrow)	0.172	0.5	48.0	0.561	0.5	173
Brain	0.551	0.5	ID	0.846	0.5	107
Cecum/colon (excluding contents)	1.84	4	157	4.17	0.5	167
Eyes (both)	0.378	0.5	840[a]	0.541	0.5	168
Fat (white)	1.29	0.5	123[a]	3.38	0.5	123
Heart	1.38	0.5	ID	1.46	0.5	ID
Kidneys	8.13	0.5	73.9	8.11	0.5	142
Liver	9.01	0.5	76.5	11.0	0.5	82.3
Lungs	1.36	0.5	158	1.95	0.5	268
Lymph nodes (mesenteric)	1.03	0.5	235	2.04	0.5	208
Muscle skeletal (left hind limb)	0.797	0.5	ID	0.933	0.5	327
Pancreas	5.53	0.5	192	2.69	0.5	330
Plasma	2.70	0.5	44.3	2.87	0.5	53.8
Salivary glands (submaxillary)	1.86	0.5	91.7	2.72	0.5	123
Sciatic nerve	0.765	0.5	ID	1.06	0.5	190
Skin (dorsal, shaved)	0.952	0.5	69.5	1.63	0.5	246
Small intestine (excluding contents)	8.97	4	129	16.3	0.5	208
Spleen	0.986	0.5	83.4	1.13	0.5	171
Stomach (excluding contents)	31.0	0.5	459	26.4	0.5	361
Testes	0.537	0.5	ID	0.789	0.5	217
Thymus	0.822	0.5	55.5	0.980	0.5	196
Thyroid	1.75	0.5	106	2.50	2	639
Trachea	1.05	0.5	387	1.59	0.5	ID
Urinary bladder (empty)	1.64	2	175	6.64	4	209

ID = Insufficient Data to define terminal phase.
[a]The terminal phase was near the limit of detection and concentration data were variable.

014:38570:0299: Distribution of radioactivity in dams and fetal rats following a single oral dose of ^{14}C-AL-6515. Vol. 3

Report Nº: 014:38570:0299
Compound: ^{14}C-AL-6515 (Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity = ' , and AL-6515 (Lot#: 6558:041, purity =
Route: Oral by gavage
Dose Level: 3 mg/kg (16.3 µCi for gestation Day 12 group and 19.3 µCi for gestation Day 18 group)

Dosing Regimen: Single
Animal: Sprague Dawley rats (0.251 ± 0.027 kg for gestation Day 12 group and 0.296 ± 0.020 for gestation Day 18 group)
Study Facility: —

GLP: No

The purpose of this study was to determine the distribution of radioactivity in pregnant and fetal rat tissues following a single 3 mg/kg oral dose of ^{14}C-AL-6515 on gestation Days 12 or 18. Three animals pre time point were sacrificed on Days 12 and 18 of gestation at 0.5, 4, 12, and 24 hr after dosing and tissues (amniotic fluid, mammary glands, brain, heart, kidney, blood, liver, lungs, placenta, ovaries, and uterus) were collected. Two whole fetuses were collected from each dam. On Day 18 of gestation, two additional fetuses were collected from each animal. Samples were analyzed for total radioactivity by LSC.

Results:

Results are summarized in the table below. Oral administration of ^{14}C-AL-6515 to pregnant rats on gestation Days 12 and 18 resulted in distribution of radioactivity to maternal tissues and placental transfer of radioactivity into the developing fetus. Cmax values were generally similar in two gestation days with the highest values seen in the liver and kidney. Radioactivity absorption and distribution were rapid with Tmax levels of 0.5 hr on both Days 12 and 18.

PK parameters in pregnant rats following a single 3 mg/kg oral dose of ^{14}C-AL-6515 on gestation Days 12 and 18

Tissue	Day 12		Day 18		
	C_{max} (ug equiv./g)	T_{max} (hours)	C_{max} (ug equiv./g)	T_{max} (hours)	$T_{1/2}$* (hours)
Amniotic fluid	0.364	0.5	0.205	4	7.3
Blood	1.12	0.5	1.19	0.5	6.9
Brain	0.482	0.5	0.522	0.5	4.1
Heart	0.864	0.5	0.926	0.5	5.6
Kidneys	4.18	0.5	3.50	0.5	7.5
Liver	4.96	0.5	4.74	0.5	9.6
Lungs	0.984	0.5	1.05	0.5	7.8
Mammary glands	0.812	0.5	0.957	0.5	7.1
Ovaries	0.887	0.5	1.27	0.5	5.7
Placenta	0.993	0.5	0.825	0.5	7.4
Plasma	1.59	0.5	1.63	0.5	6.9
Uterus	0.878	0.5	0.963	0.5	6.6
Fetal blood	ND	ND	0.422	0.5	6.6
Fetal brain	ND	ND	0.467	0.5	5.9
Fetal heart	ND	ND	0.371	0.5	2.4
Fetal liver	ND	ND	0.675	0.5	6.0
Fetal lungs	ND	ND	0.430	0.5	4.6
Whole Fetus	0.892	0.5	0.515	0.5	7.0

*Half life estimates likely underestimate true values for most matrices because samples were collected through 24 hours.
ND – Not determined due to small mass of fetus at this day of gestation.

049:38570:1298: Secretion of radioactivity in milk of lactating rats following a single oral dose of ^{14}C-AL-6515. Vol. 3

Report Nº: 049:38570:1298
Compound: ^{14}C-AL-6515 (Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity = — , and AL-6515 (Lot#: 6558:041, purity = —
Route: Oral by gavage
Dose Level: 3 mg/kg (22.3 μCi)
Dosing Regimen: Single
Animal: Lactating female Sprague Dawley rats (12 days postpartum, 0.343 ± 0.021 kg)
Study Facility: —
—

GLP: No

The purpose of this study was to determine the secretion of radioactivity in milk of the lactating rat following a single 3 mg/kg oral dose of ^{14}C-AL-6515. Three animals per time point were sacrificed at 0.5, 4, 12, 24, and 48 hr after dosing. Milk and blood samples were collected, processed, and analyzed for total radioactivity by LSC.

Results:

Results, summarized in the table below, showed that radioactivity was secreted into milk following oral administration of ^{14}C-AL-6515. The radioactivity in milk and plasma declined with similar half-lives, suggesting that radioactivity did not accumulate in milk.

Mean radioactivity concentrations in blood, plasma, and milk (µg-eq/ml, mean ± SD)

Time (hr)	Blood	Plasma	Milk
0 5	0 990±0 201	1 43±0 265	0 901±0 252
4	0 249±0 040	0 442±0 085	0 246±0 033
12	0 092±0 016	0 164±0 042	0.074
24	0 050±0 016	0 087±0 034	0 033±0 005
48	0 012±0 003	0 014±0 004	0 004±0 001
Half-life (hr)		10 0	8 4

TDOC-0002142: The *in vitro* protein binding of ^{14}C-AL-6515 in rat, monkey and human plasma. Vol. 3

Report N°: TDOC-0002142
Compound: ^{14}C-AL-6515 (Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity = — , and AL-6515 (Lot#: 6558:041, purity = —
Concentration: 10, 30, 100, 300, 1000 ng/ml
Species: Rat, monkey and human plasma
Study Facility: —
GLP: No

The purpose of this study was to determine the protein binding of ^{14}C-AL-6515 in rat, monkey and human plasma. The binding was determined at 5 concentrations in rat, monkey and human plasma using the ultrafiltration method.

Results:

Results, summarized in the table below, showed that ^{14}C-AL-6515 bound moderately to plasma proteins of rat, monkey, and human *in vitro* in a concentration-independent manner over the concentration range of 10 to 1000 ng/ml. The extents of plasma protein binding of ^{14}C-AL-6515 were similar.

% ^{14}C-AL-6515 bound to rat, monkey, and human plasma protein *in vitro* (mean ± SD)

Concentration (ng/ml)	(nM)	Rat	Monkey	Human
10	39.3	71 8±0 5	78.9±0 2	84.1±0.6
30	118	73.0±0 8	78.6±0 3	84 2±0 8
100	393	72.4±1 5	80.6±0 7	81 9±0 1
300	1180	72 8±0 9	80.4±0 2	83.6±0 4
1000	3930	73 8±0 7	80.3±0.6	83.7±0.5
Overall (mean)		**72.8±0.7**	**79.8**±0 8	**83.5**±0 8

2.6.4.5 Metabolism

TDOC-0001077: Metabolism of ^{14}C-amfenac amide (nepafenac, AL-6515) *in vitro* by human precision cut liver slices. Vol. 3.

Report N°: TDOC-0001077

Study site:
Compound: ^{14}C-amfenac amide, Lot #: CFQ13604, 5.885 x 10^5 dpm/μg, radiochemical purity =

Concentration: 0.2 and 2.0 μg/ml
Test systems: Human liver slices from three donors
GLP: No

The purpose of this study was to determine metabolic profiles of ^{14}C-amfenac amide following *in vitro* incubation with precision cut human liver slices. Radiochromatographic profiles were determined by

Results:

Incubation of ^{14}C-amfenac amide in precision-cut human liver slices resulted in substantial metabolism with approximately 12 metabolites formed (see table below). The major metabolite found was amfenac with the remaining metabolites being present in relatively low amounts. Glucuronidase mediated hydrolysis showed these metabolites were a mixture of conjugated (glucuronide or sulfate) and non-conjugated metabolites. However, both amfenac amide and amfenac were not found to be conjugated.

% Metabolites after incubation of ^{14}C-amfenac amide with human liver slices for 4 and 24 hr

	Percent of Total Radioactivity Based on Chromatographic Peak Areas					
	Liver Sample-1		Liver Sample-2		Liver Sample-3	
Metabolite	4 hour	24 hour	4 hour	24 hour	4 hour	24 hour
M1	BLQ	0.9	BLQ	1.8	BLQ	4.2
M2	BLQ	BLQ	BLQ	BLQ	BLQ	5.2
M3	BLQ	0.6	0.7	1.8	1.4	4.8
M4	BLQ	BLQ	BLQ	0.7	0.4	8.0
M5 (amfenac)	3.0	14.1	3.1	17.3	5.1	15.3
M6	0.3	1.7	0.8	3.2	4.6	12.4
M7 (AL-12384)	0.7	5.3	1.9	8.8	0.7	2.4
M8	BLQ	1.2	BLQ	1.1	BLQ	1.8
M9	BLQ	2.6	0.3	4.0	1.7	13.0
M10	0.5	10.4	BLQ	7.5	0.8	3.0
M11 (amfenac amide)	91.4	48.6	89.6	44.1	80.2	10.6
M12 (amfenac lactam)	0.9	3.6	0.7	2.0	1.3	1.4
M13	BLQ	3.6	BLQ	BLQ	BLQ	1.4

TDOC-0001353: Chromatographic profiles of radioactivity in ocular tissues following a single bilateral topical ocular dose of 0.3% ^{14}C-amfenac amide (nepafenac, AL-6515) ophthalmic suspension to New Zealand white rabbits. Vol. 3

Report Nº: TDOC-0001353
Compound: 0.3% ^{14}C-AL-6515 ophthalmic suspension (Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity =
Route: Topical ocular, both eyes
Dosing Regimen: Single dose
Animal: New Zealand white rabbits (males, 3.6 ± 0.3 kg)
Test Facility: Alcon Research, Ltd., Fort Worth, TX
GLP: No

The purpose of this study was to determine the hydrolysis of AL-6515 in ocular tissues to its pharmacologically active carboxylic acid form, amfenac, following a single bilateral topical ocular dose of 0.3% ^{14}C-AL-6515 ophthalmic suspension to male NZW rabbits. Tissue samples (cornea, aqueous humor, iris-ciliary body, retina and choroids) were collected at 0.5 hr after dosing. Radiochromatographic profiles were determined by

Results:

Amfenac amide (M2), amfenac (M1) and one unidentified metabolite (M3) were observed. Amfenac amide was present at the highest concentrations in all tissues. Amfenac was also present in all tissues at lower, but still relatively high concentrations. The results showed that amfenac amide was significantly hydrolyzed to its pharmacologically active form, amfenac, in the eye following topical ocular administration and that the major constituents of the total radioactivity in the ocular tissues were amfenac amide and amfenac.

AL-6515 and metabolite concentration in ocular tissues

	Concentration (ng eq/g)		
Ocular Tissue	M1 (Amfenac)	M2 (Amfenac amide)	M3 (Not Identified)
Cornea			
Iris-Ciliary Body			
Retina			
Choroid			
Aqueous Humor			

TDOC-0001352: Chromatographic profiles of radioactivity in plasma and urine following a 0.5 mg/kg intravenous dose of 0.3% ^{14}C-amfenac amide (nepafenac, AL-6515) in Sprague Dawley rats. Vol. 4

Report Nº: TDOC-0001352
Compound: ^{14}C-AL-6515 (Lot#: CFQ13604, 68 mCi/mmol, radiochemical purity =
Dose: 0.5 mg/kg
Route: Intravenous injection
Dosing Regimen: Single dose
Animal: Sprague Dawley rats (males, 0.319 ± 0.014 kg)
Test Facility: Alcon Research, Ltd., Fort Worth, TX
GLP: No

The purpose of this study was to determine radiochromatographic profiles of radioactivity in plasma and urine following a single 0.5 mg/kg intravenous dose of ^{14}C-amfenac amide to male SD rats. Plasma samples were collected from 5 animals per time point at 0.25, 4 and 24 hr and urine was collected between 0-24 hr after dosing. Radiochromatographic profiles were determined by

Results:

Results are summarized in the table below. The majority circulating metabolites in rat plasma and those excreted in urine were non-conjugated and more polar than amfenac amide. Plasma concentrations of radioactivity at the three sampling time points were 581+65 (0.25 hr), 57.6+14.4 (4 hr) and 5.67+0.92 (24

hr) ng eq/ml. In urine, 36.0+12.1% of the dose was excreted in the first 24 hr. Amfenac and the 5 additional metabolites were non-conjugated metabolites in plasma. Amfenac was the major circulating metabolite. At least 10 metabolites were observed in urine radiochromatographs. The majority of metabolites in the urine were non-conjugates. Amfenac was a minor metabolite in urine (< 1% of the administered dose). No amfenac amide was found in the urine indicating elimination of amfenac amide was by metabolism.

Mean rat plasma concentrations of AL-6515 and metabolites following β-glucuronidase treatment (ng-eq/ml)

Time (hr)*	MP1	MP2	Amfenac	MP4	MP5	MP6	AL-6515
0.25	33.7	114	139	1.69	72.5	8.99	1.38
4	1.14	6.70	10.2	5.13	1.85	2.57	0.48
24	BLQ (<			0.52	BLQ		

TDOC-0001076: Chromatographic profiles of radioactivity in plasma and urine following a 0.5 mg/kg intravenous dose of 0.3% ^{14}C-amfenac amide (nepafenac, AL-6515) in cynomolgus monkeys. Vol. 4

Report N°: TDOC-0001076
Compound: ^{14}C-AL-6515 (Lot#: CFQ13604, 68 mCi/mmol, radiochemical purity =)
Dose: 0.5 mg/kg
Route: Intravenous injection
Dosing Regimen: Single dose
Animal: Cynomolgus monkeys (males, 7.1 ± 0.5 kg)
Test Facility: Alcon Research, Ltd., Fort Worth, TX
GLP: No

The purpose of this study was to determine radiochromatographic profiles in plasma and urine following a single 0.5 mg/kg intravenous dose of ^{14}C-amfenac amide to male cynomolgus monkeys. Plasma samples were collected at 0.25, 4 and 24 hr and urine was collected between 0-6 and 6-24 hr after dosing. Radiochromatographic profiles were determined by

Results:

Results are summarized in the tables below. Plasma concentrations of radioactivity at three sampling time points were 989+123 (0.25 hr), 132+32.5 (4 hr) and 10.0+3.35 (24 hr) ng eq/ml. In urine, 55.0+2.0% of the dose was excreted in the first 24 hr. Both conjugated and non-conjugated metabolites were present in plasma. The major circulating radioactive components in plasma up to 4 hr after dosing were unchanged amfenac amide, amfenac and an unidentified metabolite, Mp2. The remaining 5 metabolites that were quantifiable each represented generally less than 1-5% of the total amount of radioactivity in plasma. The majority of metabolites in the urine were in the form of conjugates (e.g., glucuronide). Only a small amount of amfenac (<1% of dose) and no detectable levels amfenac amide were found in the urine indicating elimination of amfenac amide was by metabolism.

Mean plasma concentrations of AL-6515 and metabolites following β-glucuronidase treatment (ng-eq/ml, mean ± SD)

Time (hr)*	MP1	MP2	MP3	Amfenac	MP5	MP6	AL-6515	Amfenac lactam
0.25	14.4±5.1	112±65	<9.92	355±50	<12.5	<3.31	319±26	<6.05
4	3.36±0.35	7.84±3.36	5.41±2.51	26.9±12.4	13.0±3.24	6.06±3.94	8.39±0.99	6.64±1.34

* Radiochromatographic profiles of 24 hr were not available due to insufficient radioactive concentrations.

Mean percent of dose excreted as metabolites in monkey urine following β-glucuronidase treatment (%, mean ± SD)

Time (hr)	Mu1	Mu2	Mu3	Amfenac	Mu5	Mu6	Mu7	Mu8	Amfenac lactam
0-6	0.7±0.1	1.2±0.3	6.4±0.8	0.8±0.3	0.4±0.1	17.4±7.4	3.0±1.1	1.0±0.5	2.7±1.2
6-24	<0.6	<1.3	3.2±2.1	<0.2	<0.5	5.9±2.7	1.9±1.1	<1.0	<0.4
0-24	0.8±0.3	2.1±1.1	9.6±2.0	0.9±0.2	0.7±0.4	23.3±5.1	4.9±2.2	1.6±1.3	3.0±1.0

TDOC-0002143: Evaluation of the inhibitory potential of AL-6515 towards metabolic activities of cDNA-expressed human cytochrome P450 isozymes. Vol. 4

Report N°: TDOC-0002143
Study site:
Compound: AL-6515
Test systems: Human cytochrome P450 (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4)
GLP: No

The purpose of this study was to characterize the inhibitory potential of AL-6515 towards specific isozymes of human hepatic cytochrome P450 (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) *in vitro*. Each CYP isozyme-specific assay was performed using cDNA-expressed CYP isozyme and a probe substrate at a concentration that approximated the Km of that isozyme. The CYP isozyme specific marker substrates were phenacetin O-deethylase (for CYP1A2), diclofenac 4'-hydroxylase (for CYP2C9), S-mephenytoin 4'-hydroxylase (for CYP2C19), bufuralol 1'-hydroxylase (for CYP2D6), p-nitrophenol hydroxylase (for CYP2E1), testosterone 6(beta)-hydroxylase (for CYP3A4) and midazolam 1' hydroxylase (for CYP3A4). Each CYP isozyme was incubated with a probe substrate in the presence of AL-6515 at concentrations of 0 (control), 0.3, 1.0, 3.0, 10, 30, 100, 300, 1000 and 3000 ng/ml [0 (control), 1.18, 3.93, 11.8, 39.3, 118, 393, 1180, 3930 and 11800 nM].

Results:

AL-6515 at concentrations up to 1000 ng/ml [approximately 3,000-fold the observed mean plasma C_{max} in humans (0.310 ± 0.104 ng/ml; Alcon Clinical Study C-04-08)] was not an inhibitor of any of the major human cytochrome P450 (CYP1A2, 2C9, 2C19, 2D6, 2E1 and 3A4) catalytic activities *in vitro*. AL-6515 at a concentration of 3000 ng/ml showed 36% inhibition of only CYP2E1 but no meaningful inhibition of any of the other major human CYP isozymes.

Percent inhibition of cDNA-expressed CYP enzyme activities by AL-6515 *in vitro* (%)

Cytochrome P450	Probe Substrate	Concentrations of AL-6515 (ng/ml)								
		0.3	1.0	3.0	10	30	100	300	1000	3000
CYP1A2	Phenacetin	3	3	3	0	4	2	0	0	9
CYP2C9	Diclofenac	0	0	3	4	8	1	0	0	10
CYP2C19	(S)-Mephenytoin	0	6	2	0	0	0	6	0	18
CYP2D6	Bufuralol	3	0	0	0	0	1	0	1	2
CYP2E1	p-Nitrophenol	7	3	2	7	3	9	9	20	36
CYP3A4	Testosterone	4	0	0	0	0	0	Not determined		
CYP3A4	Midazolam	0	5	6	6	5	2	4	4	3

2.6.4.6 Excretion

011:38570:0299: Excretion and mass balance of radioactivity in male Sprague Dawley rats following a single intravenous dose of ^{14}C-AL-6515. Vol. 4

Report Nº: 011:38570:0299
Compound: ^{14}C-AL-6515 (Lot#: CFQ9917, 63 mCi/mmol, radiochemical purity = ' —) and AL-6515 (ERM#: 6558:041, purity = —
Dose: 0.5 mg/kg (6.73 x 10^6 DPM, 3.03 μCi)
Route: Intravenous injection
Dosing Regimen: Single dose
Animal: Sprague Dawley rats (males, 0.300 ± 0.004 kg)
Test Facility: —

GLP: No

The purpose of this study was to determine the excretion of radioactivity in urine, feces, and expired CO_2 following a single 0.5 mg/kg intravenous dose of ^{14}C-amfenac amide to male SD rats. Urine, feces, and expired CO_2 samples were collected from 5 animals per time point at 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr after dosing. Samples were analyzed for total radioactivity by LSC.

Results:

Results are summarized in the table below. Drug-derived radioactivity was rapidly and completely excreted with about 90% radioactivity recovered in excreta by 24 hr. Negligible quantities of radioactivity were recovered in traps for collection of expired CO_2 or organic volatile components of expired air. The major route of excretion was via urine. A substantial fraction was excreted from feces, suggesting that biliary excretion be an important elimination pathway.

Mean cumulative percents of radioactivity recovered after a single 0.5 mg/kg dose (iv) of ^{14}C-AL-6515 to the male SD rats

% mean ± SD	Urine	Feces	Cage rinse
0-24	54.4±2.3	35.1±4.8	0.21±0.13
0-48	56.0±2.6	39.2±2.6	0.25±0.14
0-72	56.3±2.7	39.7±2.5	0.28±0.15
0-96	56.5±2.8	39.8±2.4	0.29±0.15
0-120	56.6±2.8	39.9±2.3	0.30±0.15
0-144	56.7±2.8	40.0±2.3	0.31±0.15
0-168	56.8±2.8	40.0±2.3	

2.6.4.7 Pharmacokinetic drug interactions

No studies were provided.

2.6.4.8 Other Pharmacokinetic studies

TDOC-0001327: Toxicokinetics of nepafenac and amfenac in toxicology study N-00-309: Six-month topical ocular irritation and systemic toxicity evaluation of nepafenac ophthalmic suspension in pigmented rabbits. Vol. 4

Technical Report Nº: TDOC-0001327
Conducting laboratory and location: Alcon Research Ltd., 6201 S. Freeway, Fort Worth, TX 76134
Drug: 0.3, 1.0, or 1.5% nepafenac ophthalmic suspensions
Animals: New Zealand F1 Cross (NZW x NZR) rabbits

Route: Ocular, topical, two drops, tid, right eye only
TK study design

Group	N/sex	Treatment	Route	Sampling time Day 1	Sampling time Days 90 and 180
3	3	0.3% AL-6515 ophthalmic suspension	Ocular topical, tid	Prior to the last daily dose and 0 5, 1, and 2 hr after the last daily dose	Prior to the last daily dose and 0 75, 1 5, and 2 25 hr after the last daily dose
4	3	1 0% AL-6515 ophthalmic suspension			
5	3	1 5% AL-6515 ophthalmic suspension			

As a part of an ocular toxicity study (Study N-00-309), concentrations of AL-6515 and AL-6295 were determined in plasma samples from New Zealand F1 Cross rabbits during the 6-month ocular toxicity study. Blood samples were collected from three rabbits/sex/group immediately prior to the last dose of the day and at 0.5, 1, and 2 hr after the last dose of the day on Day 1 and at 0.75, 1.5, and 2.25 hr after the last daily dose on Days 90 and 180. Samples were analyzed by an — method with a quantitation limit of — ng/ml for both AL-6515 and AL-6295.

Results:

Results are summarized in the table below. Systemic exposure to both AL-6515 and AL-6295 was found in all dose levels and increased in a dose-dependent manner. Exposure to AL-6295 was higher than that to AL-6515. No accumulation and significant gender-related differences were noted.

Plasma TK data of AL-6515 and AL-6295 in pigmented rabbits (mean ± SD)

	AL-6515				AL-6295			
	$AUC_{0\text{-}4\,hr}$ (ng-hr/ml)		Cmax (ng/ml)		$AUC_{0\text{-}t}$ (ng-hr/ml)		Cmax (ng/ml)	
Group	Day 1	Day 180	Day 1	Day 180	Day 1	Day 180	Day 1	Day 180
3	1.03±0 54	0 90±0 58	1 76±1 19	0 89±0 49	7 83±2 29	9 79±4 22	9 32±4 08	8 36±3 20
4	1.59±0.82	1 40±0 94	2 23±0 97	1.54±0 92	39 8±60 2	16 6±4 1	18 2±7 8	16 5±6 0
5	2 67±2 38	6 01±5 98	3 96±4 01	6 01±6 03	22 0±16 9	50 6±21 2	25 4±21 5	45 4±18 0

TDOC-0001735: Toxicokinetics of nepafenac and amfenac in toxicology study N-03-090: Three-month topical ocular irritation and systemic toxicity evaluation of AL-6515 ophthalmic suspension in nonhuman primates. Vol. 4

Technical Report N^{o}: TDOC-0001735
Conducting laboratory and location: Alcon Research Ltd., 6201 S. Freeway, Fort Worth, TX 76134
Drug: 0.1, 0.3, or 1.0% nepafenac ophthalmic suspensions
Animals: Cynomolgus monkeys
Route: Ocular, topical, two drops, qid, right eye only
TK study design

Group	N/sex	Treatment	Route	Sampling time
2	4	0.1% AL-6515 ophthalmic suspension	Ocular, topical, qid	Prior to the last daily dose and 0 5, 1, 2 and 3 hr after the last daily dose on Days 1, 22 and 97
3	4	0.3% AL-6515 ophthalmic suspension		
4	4	1 0% AL-6515 ophthalmic suspension		

As a part of an ocular toxicity study (Study N-03-090), concentrations of AL-6515 and AL-6295 were determined in plasma samples from monkeys during the 3-month ocular toxicity study. Blood samples was collected from 4 animals/sex/ prior to the last dose of the day and at 0.5, 1, 2, and 3 hr after the last dose of the day on Days 1, 22, and 97. Samples were analyzed by an — method with a quantitation limit of — ng/ml for both AL-6515 and AL-6295.

Results:

Results are summarized in the table below. Systemic exposure to both AL-6515 and AL-6295 was found in all dose levels and increased in a dose-dependent manner. No accumulation and significant gender-related differences were noted.

TK data of AL-6515 and AL-6295 in Study N-03-090

Dose (%)	Study Day	Mean Cmax (ng/ml)		Mean AUC_{0-tp}(ng*h/ml)	
		Male and Female Combined		Male and Female Combined	
		Mean	SD	Mean	SD
Nepafenac (AL-6515)					
0 1	Day 1	4 82	1 20	5 57	1 43
	Day 22	2 10	0 85	2 76	0 99
	Day 97	2 95	1 62	3 89	2 03
0 3	Day 1	10 9	4 6	15 2	8 1
	Day 22	6 05	2 24	8 17	2 62
	Day 97	8 70	4 30	12 2	5 4
1 0	Day 1	23 5	4 6	41 9	10.9
	Day 22	17 4	6 2	36 4	17 2
	Day 97	17 4	5 8	35 7	12 7
Amfenac (AL-6295)					
0 1	Day 1	3.80	1 21	5 47	1 45
	Day 22	2.08	1 36	3 46	1 71
	Day 97	2 59	1 84	4 52	3 22
0 3	Day 1	10 8	6 9	17 6	10 9
	Day 22	6 17	2 58	10 6	4 0
	Day 97	7 69	4 21	13 5	6 0
1 0	Day 1	25 1	4 8	50 4	9 8
	Day 22	20 3	8 7	39 9	14 6
	Day 97	26 4	14 5	45 5	16.1

TDOC-0001557: Toxicokinetics of nepafenac (AL-6515) and afmenac (AL-6295) in toxicology study N-01-024: Six-month oral (gavage) toxicity study of in rats. Vol. 5

Technical Report N^{0}: TDOC-0001557
Conducting laboratory and location /

Drug: AL-6515
Dose: 1, 3, and 10 mg/5 ml/kg, qd x 180 days
Animals: Sprague Dawley rats
Route: Oral by gavage

TK study design

Group	Dose (mg/kg)	n/sex	Sampling time (Days 1 and 90)	Sampling time (Day 180)
2	1	27	1 and 24 hr after dosing	1, 2, 4, and 24 hr after dosing
3	3	27		
4	10	27		

As a part of a 6-month oral toxicity study in rats (Study N-01-024), concentrations of AL-6515 and AL-6295 were determined in rat plasma samples. Blood samples was collected from three animals/sex/group/time point at 1 and 24 hr after the daily dose on Days 1 and 90, and at 1, 2, 4, and 24 hr on Day 180. Samples were analyzed by an — method with a quantitation limit of — g/ml for both AL-6515 and AL-6295.

Results:

TK data are summarized in the table below. Systemic exposure to both AL-6515 and AL-6295 was found in all dose levels and increased in a dose-dependent manner. Exposure to AL-6295 was higher than that to AL-6515. No gender-related differences were noted.

Mean TK data of AL-6515 and AL-6295 in Study N-01-024 (mean ± SD)

Day	1	90	180	
Dose (mg/kg/day)	AL-6515 Cmax (ng/ml)			$AUC_{0-4\ hr}$ (ng-hr/ml)
1 0	3 79±1 36	4.19±1 45	5 93±2 96	7 08±1 22
3 0	10 2±2 4	18 7±3 7	23 9±2.4	31 7±1 2
10 0	49 5±21 9	140±35	118±32	189±22
Dose (mg/kg/day)	AL-6295 Cmax (ng/ml)			$AUC_{0-4\ hr}$ (ng-hr/ml)
1 0	35 6±9.9	54.6±17 5	47.5±15 8	85 0±7 6
3 0	101±22	204±42	186±50	369±29
10 0	388±99	866±269	670±137	1550±106

TDOC-0001901: Retrospective toxicokinetic study of nepafenac and amfenac in pregnant rats following repeated oral doses of AL-6515 (N-03-161). Vol. 5

Technical Report N^{0}: TDOC-0001901
Conducting laboratory and location: /

Drug: AL-6515 (Lot#s: 03-35079, 03-35083, and 03-35084)
Dose: 3, 10, and 30 mg/10 ml/kg, qd from gestation Days 6 through 17
Animals: Pregnant Sprague Dawley rats
Route: Oral by gavage
TK study design

Dose (mg/kg)	n/sex	Sampling time (gestation Days 6, 12, and 17)
3	12	0 (predose), 0 5, 1, 2, 4, and 6 hr after dosing
10	12	
30	12	

The purpose of this retrospective study was to describe the toxicokinetics of AL-6515 and AL-6295 in plasma following repeated oral doses of AL-6515 to pregnant rats. Blood samples was collected from four animals/group/time point prior to the daily dose and at 0.5, 1, 2, 4, and 6 hr after the daily dose on gestation Days 6, 12, and 17. Samples were analyzed by an — nethod with a quantitation limit of — ng/ml for both AL-6515 and AL-6295.

Results:

TK data are summarized in the table below. AL-6515 was rapidly absorbed with Tmax values of 0.5 or 1 hr. Systemic exposure to both AL-6515 and AL-6295 was found in all dose levels and increased with doses. Exposure to AL-6295 was higher than that to AL-6515. No accumulation was noted.

Mean TK data of AL-6515 and AL-6295 in Study N-03-161 (mean ± SD)

Gestation Day	6	12	17	6	12	17
Dose (mg/kg/day)	AL-6515 Cmax (ng/ml)			AL-6515 $AUC_{0-6\ hr}$ (ng-hr/ml)		
3 0	24 8±2 7	33 1±25 7	23 4±5 4	22 2±2 4	25 0±6.5	24 0±1 7
10 0	108±60	69 6±57 9	242±196	142±23	97 0±28 2	207±51
30 0	669±362	201±108	860±295	1160±210	518±51	1360±157
Dose (mg/kg/day)	AL-6295 Cmax (ng/ml)			AL-6295 $AUC_{0-4\ hr}$ (ng-hr/ml)		
3 0	297±72	454±378	284±33	572±53	692±115	838±76
10 0	1340±1020	793±693	1710±1620	2930±476	2340±397	4190±620
30 0	5480±3130	2650±1210	4330±1170	17500±2300	8720±673	17200±2000

TDOC-0002069: Retrospective toxicokinetic study of nepafenac and amfenac in pregnant rabbits following repeated oral doses of AL-6515 (N-03-160). Vol. 5

Technical Report N^{o}: TDOC-0002069
Conducting laboratory and location:

Drug: AL-6515 (Lot#s: 03-35085, 03-35086, and 03-35087)
Dose: 3, 10, and 30 mg/5 ml/kg, qd from gestation Days 6 through 18
Animals: Pregnant rabbits
Route: Oral by gavage
TK study design

Dose (mg/kg)	n/sex	Sampling time (gestation Days 6, 12, and 17)
3	12	0 (predose), 0 5, 1, 2, 4, and 6 hr after dosing
10	12	
30	12	

The purpose of this retrospective study was to describe the toxicokinetics of AL-6515 and AL-6295 in plasma following repeated oral doses of AL-6515 to pregnant rabbits. Blood samples was collected from four animals/group/time point prior to the daily dose and at 0.5, 1, 2, 4, and 6 hr after the daily dose on gestation Days 6, 12, and 18. Samples were analyzed by an , method with a quantitation limit of ng/ml for both AL-6515 and AL-6295.

Results:

TK data are summarized in the table below. AL-6515 was rapidly absorbed with Tmax values of 0.5 to 1.33 hr. Systemic exposure to both AL-6515 and AL-6295, found in all dose levels, was highly variable and increased with doses. Exposure to AL-6295 was higher than that to AL-6515. No accumulation was noted.

Mean TK data of AL-6515 and AL-6295 in Study N-03-160 (mean ± SD)

Gestation Day	6	12	18	6	12	18
Dose (mg/kg/day)	AL-6515 Cmax (ng/ml)			AL-6515 $AUC_{0-6\ hr}$ (ng-hr/ml)		
3.0	3 93±2 37	1 14±0 85	1 18±0 71	3 10±1 81	1.03±0.72	0.887±0 379
10.0	70 8±105 1	39 3±18 8	40 2±59 6	62 5±90 5	43 6±10 5	28 4±40 9
30.0	108±116	376±460	281±327	162±136	628±640	432±476
Dose (mg/kg/day)	AL-6295 Cmax (ng/ml)			AL-6295 $AUC_{0-4\ hr}$ (ng-hr/ml)		
3 0	710±1070	121±137	133±93	760±1150	153±174	152±118
10.0	2100±2890	1110±637	666±608	3070±4450	1340±613	663±453
30.0	1760±2110	5150±6290	2400±1980	3260±3490	9510±10300	4600±3690

2.6.4.9 Discussion and Conclusions

AL-6515 was hydrolyzed to amfenac in all species with all routes tested. Plasma exposures to amfenac were higher than that to AL-6515. The plasma half-life for amfenac amide and amfenac was short. The plasma half-lives of radioactivity were long, suggesting other uncharacterized metabolites. Following topical ocular administration of ^{14}C-AL-6515 to rabbits, radioactivity was absorbed into the eye with high concentrations of radioactivity in the conjunctiva and cornea. Radioactivity was also distributed to the posterior ocular tissues (retina and choroid). ^{14}C-AL-6515 or its radioactive drug equivalents did not bind to melanin pigmented tissues. Oral administration of ^{14}C-AL-6515 to pregnant rats resulted in placental transfer of radioactivity into the developing fetus. Radioactivity was also found in the milk of lactating rats. ^{14}C-AL-6515 bound moderately to plasma proteins of rat, monkey, and human *in vitro* (73% to 84%) in a concentration-independent manner over the concentration range of 10 to 1000 ng/ml. Incubation of ^{14}C-amfenac amide in precision-cut human liver slices produced 12 metabolites. The major metabolite was amfenac with the remaining metabolites being present in relatively low amounts. Drug-derived radioactivity was rapidly excreted after iv administration to rats. The major route of excretion was via urine. Biliary excretion was also an important elimination pathway.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Please see Module 2, Vol. 4.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

In single dose acute toxicity studies using ip or oral (gavage) route, the oral and ip LD_{50} values in rats were greater than 0.1 g/kg, the oral LD_{50} value in mice was greater than 2 g/kg, and the ip LD_{50} value in mice was greater than 1 g/kg.

Several repeated dose oral systemic toxicity studies were conducted in rats with the duration up to 6 months. In the 2-week study, GI abnormalities including jejunal serositis and mesenteric lymphoid hyperplasia were noted in animals of 25 mg/kg group. The sponsor indicated that these changes were considered to be secondary to intra-abdominal trauma, possibly associated with gavage procedures. However, distinct gavage trauma was not observed grossly or microscopically in abdominal tissues, and a relationship between drug treatment and these findings could not be entirely ruled out. In the 3-month toxicity study in SD rats, histopathological examination showed renal papillary necrosis in two of ten females at 15 mg/kg only. GI and renal lesions were common findings in animals treated with high doses of NSAIDs. In 6-month toxicity study in F344 rats, higher incidences of corneal mineralization (5 of 25 in males vs. 0 in control animals) and uterus hydrometra (5 of 25 in females vs. control's 1 of 25) were seen in HD groups (10 mg/kg/day). Similar changes were not seen in other studies, and the toxicological significance of these positive findings was not determined.

Several repeated dose ocular toxicity studies were conducted with duration up to 3 months in monkeys (concentrations up to 1.0%, qid) and NZW rabbits (concentrations up to 1.0%, qid), and 6 months in pigmented rabbits (concentrations up to 1.5%, tid). The drug was well tolerated. No drug-induced systemic and ocular toxicity was observed. In all studies, minimal to moderate conjunctival congestion and

transient and sporadic incidences of minimal conjunctival discharge were seen in the eye treated with vehicle and drugs. Because of the similar incidences and severity between control and treated eyes, these changes were not considered as drug-related. In a rabbit study in which nepafenac ophthalmic suspension (up to 1.0%) was administered prior and subsequent to a corneal incision, no significant ocular and systemic toxicity as well as postoperative ocular complications were noted.

Genetic toxicology:

AL-6515 was nonmutagenic in the Ames test and mouse lymphoma TK assay. The drug was also negative in *in vivo* micronucleus assay. AL-6515 was positive for the induction of structural chromosome aberrations in CHO cells.

Carcinogenicity:

No carcinogenicity studies were conducted on AL-6515. A waiver for carcinogenicity studies was granted by the review division in October 2004.

Reproductive toxicology:

A fertility and early embryonic development study was conducted in SD rats. At 15 mg/kg group, sperm motility and sperm concentrations were lower than in control males. Histological examination in the 15 mg/kg group showed slightly decreased spermatozoa in the duct of the epididymis and slightly more intraluminal single necrotic cells in the epididymis in two of three animals examined. In females, there were no toxicologically significant differences in copulation and fertility indices between control and treated groups. However, a decrease in the number of viable fetuses and an increase in the early resorption and post-implantation loss were noted in animals at 10 and 15 mg/kg. Oral administration of AL-6515 in rats at 3.0 mg/kg showed no developmental toxicity in this study.

In the embryo-fetal development study in pregnant rats, animals were treated orally (by gavage) with AL-6515 at 3, 10, and 30 mg/kg/day from gestation Day 6 to Day 17. Mortality and clinical signs were noted in animals at 30 mg/kg. Decreased body weight gain and food consumption were noted at doses $\geq$ 10 mg/kg. Necropsy examinations showed GI lesions in all dead animals and a few scheduled sacrifice HD animals. For reproductive evaluation, a slight decrease in fetal body weight (3.3 $\pm$ 0.5 g vs. control's 3.5 $\pm$ 0.2 g) was seen in HD group. One HD animal had 9 dead fetuses, 6 resorptions, and no viable fetuses. The observed malformations were not considered treatment-related due to the low incidence and lack of dose-dependence. Regarding developmental variations, the incidences of unossified 5th and 6th sternebrae and 7th cervical ribs were significantly higher in the HD group than in the control group. Based on the study results, the dose of 3 mg/kg was considered a NOEL for maternal toxicity and a dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

In the embryo-fetal development study in pregnant rabbits, animals were treated orally (by gavage) with AL-6515 at 3, 10, and 30 mg/kg/day from gestation Day 6 to Day 18. Maternal toxicity was seen in the 10 and 30 mg/kg groups. Abortion occurred in one MD animal and one HD animal. One HD animal had a premature delivery on gestation Day 29. Clinical signs seen in these animals prior to abortion or premature delivery included labored breathing, decreased activity, cool to touch, few or no feces, and soft or mucoid stools. HD animals showed a decrease in body weight gain and food consumption. Regarding reproductive evaluation, HD animals showed an increase in post-implantation loss which was mainly due to an increase

in early resorptions. There was a statistically significant increase in the number of litters with skeletal malformations and in the number of litters with total malformations in the 30 mg/kg/day group when compared to the controls. Low incidences of malformations were seen in the MD and LD groups and were not considered drug-related with respect to control. Based on the study results, the dose of 3 mg/kg was considered a NOEL for maternal toxicity and a dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

In the peri-natal and postnatal study in rats, AL-6515 produced dystocia and associated maternal mortality in F0 females at levels ≥ 3 mg/kg/day, and developmental toxicity in F1 offspring at levels ≥ 10 mg/kg/day. The developmental toxicity was characterized by decreased F 1 pup survival and decreased F1 pup body weights during lactation and growth phases. A no-observed-effect level (NOEL) for maternal effects in F0 females was not established in this study. The NOEL for developmental toxicity in F1 offspring was determined to be 3 mg/kg/day.

Special toxicology:

— , a known degradation product of AL-6515, was evaluated in a battery of genotoxicity studies. The compound was negative in the Ames test and *in vivo* mouse bone marrow micronucleus assay. In an *in vitro* mouse lymphoma TK assay, — , was negative for inducing forward mutations under nonactivation conditions, but the test article was positive under activation conditions. In an ocular toxicity study conducted in NZW rabbits, AL-6515 ophthalmic suspension (0.1%) with 0.003% and 0.0075% — — (tid for one month) showed no local and systemic toxicity. The drug was well tolerated.

2.6.6.2 Single-dose toxicity

129:38520:0995: Acute toxicity evaluation of AL-6515 in mice (up and down procedure). Vol. 6

Key study findings: The oral (gavage) LD_{50} value for AL-6515 in mice was greater than 2 g/kg. The IP LD_{50} value for AL-6515 in mice was greater than 1 g/kg.

Report N°: 129-:38520:0995
Compound: 10% AL-6515 suspension, Lot#: 95-13356-1
Dose: 1 and 2 g/kg
Dosing regimen: Single dose
Route: Oral (by gavage) or intraperitoneal injection
Animal: ICR mice, 25-28 g for females and 28-33 g for males, 2/sex/group
Study site: Alcon Laboratories, Inc., Fort Worth, Texas
Study initiation: 8/18/1995
GLP: Yes
Study design:

Group	n/sex	Dose (g/kg)	Dosing volume (ml/kg)	Route
1	2	1	10	Oral gavage
2	2	1	10	IP injection
3	2	2	20	Oral gavage
4	2	2	20	IP injection

The purpose of this study was to determine the toxicity potential of AL-6515 in mice following a single po or ip dose. The day of dosing was designated as Day 1. Surviving animals were observed for 14 days. Toxicity was assessed as shown below.

Clinical observations: 0.5, 1, 2, 4, and 6 hr after dosing and twice daily thereafter
Body weights: Prior to the treatment initiation and on Days 7 and 14
Necropsy: Animals that died during the observation period

Results:

Mortality: No animals treated orally with AL-6515 were found dead or moribund during the observation period. In the 2 g/kg IP group, one female was dead at the 0.5 hr check. One female and one male were found dead on Day 3. The cause of the death was not determined.

Clinical observations: Abnormal findings are summarized in the table below.

Positive findings in mice treated with AL-6515

Group/treatment	Animal #	Sex	Observations
1 (1 g/kg, oral gavage)	89928	male	Walking with a hunched gait at 0.5 hr check. Less active on Day 1
2 (1 g/kg, ip)	All 4 animals	Males and females	Less active on Day 1
4 (2 g/kg, ip)	89933	Male	Less active at 6 hr check. Abdomen appeared swollen on Day 3
	89934	Male	Less active on Days 1 and 2. Abdomen appeared swollen on Day 2. found dead on Day 3
	89907	Female	Walking with hunched gait at 4 hr check. Less active, weak on Days 1 and 2. Found dead on day 3
	89908	Female	Found dead at 0.5 hr check

Body weights: Positive growth curve was seen in all surviving animals during the observation period.

Gross necropsy: One Group 4 female (animal#: 89907) showed stomach distension with gas. No treatment-related changes were noted in the other dead animals.

In conclusion, the oral (gavage) LD_{50} value for AL-6515 in mice was greater than 2 g/kg. The IP LD_{50} value for AL-6515 in mice was greater than 1 g/kg.

130:38520:0995: Acute oral toxicity evaluation of AL-6515 in rats (up and down procedure). Vol. 6

Key study findings: The oral (gavage) and IP LD_{50} values for AL-6515 in rats were greater than 0.1 g/kg.

Report N^{o}: 130:38520:0995
Compound: 10% AL-6515 suspension, Lot#: 95-13356-1
Dose: See table below
Dosing regimen: Single dose
Route: Oral (by gavage) or intraperitoneal injection
Animal: Sprague Dawley rats, 181-207 g for females and 208-245 g for males, 2/sex/group
Study site: Alcon Laboratories, Inc., Fort Worth, Texas
Study initiation: 8/18/1995
GLP: Yes

Study design:

Group	n/sex	Dose (g/kg)	Dosing volume (ml/kg)	Route
1	2	0.1	10	IP injection
2	2	0 25	10	IP injection
3	2	0 5	10	IP injection
4	2	0 1	10	Oral gavage
5	2	0 5	10	Oral gavage
6	2	1	10	Oral gavage

The purpose of this study was to determine the toxicity potential of AL-6515 in rats following a single po or ip dose. The day of dosing was designated as Day 1. Surviving animals were observed for 14 days. Toxicity was assessed as shown below.

Clinical observations: 0.5, 1, 2, 4, and 6 hr after dosing and twice daily thereafter
Body weights: Prior to the treatment initiation and on Days 7 and 14
Necropsy: Animals that were found dead during the observation period

Results:

Mortality: No animals treated with AL-6515 at 0.1 g/kg via either oral route or ip route were found dead or moribund during the observation period. Mortality was seen in all other groups and is summarized in the table below.

Summary of mortality

Group	n/sex	Dose (g/kg)	Route	Mortality
1	2	0 1	IP injection	0
2	2	0 25	IP injection	1 ♀ (Day 6)
3	2	0 5	IP injection	1 ♂ (Day 7) and 2 ♀ (Days 6 and 7)
4	2	0 1	Oral gavage	0
5	2	0 5	Oral gavage	2 ♂ (Days 8 and 9)
6	2	1	Oral gavage	2♀ (Days 6 and 7)

Clinical observations: Abnormal findings, summarized in the table below, included red exudates on face, less activity, swollen abdomen, and stool changes (no stool, little stool, or loose stool).

Summary of positive clinical observations

Group	n/sex	Dose (g/kg)	Route	Less active	Red exudates on face	Abdomen appeared swollen
1	2	0 1	IP injection		2♀ (Days 3-5) and 1♂ (Days 3-5, 12-14)	1♂ (Days 13 and 14)
2	2	0.25	IP injection	All animals, Day 1 1♀, Days 4 and 5	All animals, 3-12 days	2♂ (Days 8-10)and 1 ♀ (Days 8-14)
3	2	0 5	IP injection	1♂(Day 1) and 2♀ (Days 1-3)	All animals, 2-11 days	2♂ (Days 6 and 7) and 1♀(Days 5-14)
4	2	0 1	Oral gavage		2♀ (Days 3-5)	
5	2	0 5	Oral gavage	2 ♂ (Day 4 and Days 7-9)	All animals, 1-7 days	All animals (Days 7-12)
6	2	1	Oral gavage	1♀ (Day 6)	All animals, 3-11 days	All animals, 1-9 days

Body weights: No abnormal findings were seen in animals at 0.1 g/kg by either po or ip route. In all other groups, body weight was decreased in the first week and went back in the second week.

Gross necropsy: Seven of eight dead animals showed stomach distended with fluid. One 0.5 g/kg IP female showed peritoneal cavity with clear fluid, distended intestine and enlarged spleen.

In conclusion, the oral (gavage) and IP LD_{50} values for AL-6515 in rats were greater than 0.1 g/kg.

2.6.6.3 Repeated-dose toxicity

044:30:0501: Topical ocular irritation evaluation of nepafenac ophthalmic suspension used in conjunction with corneal incisions in New Zealand white rabbits. Vol. 6

Key study findings: Nepafenac ophthalmic suspensions at concentrations up to 1.0% exhibited a low ocular irritation potential when topically administered prior and subsequent to a corneal incision. No postoperative ocular complications were observed.

Report no.: 044:30:0501
Conducting laboratory and location: Alcon Research, Ltd., 6201 South freeway, Fort Worth, TX 76134
Date of study initiation: 3/1/2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Nepafenac ophthalmic suspension
Study Design:

Groups	Concentration (%)	Batch #	Dosing volume (µl)	N/sex
1 sham control				4
2 Control (vehicle)	0	00-27883	80	4
3 Nepafenac ophthalmic suspension	0.1	00-27875	80	4
4 Nepafenac ophthalmic suspension	0.3	00-27877	80	4
5 Nepafenac ophthalmic suspension	1.0	01-28142	80	4

The purpose of this study was to evaluate the potential ocular effects of various concentrations of nepafenac ophthalmic suspension prior and subsequent to a corneal incision in rabbits. Animals were treated with AL-6515 starting 7 days prior to corneal incision and continuing for a total of 34 days of treatment. The day of the first dosing was designated as Day 1. The incision, made on Day 8, was an approximately 3-5 mm partial depth (approximately 60%) corneal incision in the right eye parallel to the limbus at approximately 9 o'clock. The left eye was not treated and served as an untreated contralateral control. The sponsor did not indicate if anesthesia was used during the ocular incision surgery.

Methods

Doses: 0 (vehicle), 0.1, 0.3, or 1.0% nepafenac ophthalmic suspension, qid (at 2.5 hr intervals). A sham control group receiving the incision but no test article was added for comparative purpose.

Species/strain: New Zealand white rabbits,

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

Route, formulation, volume, and infusion rate: Topical ocular (right eye only), 80 µl/eye

Satellite groups used for toxicokinetics or recovery: N/A

Age: 2-4 months old

Weight (nonrodents only): 2.9-3.5 kg

Unique study design or methodology (if any): A sham control group was included.

Composition of nepafenac ophthalmic suspensions and vehicle

Concentration (w/v %)				
Ingredients	Vehicle	0 1% Nepafenac	0 3% Nepafenac	1% Nepafenac
AL06515,	-	0 1	0 3	1 0 +
Carbopol 974P,				
Tyloxapol, USP				
Glycerin, USP				
Disodium EDTA (edetate disodium), USP				
Benzalkonium Chloride Solution, NF				
Sodium Hydroxide, NF	Adjust pH	Adjust pH	Adjust pH	Adjust pH
Hydrochloric Acid, NF	Adjust pH	Adjust pH	Adjust pH	Adjust pH
Purified Water, USP	QS to 100	QS to 100	QS to 100	QS to 100

Observation times

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Prior to the first treatment, on slit-lamp days, and again immediately prior to necropsy
Biomicroscopic examinations: Pretest and on Days 5, 9, 14, 20, 29 and 34, both eyes
Indirect ophthalmoscopic examinations: Prior to initiation of dosing and on Day 34, both eyes
Corneal pachymetry: Prior to initiation of treatment and on Days 7, 15, and 28, both eyes
Necropsy: All animals, Day 35
Organ weights (specify organs weighed if not in histopath table): The following organs from each animal were weighed: adrenal, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroid and parathyroids, and uterus.
Histopathology: The eyes and adnexa from all animals

Results:

Mortality: No mortality was seen during the observation period.

Clinical observations: No treatment-related abnormal findings were noted.

Body weights: No treatment-related differences in body weights were noted.

Ocular evaluations:

Biomicroscopic evaluations: No drug-related abnormal findings in conjunctival swelling, light reflex, aqueous flare, iritis, fluorescein staining, lens changes, and neovascularization were observed. Minimal to moderate conjunctival congestion (hyperemia, score = 1 or 2) was seen in the eye treated with vehicle and drugs (see table below). Minimal hyperemia (score = 1) was seen in sham control and untreated eyes. Transient and sporadic incidences of minimal conjunctival discharge (score = 1) were noted in the treated eye in Group 2 to 5 animals. A single incidence was seen in the untreated eye in one Group 5 animal on Day 9. In each case, the discharge was resolved by the next biomicroscopic examination. On Day 14, corneal cloudiness was seen in three Group 1 animals, two Group 2 and Group 3 animals, and three Group 4 animals. The cloudiness was minimal (score = 1) and involved the incision site.

Positive findings in slit-lamp biomicroscopic examinations

Group	Day	0	5	9	14	20	29	34	0	5	9	14	20	29	34
Conjunctival congestion, OD		Mean score							Incidence						
1	Female	1.0	0 5	0 8	1 0	0 5	0 5	0 5	4/4	2/4	¼	4/4	2/4	2/4	2/4
1	Male	1 0	0	0 5	0 8	1 0	0 8	0 5	4/4	0/4	2/4	¼	4/4	¼	2/4
2	Female	1.0	0 3	1 0	1 0	0 8	0 8	1 0	4/4	¼	4/4	4/4	¾	2/4	4/4
2	Male	0 3	0 3	0 5	1 0	0 5	0 8	0 5	¼	¼	2/4	¾	2/4	¾	2/4
3	Female	0 8	0.5	1 0	1 3	1 3	1 3	1 0	¾	2/4	¾	4/4	4/4	4/4	¾
3	Male	0 8	0 5	1.0	1 3	1 0	1 0	1 3	¾	2/4	4/4	4/4	4/4	4/4	4/4
4	Female	0 8	0 8	1.3	1 0	1 0	1 3	1 5	3/4	3/4	4/4	4/4	4/4	4/4	4/4
4	Male	0 8	0	1 0	1 3	1 3	1 3	0 8	¾	0	2/4	4/4	4/4	4/4	¾
5	Female	0 8	0 8	1 3	1 0	1 3	1 0	1 5	¾	¾	4/4	4/4	4/4	4/4	4/4
5	Male	0 8	0 5	1 0	1 0	1 0	1 6	0 8	¾	2/4	¾	4/4	4/4	4/4	3/4
Conjunctival discharge, OD															
1	Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Female	0	0	0.5	0	0	0	0	0	0	2/4	0	0	0	0
2	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Female	0	0	0	0 3	0	0	0 3	0	0	0	¼	0	0	¼
3	Male	0	0	0	0	0 3	0 3	0 3	0	0	0	0	¼	¼	¼
4	Female	0	0	0	0	0	0 3	0	0	0	0	0	0	¼	0
4	Male	0	0	0 5	0	0	0	0	0	0	2/4	0	0	0	0
5	Female	0	0	0	0 3	0	0	0	0	0	0	¼	0	0	0
5	Male	0	0	0	0	00	0	0	0	0	0	0	0	0	0

Indirect ophthalmoscopy: No treatment-related, toxicologically significant abnormal findings were noted.

Corneal pachymetry: No treatment-related changes were noted.

Gross examinations: No treatment-related changes were noted in gross examinations.

Histopathological examinations: Minimal to mild corneal epithelial hyperplasia (consistent with regeneration following a surgical incision) was seen in Groups 2, 3, 4, and 5 animals in the treated eyes. Since the incidences were only slightly higher in Groups 4 and 5, there were no clear dose-related increases in severity, and no other lesions (inflammation, ulceration, neovascularization, and so on) related to prolonged irritation were seen, the finding might not be toxicologically significant. The sponsor indicated that the marginal increases in the incidences were more likely related to fortuitous sectioning of small localized incision sites. No other drug-related histopathological findings were noted.

Incidence of corneal epithelial hyperplasia in histologic examination in male and female rabbits

Treatment group	1l	1r	2l	2r	3l	3r	4l	4r	5l	5r
N	8	8	8	8	8	8	8	8	8	8
Corneal hyperplasia	0	0	0	1m*	0	1m	0	2d**	0	3m

* m = minimal; ** d = mild

In summary, NZW rabbits were treated topically with AL-6515 ophthalmic suspension (0.1%, 0.3%, and 1.0%) qid for 34 days. The drug was well tolerated. No toxicologically significant, treatment-related clinical signs, body weight changes, and necropsy and histopathological findings were noted. Minimal to moderate conjunctival congestion and transient and sporadic incidences of minimal conjunctival discharge were seen in the eye treated with vehicle and drugs. In conclusion: nepafenac ophthalmic suspension at concentrations up to 1.0% exhibited a low ocular irritation potential when topically administered prior and subsequent to a corneal incision. No postoperative ocular complications were observed.

140:38520:1195: Three-month topical ocular irritation and systemic toxicity evaluation of AL-6515 ophthalmic suspension in rabbits (one-month interim). Vol. 7

[**Reviewer's comments**: This study was reviewed by Dr. Asoke Mukherjee in February 1996. The following is his review. Minor modifications were made by the current reviewer.]

Key study findings: AL-6515 ophthalmic suspension at concentrations up to 1.0% produced no ocular irritation and systemic toxicity following one month qid topical ocular administration in NZW rabbits.

Report no.: 140:38520:1195
Protocol #: N-95-142
Conducting laboratory and location: Alcon Research, Ltd., 6201 South freeway, Fort Worth, TX 76134
Date of study initiation: 8/31/1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AL-6515 ophthalmic suspension (0.1, 0.3, and 1.0%)

The experiment was conducted in New Zealand white rabbits weighing 2-2.2 kg and 2 months of age at initiation of the experiment. Animals were screened for ophthalmological changes prior to the inclusion in the study. Furthermore, slit-lamp biomicroscopic examinations were conducted on Days 3, 7, 21 and 30. Examinations were related to the changes in conjunctiva, cornea, anterior chamber, light reflex, lens and iris. A pupil was dilated for the evaluation of the lens before the treatment and on Day 30. On Days 0 and 28, an indirect ophthalmic examination was conducted. Animals with any abnormality during the pretreatment screening were excluded from the study. Ultrasound pachymetry was conducted on Days 0 and 28. The test substance or the placebo was applied 4 times a day, one drop per eye for the duration of experiment. The drug formulations were similar to the proposed clinical formulation. All animals were observed twice daily for clinical signs and for mortality. Body weights were recorded weekly. A fasting blood sample was collected on Day 29 for hematology and clinical chemistry from those animals scheduled for the necropsy. Animals designated for interim sacrifice were terminated on Day 31 for organ weight determination, gross and microscopic pathology.

Study design

Group	n/sex	Dosing volume (µl)*	Daily dose (mg)	Treatment days
Untreated control	4	0	0	30
Vehicle control	4	80	0	30
0.1% AL-6515	4	80	0.32	30
0.3% AL-6515	4	80	0.96	30
1.0% AL-6515	4	80	3.2	30

* both eyes, 40 µl for each eye

Results:

One rabbit at 0.3% dose showed ocular discharges from the left eye on Day 27. Other than that there were no abnormal clinical signs noted in these animals. There was no treatment-related loss of the body weight or body weight gain in these animals.

The slit-lamp biomicroscopy study did not show any compound-related abnormalities. Minimal conjunctival congestion (score = 1) was noted in all animals except one Group 5 female. One Group 1 male

showed moderate congestion (score = 2) on Day 7. Severe congestion (score = 3) was seen in one Group 3 male on Day 7 with focal hemorrhage on the third eyelid's anterior surface. These changes resolved by the next slit-lamp evaluation. Two Group 2 animals (one male and one female), one Group 3 male, and three Group 4 animals (one male and two females) showed minimal conjunctival discharge (score = 1). All of these findings were considered as incidental. Data for the indirect ophthalmoscopic and pachymetry examinations did not show significant treatment-related changes.

There were no remarkable changes in the blood chemistry and hematology data.

Gross necropsy examinations showed no drug-related abnormal findings. Organ weight data showed that the weight of spleen was increased in the vehicle control and HD groups for male animals. The weight of spleen in male rabbits was 0.823, 1.215, 1.025, 0.885, and 1.198 g in Groups 1-5. Due to the lack of dose-response, it was considered incidental.

There was no evidence of drug-related histopathological changes in any animals examined. Minimal chronic keratitis was observed at 1.0% dose in one male rabbit.

In summary, NZW rabbits treated topically with AL-6515 ophthalmic suspension (0.1%, 0.3%, and 1.0%) qid for 30 days showed no local and systemic toxicity. The drug was well tolerated. No toxicologically significant, treatment-related findings in clinical observations, body weight changes, ophthalmic examinations, clinical pathological examinations, and necropsy and histopathological examinations were noted.

032:38520:0196: Three-month topical ocular irritation and systemic toxicity evaluation of AL-6515 ophthalmic suspension in rabbits. Vol. 8

Key study findings: AL-6515 ophthalmic suspension at concentrations up to 1.0% produced no significant ocular irritation and systemic toxicity following three-month qid topical ocular administration in NZW rabbits.

Report no.: 032:38520:0196
Protocol #: N-95-142
Conducting laboratory and location: Alcon Research, Ltd., 6201 South freeway, Fort Worth, TX 76134
Date of study initiation: 8/31/1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AL-6515 ophthalmic suspension (0.1, 0.3, and 1.0%)
Study design

Group	n/sex	Dosing volume (µl)*	Daily dose (mg)	Treatment days
Untreated control	6	0	0	91♂, 92♀
Vehicle control	6	80	0	91♂, 92♀
0.1% AL-6515	6	80	0.32	91♂, 92♀
0.3% AL-6515	6	80	0.96	91♂, 92♀
1.0% AL-6515	6	80	3.2	91♂, 92♀

* both eyes, 40 µl for each eye

The purpose of this study was to determine the ocular irritation potential of AL-6515 ophthalmic suspension resulting from qid topical ocular administration to NZW rabbits for three months.

Methods

Doses: 0.1%, 0.3%, and 1.0% AL-6515 ophthalmic suspensions, one drop per eye, both eyes, qid x 3 months

Species/strain: New Zealand white rabbits

Number/sex/group or time point (main study): 10 (4/sex/group rabbits were assigned to the one-month interim evaluation. See review for Study 140:38520:1195.)

Route, formulation, volume, and infusion rate: Topical ocular, 40 µl/eye, both eyes, qid x 3 months

Satellite groups used for toxicokinetics or recovery: N/A

Age: 3 months old

Weight (nonrodents only): 2.0-2.3 kg

Unique study design or methodology (if any): No

Composition of AL-6515 ophthalmic suspensions and vehicle

Component (%, w/v)	Vehicle	0.1% AL-6515	0.3% AL-6515	1.0% AL-6515
Lot#	95-13367-1	95-13368-1	95-13369-1	95-13370-1
AL-6515		0.1	0.3	1.0
Benzalkonium chloride				
Carbomer 974P				
Tyloxapol				
Edentate disodium				
Mannitol				
Sodium chloride				
NaOH or HCl	pH	pH	pH	pH
Purified water	qs 100	qs 100	qs 100	qs 100

Observation times

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Biomicroscopic examination: Days 0, 3, 7, 21, 30, 42, 56, 70, 84 and 91
Indirect ophthalmoscopy and pachymetry: Days 0, 28, and 91
Hematology: Day 90/91
Clinical chemistry: Day 90/91
Gross pathology: All animals, Day 91 (males) or 92 (females)
Organ weights: See Histopathology Inventory Table
Histopathology: The eyes, adnexa and nasal lacrimal tissues from all animals along with the tissues listed in the Histopathology Inventory Table from Groups 1 and 5 animals, along with any gross lesions from Groups 2, 3 and 4 animals were examined microscopically.

Results:

Clinical observations: No mortality occurred during the observation period. Ocular discharge was seen for only one or two days in one Group 2 females, three Group 3 males, one Group 4 male and one Group 5 female. Because of the very low incidence, it was not judged as biologically relevant. One Group 4 female showed a swollen and matted shut left eye. Slit-lamp examination showed moderate congestion and swelling(score = 2), severe discharge (score = 3), moderate flare and iritis (score = 2). A slit-lamp examination on Day 30 determined the site resembled a healed corneal ulcer. This incident was not

considered as drug-related. In conclusion, No toxicologically significant, treatment-related clinical signs were noted in clinical observations.

Body weights: No toxicologically significant differences in body weight changes were noted between control and drug-treated animals.

Ocular evaluations:

Biomicroscopic evaluations: No toxicologically significant, drug-related abnormal findings in slit-lamp biomicroscopic examinations were observed. Minimal conjunctival congestion (hyperemia, score = 1) was seen all control and treated animals. Other abnormal findings are summarized in the table below. All these findings were resolved by the next slit-lamp examination and were considered as incidental.

Positive findings in slit-lamp biomicroscopic examinations

Group	1	2	3	4	5
Minimal conjunctival discharge		2♂	1♂, 1♀	1♀	2♂
Moderate conjunctival discharge	1♂ (Day 3)				
Minimal flare	1♂, 2♀ (Day 70)			1♀ (Day 70)	
Moderate corneal cloudiness			1♀ (Day 70)	1♀ (Day 30)	

Indirect ophthalmoscopy and corneal pachymetry: No treatment-related, toxicologically significant abnormal findings were noted.

Clinical pathology: No treatment-related, toxicologically significant abnormal findings in hematology and clinical chemistry examinations were noted.

Gross examinations: Positive findings are summarized in the table below. No treatment-related changes were noted in gross examinations.

Positive gross necropsy findings

Group	1	2	3	4	5
Bilateral mildly pitted kidneys	1♂				
Para-ovarian cyst		1♀ (several pinpoints, bilateral)			1♀ (0.1 cm^3, left ovary)
Bladder thickened and filled with 15 ml thick yellow pasty material			1♂*		
Urinary bladder contained bone-like substance					1♀*
Serrus atrophy of fat adjacent to urinary bladder				1♂	

* Histopathological examination showed urinary bladder calculi.

Organ weights: No treatment-related, toxicologically significant abnormal findings in organ weights were noted

Histopathological examinations: No drug-related histopathological findings in ocular or systemic tissues were noted. Higher incidences of minimal urinary calculi (urinary bladder and urethra) were seen with treated animals than with untreated control (see table below). The sponsor indicated that spontaneous urolithiasis was common in laboratory rabbits, and there was no distinct evidence of treatment-related renal changes in any animals.

Incidence of urinary and urethra calculi in histological examination in male and female rabbits

Treatment group	1♂	5♂	1♀	5♀
Urinary bladder calculi*	1/6	4/6	0/6	3/6
Urethra calculi*	0/6	1/5	1/5	0/6

* The severity in all animals was rated minimal.

In summary, NZW rabbits treated topically with AL-6515 ophthalmic suspension (0.1%, 0.3%, and 1.0%) qid for 90 days showed no local and systemic toxicity. The drug was well tolerated. No toxicologically significant, treatment-related findings in clinical observations, body weight changes, ophthalmic examinations, clinical pathological examinations, necropsy, and histopathological examinations were noted.

TDOC-0001960: Six-month topical ocular irritation and systemic toxicity evaluation of nepafenac ophthalmic suspension in pigmented rabbits. Vol. 9

Key study findings: AL-6515 ophthalmic suspension at concentrations up to 1.5% exhibited a low ocular irritation potential and did not elicit any signs of ocular or systemic toxicity.

Report no.: TDOC-0001960
Protocol #: N-00-309
Conducting laboratory and location: Alcon Research, Ltd., 6201 South freeway, Fort Worth, TX 76134
Date of study initiation: 1/12/2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AL-6515 ophthalmic suspension (0.3, 1.0 and 1.5%)
Study design

Group	n/sex	Dosing volume (µl)*	Treatments/day	Treatment days
Untreated control	7	0	0	180-181
Vehicle control	7	80	3	180-181
0.3% AL-6515	7	80	3	180-181
1.0% AL-6515	7	80	3	180-181
1.5% AL-6515	7	80	3	180-181

* right eyes only

The purpose of this study was to determine the ocular irritation and systemic toxicity potential of various concentrations of AL-6515 ophthalmic suspension resulting from tid topical ocular administration to pigmented rabbits for 6 months.

Methods

Doses: 0.3, 1.0 and 1.5% AL-6515 ophthalmic suspensions, two drops per eye, right eyes only, tid x 6 months

Species/strain: F1 Cross (NZW x NZR) rabbits

Number/sex/group or time point (main study): 7

Route, formulation, volume, and infusion rate: Topical ocular, 80 µl/eye, right eyes only, tid (at 3.5 hr intervals) x 6 months. The left eye was untreated and served as a contralateral control.

Satellite groups used for toxicokinetics or recovery: N/A

Age: 4-5 months old

Weight: 2.4-3.5 kg

Unique study design or methodology (if any): No

Composition of AL-6515 ophthalmic suspensions and vehicle

Ingredients	Concentration (w/v%)			
	Vehicle	0.3% AL-6515	1.0% AL-6515	1.5% AL-6515
Lot#	00-27883	00-27877	00-27879, 01-21083	00-27881, 01-28085
AL-6515	-	0.3	1.0*	1.5*
Carbopol 974P				
Tyloxapol				
Glycerin				
Disodium EDTA				
Benzalkonium chloride				
Sodium Hydroxide	q.s. pH	q.s. pH	q.s. pH	q.s. pH
Hydrochloric Acid	q.s. pH	q.s. pH	q.s. pH	q.s. pH
Purified Water, USP	q.s. 100	q.s. 100	q.s. 100	q.s. 100

Observation times

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Prior to the first treatment, during Weeks 1, 2, 4, 6, 8, 10 and 12, and then monthly to study completion
Biomicroscopic examination: Weeks 1, 2, 4, 6, 8, 10 and 12, and then monthly to study completion
Indirect ophthalmoscopy: After approximately three and six months of treatment
Pachymetry and IOP: Prior to the initiation of dosing and at approximately 1, 3 and 6 months of treatment
Specular microscopy: During prescreen activities and near completion of the six-month study
Clinical pathology: After approximately 3 and 6 months of treatment
Gross pathology: All animals
Organ weights: See Histopathology Inventory Table
Histopathology: The eyes, and adnexa tissues from all animals along with the tissues listed in the Histopathology Inventory Table from Groups 1 and 5 animals, along with any gross lesions from Group s 2, 3 and 4 animals were examined microscopically.
TK: Please see Pharmacokinetics section

Results:

Clinical observations: No mortality occurred during the observation period. No toxicologically significant, treatment-related clinical signs were noted in clinical observations.

Body weights: No toxicologically significant differences in body weight changes were noted between control and drug-treated animals.

Ocular evaluations:

Biomicroscopic evaluations: No toxicologically significant, drug-related abnormal findings in slit-lamp biomicroscopic examinations were observed. Transient and sporadic instances of minimal conjunctival congestion (hyperemia, score = 1) were observed in both treated and untreated eyes in vehicle control, untreated control, and drug-treated animals with the similar incidence. A single instance of minimal conjunctival discharge was noted in one Group 1 animal (left eye, Day 40), one Group 3 animal (right eye, Day 54), and one Group 5 animal (right eye, Day 40). Low incidences of minimal aqueous flare were seen in both control and treated animals (see table below). All of these findings (conjunctival congestion, discharge and aqueous flare) were not drug-related. There were no conjunctival swelling, light reflex

changes, iritis, neovascularization, corneal cloudiness, fluorescein staining, and lenticular changes in any of the control and treated groups throughout the study.

Positive findings of minimal aqueous flare in slit-lamp biomicroscopic examinations

Group	1	2	3	4	5
Minimal conjunctival discharge	1♀ (OS, Day 40)		1♀ (OD, Day 54)		1♂ (OD, Day 40)
Minimal aqueous flare	OD 3♂ 2♀, Days 82, 110, 1♀, Day 180, OS 1♀ 3♂, Day 82, 2♂2♀, Day 110	OS 1♂, Day 82), OD 1♂, Day 82, 1♀, Day 110	OD 1♂, Days 68, 82, 1♂1♀, Day 110, OS 1♂, Days 68, 82, 1♀2♂, Day 110	OD 1♂, Day 68, 1♂1♀, Day 82, OS 1♀, Day 82	OD 1♂, Day 110; OS 1♀, Day 82, 1♂, Day 110

Indirect ophthalmoscopy: Indirect ophthalmoscopy examinations demonstrated that the optic nerve head and major retinal and choroidal vessels remained within normal limits for both treated and untreated control eyes during the six month treatment phase.

Pachymetry: No treatment-related, toxicologically significant abnormal findings were noted.

IOP: No treatment-related, toxicologically significant differences between control and treated animals were noted.

Specular microscopy: No treatment-related, toxicologically significant abnormal findings in corneal endothelial cell density were noted.

Clinical pathology: No treatment-related, toxicologically significant abnormal findings in hematology and clinical chemistry examinations were noted.

Gross examinations: No treatment-related changes were noted in gross examinations.

Organ weights: No treatment-related, toxicologically significant abnormal findings in organ weights were noted

Histopathological examinations: No drug-related histopathological findings in ocular or systemic tissues were noted.

In summary, pigmented rabbits treated topically with AL-6515 ophthalmic suspensions (0.3%, 1.0%, and 1.5%) tid for 6 months showed no local and systemic toxicity. The drug was well tolerated. No toxicologically significant, treatment-related findings in clinical observations, body weight changes, ophthalmic examinations, clinical pathological examinations, necropsy, and histopathological examinations were noted.

TDOC-0001434: Three-month topical ocular irritation and systemic toxicity evaluation of nepafenac ophthalmic suspension in nonhuman primates. Vol. 10

Key study findings: AL-6515 ophthalmic suspension at concentrations up to 1.0% exhibited a low ocular irritation potential and did not elicit any signs of ocular or systemic toxicity. The NOEL of nepafenac (AL-6515) ophthalmic suspension was greater than 1%.

Report no.: TDOC-0001434

Protocol #: N-03-090
Conducting laboratory and location: Alcon Research, Ltd., 6201 South Freeway, Fort Worth, TX 76134
Date of study initiation: September 2003
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AL-6515 ophthalmic suspension (0.1, 0.3 and 1.0%)
Study design

Group	n/sex	Dosing volume (µl)*	Treatments/day	Treatment days
1 Vehicle control	4	80	4	113
2 0.1% AL-6515	4	80	4	113
3 0.3% AL-6515	4	80	4	113
4 1.0% AL-6515	4	80	4	113

* right eyes only

The purpose of this study was to determine the ocular irritation and systemic toxicity potential of various concentrations of AL-6515 ophthalmic suspensions resulting from qid topical ocular administration to monkeys for three months.

Methods

Doses: 0.1, 0.3 and 1.0% AL-6515 ophthalmic suspensions, two drops per eye, right eyes only, qid x 3 months

Species/strain: Cynomolgus monkeys

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

Route, formulation, volume, and infusion rate: Topical ocular, 80 µl/eye (two drops), right eyes only, qid x 3 months. The left eye was untreated and served as a contralateral control.

Satellite groups used for toxicokinetics or recovery: N/A

Age: Young adults

Weight (nonrodents only): 2.2-3.1 kg for females and 2.0-3.0 kg for males

Unique study design or methodology (if any): No

The formulations listed below were the same as the proposed clinical formulation with the exception of AL-6515 concentrations.

Composition of AL-6515 ophthalmic suspensions and vehicle

Ingredients	Concentration (w/v%)			
	Vehicle	0.3% AL-6515	1.0% AL-6515	1.5% AL-6515
AL-6515	-	0.1	0.3	1.0
Carbopol 974P				
Tyloxapol				
Mannitol				
Disodium EDTA				
Benzalkonium chloride				
NaCl				
Sodium Hydroxide	q.s. pH	q.s. pH	q.s. pH	q.s. pH
Hydrochloric Acid	q.s. pH	q.s. pH	q.s. pH	q.s. pH
Purified Water, USP	q.s. 100	q.s. 100	q.s. 100	q.s. 100
Lot#	03-500531-1	03-500529-1	03-34551	03-34705

Observation times

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Prior to the first treatment, on Days 6, 13, 27, 41, 55, 69, 83 and 104

Biomicroscopic examination: At prescreen and on Study Days 6, 13, 27, 41, 55, 69, 83 and 104
Indirect ophthalmoscopy: Prior to the initiation of dosing (prescreen) and on Study Day 104
Pachymetry and IOP: Prior to the initiation of dosing and on Days 30 and 103
Specular microscopy: Prior to the initiation of dosing and on Day 93
Clinical pathology: Prior to the initiation of dosing and on Day 103
Gross pathology: All animals
Organ weights: See Histopathology Inventory Table
Histopathology: The eyes and adnexa tissues from all animals along with the tissues listed in the Histopathology Inventory Table from all animals were examined microscopically.
TK: Please see Pharmacokinetics section

Results:

Clinical observations: No mortality occurred during the observation period. No toxicologically significant, treatment-related clinical signs were noted in clinical observations.

Body weights: No toxicologically significant differences in body weight changes were noted between control and drug-treated animals.

Ocular evaluations:

Biomicroscopic evaluations: No toxicologically significant, drug-related abnormal findings in slit-lamp biomicroscopic examinations were observed. There were no conjunctival congestion, swelling, light reflex changes, iritis, neovascularization, corneal cloudiness, fluorescein staining, and aqueous flare in any of the control and treated groups throughout the study. Posterior axial capsular and subcapsular cataracts were observed beginning on Day 41 until study completion in both eyes of one LD male animal. Since the lenticular changes were seen in both treated and untreated eyes, and similar changes were not seen in any other animals, they were not attributed to treatment with AL-6515. A few incidences of conjunctival discharge were noted in treated animals (see table below). Since the incidences were low and were noted in both treated and untreated eyes, these changes were not considered drug-related.

Positive findings of conjunctival discharge in slit-lamp biomicroscopic examinations

Group	1	2	3	4
Minimal conjunctival discharge		1♀, OS, Day 6	1♀, OD, Day 6	1♂, OD, Day 6, 1♀*, OD, Day 6
Moderate conjunctival discharge				1♀*, OS, Day 6

* Same animal

Indirect ophthalmoscopy: The optic nerve head and major retinal and choroidal vessels remained within normal limits for both treated and untreated control eyes during the three month treatment phase.

Pachymetry: No treatment-related, toxicologically significant abnormal findings were noted.

IOP: No treatment-related, toxicologically significant differences between control and treated animals were noted.

Specular microscopy: No treatment-related, toxicologically significant abnormal findings in corneal endothelial cell density were noted.

Clinical pathology: No treatment-related, toxicologically significant abnormal findings in hematology and clinical chemistry examinations were noted. The mean activated partial thromboplastin time (APTT) for HD animals was higher than those in vehicle control (see table below). However, compared with pretest data, vehicle control animals also showed an increase in APTT levels. Similar results were not seen in other studies with AL-6515. The toxicological significance was not determined.

Mean APTT data (sec)

Group	1	2	3	4	1	2	3	4
	Males				Females			
Pretest	23.45	22.9	21.1	23.25	20.95	21.875	20.95	24.625
SD	5.455578	1.823915	1.283225	3.309078	1.181807	2.106142	1.982423	1.466004
Day 103	26.975	27.85	28.9	29.25	26.725	27.975	27.075	29.4
SD	8.546491	3.54542	5.705553	4.18051	3.874167	4.58939	4.68855	1.462874

Gross examinations: No treatment-related changes were noted in gross examinations.

Organ weights: No treatment-related, toxicologically significant abnormal findings in organ weights were noted

Histopathological examinations: No drug-related histopathological findings in ocular or systemic tissues were noted.

In summary, cynomolgus were treated topically with AL-6515 ophthalmic suspensions (0.1%, 0.3%, and 1.0%) qid for 3 months. The drug was well tolerated. No toxicologically significant, treatment-related findings in clinical observations, body weight changes, ophthalmic examinations, clinical pathological examinations, necropsy, and histopathological examinations were noted. The coagulation assay showed an increase in APTT values. The toxicological significance was not determined for this finding since APTT values in the vehicle animals also increased compared with the pretest values. In conclusion, AL-6515 ophthalmic suspensions at concentrations up to 1.0% exhibited a low ocular irritation potential and did not elicit any signs of ocular or systemic toxicity following repeated topical ocular administration to monkeys. An NOEL value was determined greater than 1.0% AL-6515 ophthalmic suspension in this study.

131:38520:0995: Two-week oral toxicity evaluation of AL-6515 in rats. Vol. 11

[**Reviewer's comments**: This study was reviewed by Dr. Asoke Mukherjee in February 1996. The following is Dr. Mukherjee's review. Minor modification was made by the current reviewer.]

Key study findings: Daily oral dosing of AL-6515 at 2.5, 7.5, and 25 mg/kg/day for two weeks produced no significant systemic toxicity potential in SD rats.

Report no.: 131:38520:0995
Protocol #: N-95-141
Conducting laboratory and location: Alcon Research, Ltd., 6201 South freeway, Fort Worth, TX 76134
Date of study initiation: 8/14/1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot#s: 95-13353-1, 95-13354-1, and 95-13355-1

The purpose of this study was to determine the systemic toxicity potential of AL-6515 following once daily oral administration of the drug to rats for two weeks. The study was conducted at Alcon Laboratories according to the GLP. Sprague-Dawley rats with the initial body weight between 245 g and 290 g for males, 196 g and 250 g for females were used in the study. All animals were screened for ophthalmological changes before enrollment in the study. The drug substance was suspended in a vehicle composed of ___ carbopol, 0.05% polysorbate and ___ sodium chloride. Rats were treated by oral gavage. The doses are shown in the following table.

Treatment	n/sex	Dose (mg/5 ml/kg/day)
1 Vehicle	10	0
2 AL-6515	10	2.5
3 AL-6515	10	7.5
4 AL-6515	10	25

Animals were examined twice daily for clinical signs. The body weights were recorded on Days 0, 7, and 14. Eyes were examined by slit-lamp on the initiation of the study and on Day 14. Fluorescein staining was not evaluated. Blood chemistry and hematology were done on the Days 15 and 16 for males and females, respectively. Animals were sacrificed on Day 15 (for males) and Day 16 (for females) for organ weight determination, gross and histological examinations. Microscopic changes were evaluated for the vehicle control and high dose group only. The histopathological evaluation of the liver, spleen, mesenteric lymph node, jejunum and uterus from LD and MD females was also performed.

Results:

There was no abnormal clinical sign recorded during the treatment period. The mean body weights of the rats on Days 0 and 14 are shown in the following table.

Body weight data in rats treated orally with AL-6515 (g)

Treatment	Day 0 ♂	Day 0 ♀	Day 14 ♂	Day 14 ♀
1 Vehicle	264	223	307	230
2 AL-6515	271	215	317	223
3 AL-6515	272	219	322	234
4 AL-6515	267	220	309	223

The body weight change was not significant. There were no ophthalmological changes except corneal opacity observed in the right eye of one MD male, which was considered as incidental.

Among blood chemistry parameters, no toxicologically significant changes were noted. Hematology data showed that hemoglobin levels and RBC counts were reduced in male and female rats at the high dose (see table below). These changes might reflect NSAID-induced hemorrhage. The sponsor indicated that the changes were within the laboratory's reference range.

Mean RBA and HB values in rats treated with AL-6515

RBC 10^6/µl	Males				Females			
Group	1	2	3	4	1	2	3	4
Mean	8.951	8.704444	8.988	7.72	7.61	7.587778	7.331111	5.8975
SD	1.192741	0.965843	0.708642	0.739519	0.369534	0.625176	0.499085	0.850592
HB g%								
Mean	19.36	18.81111	19.87	17.21	16.62	17.15556	17.74444	14.85
SD	1.404912	1.69591	1.911399	1.522753	1.539697	1.333333	1.100126	1.823654

The ovary weight was increased in MD and HD animals (see table below). The individual values for these two groups were generally within the vehicle control group range. The liver weight was also slightly increased in HD females.

Ovary and liver weights in rats treated with AL-6515 (mean ± SD)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Ovary (g)					0 118±0 027	0 122±0 028	**0.149±0.019**	**0.151±0.030**
%					0 056±0 012	0 059±0.014	**0.070±0.012**	**0.075±0.015**
Liver (g)	10 512±1 224	10.980±1 049	11 133±0 970	11 352±0 897	6 548±0 931	6 077±0 546	6 776±0 527	**7.282±0 849**
%	3 647±0 297	3 741±0 243	3 737±0 206	3 965±0 239	3 070±0 307	2 957±0 227	3 165±0 147	**3.614±0.356**

Gross necropsy data are summarized in the table below. No biologically relevant changes were noted.

Gross necropsy findings

Group	1	2	3	4
Lungs mottled yellow, red and tan	1♂ and 1♀ Histology chronic inflammation			
Lungs nodules, multiple, 0.5-5 mm in diameter, firm, irregular, white				1♂ Histology chronic inflammation
Diaphragmatic hernia with entrapped lobe	1♀ Histology. hepato-diaphragmatic nodule			
Kidneys irregular and mottled white and brown, bilateral			1♀ No microscopic correlation	
Uterus moderately diluted fluid filled uterine horns				1♀ Histology dilatation

Possibly treatment-related histopathological findings are summarized in the table below. Histological examinations showed jejunal serositis, extramedullary hematopoiesis (EMH) in the liver and spleen in HD females. Mesenteric lymphoid hyperplasia and dilatation of a uterus were seen in all treated groups in a dose-dependent manner. The sponsor indicated that many of the changes were considered to be secondary to intraabdominal trauma, possibly associated with gavage procedures. However, distinct gavage trauma was not observed grossly or microscopically in abdominal tissues, and a relationship between drug treatment and these findings could not be entirely ruled out.

Histopathological data

	Males				Females			
Group	1	2	3	4	1	2	3	4
Liver: extramedullary hematopoiesis								5/10, (5m)
Spleen: extramedullary hematopoiesis								6/10 (4m2d)
Mesenteric lymph nodes: hyperplasia						1/10(1m)	3/10(3m)	9/10(4m4d1o)
Uterus: dilatation						2/10(2d)	3/10(2m1d)	5/10 (4d1o)
Jejunum: serositis								5/10 (1m2d2o)

m: minimal; d: mild; o: moderate; k: marked

To summarize the data, it can be concluded that AL-6515 up to 25 mg/kg/day oral doses for two weeks did not induce clear systemic toxicity. A relationship between drug treatment and liver/splenic EMH, jejunal serositis, mesenteric lymphoid hyperplasia, and uterine dilatation in HD females could not be entirely ruled out.

025:38520:0196: Three month oral toxicity evaluation of AL-6515 in rats. Vol. 12

Key study findings: Daily oral dosing of AL-6515 at 1 and 5 mg/kg/day for three months produced no significant systemic toxicity potential in SD rats. Renal papillary necrosis was seen in 2 of 10 female HD rats. The dose of 5 mg/kg/day was considered as an NOAEL in this study.

Report no.: 025:38520:0196
Protocol #: N-95-160
Conducting laboratory and location: Alcon Research, Ltd., 6201 South freeway, Fort Worth, TX 76134
Date of study initiation: September 1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot#s: 95-13612-1, 95-13613-1, and 95-13614-1

The purpose of this study was to determine the systemic toxicity potential of AL-6515 following once daily oral administration of the drug to rats for three months. The doses are shown in the following table.

Treatment	n/sex	Dose (mg/5 ml/kg/day)	Treatment days
1 Vehicle	10	0	♂91, ♀92
2 AL-6515	10	1	♂91, ♀92
3 AL-6515	10	5	♂91, ♀92
4 AL-6515	10	15	♂91, ♀92

Methods
Doses: 0, 1, 5, and 15 mg/kg/day, qd x 3 months
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: Oral by gavage, 5 ml/kg/day
Satellite groups used for toxicokinetics or recovery: N/A
Age: Not indicated
Weight: 180-228 g for females and 213-250 g for males
Unique study design or methodology (if any): No

Observation times

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Indirect ophthalmoscopy: Days 0 and 91
Clinical pathology: Day 92 for males and Day 93 for females
Gross pathology: All animals Day 92 for males and Day 93 for females
Organ weights: See Histopathology Inventory Table
Histopathology: Tissues listed in the Histopathology Inventory Table were examined microscopically for control and HD animals.

Results:

Clinical observations: No treatment-related clinical signs were noted.

Body weight: MD and HD males showed a decrease in body weight gain (10%) at the end of the treatment (see table below). However, the final body weights of MD and HD males were about 95% of vehicle control animals. Similar changes were not seen in females. The decreased body weight gain might not be toxicologically significant.

Body weight data in male rats treated orally with AL-6515 (g, mean ±SD)

	Body weight			Body weight gain	
Treatment	Day 0	Day 91	% control	Day 0-91	% control
1 Vehicle	236±7 5	418+27 3	100	182	100
2 AL-6515, 1 mg/kg	236±8 9	423±31 6	100	187	100
3 AL-6515, 5 mg/kg	231±7 2	396+28 5	94 7	165	90 7
4 AL-6515, 15 mg/kg	235±8 7	397±30 6	95	162	89

Indirect ophthalmoscopic examination: One Group 4 female showed a lesion resembling segmental atrophy of the temporal optic disc and adjacent retina on Day 91. The cause of the lesion was unknown. Histopathological examination showed no treatment-related findings in the retina. This finding might not be drug-related.

Clinical pathology: No toxicologically significant abnormal findings in clinical chemistry and hematology examinations were noted.

Gross pathology: Gross necropsy data are summarized in the table below. None of the findings were considered treatment-related.

Gross necropsy findings and corresponding histologic changes

Group	1	2	3	4
Lungs mottled, red and tan, slightly firm				1♀ Histology inflammation, granulomatous
Lungs nodules, multiple, 1-5 mm in diameter, solid, hard, white	1♂ and 1♀ Histology inflammation, granulomatous			1♂ Histology inflammation, granulomatous 1♀ Histology alveolar macrophages
Thick green exudates in region of preputial gland		1♂ Histology chronic inflammation	3♂ Histology chronic inflammation	
Lungs: all lobes, multiple foci, 5 mm in diameter, white				1♀ Histology no corollary changes

Organ weights: The adrenal (in males) and ovary (in females) weight was increased in treated animals (see table below). Without corresponding histological findings, the toxicity significance was not determined.

Ovary and adrenal weights in rats treated with AL-6515 (mean ± SD)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Ovary (g)					0 109±0 036	**0.138±0.032**	**0.137±0.047**	**0.131±0.024**
%					0 041±0.013	**0.053±0.012**	**0.053±0.019**	**0.051±0.008**
Adrenal (g)	0 056±0.020	**0.065±0.022**	**0.067±0.021**	**0.071±0.028**	0 081±0 023	0 084±0.013	0 088±0 014	0 081±0 010
%	0.014±0.005	**0.016±0.005**	**0.017±0.005**	**0.018±0.007**	0.031±0.010	0 032±0.006	0.034±0 005	0 032±0 005

Histopathology: Renal papillary necrosis was seen in two HD females (one minimal and one mild). Similar findings were not seen in male animals and in MD and LD females. The sponsor indicated that renal papillary necrosis was a common finding with NSAIDs. No other drug-related findings were noted.

In summary, SD rats were treated orally (by gavage) with AL-6515 at 1, 5, and 15 mg/kg/day for three months. No toxicologically significant drug-related abnormal findings in clinical observations, indirect

ophthalmoscopic evaluation, clinical chemistry and hematology, and gross necropsy examinations. Slight body weight gain decrease was seen in MD and HD males. Histopathological examination showed renal papillary necrosis in two HD females. The dose of 5 mg/kg/day was determined as the NOAEL in this study.

TDOC-0001935: Six-month oral (gavage) toxicity study of AL-6515 in rats. Vol. 13

Key study findings: Daily oral dosing of AL-6515 at 1, 3 and 10 mg/kg/day for 6 months produced no significant systemic toxicity potential in rats. The NOAEL was greater than 10 mg/kg/day in this study.

Report no.: TDOC-0001935
Protocol #: N-01-024
Conducting laboratory and location:

Date of study initiation: March 21, 2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot#s: 8443:024 and 8443:090

The purpose of this study was to determine the systemic toxicity potential of AL-6515 following once daily oral administration of the drug to rats for 6 months. The doses are shown in the following table.

Treatment	n/sex (main study)	n/sex (TK)	Dose (mg/5 ml/kg/day)
1 Vehicle	25		0
2 AL-6515	25	27	1
3 AL-6515	25	27	3
4 AL-6515	25	27	10

Methods

Doses: 0, 1, 3, and 10 mg/kg/day, qd x 6 months
Species/strain: Fisher F344 rats [— (F344) —]
Number/sex/group or time point (main study): 25
Route, formulation, volume, and infusion rate: Oral by gavage, 5 ml/kg/day, AL-6515 was suspended in 0.5% carboxymethylcellulose
Satellite groups used for toxicokinetics or recovery: 27/sex/group
Age: 6-8 weeks old
Weight (nonrodents only): Not indicated
Unique study design or methodology (if any): No

Observation times

Mortality: At least once daily
Clinical observations: Weekly
Body weights: Weekly during the first 14 weeks and every other week thereafter
Food consumption: Weekly during the first 14 weeks and biweekly thereafter
Indirect ophthalmoscopy: Pretest and Week 25
TK: See Pharmacokinetics section
Clinical pathology: Weeks 13 (10 rats/sex/group) and 27

Gross pathology: All animals
Organ weights: See Histopathology Inventory Table
Histopathology: Tissues listed in the Histopathology Inventory Table were examined microscopically for control and HD animals.

Results:

Mortality: No mortality occurred during the study period in the main study groups. The sponsor indicated that one TK rat died but no detailed information was provided.

Clinical observations: No treatment-related clinical signs were noted. Clinical signs including alopecia (forelimb), chromodacryorrhea, red material around eye and/or nose, discolored and/or wet inguinal fur, discoloration around the mouth and discolored paws were seen in both control and treated groups with similar incidences.

Body weight: No treatment-related differences in body weights were noted between control and treated animals.

Food consumption: No treatment-related differences in food consumption were noted between control and treated animals.

Indirect ophthalmoscopic examination: No treatment-related abnormalities were observed.

Clinical pathology: No toxicologically significant abnormal findings in clinical chemistry, hematology, coagulation, and urinalysis examinations were noted.

Gross pathology: Gross necropsy data are summarized in the table below. None of the findings were considered treatment-related.

Gross necropsy findings

Group	1		2		3		4	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
No gross lesions	18	23	19	20	21	14	19	18
Lung, focus	1		1		1		1	
Thymus, small	4							
Thymus, pigmentation, mottled/red			2	1	2		1	
Thymus, focus, red						1	1	
Liver, median lobe, nodule*	1					5	3	1
Lymph node, mediastinal, enlarged, red	1							
Lymph node, mandibular, pigmentation, red		1	1			1	1	
Lymph node, mandibular, enlarged, red		1			1			
Mesentery, nodule, mottled, tan				1		1		1
Kidney, left, deformity			1					
Skin, inguinal, nodule			1					
Eye, pigmentation, opaque			1	1			2	
Eye, pigmentation, red								1
Eye, small						1		
Uterus, bilateral, dilatation				1		2		2
Ovary, cyst				1		3		3

* This was diagnosed as accessory hepatic tissues.

Organ weights: No toxicologically significant differences in organ weights were noted between control and treated animals.

Histopathology: Infrequent histopathological findings were seen in both control and treated animals with similar incidence and low severity. No positive histology findings were considered as drug-related. Higher incidences of corneal mineralization (5 of 25 in males vs. 0 in control animals) and uterus hydrometra [5 of 25 in HD females (3 minimal, 1 mild, and 1 moderate) vs. control's 1 of 25 (mild)] were seen in HD groups (10 mg/kg/day). Similar changes were not seen in other studies including 6-month ocular toxicity in which 1.0% AL-6515 ophthalmic suspension was used. Corneal and uterus abnormalities were not listed in the common adverse events seen in clinical studies. In addition, the systemic exposure to AL-6515 and AL-6295 at 10 mg/kg/day was much higher than that in humans. These findings might not be toxicologically significant. Mineralization in the arterial walls and kidney cortex was also seen with similar incidences and severity in control and treated groups.

Summary: F344 rats were orally (by gavage) treated with AL-6515 at 1, 3, and 10 mg/kg/day for 26 weeks. The drug was well tolerated. No treatment-related mortality, clinical signs, body weight changes, food consumption changes, abnormal clinical pathology findings, gross and histopathological findings were noted. The dose of 10 mg/kg/day was determined as the NOAEL in this study.

Histopathology inventory

Study	032 38520/0196	TDOC-0001434	TDOC-0001960	131:38520 0995	025 38520/0196	TDOC-0001935
Species	NZW rabbit	Monkeys	F1 rabbits	SD rats	SD rats	F344 rats
Adrenals	+*	+*	+*	+*	+*	+*
Aorta	+	+	+	+	+	+
Bone marrow smear	+	+	+	+	+	+
Bone	+	+	+	+	+	+
Brain	+*	+*	+*	+*	+*	+*
Cecum	+	+	+	+	+	+
Cervix	+	+	+	+	+	+
Colon	+	+	+	+	+	+
Duodenum	+	+	+	+	+	+
Epididymis	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+
Eye	+	+	+	+	+	+
Gall bladder	+	+	+			
Gross lesions	+	+	+	+	+	+
Harderian gland						
Heart	+*	+*	+*	+*	+*	+*
Ileum	+	+	+	+	+	+
Injection site						
Jejunum	+	+	+	+	+	+
Kidneys	+*	+*	+*	+*	+*	+*
Lachrymal gland	+	+	+	+	+	
Larynx	+	+	+	+	+	
Liver	+*	+*	+*	+*	+*	+*
Lungs	+	+	+	+	+	+
Lymph nodes mandibular						+
Lymph nodes, mesenteric	+	+	+	+	+	+
Lymph nodes, cervical	+	+	+	+	+	
Mammary Gland	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	
Optic nerves	+	+	+	+	+	+
Ovaries	+*	+*	+*	+*	+*	+*
Oviduct	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+
Parathyroid	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	
Pharynx						
Pituitary	+	+	+	+	+	+
Prostate	+	+	+	+	+	+
Rectum	+	+	+	+	+	+
Salivary gland	+	+	+	+	+	+
Sciatic nerve		+	+	+	+	+
Seminal vesicles	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+
Skin	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+
Spleen	+*	+*	+*	+*	+*	+*
Sternum	+	+	+	+	+	+
Stomach	+	+	+	+	+	+
Testes	+*	+*	+*	+*	+*	+*
Thymus	+	+	+	+	+	+*
Thyroid	+	+	+	+	+	+*
Tongue	+	+	+	+	+	+
Trachea	+	+	+	+	+	+

Ureters		+	+	+	+	
Urethra		+	+	+	+	
Urinary bladder	+	+	+	+	+	+
Uterus	+	+	+	+	+	+
Vagina	+	+	+	+	+	+
Zymbal's gland						+

+, histopathology performed; *, organ weight obtained

6.6.6.4 Genetic toxicology

141:38520:1195: Mutagenicity test with AL-6515 in the *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay. Vol. 14

Key findings: AL-6515 is not mutagenic in the Ames assay in the presence or absence of S-9 mixtures.

[Reviewer's comments: This study was reviewed by Dr. Asoke Mukherjee in February 1996. The following is his review. Minor modifications were made by the current reviewer.]

The experiment was conducted at ,ccording to the GLP. The tester strains used were several mutants of *Salmonella typhimurium*, i.e., TA98, TA100, TA1535, and TA1537. The strain used for *E. Coli* assay was WP2uvrA. Doses used in the study were 100, 250, 500, 1000, 2500, and 5000 µg/plate in the presence and absence of metabolic activation systems. Three plates were used for each concentration. Positive controls are shown in the following table.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)	Dose µg/plate (w/o S9)
Salmonella typhimurium	TA-1535	2-aminoanthracene 2.5	Sodium azide 2.0
	TA-1537	2-aminoanthracene 2.5	ICR-191 2.0
	TA-98	2-aminoanthracene 2.5	2-nitrofluorene 1.0
	TA-100	2-aminoanthracene 2.5	Sodium azide 2.0
Escherichia coli	WP2uvrA	2-aminoanthracene 25	4-nitroquinoline-N-oxide 1.0

DMSO was used as the vehicle. It is not clear from the report whether the drug solution was stable in the plate so as to prevent a hydrolysis of AL-6515 to amfenac. There was no cytotoxicity to the compound in the Ames assay at the highest dose tested. AL-6515 did not show any increase in the number of revertant colonies in the presence or absence S9 activation. All positive controls showed an increase in the number of colonies. Therefore, it was concluded that AL-6515 is not mutagenic in the Ames assay in the presence or absence of S-9 mixtures.

007:38520:0298: *In vitro* mammalian cell gene mutation test with an independent repeat assay with AL-6515. Vol. 14

Key findings: AL-6515 was not considered mutagenic at the TK locus of mouse lymphoma cells in the presence and absence of S9 activation under the present testing conditions.

Study no.: 007:38520:0298
Protocol #: N-98-021
Conducting laboratory and location

Date of study initiation: 7/23/1996
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot#: 5198:088, purity =

Methods

Strains/species/cell line: Mouse lymphoma L5178Y cell line

Doses used in definitive study: 50 to 2500 μg/ml without or with S9 activation. In the preliminary toxicity assay, a visible precipitation was seen at 2500 μg/ml. At 1000 μg/ml, the medium was cloudy with no visible precipitation. Concentrations of ≤ 500 μg/ml were soluble. Substantial toxicity was noted at ≥ 500 μg/ml with or without S9 activation. Based on these findings, concentrations of 50 to 2500 μg/ml were selected for both nonactivated and S9 activated cultures.

Basis of dose selection: Solubility and cytotoxicity in the preliminary study

Negative controls: DMSO

Positive controls: Methyl methanesulfonate (MMS, 1 and 2 mg/ml) w/o S9 activation; 7, 12-Dimethyl-benz(a)anthracene (7,12-DMBA, 250 and 400 μg/ml) w/ S9 activation

Results

Study validity: The doses used in this study were up to 2500 μg/ml. Cytotoxicity evidenced by 80% of inhibition in total growth was seen at concentrations ≥ 300 μg/ml in both S9-activated or nonactivated cultures. The positive controls produced typical positive results. The mutant colonies from vehicle controls were within the historical control range. The study was valid.

Study outcome: More than 80% of the relative total growth inhibition as cytotoxicity was produced in the presence and absence of S9 activation, respectively. In both initial assay and independent repeat assay, AL-6515 did not increase the mutant frequency. AL-6515 was not considered mutagenic at the TK locus of mouse lymphoma cells in the presence and absence of S9 activation under the present testing conditions.

008:38520:0298: *In vitro* mammalian cytogenetic test with an independent repeat assay with AL-6515. Vol. 14

Key findings: AL-6515 was positive for the induction of structural chromosome aberrations in this study.

Study no.: 008:38520:0298
Protocol #: N-98-020
Conducting laboratory and location:

Date of study initiation: 8/12/1996
GLP compliance: yes
QA reports: yes (X) no ( )

Drug, lot #, and % purity: AL-6515, Lot#: 5198:088, purity = —

Methods

Strains/species/cell line: Chinese hamster ovary ($CHO\text{-}K_1$) cells

Doses used in definitive study: 40, 79, 157, 313, 625, 1250, and 2500 µg/ml with or without S9

Basis of dose selection: Cytotoxicity after the test article treatment in the initial assay

Negative controls: DMSO

Positive controls: Mitomycin C (MMC) 0.08 and 0.15 µg/ml w/o S9 activation; cyclophosphamide (CP) 5, 10, and 20 µg/ml w/ S9 activation

Incubation and sampling times:

	Treatment time (hr)	Harvest time
Initial test with or without S9 activation	6	20 hr after the initiation of treatment
Repeat test without S9 activation	20 or 44	20 or 44 hr after the initiation of treatment
Repeat test with S9 activation	6	20 or 44 hr after the initiation of treatment

Results

Study validity: The doses used in this study were up to 2500 µg/ml. Cytotoxicity evidenced by about 50% of cell growth inhibition was seen at all groups with only one exception in the initial assay with S9 activation in which only 20% inhibition was seen at 2500 µg/ml. The positive controls produced typical positive results. The frequency of cells with structural chromosome aberrations in vehicle and untreated controls were within the historical control range. The study was valid.

Study outcome: In the initial assay, the percentage of cells with structural aberrations in AL-6515-treated groups was not significantly increased above solvent control at any dose levels with or without S9. In the repeat assay, the percentage of cells with structural aberrations in AL-6515-treated groups was not significantly increased above solvent control at any dose levels at the 20 hr harvest time. However, at 44 hr harvest time, a significant increase in the percentage of cells with structural aberrations in AL-6515-treated groups was seen (see table below). In conclusion, under the conditions in this study, AL-6515 was positive for the induction of chromosome aberrations.

Chromosomal aberration data from the repeat assay (% structural aberrations)

	20 hr harvest		44 hr harvest	
	Without S9	With S9	Without S9	With S9
Untreated	3.5	3.0	1.5	1.0
DMSO	2.5	3.5	0.5	2.5
AL-6515 313 µg/ml	3.5	4.0	**14.0**	**11.5**
AL-6515 625 µg/ml	3.5	3.5	**23.5**	**7.0**
AL-6515 1250 µg/ml	3.5	3.0	**11.5**	**4.0**
AL-6515 2500 µg/ml	4.0	3.5	**15.5**	**10.0**
Positive control (MMC 0.08 µg/ml or CP 5 µg/ml)	18.0	29.0	47.0	48.0

006:38520:0298: Micronucleus cytogenetic assay in mice with AL-6515. Vol. 14

Key findings: AL-6515 was negative in the micronucleus assay under the experiment conditions in this study.

Study no.: 006:38520:0298
Protocol #: N-98-022
Conducting laboratory and location: [redacted]
Rockville, MD 20850
Date of study initiation: 7/31/1996
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot#: 5198:088, purity =

Methods

Strains/species/cell line: ICR mice, 29.6-36.1 g for males and 26.0-32.8 g for females for pilot test, 28.8-35.7 g for males and 24.6-29.8 g for females for micronucleus assay, 5/sex/group

Doses used in definitive study: 1250, 2500, and 5000 mg/20 ml/kg, oral by gavage, single dose

Basis of dose selection: Pilot test in which mice were treated with a single oral dose at 1 to 5000 mg/kg and were observed for 3 days. No mortality occurred.

Negative controls: Amfenac oral suspension vehicle

Positive controls: Cyclophosphamide (CP) 60 mg/kg, po (gavage), single dose

Incubation and sampling times: Animals (5/sex/dose/time point) were terminated at 24, 48 and 72 hr after dosing and bone marrow samples were prepared. Positive control animals were sacrificed at 24 hr after dosing. The number of micronucleated polychromatic erythrocyte (MPCE) then was determined for 1000 polychromatic erythrocytes (PCE) per animal. The proportion of polychromatic erythrocytes to total erythrocytes was determined per 1000 erythrocytes.

Results

Study validity: The positive controls produced a significant increase in the number of MPCE. The number of MPCE from vehicle controls was within the historical control range. The study was valid.

Study outcome: All animals appeared normal. AL-6515 at 1250, 2500, and 5000 mg/kg (po by gavage) caused no significant decrease in PCE ratio (PCE/total erythrocytes) and no increase in the number of MPCE. In conclusion, AL-6515 showed no clastogenic effects in this *in vivo* micronucleus assay under the experiment conditions in this study.

Summary of genotoxicity studies:

AL-6515 was not genotoxic in the Ames test nor in L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay. The drug was also negative in *in vivo* micronucleus assay. AL-6515 was positive for the induction of structural chromosome aberrations in CHO cells.

2.6.6.5 Carcinogenicity

A waiver for carcinogenicity studies was granted in October 2004 by Dr. Asoke Mukherjee. No carcinogenicity studies were conducted.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

155:30:0801: A fertility and general reproduction study in rats with AL-6515

Key study findings: Decreased sperm motility, increased early resorption and decreased viable fetuses were observed at doses ≥ 10 mg/kg. The NOEL for reproduction in males and females was determined as 3 mg/kg/day.

Study no.: 155:30:0801
Protocol #: N-96-275
Conducting laboratory and location:

Date of study initiation: October 31, 1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot #: ERM 5198:088

The purpose of this study was to determine the potential toxic effects of AL-6515 when administered orally to rats before mating and through mating, implantation, and early gestation. Following 4 weeks of dosing in males and two weeks of dosing in females, each female was cohabited with a single male from the same dose group and the mating pair was examined daily for evidence of copulation. The day evidence of copulation was confirmed was designated as gestation Day 0.

Methods

Doses: 0, 3, 10, and 15 mg/10 ml/kg/day qd [The males were dosed for 4 weeks prior to mating and dosing was continued until the day prior to scheduled termination (minimum of 11 weeks). The females were dosed for two weeks prior to mating and dosing was continued until gestation Day 6.]

Species/strain: Sprague Dawley rats, males: 10-11 weeks old, 330-466 g; females: 12 weeks old, 210-319 g

Number/sex/group: 25/group

Route, formulation, volume, and infusion rate: Oral (by gavage), 10 ml/kg/day

Satellite groups used for toxicokinetics: No

Study design: See below

Group	Treatment	n/sex	Dosage (mg/kg/day)	Dosing volume (ml/kg/day)
1	Vehicle	25	0	10
2	AL-6515	25	3	10
3	AL-6515	25	10	10
4*	AL-6515	25	30	10
5*	Vehicle	25	0	10
6*	AL-6515	25	15	10

* Due to high toxicity in 30 mg/kg females, the male and female animals were terminated on study Days 29 and 21, respectively. Two additional groups (Groups 5 and 6) were added to the study.

Parameters and endpoints evaluated: See below

Mortality: Twice daily
Clinical observations: Twice daily
Body weights: Twice per week
Food consumption: Twice per week
All males in Groups 1, 2, 3, 5, and 6 were terminated after a minimum of 11 weeks of treatment (study Days 78-81). Organ weights were obtained for the brain, testes, prostate, epididymis, and seminal vesicle. Total sperm count, sperm motility and sperm concentration were also determined.
Pregnant females from Groups 1, 2, 3, 5, and 6 were terminated on gestation Day 15. The uterus and ovaries were examined for reproductive parameters including the number of viable and nonviable fetuses, early resorptions, and the number of corpora lutea.
Histopathology: Male reproductive organs (testes, epididymides, prostate, and seminal vesicles) and gross internal lesions were examined histopathologically from three males of 15 mg/kg group which exhibited the lowest sperm motility and concentration values.

Results

Mortality and clinical observations (dams): Five females at 30 mg/kg died spontaneously during the study. Remarkable clinical signs in the 30 mg/kg group females included decreased activity, convulsion, abnormal excreta, pale extremities, distended abdomen, emaciation, and urine stain. Clinical signs were less severe in the males at 30 mg/kg with no mortality. In other groups, no toxicologically significant findings were noted. One female of 3 mg/kg group was found dead on study Day 16. The death was due to a gavage error.

Body weight (dams): A decrease in body weight and body weight gain was noted in males (first 15 days only) and females in 15 mg/kg and 30 mg/kg groups (see table below). No toxicologically significant findings were noted in males and females at 3 and 10 mg/kg groups.

Body weight data in rats (g, mean ± SD)

Group	1 (vehicle)	2 (3 mg/kg)	3 (10 mg/kg)	4 (30 mg/kg)	5 (vehicle)	6 (15 mg/kg)
Males	**Body weight**					
Day 1	395±29 8	397±32 2	398±29 9	394±28 0	353±9 6	352±9.7
Day 15	441±37 8	442±35.7	445±40 0	429±41 3	407±22.9	396±22.8
Day 29	489±44 4	493±41.9	497±46 9	483±46.7	456±31.6	444±30.3
Day 78	584±57 9	599±55 3	603±55.8		559±48.8	553±44.4
	Body weight gain					
Days 1-4	12±5 1	12±6 4	9±7 4	8±9 9	14±6 6	7±5.1
Days 4-8	10±4 6	11±5 7	11±5.3	9±7 0	17±7.5	14±7 1
Days 8-11	12±5 2	11±5 3	15±5 2	8±5 8	13±5 7	10±5.3
Days 11-15	11±5.8	11±3 6	13±3.6	10±9.4	11±5.8	11±6 9
Days 15-18	17±5 4	19±5 6	18±5.4	16±5 8	10±4.1	9±4 1
Females	**Body weight**					

Day 11 (Day 1 of treatment)	275±25.7	273±24 8	276±23 4	277±23 9	248±8 5	250±8 3
Day 18	281±27 4	277±23 9	279±23 3	259±20 0	255±10.1	252±9 4
Day 25	292±28 1	285±25 9	287±25 3		265±11 9	259±9 9
	Body weight gain					
Days 11-15	7±3 3	3±8 9	3±4 3	-14±13 2	4±3 3	-1±4 6
Gestation	**Body weight**					
Day 0	293±27 8	287±26 0	287±28 4		266±12 8	260±11 2
Day 3	310±27 2	303±27 0	304±26 4		282±12 0	271±13 4
Day 15	361±27 7	361±25 9	364±31 2		330±16 2	322±19 9
	Body weight gain					
Days 0-3	18±6 1	16±11 6	17±7 3		16±3 6	10±5 4
Days 3-7	12±6 7	12±7 5	14±6 2		10±6 5	10±4 2

Food consumption (dams): Similar to the body weight findings, a decrease in food consumption was seen in males and females in 15 and 30 mg/kg/day groups (see table below). No toxicologically significant changes in food consumption were noted in other groups.

Food consumption data in rats (g/animal/day, mean ± SD)

Group	1 (vehicle)	2 (3 mg/kg)	3 (10 mg/kg)	4 (30 mg/kg)	5 (vehicle)	6 (15 mg/kg)
Males						
Days 1-4	30±3 9	29±2 8	28±3 0	27±3 5	27±2 6	26±2 4
Days 4-8	29±2 8	28±2 6	27±3 4	27±3 1	27±3 0	26±2 9
Days 8-11	30±3 8	29±2 6	29±3 7	28±4 1	26±2 5	25±2 7
Days 15-18	31±4 3	30±2 9	30±3 9	28±4 0	27±2 4	26±2 6
Females						
Days 11-15 (treatment init)	20±2 2	18±4 0	19±2 2	12±3 8	18±1 7	16±2 1
Days 15-18	20±3 1	20±3 2	20±2 7	13±5 8	19±2 0	18±1 7
Days 18-22	21±2 2	20±3 2	20±2 6		19±2 5	18±1 5
Gestation						
Days 0-3	23±3 0	22±5 6	21±2 8		22±2 3	19±3 1
Days 3-7	24±3 3	24±2 8	25±3 1		23±2 6	21±2 1
Days 7-12	25±3 3	26±3 4	27±2 4		24±2 6	24±2 0

Necropsy (dams): No drug-related abnormal findings were noted in scheduled sacrifice animals. In the 5 animals of 30 mg/kg group that died during the study period, the most notable necropsy findings included abnormal mucoid contents in the stomach and small intestine, abnormal fluid (5/5), granular or gelatinous material in the abdominal cavity (5/5), small thymus (2/5), blackish-purple spleen (2/5), and abdominal adhesions (4/5). In the remaining 20 animals of 30 mg/kg group sacrificed on Day 21, two animals showed abnormal intestinal contents and three animals had abdominal adhesions. Esophageal perforation was seen in two animals that died during the study period, one in the 3 mg/kg group and one in the 30 mg/kg group.

Male organ weights: Animals of 30 mg/kg group were sacrificed on Day 29. The other animals were terminated on days 78-81. No toxicologically significant changes in organ weights were noted.

Male sperm analysis: Data are summarized in the table below. Sperm motility and concentrations were decreased in animals of 15 mg/kg group.

Sperm analysis data in male rats (mean ± SD)

Group	1 (vehicle)	2 (3 mg/kg)	3 (10 mg/kg)	5 (vehicle)	6 (15 mg/kg)	Historical control
% motility	88±16 4	88±6 3	86±15 4	88±6 9	**72±28.1**	85.0-88 0
Concentration (m/ml)	4 8±1 33	5 3±1.19	5.1±1 13	4 9±1 07	**4.4±1.28**	4.2-5 3
Total sperm (m/g)	981 1±228 847	1061 13±221 495	985.31±200 378	1021 92±239.270	938.71±248 776	877.9-1198.7

Histopathology (3 male animals with the lowest sperm motility and concentration values): Some abnormal findings in the epididymides and testes (see table below) were noted in animals at 15 mg/kg/day. One

animal showed spermatic granuloma in the left epididymis and degeneration in the testes. The sponsor indicated that these changes were incidental as they were associated with an apparent gross abscess adhered to the left cauda epididymis of this animal.

Abnormal histology findings in rats

	Group 5	Group 6
N	3	3
Epididymis		
Decreased intraductal spermatozoa, minimal	0	2
Granuloma, spermatic, unilateral, moderate	0	1
Necrosis, single cell, intraluminal, minimal	0	2
Testis		
Degeneration, seminiferous tubule, unilateral, mild	0	1
Edema, unilateral, mild	0	1

Estrous cyclicity, copulation, fertility and pregnancy: Results are summarized in the table below. No biologically relevant differences in copulation and fertility indices, and mean precoital intervals, estrous cyclicity were noted.

Copulation, fertility and pregnancy data in rats (g, mean ± SD)

Group	1 (vehicle)	2 (3 mg/kg)	3 (10 mg/kg)	5 (vehicle)	6 (15 mg/kg)	Historical control
Copulation index	25/25 (100%)	24/24 (100%)	25/25 (100%)	24/25 (96 0%)	25/25 (100%)	90-100%
Fertility index	25/25 (100%)	23/24 (95 8%)	23/25 (92%)	23/24 (95 8%)	22 25 (88%)	77 8-100%
Precoital interval (days)	2 8±3 8	2 0±2 7	2 4±3 1			2 6-3 7
	1 (vehicle)	2 (3 mg/kg)	3 (10 mg/kg)	4 (30 mg/kg)	5 (vehicle)	6 (15 mg/kg)
Females found dead prior to mating	0	1	0	5	0	0
Females with no evidence of mating	0	1	0	0	1	0
Gravid	0	1	0	0	0	0
Females at scheduled necropsy	25	23	25	0	24	25
Nongravid	0	1	2	0	1	3
Gravid	25	22	23	0	23	22
With resorptions only	0	0	0	0	1	0
With viable fetuses	25	22	23	0	22	22
Total females gravid	25 (100%)	22 (88 0%)	23 (92 0%)		23 (92 0%)	22 (88 0%)

C-section data (implantation sites, pre- and post-implantation loss, etc.): Results are summarized in the table below. At doses ≥ 10 mg/kg/day, early resorptions and post-implantation loss were increased, and viable fetuses were decreased. No drug-related changes in C-sectioning and litter parameters were observed in animals at 3 mg/kg.

Reproductive parameters in rats treated with AL-6515 (mean ± SD)

Group	1	2	3	5	6	Historical control
Dose (mg/kg)	Control	3	10	control	15	
Pregnant animals examined	25	22	23	23	22	
Corpora lutea/dam	17.6±1 9	16.5±2.3	16 9±2 0	15.9±2.1	15.7±2 0	13 6-19 3
Implantations/dam	16 5±2.3	15 5±1.6	15 0±3 3	14 4±3.6	14 5±2 5	11 4-18 4
Preimplantation loss/dam	1 0±1 9	0.9±1 8	1 9±2 9	1.5±2 6	1 2±1 8	
Viable fetuses/dam	14 9±2 2	14.1±1.9	12 2±3 8	13 7±4 0	12.2±3 0	11 0-17.3
Dead fetuses	0	0	0	0	0	
Early resorptions	1 6±1.6	1 4±1 6	2 8±2 6	0 7±0.8	2 4±2 6	
Late resorptions	0	0	0	0	0	
Post-implantation loss	1 6±1.6	1 4±1 6	2 8±2 6	0.7±0.8	2.4±2.6	0.4-2.4

In summary, SD rats were treated orally by gavage with AL-6515 at 3, 10, 15, and 30 mg/kg/day before mating and through mating, implantation and early gestation. The 30 mg/kg group was terminated between Weeks 3 and 4 (prior to mating) due to excessive toxicity (mortality and clinical signs) in females. No drug-

related mortality and clinical observations were seen in animals treated at doses up to 15 mg/kg. At doses ≥ 15 mg/kg, decreased body weight gain and food consumption were noted in both male and female animals. Gross necropsy showed no toxicologically significant findings in animals sacrificed at the termination.

At 15 mg/kg group, sperm motility and sperm concentrations were lower than control males. Histological examination in three males in the 15 mg/kg group showed slightly decreased spermatozoa in the duct of the epididymis and slightly more intraluminal single necrotic cells in the epididymis in two animals.

There were no toxicologically significant differences in copulation and fertility indices, and mean precoital intervals between control and treated groups. However, a decrease in the number of viable fetuses and an increase in the early resorption and post-implantation loss were noted in animals at 10 and 15 mg/kg. In conclusion, oral administration of AL-6515 in rats at 3.0 mg/kg showed no developmental toxicity in this study.

Embryofetal development

153:30:0801: A range finding development toxicity study in rats with AL-6515. Vol. 16

Key study findings: Dose-related maternal toxicity (clinical signs, body weight changes, and GI lesions) was noted in all dose groups (50-1000 mg/kg/day, po by gavage, gestation Days 6-17). Doses of 3, 10 and 30 mg/kg/day were selected for a definitive development study in rats.

Study no.: 153:30:0801
Protocol #: N-96-252
Conducting laboratory and location: —

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Date of study completion: 2/16/1998
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot #: ERM 5198:088
Vehicle: — Carbopol 974P and 0.05% Tween 80 in — NaCl for injection

The purpose of this study was to determine the potential toxic effects of AL-6515 when administered orally to pregnant rats for selecting doses for a definitive development study in rats. The day evidence of copulation was confirmed was designated as gestation Day 0.

Methods

Doses: 0, 50, 125, 250, 500, and 1000 mg/10 ml/kg on gestation Days 6 to 17
Species/strain: Sprague Dawley - CD®BR VAF/Plus rats
Number/sex/group: 6/group, 79 days old, 243-295 g on gestation Day 0
Route, formulation, volume, and infusion rate: Oral by gavage, — carbopol 974P and 0.05% Tween 80 in — NaCl for injection, 10 ml/kg
Satellite groups used for toxicokinetics: No
Parameters and endpoints evaluated: See below.

Mortality: Twice daily

Clinical observations: Daily
Body weights: Gestation Days 0, 6, 9, 12, 15, 18 and 20
Necropsy: All animals, gestation Day 20. Animals in any group with 50% mortality were terminated and necropsied.

Results

Mortality: Mortality (found dead and euthanized moribund) occurred in 6, 4, 3, 3, and 3 animals at 50, 125, 250, 500 and 1000 mg/kg/day groups, respectively. Because animals in any group with 50% mortality were terminated and necropsied, all animals in AL-6515-treated groups were dead or euthanized prior to scheduled cesarean section. No mortality occurred in the control animals. The pregnancy rate was 100% in control and the 50 mg/kg groups, and 83.3% in the other groups.

Clinical signs: Clinical signs, including decreased activity, cool to touch, slow breathing/hunched posture, rough coat, distended abdomen, few/no feces, urine stain, pale eyes, and pale extremities were found in AL-6515 treated animals in a dose-dependent manner.

Body weight: Decreased body weights were noted in all measurable drug-treated groups (gestation Days 6-9: +11 g, -28 g, -39 g, -41 g, -34 g at 0, 50, 125, 250, and 5000 mg/kg doses, respectively). All animals at 1000 mg/kg were either dead or euthanized on gestation Day 9.

Necropsy: All AL-6515-treated animals had abnormal yellow or greenish mucoid contents in the GI tract. A majority of animals showed lesions or eroded areas on the small intestine wall, and areas of adhesions in the abdomen. No abnormal findings were noted in control animals.

In summary, pregnant rats were treated orally (by gavage) with AL-6515 at 50 to 1000 from gestation Day 6 to Day 17. Mortality (50% to 100%) was seen in all treated groups. Clinical signs were seen in all treated groups in a dose-dependent manner. Body weight decreases were seen in all measurable treated groups. Necropsy examinations showed GI lesions in all treated animals. In conclusion, dose-related maternal toxicity (mortality, clinical signs, body weight changes, and GI lesions) was noted in all dose groups. Based the study results, doses of 3, 10 and 30 mg/kg/day were selected for a definitive development study in rats.

154:30:0801: A range finding development toxicity study in rabbits with AL-6515. Vol. 18

Key study findings: The number of early resorption and post-implantation loss was increased in animals at 25, 50 and 100 mg/kg. The number of viable fetuses was decreased in animals at 100 mg/kg. One fetus from a 50 mg/kg dam had multiple craniofacial and CNS malformations. Another fetus from the same dam had omphalocele. Based the study results, doses of 3, 10 and 30 mg/kg/day were selected for a definitive development study in rabbits.

Study no.: 154:30:0801
Protocol #: N-96-253
Conducting laboratory and location: [redacted]

Date of study completion: 2/16/1998
GLP compliance: Yes

QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot #: ERM 5198:088
Vehicle: — Carbopol 974P and 0.05% Tween 80 in — NaCl for injection

The purpose of this study was to determine the potential toxic effects of AL-6515 when administered orally to pregnant rabbits for selecting doses for a definitive development study. The day evidence of copulation was confirmed was designated as gestation Day 0.

Methods

Doses: 0, 5, 10, 25, 50 and 100 mg/5 ml/kg on gestation Days 6 to 18
Species/strain: New Zealand white rabbits, 5.5 months old, 3.203-3.279 kg on gestation Day 0
Number/sex/group: 5/group
Route, formulation, volume, and infusion rate: Oral by gavage. — Carbopol 974P and 0.05% Tween 80 in — NaCl for injection, 5 ml/kg
Satellite groups used for toxicokinetics: No
Parameters and endpoints evaluated: See below.

Mortality: Twice daily
Clinical observations: At least once daily
Body weights: Gestation Days 0, 4, 6, 9, 12, 15, 19, 24 and 29
Scheduled euthanized and cesarean section: All animals, gestation Day 29
Fetal morphological examination: All animals

Results

Mortality: One animal each in the 25 and 100 mg/kg/day groups was found dead on gestation Days 18 and 13, respectively. The cause of the death for the 25 mg/kg animal was unknown, while the 100 mg/kg animal died as a result of an intubation error. The pregnancy rate was 100% in the 5, 15, 25, and 100 mg/kg groups, and 80% in the control and 50 mg/kg groups.

Clinical signs: Animals with few feces or no feces were seen in all groups but a higher incidence was seen in animals at 100 mg/kg.

Body weight: Decreased body weights were noted in animals at 100 mg/kg/day (-351 g from gestation Days 6-18). After the treatment, there appeared to be a recovery period between gestation Days 19 and 29, during which the mean body weight gain was 502 g in 100 mg/kg animals while in the control animals the body weight gain was 150 g. Throughout the study, mean body weights and body weight gain in all other treated groups were similar to the controls.

Necropsy: No toxicologically significant findings were noted.

Cesarean section observations: The number of early resorption and post-implantation loss was increased in animals at 25, 50 and 100 mg/kg. The number of viable fetuses was also low in animals at 100 mg/kg (see table below). No toxicologically significant findings in other cesarean parameters were noted.

Summary of cesarean section data (mean ± SD)

Dose (mg/kg)	Vehicle	5	10	25	50	100
Corpora lutea	10 0±2 4	8 6±3 2	10 8±2 5	10 3±1 5	9 8±1.7	8 3±1 7
Implantation sites	7 8±2 9	7 0±4 3	10 2±2 9	9 8±1 7	9.3± 5	7 3±2 9
Pre-implantation loss	2 3±0 5	1 6±2 5	0 6±0 5	0 5±0 6	0 5±0 6	1 0±1.4
Viable fetuses	7 3±3 1	5 8±4 9	9 8±3 2	7 5±1 9	7 3±0 5	**0.5±1.0**
Dead fetuses	0	0	0	0	0	0
Early resorption	0 5±0 6	1 2± 6	0 4±0 5	**1.8±1.3**	**1.8±1.5**	**6.8±2.6**
Post-implantation loss	0 5±0 6	1 2±1 6	0 4±0 5	**2.3±1.7**	**2.0±1.8**	**6.8±2.6**

Fetal morphological observations: One fetus from a 50 mg/kg dam had multiple external malformations including craniorachischisis, exencephaly, open eyelids, fixed forepaws and clubfoot. Another fetus from the same dam had omphalocele. No other abnormal findings were noted.

In summary, pregnant rabbits were treated orally (by gavage) with AL-6515 at 5 to 100 from gestation Day 6 to Day 18. Mortality was seen in one animal each in the 25 and 100 mg/kg groups, respectively. A decrease in body weight was seen in the 100 mg/kg animals. The number of early resorption and post-implantation loss was increased in animals at 25, 50 and 100 mg/kg. The number of viable fetuses was decreased in animals at 100 mg/kg. One fetus from a 50 mg/kg dam had multiple craniofacial and CNS malformations. Another fetus from the same dam had omphalocele. Based the study results, doses of 3, 10 and 30 mg/kg/day were selected for a definitive development study in rabbits.

156:30:0801: A development toxicity study in rats with AL-6515. Vol. 17

Key study findings: A slight decrease in fetal body weight was seen in HD (30 mg/kg) group. One HD animal had 9 dead fetuses, 6 resorptions, and no viable fetuses. The dose of 3 mg/kg was considered a NOEL for maternal toxicity and the dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

Study no.: 156:30:0801
Protocol #: N-96-254
Conducting laboratory and location: —

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Date of study initiation: 9/18/1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot #: ERM 5198:088, purity = —
Vehicle: — Carbopol 974P and 0.05% Tween 80 in — NaCl for injection

The purpose of this study was to determine the potential fetotoxic or developmental effects of AL-6515 when administered orally to pregnant rats during the period of major organogenesis. The day evidence of copulation was confirmed was designated as gestation Day 0.

Methods

Doses: 0, 3, 10, and 30 mg/10 ml/kg on gestation Days 6 to 17
Species/strain: Sprague Dawley — CD®BR VAF/Plus rats, 100 days old, 240-312 g on gestation Day 0
Number/sex/group: 25/group

Route, formulation, volume, and infusion rate: Oral by gavage, — carbopol 974P and 0.05% Tween 80 in — , NaCl for injection, 10 ml/kg
Satellite groups used for toxicokinetics: 8/group

	n/group		Dose	Dosing volume
Group	Main study	TK	(mg/kg/day)	(ml/kg/day)
1 (Vehicle)	25		0	10
2 (AL-6515)	25	8	3	10
3 (AL-6515)	25	8	10	10
4 (AL-6515)	25	8	30	10

Parameters and endpoints evaluated: See below.

Mortality: Twice daily
Clinical observations: Twice daily
Body weights: Gestation Days 0, 6, 9, 12, 15, 18 and 20
Food consumption: Gestation Days 0, 6, 9, 12, 15, 18 and 20
TK: Blood samples were collected prior to dosing (trough) and one hr after dosing (from four animals at each time point) on gestation Days 6, 12 and 17.
Termination and cesarean section: Gestation Day 20, all animals. The thoracic, abdominal, and pelvic cavities were exposed and viscera examined. The uterus, ovary and placenta were examined. The numbers of viable and nonviable fetuses, corpora lutea, and resorptions were recorded.
Fetal morphological examination: Fetuses were examined for external (all fetuses), internal (half fetuses) and skeletal (half fetuses) abnormalities.

Results

Mortality and pregnancy: Mortality occurred in five animals of the 30 mg/kg group (one on gestation Days 15, 17, and 19, two on gestation Day 20). Pregnancy data are summarized in the table below.

Summary of pregnancy data

Group	**1**	**2**	**3**	**4**
Females on study	**25**	**25**	**25**	**25**
Dead	0	0	0	5
--Gravid	0	0	0	5
Females examined at scheduled necropsy	**25**	**25**	**25**	**20**
--Nongravid	0	0	1	0
--Gravid	25	25	24	20
-----With viable fetuses	25	25	24	19
-----With resorptions only	0	0	0	1
Total females gravid	**25**	**25**	**24**	**25**

Clinical observations: Drug-related clinical signs were noted in animals of the 30 mg/kg group. Prior to death, the most notable clinical signs for the HD females found dead during the study included urine staining or colored staining in the urogenital area, pale in color, dark material around nose, hunched posture, cool to touch, and wobbly gait. For surviving HD animals, clinical signs included urine staining or colored staining in the urogenital area, pale in color, dark material around nose or mouth, cool to touch, few or no feces, and decreased activity. The yellow drug suspensions resulted in a post-dose observation of bright yellow urine in 6, 9 and 8 animals in the 3, 10 and 30 mg/kg/day groups, respectively. No toxicologically significant findings were seen in LD and MD animals.

Body weight: Decreased body weight gain was noted in HD and MD animals (see table below).

Summary of body weight data (g, mean ± SD)

Group	1	2	3	4
Gestation day 6	294±14 5	296±16.3	293±12 9	295±16 0
Gestation day 18	374±19 8	376±21 0	360±16 2	322±35 6
% control	100	100	96 3	86 1
Body weight gain (GDs 6-18)	79±12 1	80±10 4	67±10 8	28±37 8
% control	100	100	84 8	35 4

Food consumption: A decrease in daily food consumption was noted in HD and MD animals (see table below).

Summary of food consumption data (g/animal/day, mean ± SD)

Group	1	2	3	4
Gestation Day 6-9	25±2.6	24±2 6	21±1 8	16±4 4
Gestation Day 9-12	26±2 8	25±2 8	22±2 4	18±6 0
Gestation Day 12-15	28±3 1	26±1 7	24±1 9	20±5 8
Gestation Day 15-18	30±2 4	29±2 6	26±2 1	18±8 0
Gestation Day 18-20	30±2 9	30±2 9	29±2 3	20±10 2
Gestation Day 6-18	27±2 6	26±2 2	23±1 4	18±4 7

Necropsy: All five dead HD animals showed red, yellow or amber fluid in the abdomen and yellow or greenish mucoid material in the GI tract. Four of these animals have tan, greenish-tan or yellow fibrous materials adhered to the abdominal viscera, and three animals had perforated or eroded mucosa of the GI tract. For the scheduled sacrifice animals, one HD animal had amber fluid in the abdomen and greenish-tan material adhered to the abdominal viscera. Another HD animal showed brown or yellow mucoid material in the GI tract and an eroded small intestine. Three other HD animals showed enlarged mediastinal lymph nodes. No remarkable necropsy findings were seen in other animals.

Cesarean section observations: A slight decrease in fetal body weight (3.3 ± 0.5 g vs. control's 3.5 ± 0.2 g) was seen in HD group. One HD animal had 9 dead fetuses, 6 resorptions, and no viable fetuses. No toxicologically significant findings were seen in all other C-section parameters including the number of corpora lutea, viable fetuses, early and late resorptions, post-implantation loss, and fetal sex ratios.

Fetal morphological observations: Results are summarized in the table below. Malformations were seen in three, four, and four fetuses in Groups 1, 2, and 3, respectively. No malformations were seen in the HD group. Because there was no dose-dependence, the malformations were not considered as treatment-related. Regarding developmental variations, the incidences of unossified 5th and 6th sternebrae and 7th cervical ribs were significantly higher in the HD group than in the control group.

Summary of fetal observations

	Fetuses				Litters			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Total malformations								
Number with external malformations	**2**	**3**	**2**	**0**	**2**	**3**	**2**	**0**
Number with soft tissue malformations	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**0**
Number with skeletal malformations	**0**	**1**	**2**	**0**	**0**	**1**	**2**	**0**
Total fetuses with malformations	**3**	**4**	**4**	**0**	**3**	**4**	**4**	**0**
Fetuses examined externally	**337**	**378**	**344**	**279**	**25**	**25**	**24**	**19**
Filamentous tail	0	1	1	0	0	1	1	0
Anophthalmia and/or microphthalmia	1	1	1b	0	1	1	1	0
Exencephaly	0	0	1b	0	0	0	1	0
Short tail	1	0	1b	0	1	0	1	0
Micrognathia	0	0	1b	0	0	0	1	0

Omphalocele	0	0	1b	0	0	0	1	0
Club foot	0	0	1b	0	0	0	1	0
High-arched palate	0	0	1b	0	0	0	1	0
Anal atresia	0	0	1b	0	0	0	1	0
Fetal edema	0	1a	0	0	0	1	0	0
Fetuses examined viscerally	**169**	**187**	**172**	**139**	**25**	**25**	**24**	**19**
Right sided aorta arch	0	1a	0	0	0	1	0	0
Common truncus arteriosus	0	1a	0	0	0	1	0*	0
Incomplete nasal septation	0	0	1b	0	0	0	1	0
Nasal atresia	0	0	1b	0	0	0	1	0
Kidney, fused	0	0	1b	0	0	0	1	0
Diaphragmatic hernia	0	0	1b	0	0	0	1	0
Situs inversus	1	0	0	0	1	0	0	0
Fetuses examined skeletally	**168**	**191**	**172**	**140**	**25**	**25**	**24**	**19**
Atlas-occipital defect	0	1	0	0	0	1	0	0
Costal cartilage fused	0	0	1	0	0	0	1	0
Costal cartilage malaligned, severe	0	0	1	0	0	0	1	0
Variation								
Fetuses examined viscerally	**169**	**187**	**172**	**139**	**25**	**25**	**24**	**19**
Distended ureter	21	23	15	7	13	9	12	4
Renal, papilla, not developed	1	0	0	0	1	0	0	0
Fetuses examined skeletally	**168**	**191**	**172**	**140**	**25**	**25**	**24**	**19**
Sternebra malaligned (slight or moderate)	52	57	66	55	21	24	23	17
Reduced ossification of the vertebral arches	0	1	1	2	0	1	1	2
Reduced ossification of the skull	11	14	6	7	10	9	4	6
14th rudimentary ribs	3	5	1	2	3	4	1	2
Sternebra #5 and/or #6 unossified	5	7	8	24	4	7	6	8
Bent rib	3	1	2	3	2	1	2	2
7th cervical rib	0	2	0	4	0	2	0	4
Costal cartilage malaligned	2	2	2	1	2	2	2	1
Hyoid unossified	4	5	7	3	2	4	3	3
Reduced ossification of the 13th rib	4	5	3	0	3	3	2	0
27 presacral vertebrae	0	0	0	1	0	0	0	1
Unco-ossified vertebral centra	0	0	0	1	0	0	0	1

a, b: These findings were observed from one animal.

TK animal observations: TK data were reported separately. Abnormal findings were noted in HD TK animals. One gravid animal was found dead on gestation Day 15. Another nongravid animal died during blood collection on gestation Day 17. Necropsy examination in these two animals showed cloudy amber fluid in the abdomen, greenish-tan fibrous material covering the abdominal viscera, and dark yellow mucoid material in the GI tract. Clinical signs in HD TK animals were similar to those seen with main study HD animals. Body weight examination showed reduced body weight and body weight gain during the treatment period.

In summary, pregnant rats were treated orally (by gavage) with AL-6515 at 3, 10, and 30 mg/kg/day from gestation Day 6 to Day 17. Mortality was seen in five HD animals. Clinical signs including urine staining or colored staining in the urogenital area, pale in color, dark material around nose, hunched posture, cool to touch, decreased activity, and wobbly gait were seen in HD animals. Decreased body weight gain and food consumption were noted in MD and HD animals. Necropsy examinations showed GI lesions in all dead animals and a few scheduled sacrifice HD animals that included abnormal fluid in the abdomen, yellow or greenish mucoid material in the GI tract, tan, greenish-tan or yellow fibrous materials adhered to the abdominal viscera, and perforated or eroded mucosa of the GI tract. For reproductive evaluation, a slight decrease in fetal body weight (3.3 ± 0.5 g vs. control's 3.5 ± 0.2 g) was seen in HD group. One HD animal had 9 dead fetuses, 6 resorptions, and no viable fetuses. The observed malformations were not considered treatment-related due to the low incidence and lack of dose-dependence. Regarding developmental variations, the incidences of unossified 5th and 6th sternebrae and 7th cervical ribs were significantly higher in the HD group than in the control group. Based on the study results, the dose of 3 mg/kg was considered a

NOEL for maternal toxicity and a dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

157:30:0801: A development toxicity study in rabbits with AL-6515. Vol. 18

Key study findings: Abortion occurred in one MD animal on gestation Day 18 and one HD animal on gestation Day 21. One HD animal had a premature delivery on gestation Day 29 and another HD animal was found dead on gestation Day 19. The cause of death of the HD animal might be due to aspiration of the test article into the respiratory system. HD animals showed a statistically increase in post-implantation loss which was mainly due to a statistically increase in early resorptions. There was a statistically significant increase in the number of litters with skeletal malformations and in the number of litters with total malformations in the 30 mg/kg/day group when compared to the controls. The dose of 3 mg/kg was considered a NOEL for maternal toxicity and a dose of 10 mg/kg was considered a NOEL for developmental toxicity in rabbits.

Study no.: 157:30:0801
Protocol #: N-96-255
Conducting laboratory and location:

Date of study initiation: 9/19/1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot #: ERM 5198:088, purity =
Vehicle: Carbopol 974P and 0.05% Tween 80 in NaCl for injection

The purpose of this study was to determine the potential fetotoxic or developmental effects of AL-6515 when administered orally to pregnant rabbits during the period of major organogenesis. The day evidence of copulation was confirmed was designated as gestation Day 0.

Methods

Doses: 0, 3, 10, and 30 mg/5 ml/kg on gestation Days 6 to 18
Species/strain: Time-mated New Zealand white rabbits, 100 days old, 3.254-4.377 kg on gestation Day 0
Number/sex/group: 20/group
Route, formulation, volume, and infusion rate: Oral by gavage, Carbopol 974P and 0.05% Tween 80 in NaCl for injection, 5 ml/kg
Satellite groups used for toxicokinetics: 4/group

	n/group		Dose	Dosing volume
Group	Main study	TK	(mg/kg/day)	(ml/kg/day)
1 (Vehicle)	20		0	5
2 (AL-6515)	20	4	3	5
3 (AL-6515)	20	4	10	5
4 (AL-6515)	20	4	30	5

Parameters and endpoints evaluated: See below.

Mortality: Twice daily
Clinical observations: Twice daily

Body weights: Gestation Days 0, 4, 6, 9, 12, 15, 19, 24 and 29
Food consumption: Daily
TK: Blood samples were collected prior to dosing (trough) and one hr after dosing on gestation Days 6, 12 and 18.
Termination and cesarean section: Gestation Day 29, all animals. The thoracic, abdominal, and pelvic cavities were exposed and viscera examined. The uterus, ovary and placenta were examined. The number of viable and nonviable fetuses, corpora lutea, and resorptions were recorded.
Fetal morphological examination: Fetuses were examined for external, internal and skeletal abnormalities.

Results

Mortality and pregnancy: Results are summarized in the table below. One MD animal aborted on gestation Day 18. In the HD group, one animal was found dead on gestation Day 19, one animal aborted on gestation Day 21, and one animal had a premature delivery on gestation Day 29.

Summary of pregnancy data

Group	1	2	3	4
Females on study	20	20	20	20
Dead	0	0	0	1
--Gravid	0	0	0	1
Females that aborted	0	0	1	1
Females with premature delivery	0	0	0	1
Females examined at scheduled necropsy	20	20	19	17
--Nongravid	1	0	1	2
--Gravid	19	20	18	15
-----With viable fetuses	19	20	18	15
-----With resorptions only	0	0	0	0
Total females gravid	19 (95%)	20 (100%)	19 (95%)	18 (90%)

Clinical observations: The aborted MD animal had few feces, soft stool and fecal stains in the anogenital area prior to euthanasia. The aborted HD animal showed labored breathing, few feces and reddish fluid in the cage/tray prior to euthanasia. Prior to the premature delivery, the HD animal showed decreased activity, cool to touch, few or no feces, soft or mucoid stools, fecal stains in the anogenital area. No toxicologically significant findings were seen in the HD animal found dead on gestation Day 19. For surviving animals, low incidences of clinical signs including few feces and soft or mucoid stools were noted in both control and treated groups.

Body weight: Results are summarized in the table below. Decreased body weight gain was noted in HD animals. However, the body weights between control and HD groups were similar on gestation Day 19 and Day 29. The decreased body weight gain in the LD group was not toxicologically significant since there was no dose-dependence.

Summary of body weight data (g, mean ± SD)

Group	1	2	3	4
Gestation day 6	3949±318 1	3932±284.8	3960±312.4	3964±240 8
Gestation day 19	4165±319 3	4112±281 5	4234±343 9	4098±307 8
% control	100	98 7	100	98.4
Gestation Day 29	4375±354 4	4194±325.5	4393±400 3	4389±254 7
Body weight gain (GDs 6-19)	216±83 9	180±98 5	251±136 2	155±05.1
% control	100	83.3	100	71.8

Food consumption: A slight decrease in daily food consumption was noted in HD animals on gestation Days 12-24 (see table below).

Summary of food consumption data (g/animal/day, mean ± SD)

Group	1	2	3	4
Gestation Day 12-15	182±19 2	161±40 9	186±52 9	171±63 7
Gestation Day 15-19	183±17 5	172±29 5	192±36 9	169±51 5
Gestation Day 19-24	178±35 1	170±32 3	172±47 3	169±54 4

Maternal necropsy: The HD animal that died on gestation Day 19 had dark mottled lung lobes and red foam in the trachea. The cause of death might be due to aspiration of the test article into the respiratory system. The remaining two HD animals that aborted or delivered prematurely showed no remarkable necropsy findings. The MD animal that aborted on gestation Day 18 had dark red, mottled and consolidated lung lubes. The necropsy findings for the scheduled sacrifice animals are summarized in the table below. Because of the low incidences and lack of dose-response relationship, these findings were not considered drug-related.

Summary of maternal necropsy observations

Group	1	2	3	4
N	20	20	19	17
Nongravid	1	0	1	2
Greenish-brown fluid in the cecum	0	1	1	0
Blood and blood clots in the right uterine horn	0	1	0	0
Cyst in the right kidney	0	1	0	0
Oviduct cyst	3	5	5	5

Cesarean section observations: Results are summarized in the table below. An increase in post-implantation loss and early resorptions was seen in HD animals. These changes were within the historical control range in this laboratory. No other toxicologically significant findings were noted.

Summary of cesarean section data (mean ± SD)

Group	**1**	**2**	**3**	**4**	**Historical control**
Females gravid	**19**	**20**	**18**	**15**	
Corpora lutes	9 5±2 3	9 8±2 2	10 5±2 4	9 9±2 6	8 4-13.4
Implantation site	8 4±3 0	8 7±2 3	9 7±2 6	9.1±2 5	5 5-9 3
Pre-implantation loss	1 1±1 8	1 1±1 1	0 8±1 0	0 8±1 2	
Viable fetuses	8 1±2 9	8 4±2 3	8 7±2 2	7.7±2 8	4.6-9 1
Late resorption	0 2±0 5	0 1±0 3	0 8±1 6	0 5±0 6	
Early resorption	0.2±0 9	0 3±0 6	0.1±0 3	**0.9±0.3**	
Post-implantation loss	0 4±1 0	0 4±0 6	0 9±1 6	**1.4±1.5**	0.2-1 9
Fetal weight (g)	44.2±3 6	42 0±4.9	43 5±5 2	43.4±3.7	40.6-51.2

Fetal morphological observations: Results are summarized in the table below. In the HD group, there was a significant increase in the total number of litters with skeletal malformations, and in the total number of litters with all malformations. The malformations noted in LD and MD groups were not considered to be drug-related because they occurred at a low incidence, were dissimilar in nature, and were not statistically different from the controls. There were no meaningful differences in developmental variations among different groups.

Summary of fetal observations

	Fetuses				**Litters**			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Total malformations								
Number with external malformations	**0**	**2**	**2**	**3**	**0**	**1**	**2**	**3**

Number with soft tissue malformations	1	1	1	2	1	1	1	2
Number with skeletal malformations	1	5	1	9	1	3	1	7*
Total fetuses with malformations	2	5	3	11	2	3	3	8*
Fetuses examined externally	153	167	157	115	19	20	18	15
Flexed paw	0	0	0	1	0	0	0	1
Clubfoot	0	0	0	1	0	0	0	1
Filamentous tail	0	0	0	1	0	0	0	1
Omphalocele	0	1	0	1	0	1	0	1
Spina bifida	0	0	1	0	0	0	1	0
Short tail	0	2	1	0	0	1	1	0
Open eyelid(s)	0	1	0	0	0	1	0	0
Hemimelia	0	1	0	0	0	1	0	0
Micrognathia	0	1	0	0	0	1	0	0
Gastroschisis	0	1	0	0	0	1	0	0
Craniorachischisis	0	1	0	0	0	1	0	0
Syndactyly	0	1	0	0	0	1	0	0
Brachydactyly	0	1	0	0	0	1	0	0
Cleft palate	0	1	0	0	0	1	0	0
Microtia	0	1	0	0	0	1	0	0
Fetuses examined viscerally	153	167	157	115	19	20	18	15
Hydrocephaly	1	0	0	0	1	0	0	0
Heart or great vessel anomaly	0	0	0	1	0	0	0	1
Bulbous aortic arch	0	0	1	1	0	0	1	1
Interventricular septal defect	0	0	0	1	0	0	0	1
Stenotic pulmonary trunk	0	0	0	1	0	0	0	1
Kidney malpositioned	0	1	0	0	0	1	0	0
Fetuses examined skeletally	153	167	157	115	19	20	18	15
Extra site of ossification anterior to sternebrae #1	0	3	0	3	0	2	0	3
Nasal bone fused	0	0	0	1	0	0	0	1
Costal cartilage malaligned (severe)	0	0	0	1	0	0	0	1
8 cervical vertebrae	0	0	0	1	0	0	0	1
Thoracic vertebrae anomaly	0	1	0	3	0	1	0	3
Interparietal bone formed in two distinct pieces	0	0	1	0	0	0	1	0
Bent limb bone	0	1	0	0	0	1	0	0
Sacral vertebrae anomaly	1	0	0	0	1	0	0	0
Rib anomaly	0	1	0	3	0	1	0	3
Costal cartilage anomaly	0	0	0	2	0	0	0	2
Thoracic centra anomaly	0	0	0	1	0	0	0	1
Lumbar centra anomaly	0	0	0	1	0	0	0	1

* Significantly different from control, $P < 0.05$

In summary, pregnant rabbits were treated orally (by gavage) with AL-6515 at 3, 10, and 30 mg/kg/day from gestation Day 6 to Day 18. Maternal toxicity was seen in the 10 and 30 mg/kg groups. Abortion occurred in one MD animal on gestation Day 18 and one HD animal on gestation Day 21. One HD animal had a premature delivery on gestation Day 29 and another HD animal was found dead on gestation Day 19. With the exception of the animal found dead, all the above animals showed clinical signs prior to abortion or premature delivery including labored breathing, decreased activity, cool to touch, few or no feces, and soft or mucoid stools. The cause of death of the HD animal might be due to aspiration of the test article into the respiratory system. HD animals showed a decrease in body weight gain and food consumption. The MD animal that aborted on gestation Day 18 had dark red, mottled and consolidated lung lubes in necropsy examinations. No remarkable necropsy findings were noted in surviving animals. Regarding reproductive evaluation, HD animals showed a statistically increase in post-implantation loss which was mainly due to a statistically increase in early resorptions. No abnormal findings were noted in other C-section parameters including the number of corpora lutea, implantation, viable fetuses, fetal sex ratio, and fetal weight. There was a statistically significant increase in the number of litters with skeletal malformations and in the number of litters with total malformations in the 30 mg/kg/day group when compared to the controls. Low incidences of malformations were seen in the MD and LD groups and were not considered drug-related because they occurred at a low incidence, were dissimilar in nature, and were not statistically different from

the controls. Based on the study results, the dose of 3 mg/kg was considered a NOEL for maternal toxicity and a dose of 10 mg/kg was considered a NOEL for developmental toxicity in rabbits.

158:30:0801: A perinatal and postnatal study in rats with AL-6515. Vol. 19

Key study findings: AL-6515 produced dystocia and associated maternal toxicity in F0 females at doses ≥ 3 mg/kg/day, and developmental toxicity (decreased F1 pup survival and decreased F1 pup body weight during lactation) at doses ≥ 10 mg/kg/day. The NOEL for maternal toxicity was not determined. The dose of 3 mg/kg was considered a NOEL for developmental toxicity in F1 offspring.

Study no.: 158:30:0801
Protocol #: N-96-276
Conducting laboratory and location: —

Date of study initiation: 11/4/1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AL-6515, Lot #: ERM 5198:088, purity = —
Vehicle: — Carbopol 974P and 0.05% Tween 80 in — NaCl for injection

The purpose of this study was to determine the potential toxic effects of AL-6515 on parturition and lactation of F0 females, and survival, growth, development, behavior, productive capability of F1 offspring. The drug will be administered orally to pregnant rats from gestation Day 6 through lactation Day 20.

Methods

Doses: 0, 3, 10, 15 and 30 mg/10 ml/kg on gestation Days 6 to lactation Day 20
Species/strain: Sprague Dawley — CD®BR VAF/Plus rats, 88-105 days old and 220-288 g at the initiation of breeding
Number/sex/group: 25/group
Route, formulation, volume, and infusion rate: Oral by gavage, — Carbopol 974P and 0.05% Tween 80 in — NaCl for injection, 10 ml/kg
Satellite groups used for toxicokinetics: No

	n/group		Dose	Dosing volume
Group	Main study	TK	(mg/kg/day)	(ml/kg/day)
1 (Vehicle)	25	0	0	10
2 (AL-6515)	25	0	3	10
3 (AL-6515)	25	0	10	10
4 (AL-6515)	25	0	30	10
5 (vehicle)*	25	0	0	10
6 (AL-6515)*	25	0	15	10

*Due to higher than expected toxicity in the 30 mg/kg group, the surviving animals were euthanized between the first and second weeks of lactation and two additional groups were added to the study.

Parameters and endpoints evaluated: See below.
F0 generation:

Mortality: Twice daily
Clinical observations: Twice daily
Body weights: Gestation Days 0, 6, 9, 12, 15, 18 and 20; lactation Days 1, 4, 7, 10, 14, 17 and 21

Food consumption: Gestation Days 0-6, 6-9, 9-12, 12-15, 15-18, 18-20
Parturition and lactation: Signs of difficult or prolonged delivery were recorded. The females and their offspring remained together until completion of weaning on lactation Day 21.
Litter retrieval: On lactation Day 6. The number of pups retrieved with 5 min was recorded.
Euthanasia and necropsy: Lactation Day 21, all F0 females

F1 generation

Pup identification: Lactation Day 0
Standardization of litter size: On lactation Day 4, each litter was randomly culled to a maximum of 8 pups, 4/sex. The culled pups were euthanized.
Litter data: Viability: daily; detailed examination: lactation Days 0, 4, 7, 14 and 21; sex of each pup: lactation Days 0, 4 and 21
Developmental landmarks, functional and behavioral testing:

Pinnae detachment: After culling on lactation Day 4
Surface righting response: Daily, beginning on lactation Day 5, until a positive response was seen
Cliff aversion: Daily, beginning on lactation Day 9, until a positive response was seen
Eye opening: Daily, beginning on lactation Day 14, until complete opening of both eyes was seen
Startle response: Daily, beginning on lactation Day 14, until a positive response was seen
Auditory response: On lactation Day 21
Vaginal opening: Female pups selected for mating, daily, beginning on postpartum Day 33, until vaginal opening was seen
Preputial separation: Male pups selected for mating, daily, beginning on postpartum Day 40, until complete separation was seen
Open field test: Between 35 and 45 days of age, selected F1 pups
Water maze test: Testing was initiated for pups between 45 and 55 days of age. The testing phases included swimming ability, learning trials, and memory recall.

Selection of parental animals: Between postpartum Days 17 and 23, 20 pups/sex/group were randomly selected as the F1 parental animals to produce an F2 generation. When it was possible, one male and one female were selected from each litter. Non-selected F1 pups were euthanized and necropsied.
Breeding phase: At approximately 10 weeks of age, each selected F1 female was cohabitated with a single selected male from the same treatment group.
Gestation clinical observations and body weights: The F1 females were observed daily throughout gestation and lactation. Body weight was observed on gestation Days 0, 4, 7, 10, 14, 17 and 20, and on lactation Days 1, 4, 7, 10, 14, 17 and 21.
Parturition and lactation: Signs of difficult or prolonged delivery were recorded. The females and their offspring remained together until completion of weaning on lactation Day 21.
Euthanasia and necropsy: Lactation Day 21

F2 generation:

Pup identification: Lactation Day 0
Standardization of litter size: On lactation Day 4, each litter was randomly culled to a maximum of 8 pups, 4/sex. The culled pups were euthanized.

Litter data: Viability: daily; detailed examination: lactation Days 0, 4, 7, 14 and 21; sex of each pup: lactation Days 0, 4 and 21. All surviving pups were euthanized on lactation Day 21 and necropsied.

Results

F0 generation:

Mortality and pregnancy: Results are summarized in the table below. A high mortality rate was seen in animals at 15 and 30 mg/kg/day. At 30 mg/kg/day, ten animals died prior to signs of delivery. Eight animals were found dead on lactation Days 0 to 3. Two additional females were euthanized following the death of all pups in their respective litters. Three other females with evidence of mating failed to deliver and were euthanized on post-breeding Day 25. All remaining F0 animals at 30 mg/kg/day were euthanized between the first and second weeks of lactation due to excessive toxicity. No females at 30 mg/kg finished the lactation phase of the study. At 15 mg/kg, one female died spontaneously prior to delivery on gestation Day 22. Three animals died or euthanized moribund on lactation Day 0 or 1. Two additional animals were euthanized following the death of all pups in their respective litters. At 10 mg/kg, two animals died on lactation Day 0. At 3 mg/kg, one animal was found dead on lactation Day 1. On Group 1, one animal was euthanized following the death of all pups in her litter.

Summary of mortality data

Group	1	2	3	4	5	6
Dose (mg/kg)	0	3	10	30	0	15
Females on study	25	25	25	25	25	25
Females found dead prior to delivery	0	0	0	10	0	1
Females that delivered	25	23	25	12	25	24
Females with total litter loss	1	0	2	2	0	2
Females found dead	0	1	2	8	0	1
Females euthanized moribund	0	0	0	0	0	2
Females that did not deliver	0	2	0	3	0	0
--Gravid	0	0	0	1	0	0
--Nongravid	0	2	0	2	0	0
Total females gravid	25	23	25	23	25	25

Clinical observations: Drug-related clinical signs were noted in animals of the 15 and 30 mg/kg groups. The clinical signs included decreased activity, wobbly gait, hunched posture, cool to touch, abnormal excreta, extremities pale in color, eyes pale in color, urine staining, dark material on the forelimbs and facial region, slow breathing, and bright yellow color urine. Clinical signs in the control, 3, and 10 mg/kg/day groups were unremarkable although bright yellow color urine was seen as a post-dose finding in animals of the 3 and 10 mg/kg groups.

Body weight: Results are summarized in the table below. Decreased body weight gain and lower body weights were noted in animals at 10, 15 and 30 mg/kg/day.

Summary of body weight data (g, mean ± SD)

Group	1	2	3	4	5	6
Dose (mg/kg)	0	3	10	30	0	15
Gestation day 6	289±15 6	289±15 0	287±16 5	289±17 8	268±9 5	269±10 6
Gestation day 20	383±33 5	391±22 9	373±24 2	**316±30.0**	379±16 4	355±18.0
% control	100	100	97 4	**82.5**	100	93 7
Body weight gain (GDs 6-9)	9±3 7	8±3 9	**3±3.8**	**-14±15.0**	13±4 4	**6±6.9**
Body weight gain (GDs 9-12)	16±4 7	16±5 7	**13±5.4**	**6±13.0**	14±5 5	**11±5.6**
Body weight gain (GDs 15-18)	35±8.5	37±6 5	33±8 4	**17±6.8**	38±5 2	**32±6.6**
Body weight gain (GDs 18-20)	19±14 6	23±5 8	21+7 3	**-2±12.4**	28+5 1	**20±6.6**
Lactation Day 1	299±21 8	300±17 6	286±18 0	**256±15 4**	290±12 0	**277±12.2**
Lactation Day 7	319±25 9	323±16 4	309±20 2	**270**	313±14 9	**303±14.0**
Lactation Day 21	323±21 2	322±19 7	324±21 2		325±18 2	318±12 9
Body weight gain (LDs 1-4)	16±8 5	16±5 9	16±7 2	**13**	17±8 3	**11±8.1**
Body weight gain (LDs 4-7)	4±10 4	6±5 5	8±6 9	10	7±11 0	15±8 0

Food consumption: A decrease in daily food consumption was noted in animals at 10, 15 and 30 mg/kg/day during gestation (see table below).

Summary of food consumption data (g/animal/day, mean ± SD)

Group	1	2	3	4	5	6
Dose (mg/kg)	0	3	10	30	0	15
Gestation Day 6-9	25±2 0	24+2 2	**21±2.4**	**14±3.8**	22±2.4	**19±2.8**
Gestation Day 9-12	25±2 0	24±2 4	**22±2.0**	**15±5.7**	23±2 2	**22±2.3**
Gestation Day 12-15	26±2 4	25±2 5	**23±1.9**	**19±5.8**	24±1 2	**23±2.6**
Gestation Day 15-18	27+2 5	27+3 0	**25±3.0**	**17±6.1**	28±2 3	**26±2.9**
Gestation Day 18-20	22± 7	23+2 7	**20±3.8**	**7±4.9**	24+2 1	**19±5.0**

Parturition and lactation: The table below shows the mean gestation length. Although Group 6 animals showed a statistically significant increase ($P < 0.01$), the value (22 days) was within the laboratory's historical range (21.7-22.0 days).

Summary of gestation length data (days, mean ± SD)

Group	1	2	3	4	5	6
Dose (mg/kg)	0	3	10	30	0	15
Gestation length	21 9+0 3	22 1+0 3	**22.0±0 2**	**21.8±0 8**	21 6+0 5	**22.0±0 4***

* Significantly different from control: $P < 0.01$

Litter retrieval: Litter retrieval was decreased in Group 6 animals (15 mg/kg, 85.7%) compared to control Group 5 (100%). Two Group 6 animals failed to retrieve any pups, and one other Group 5 animal retrieved less than one-half of her litter. The sponsor indicated that this finding was not considered toxicologically significant because two control Group 1 animals failed to retrieve any of their pups. The reviewer agrees with the sponsor since no dose-dependence was noted.

Summary of F0 litter retrieval data

Group	1	2	3	4	5	6
Dose (mg/kg)	0	3	10	30	0	15
Retrieval data	174/186, 93 5%	169/176, 96%	**175/182, 96.2%**	**16/16, 100%**	198/198, 100%	**126/147, 85.7%**

Necropsy: Results are summarized in the table below. Similar necropsy findings were noted in the treated animals that were found dead or euthanized moribund during the study that included abnormal fluid in the abdominal cavity, abdominal adhesions, mottled adrenals, tan areas in the liver, mottled liver and lungs, reddened mediastinal lymph nodes, mucoid or gelatinous material in the small intestine, dark red areas in the small intestine, abnormal fluid, mucoid or paste-like material in the stomach, dark red foci in the

stomach, smooth stomach mucosa, small thymus, dark red foci on the thymus, and whole.body pale in color.

Seven 30 mg/kg/day females, three 15 mg/kg/day females, two 10 mg/kg/day females and one 3 mg/kg/day female which were found dead or euthanized moribund during early lactation had retained fetuses in uteri, indicating that the death occurred prior to completion of parturition.

In F0 females that survived to scheduled euthanasia, gross necropsy findings were generally unremarkable. In F0 females that failed to deliver and were euthanized on post-breeding Day 25, significant gross necropsy findings were limited to the 30 mg/kg/day group and included abdominal adhesions, abnormal contents in the abdominal cavity, and fetuses retained in the uterus.

In F0 females that were euthanized following total litter loss, the most notable gross necropsy findings included adhesions and/or abnormal contents in the abdominal cavity and stomach at the 15 and 30 mg/kg/day levels, and depletion of body fat, tan areas on the kidneys, and abnormal stomach contents in one control female.

Summary of F0 gross necropsy observations

	Found dead or euthanized moribund						Scheduled sacrifice					
Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
N	**0**	**1**	**2**	**18**	**0**	**4**	**24**	**22**	**23**	**2**	**25**	**19**
Haircoat, wet matting	0	1	2	18	0	3						
Hair coat, dark material	0	1	2	15	0	3						
Abdominal cavity, abnormal content	0	0	0	12	0	1						
Abdominal cavity, adhesion	0	0	0	10	0	1						
Adrenal gland, mottled	0	0	0	3								
Adrenal gland, reddened	0	0	2	0								
Kidney, mottled pitted	0	0	0	1			2	1	1	0		
Kidney, dilated pelvis	0	0	0	1			0	0	2	0		
Liver, tan areas	0	0	1	7			0	0	1	0		
Liver, mottled	0	1	2	4								
Lungs, mottled	0	0	1	7								
Lungs, consolidated	0	0	0	1								
Lymph node, mediastinal, reddened	0	0	2	9								
Salivary gland	0	0	0	1								
Small intestine, abnormal content	0	1	2	15	0	1						
Small intestine, adhesion	0	0	0	1								
Small intestine, dark red area	0	0	0	5								
Small intestine, reddened mucosa	0	0	0	1								
Small intestine, tan area	0	0	0	1								
Small intestine, Peyer's patch, accentuated											0	1
Spleen, blacklish-purple	0	0	0	2								
Spleen, enlarged											0	1
Stomach, abnormal content	0	1	2	16	0	3						
Stomach, perforation	0	0	0	1								
Stomach, dark red area	0	0	0	4								
Stomach, mucosa smooth	0	0	0	3								
Stomach, eroded area	0	0	0	1								
Stomach, foci											2	0
Stomach, thickened											0	1
Thymus, small	0	0	0	8								
Thymus, dark red foci	0	1	2	2	0	2						
Uterus, endometrium dark red	0	0	0	1								
Whole body, pale	0	1	0	6								
Uterus, retained fetuses					0	4						
Vagina, retained fetuses					0	1						
Uterine horns, abnormal content							0	1	0	0		
Thoracic cavity, adhesion											0	1
Thoracic cavity, lesion							1	0	0	0	0	0

	Euthanized post breeding Day 25						Euthanized, total litter loss					
Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
N	**0**	**2**	**0**	**3**			**1**	**0**	**0**	**2**	**0**	**2**
Haircoat, wet matting	0	0	0	1			1	0	0	1	0	1
Hair coat, dark material							1	0	0	1	0	2
Abdominal cavity, abnormal content	0	0	0	1			0	0	0	1	0	1
Abdominal cavity, adhesion	0	0	0	1			0	0	0	1	0	1
Abdominal cavity, body fat depletion							1	0	0	0		
Uterine horns, nongravid, ammonium sulfide negative	0	2	0	2								
Uterine, gravid, fetuses in uterine horns	0	0	0	1								
Kidney, tan area							1	0	0	0		
Kidney, pale							0	0	0	1		
Lymph node, enlarged											0	1
Vagina, abnormal content							1	0	0	0		
Thymus, small							0	0	0	1		
Stomach, abnormal content							1	0	0	1	0	1
Small intestine, perforation							0	0	0	1		
Small intestine, adhesion							0	0	0	1		

F1 generation:

F1 pup mortality: Results are summarized in the table below. There was an increase in the number of dead F1 pups and concomitant decrease in mean live litter size at the 15 and 30 mg/kg/day levels on lactation Day 0. Pup viability continued to decrease at these doses during the first four days of lactation. Following culling on lactation Day 4, pup viability was stabilized and no further spontaneous pup mortality occurred in either group. At the 30 mg/kg/day, pup viability was not assessed beyond lactation Day 7 as all remaining animals in this group were euthanized due to excessive toxicity in F0 dams. No significant differences in F1 pup viability were observed at the 3 and 10 mg/kg/day levels.

Summary of F1 pup viability

Group	**1**	**2**	**3**	**4**	**5**	**6**
Dose (mg/kg)	**0**	**3**	**10**	**30**	**0**	**15**
Number of dead pups on LD 0	17	13	13	**31**	9	**40**
Number of live pups LD 0	322	310	338	**60**	346	**253**
Number of litters with live offspring	25	23	25	**10**	25	**23**
Mean live litter size	12.9	13.5	13.5	**6**	13.8	**11.0**
Sex ratio (m/f)	159/163	147/163	170/168	**30/30**	182/164	**134/119**
LD 1 # alive/total pups (% survival)	315/322(97.8)	298/303(98.3)	317/328(96.6)	**25/42(59.5)**	342/346(98.8)	**212/235(90.2)**
LD 4 before culling	315/322(97.8)	298/303(98.3)	313/328(95.4)	**20/42(47.6)**	342/346(98.8)	**208/235(88.5)**
LD 4 after culling	186	176	182	16	200	**147**
LD7 #alive/total (% survival)	186/186(100)	176/176(100)	182/182(100)	16/16(100)	198/200(99)	147/147(100)
LD21	186/186(100)	176/176(100)	182/182(100)		195/200(97.5)	147/147(100)

F1 pup observations: Abnormal observations in pups in the 15 and 30 mg/kg/day groups included increased mortality, cool to touch, gasping and slow breathing. No toxicologically significant findings were noted in pups in the 3 and 10 mg/kg/day group.

Summary of F1 pup observations during lactation

Group	**1**	**2**	**3**	**4**	**5**	**6**
Dose (mg/kg)	**0**	**3**	**10**	**30**	**0**	**15**
Found dead	20	14	20	**43**	14	**43**
Cannibalized	0	1	2	**6**	0	**10**
Missing, presumed cannibalized	4	3	6	**4**	4	**14**
Body, cool to touch	11/11*	8/8	12/12	**36/36**	0	**34/34**
Activity, gasping	0	0	0	**2/2**		
Activity, slow breathing	0	1/1	0	**4/4**		

* Total incidences/animals affected

F1 pup body weights: Lower pup body weights were noted in Groups 3 and 4 pups on lactation Days 1, 4 and 7 (10 and 30 mg/kg/day, see table below). For Group 3 pups, the mean body weight was comparable to controls on lactation Days 14 and 21. No toxicologically significant differences in pup body weights were seen in pups in the 3 or 15 mg/kg/day group.

Summary of F1 pup body weight during lactation (g, mean ± SD)

Group	1	2	3	4	5	6
Dose (mg/kg)	0	3	10	30	0	15
Lactation Day 1	6 9±0 72	6 9±0 46	6.2±0.59	4.8±0 96	6 5±0 50	6 2±0 88
Lactation Day 4	10 0±1 35	10 0±0 76	9 1±0 96	6.7	9 5±0 99	9 5±1.11
Lactation Day 7	16 0±2 14	16 5±1 03	14 6±1 96	9.6	15 7±1 64	15 3±1 54
Lactation Day 21	52 9±5 18	54 3±3 92	50 0±4.52		51 7±4 22	50 4±4 22

F1 pup gross necropsy observations: No toxicologically significant differences were noted between control and treated groups.

F1 pup developmental landmarks and functional testing: There were no toxicologically significant differences among the groups in F1 developmental and functional indices including pinnae detachment, surface righting response, cliff aversion, eye opening, startle response, auditory response, vaginal opening and preputial separation.

F1 pup behavioral testing: No toxicologically meaningful differences were observed among the groups with respect to open-field testing parameters. Evaluation of T-maze testing data did not reveal any changes that would indicate a treatment-related effect on learning or memory recall.

F1 adult survival, growth and development: All selected F1 animals survived and no treatment-related clinical observations were observed during the growth phase at dose levels up to 15 mg/kg/day. However, during the growth phase mean body weights were significantly decreased on a number of days in F1 males at the 10 and 15 mg/kg/day levels.

Summary of F1 body weight data (g, mean ± SD)

Group	1	2	3	5	6	1	2	3	5	6
Dose (mg/kg)	0	3	10	0	15	0	3	10	0	15
	Males					Females				
Day 27/28	79±8 9	85±5 2	73±10 9	83±7 8	78±8 0	75±8 0	80±7 3	71±8 6	75±6 7	71±7 9
Day 30/31	98±10 1	93±11.6	88±8.7	104±9.4	99±9 9	86±8.4	88±10 6	82±7 9	92±7 8	87±10 2
Day 41/42	200±17 6	194±19.7	181±15.1	206±16.1	195±16 5	145±13 0	149±13.3	140±9 2	152±9.7	145±13.1
Day 69/70	419±35.4	411±37 3.	387±26.3	430±30 0	406±27.7	233±17.8	238±19 6	224±15 1	233±16.0	231±20 7
Day 100/101	538±51 6	536±51 7	505±36.6	546±45 5	518±41.1	250±21 9*	259±22.5	244±17 0	240±17.0	237±19 8
Day 114/126	571±56 0	568±53.9	540±40.5	603±52 7	569±47.0					
Body weight gain	492	483	467	520	491	175	179	173	165	166
% control	100	98.2	94.9	100	94.4	100	100	98.9	100	100

Last measurement was on Days 76 (Groups 1, 2 and 3) and 73 (Groups 5 and 6)

F1 adult copulation, fertility, gestation, parturition, and lactation: There were no significant differences among the groups in the F 1 copulation and fertility indices, precoital intervals or mean gestation lengths. There were no differences among different groups regarding body weights and body gain in F1 animals during gestation and lactation periods. The F1 group pregnancy rate ranged from 85 to 100%.

Summary of F 1 copulation, fertility, precoital interval and gestation length data (mean ± SD)

Group	1	2	3	5	6
Dose (mg/kg)	0	3	10	0	15
Copulation index.# of animals paired (%)	20/20 (100)	20/20 (100)	20/20 (100)	19/20 (95)	19/20 (95)
Fertility index (%)	95	95	100	89.5	94 7
Precoital interval (days)	3.3±2 0	2 2±1 4	2.8±2 1	2.5±3.2	3.3±2 5
Gestation length (days)	21 8±0 4	21 8±0 4	21 6±0 5	21 8±0.4	21 7±0 5

F1 gross necropsy: No toxicologically significant findings in F1 necropsy examinations were noted.

F2 generation: No toxicologically significant findings in F2 pup viability, observations, body weight, and gross necropsy examinations were noted.

In summary, pregnant F0 rats were treated orally (by gavage) with AL-6515 at 3, 10, and 30 mg/kg/day from gestation Day 6 to lactation Day 20. The 30 mg/kg/day group was terminated between the first and second weeks of lactation due to excessive toxicity and two additional groups (a second vehicle group and a 15 mg/kg/day group) were added to the study. Mortality occurred at all dose levels (1, 2, 4, and 18 at 3, 10, 15, and 30 mg/kg/day level, respectively). The majority of these deaths occurred following initiation of parturition. Marked clinical signs of toxicity were observed in F0 females at the 15 and 30 mg/kg/day levels including decreased activity, hunched posture, abnormal excreta, eyes pale in color, urine staining, slow breathing, cool to touch, extremities pale in color, and dark material on the forelimbs and facial region. Retained fetuses in uteri were detected at necropsy in seven 30 mg/kg/day females, three 15 mg/kg/day females, two 10 mg/kg/day females, and one 3 mg/kg/day female which were found dead or euthanized moribund following initiation of parturition. No mortality or significant clinical signs were noted in control females. Mean body weight, weight gain and food consumption were significantly decreased in F0 females at the 10, 15 and 30 mg/kg/day levels. There were no toxicologically significant findings in the mean gestation length and litter retrieval examinations. Various gross abnormalities were noted at necropsy in the treated females that died during the study. These included abnormal fluid in the abdominal cavity, abdominal adhesions, mottled adrenals, mottled liver and lungs, and various GI abnormalities. In F0 females which survived to scheduled euthanasia, gross necropsy findings were unremarkable.

The number of dead F1 pups was increased and mean live litter size was decreased at the 15 and 30 mg/kg/day levels on lactation Day 0. Pup viability continued to decrease at the same levels during the first four days of lactation. Following culling on lactation Day 4, pup viability was stabilized. Mean F1 pup body weights were decreased at the 10 and 30 mg/kg/day levels. There were no toxicologically meaningful differences among the groups in F1 developmental or functional indices, and behavioral, learning, and memory recall testing. During the F1 growth phase, all selected F1 animals survived and no treatment-related clinical observations were observed. However, mean body weight was decreased on a number of days in F1 males at the 10 and 15 mg/kg/day levels. No significant differences were observed in F1 parental animals with respect to copulation and fertility indices, precoital intervals, or mean gestation lengths. Gross necropsy findings in F1 parental animals were generally unremarkable.

For F2 pup data, there were no significant differences among the groups in pup viability, mean live litter size, male-to-female sex ratios, and necropsy observations.

In conclusion, AL-6515 produced dystocia and associated maternal mortality in F0 females at levels ≥ 3 mg/kg/day, and developmental toxicity in F1 offspring at levels ≥ 10 mg/kg/day. The developmental toxicity was characterized by decreased F 1 pup survival and decreased F1 pup body weights during

lactation and growth phases. The sponsor indicated that the findings observed in this study were very similar to those elicited by other nonsteroidal anti-inflammatory drugs (NSAIDs) which had been shown to cause prolonged gestation, delayed parturition, reduced offspring weights, and reduced offspring survival in rats. A NOEL for maternal effects in F0 females was not established in this study. The NOEL for developmental toxicity in F1 offspring was determined to be 3 mg/kg/day. No adverse effects were observed with respect to F1 reproductive parameters or F2 viability and growth.

2.6.6.7 Local tolerance

See repeated dose topical ocular toxicity studies.

2.6.6.8 Special toxicology studies

TDOC-0001891: A skin sensitization study of AL-6515 (nepafenac) in guinea pigs using the maximization method

Key Findings: AL-6515 is a non-sensitizer in the guinea pig maximization study.

Report Number:	TDOC-0001891
Protocol Number:	N-04-105
Conducting Laboratory/Location:	—
	49070-9399
Study Initiation:	9/13/2004
GLP Compliance:	Yes
Quality Assurance:	Yes
Test substance:	AL-6515, Lot No. 990802, purity = —
Positive Control:	Hexylcinnamic aldehyde (HCA)

The purpose of this study was to evaluate the potential of AL-6515 (nepafenac, amfenac amide) to elicit skin sensitization (allergic contact dermatitis) via intradermal injection and topical patch applications.

Methods:

Species/Strain/Age/Gender: Guinea pig, albino, — (HA)BR, 5-6 weeks old, male, 358-482 g

Irritation Screening:

Intradermal injection: 0.1 ml of 0.5, 1, 2, 4 and 5% AL-6515 in mineral oil, two animals/sex (n = 4). Each animal was given all doses in duplicate injections (10 in total) to five separate regions located in the shoulder area. Irritation scores were recorded at approximately 24 and 48 hr after injection. A concentration of 2% was selected based on the test article handling consideration.

Topical application: 3, 6, 12 and 25% AL-6515 in mineral oil, 2 animals/sex (n = 4). Each animal was given all doses. The dressings covering the test article patches were left in place approximately 24 hr. Irritation scores were recorded at approximately 24 and 48 hr after patch removal. Two additional animals/sex were dosed at 0.5, 1, 1.5, and 2% AL-6515 to confirm the challenge concentration. Based on the findings of these two topical irritation screens, a topical induction concentration and a topical challenge concentration of 25% and 2% AL-6515, respectively, were selected.

Definitive Study:

Intradermal injection: On Day 1, animals received two series of three injections (6 in total) on the left and right shoulder/dorsal trunk area. For each animal two sites received either the test article (2% AL-6515), positive control (5% HCA), or vehicle. Two injection sites received the test material, vehicle, or positive control mixed with 50% Fruend's Complete Adjuvant (FCA) in a one to one ratio. Two sites received 50% FCA (see table below). Injection volume was 0.1 ml.

Induction phase: intradermal injections

Group	Region number	Treatment
Vehicle control (n = 5/sex)	1	50% FCA
	2	Vehicle
	3	Vehicle/50% FCA
Positive control (n = 5/sex)	1	50% FCA
	2	5% HCA
	3	5% HCA/50% FCA
AL-6515 (n = 10/sex)	1	50% FCA
	2	2% AL-6515
	3	2% AL-6515/50% FCA

Topical application: On Day 8, topical patches were applied to the same area of the shoulder that received the injections. AL-6515 (25%) was applied to the treatment group, HCA (100%) was applied to the positive control group, and the vehicle control group received mineral oil. The topical dressings were left in place for approximately 48 hr.

Challenge: On Day 22, animals were challenged by placement of vehicle, 50% HCA or AL-6515 (2%) on the left and right flanks, respectively (see table below). The dressings were left in place for approximately 24 hr. Application sites were scored for erythema and edema at 24 and 48 hr after application. Three animals in the vehicle control group, one animal in the positive control group, and one animal in the test article group escaped their wraps prior to the 24-hr time point. The data for these animals were not included in the final evaluation.

Challenge phase: topical patch

Group	Left trunk	Right trunk
Vehicle control	AL-6515	Vehicle
Positive control	50% HCA	Vehicle
AL-6515	AL-6515	Vehicle

Parameters Evaluated:

Dermal Observations: Skin sensitization was evaluated during the irritation screens and challenge phase. The irritation and challenge sites were examined 24 and 48 hr after removal of patches or after the last injection of each region. Skin sensitization scores are listed below:

0: No reaction
1: Scattered mild erythema
2: Moderate and diffuse erythema
3: Intense erythema and edema

Two indices were used in sensitization evaluation:

SI (severity index) = Sum of the grades at interval/total # of animals
SII (sensitization index) = # of animals showing a positive response at 24 and/or 48 hr x 100/total # of animals

Results:

In intradermal irritation screen, mild to moderate erythema was noted in most animals at all concentrations. Higher concentrations (4 and 5%) required a larger gauge needle that caused some leakage from the dosing site. As a result, 2% was chosen as the concentration used in the intradermal induction phase.

In the initial topical irritation screen (3 to 25%), slight to moderate erythema was noted in most animals at all concentrations and 25% was chosen as the concentration used in the topical induction phase. To determining the concentration in the challenge phase, a second topical screening study was conducted on four additional animals at 0.5, 1, 1.5, and 2% AL-6515. In this study, slight erythema was noted and 2% concentration was chosen as the concentration used in the challenge phase.

In the challenge phase of the study, slight erythema was noted in 1 of 7 animals in the vehicle control group and in 3 of 19 animals in the AL-6515 group at 24 hr. There was no evidence of erythema in either group at 48 hr. The sensitization index (SII) for the vehicle control and AL-6515 groups was 14% and 16%, respectively. No increase in the sensitization potential was noted in the AL-6515 group when compared to the vehicle control group. In the positive control group, mild to moderate erythema was seen in all animals at both 24 and 48 hr resulting in a SII of 100%. In conclusion, under the conditions of this study AL-6515 was a non-sensitizer in the guinea pig maximization study.

TDOC-0001457: Four-week topical ocular irritation and toxicity evaluation of nepafenac (AL-6515) ophthalmic suspension degradation product — , in New Zealand white rabbits. Vol. 22

Key study findings: Nepafenac ophthalmic suspension degradation product, —, in AL-6515 ophthalmic suspension exhibited a low ocular irritation potential and did not elicit any signs of ocular or systemic toxicity.

Report no.: TDOC-0001457
Protocol #: N-04-034
Conducting laboratory and location: Alcon Research, Ltd., 6201 South freeway, Fort Worth, TX 76134
Date of study initiation: 5/11/2004
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, and % purity: AL-6515 ophthalmic suspension (0.1, 0.3, and 1.0%)
Study design

Group	n/sex	Dosing volume (µl)	Total daily dose of AL-6515 (µg)	Total daily dose of — (µg)
1. Vehicle control	4	80	0	0
2. 0.1% AL-6515	4	80	240	0
3. 0.1% AL-6515 with 0.003%	4	80	240	7.2
4. 0.1% AL-6515 with 0.0075%	4	80	240	18

The purpose of this study was to determine the ocular irritation and toxicity potential of — , a degradation product of AL-6515 in AL-6515 ophthalmic suspension resulting from tid topical ocular administration to NZW rabbits for one month. The day of treatment initiation in male animals was designated as Day 1. The first day of dosing for female animals was Study Day 2.

Methods

Doses: 0.1% AL-6515 ophthalmic suspension with 0.003% or 0.0075% — two drop per eye (80 µl), right eye only, tid x one month. The left eye served as the untreated control.

Species/strain: New Zealand white rabbits

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

Route, formulation, volume, and infusion rate: Topical ocular, 80 µl/eye, right eye only, tid x 1 month

Satellite groups used for toxicokinetics or recovery: N/A

Age: 3-4 months old

Weight; 2.5-3.5 kg

Unique study design or methodology (if any): No

The formulation of 0.1% AL-6515 ophthalmic suspension was similar to the proposed clinical formulation.

Composition of AL-6515 ophthalmic suspensions and vehicle

Component (%, w/v)	Vehicle	0.1% AL-6515	0.3% AL-6515	1.0% AL-6515
Lot #	03-500531-1	03-500529-1	04-36305	04-36287
AL-6515		0.1	0.1	0.1
—	0	0	0.003	0.0075
Benzalkonium chloride	0.005	0.005	0.005	0.005
Carbomer 974P				
Tyloxapol				
Edentate disodium				
Mannitol				
Sodium chloride				
NaOH or HCl	pH	pH	pH	pH
Purified water	qs 100	qs 100	qs 100	qs 100

In formulations labeled with 0.003% [redacted] (Group 3), the post-study analysis for — showed the concentration of — was — of the labeled strength). In formulations labeled 0.0075% [redacted] (Group 4), the post-study analysis for — showed the concentration was — of the labeled strength).

Observation times

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Biomicroscopic examination: Prescreen and on Days 7, 14, 21, 28, and 35
Indirect ophthalmoscopy: Day 35
Pachymetry and IOP: Days 14 and 28
Specular microscopy: Day 29
Hematology: Day 30
Clinical chemistry: Day 30
Gross pathology: All animals, Day 36
Organ weights: Adrenal, brain, ovaries, testes, heart, kidneys, liver, pituitary, and spleen from all animals
Histopathology: The following organ and tissues from all animals were examined histopathologically: adrenal, aorta, bone, bone marrow, brain, epididymis, eyes with optic nerve and adnexa, esophagus, stomach, small intestine, cecum, colon, rectum, ovaries, testes, heart, kidneys, lungs, liver, larynx, gall bladder, mesenteric lymph node, cervical lymph node, mammary gland and skin, nasal-lacrimal tissues,

oviduct, pancreas, peripheral nerve, pituitary, prostate, ribs/stern, salivary glands, seminal vesicle, skeletal muscle, spinal cord, spleen, thymus, thyroid/parathyroid, trachea, tongue, urinary bladder/urethra, ureters, uterus/cervix/vagina, and any gross lesion.

Results:

Clinical observations: No mortality occurred during the observation period. No toxicologically significant, treatment-related clinical signs were noted in clinical observations.

Body weights: No toxicologically significant differences in body weight changes were noted between control and drug-treated animals.

Ocular evaluations:

Biomicroscopic evaluations: No treatment-induced abnormal light reflex, aqueous flare, iritis, corneal or lenticular abnormalities were observed during the course of this study. Minimal conjunctival congestion was observed during the course of this study in all control and treatment groups. The only incident of moderate congestion (score = 2) was noted in a Group 2 animal on Day 14 only. Conjunctival discharge was observed in the treated eye of both vehicle-treated and test article treated rabbits. Because of the low incidence and severity, all these changes were not considered as incidental.

Conjunctival discharge findings in slit-lamp biomicroscopic examinations

Group	1	2	3	4
Minimal conjunctival discharge	1 (Day 35)	1 (Day 35)	1 (Day 35), 1 (Days 28)*	1 (Days 28 and 35), 1 (Days 21 and 35)
Moderate conjunctival discharge	1 (Day 21)		1 (Day 28), 1 (Day 35)*	

* Same animal

Indirect ophthalmoscopy and corneal pachymetry: No treatment-related, toxicologically significant abnormal findings were noted.

Intraocular pressure: No treatment-related, toxicologically significant abnormal findings were noted.

Specular microscopy: Specular microscopy and photography of the central corneal endothelium of both the right and left eyes showed no drug-related, toxicologically significant differences.

Clinical pathology: No treatment-related, toxicologically significant abnormal findings in hematology, clinical chemistry, and coagulation examinations were noted.

Gross examinations: No treatment-related changes were noted in gross examinations.

Organ weights: The absolute and relative weight of the liver and spleen in male rabbits treated with AL-6515, AL-6515 with 0.003% — and AL-6515 with 0.0075% were increased (see table below). A slight increase in females was also noted. Clinical pathology did not reveal changes in liver enzymes associated with AL-6515 and — treatment, and no treatment related liver and spleen histopathology findings were noted. The liver and spleen weight increase noted in Groups 2 to 4 was considered an incidental finding since AL-6515 caused no liver and spleen weight increases in several other

topical ocular and systemic exposure studies utilizing equivalent doses, higher doses, and longer exposure periods.

Liver and spleen weight data in rabbits (mean ± SD)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Liver (g)	89 8±3 91	106.9±8.13	104 7±8.0	111.7±8.7	86 9±3 27	96 5±7 67	94.4±10 14	103 0±16 6
(%)	2 6±0 13	3.1±0.25	3.1±0 22	3 3±0 22	2.5±0 08	2 8±0.32	2 8±0 18	3 0±0.49
Spleen (g)	1.02±0.09	1 42±0.49	1.23±0 37	1 67±0 59	1.32±0.51	1 25±.19	1 68±0 43	1 43±0.38
(%)	0 029±0 003	0 041±0.012	0.036±0 012	0 049±0 017	0 038±0 015	0 036±0 005	0 051±0 013	0 042±0 012

Histopathological examinations: No drug-related histopathological findings in ocular or systemic tissues were noted.

In summary, NZW rabbits treated topically with AL-6515 ophthalmic suspension (0.1%), AL-6515 ophthalmic suspension (0.1%) with 0.003% and AL-6515 ophthalmic suspension (0.1%) with 0.0075% — tid for one month showed no local and systemic toxicity. The drug was well tolerated. No toxicologically significant, treatment-related findings in clinical observations, body weight changes, ophthalmic examinations, clinical pathological examinations, necropsy, and histopathological examinations were noted.

TDOC-0001324: Bacterial reverse mutation assay with a confirmatory assay using — — a degradation product of AL-6515 (nepafenac). Vol. 22

Key findings: — was not mutagenic in the Ames assay in the presence or absence of S9 activation under the conditions of this study.

Study no.: TDOC-0001324
Protocol #: N-04-063
Conducting laboratory and location: —
Date of study initiation: June 9, 2004
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Lot#: ERM# 10195:033, purity = —

Methods

Strains/species/cell line: *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A

Doses used in definitive study: 100, 333, 1000, 3330, and 5000 µg/plate with or without S9

Basis of dose selection: Cytotoxicity after the test article treatment in a dose range-finding assay

Negative controls: DMSO

Positive controls: See table below.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)	Dose µg/plate (w/o S9)
Salmonella typhimurium	TA-1535	2-aminoanthracene 2.5	Sodium azide 2.0
	TA-1537	2-aminoanthracene 2.5	ICR-191 2.0
	TA-98	Benzo(a)pyrene 2.5	2-nitrofluorene 1.0
	TA-100	2-aminoanthracene 2.5	Sodium azide 2.0
Escherichia coli	WP2uvrA	2-aminoanthracene 2.5	4-nitroquinoline-N-oxide 1.0

Incubation and sampling times:

The tester strains were exposed to test article via the plate incorporation methodology. Following incubation (52 ± 4 hr), revertant colonies were counted by automated colony counter and by hand. All doses of the test article, the vehicle controls, and the positive controls were plated in triplicate.

Results

Dose range-finding study: Doses tested in the mutagenicity assay were based on the results of the dose range-finding assay using tester strains TA100 and WP2*uvr*A with or without S9 with one plate per dose. Ten doses of test article ranging from 6.67 to 5000 µg per plate were tested. No cytotoxicity was observed with either strain in the presence or absence of S9 mix. As the result, the doses tested in the mutagenicity assay in both the presence and absence of S9 mix were 100, 333, 1000, 3330, and 5000 µg per plate.

Mutagenicity assay: In both initial and confirmatory mutagenicity assays, no positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 activation.

Study validity: The doses used in this study were up to 5000 µg/plate. The positive controls produced typical positive results. The number of revertants per plate in vehicle controls was within the historical control range. The study was valid.

In conclusion, the results of the Ames assay indicated that under the conditions of this study, — did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of S9.

TDOC-0001325: L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay with a confirmatory assay using — , a degradation product of AL-6515 (nepafenac). Vol. 23

Key findings: — was not considered mutagenic at the TK locus of mouse lymphoma cells in the absence of S9 activation under the present testing conditions. However, the compound was positive in the presence of S9 activation.

Study no.: TDOC-0001325
Protocol #: N-04-064
Conducting laboratory and location: —
Date of study initiation: 6/7/2004
GLP compliance: yes
QA reports: yes (X) no ( )

Drug, lot #, and % purity: — , Lot#: EMR # 10195:033, purity = —

Methods

Strains/species/cell line: Mouse lymphoma L5178Y cell line

Doses used in definitive study: Initial assay without S9: 50, 100, 200, 400, 500, 600, 800 μg/ml; confirmatory assay without S9: 5, 10, 25, 50, 75, 100, 125 and 150 μg/μl; Initial assay with S9: 150, 300, 350, 400, 450, 500, 600, and 700 μg/ml; confirmatory assay with S9: 200, 400, 500, 550, 600, 700 and 800 μg/μl

Basis of dose selection: Solubility and cytotoxicity in the preliminary study

Negative controls: Water

Positive controls: Methyl methanesulfonate (MMS, 1 and 2 μg/ml) w/o S9 activation; methylcholanthrene (MCA, 2 and 4 μg/ml) w/ S9 activation

Incubation times:

	Treatment time (hr)
Initial test with or without S9 activation	4
Repeat test without S9 activation	24
Repeat test with S9 activation	4

Results

—.90 to 3000 μg/ml) was tested in a preliminary dose range finding assay with a treatment period of 4 hr with and without S9 activation and a dose range finding nonactivation assay with a treatment period of 24 hr. Severe cytotoxicity was seen at 1500 μg/ml for the 4-hr assay without S9 (relative total growth: 1.5%), 750 μg/ml for the 4-hr assay with S9 (relative total growth: 3.4%) and 188 μg/ml for the 24-hr assay without S9 (relative total growth: 2.6%). These results were used to select doses for the mutation assays.

Results of initial and confirmatory assays are summarized in the table below. For initial assays with or without S9 and for the confirmatory assay with S9, higher concentrations were not analyzed due to excessive cytotoxicity.

In both initial and confirmatory mutation assays in the absence of S9 with a treatment period of 4 hr and 24 hr, none of the treatments analyzed induced mutant frequencies that exceeded the minimum criteria for a positive response (see table below).

The initial and confirmatory mutation assays performed in the presence of S9 were conducted with a treatment period of 4 hr. In the initial assay, treatments at 450, 500 and 600 μg/ml induced mutant frequencies that exceeded the minimum criteria for a positive response with values 2.2- to 2.6-fold above the average vehicle control value. In the confirmatory mutation assay, treatment at 400, 500, 550, 600, and 650 μg/ml induced mutant frequencies that exceeded the minimum criteria for a positive response, with

values 2.4- and 4.4-times the average vehicle control value. Therefore, the test article was evaluated as positive with metabolic activation. In sizing analysis, positive concentrations induced a preferential increase in small colonies. The large colonies presumably aroused from point mutations and the small colonies from chromosome changes.

Summary of L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay results

	Initial assay				Confirmatory assay			
Treatment	**With S9**		**Without S9**		**With S9**		**Without S9**	
Concentration (μg/ml)	Relative growth (%)	Mutant frequency (10^{-6})	Relative growth (%)	Mutant frequency (10^{-6})	Relative growth (%)	Mutant frequency (10^{-6})	Relative growth (%)	Mutant frequency (10^{-6})
Vehicle	117 5	81 4	112 9	69 9	99 7	84 3	88 0	59 9
Vehicle	94 7	71 5	83 0	82.6	99 9	90 1	105 0	64 3
Vehicle	86 2	85.2	104 2	74 8	100 4	80 7	105 3	71.1
Positive	70 7***	289 6	27 2*	348 4	40 0***	478 1	4 5**	636 8
Positive	52 5****	347 3	29 0*	367 4	15 8****	549 8	5 3**	653 5
5							89 3	71 9
10							89.8	57 0
25							92 9	62.7
50			98 3	81 7		110 5	87 3	73 0
75							77 3	52 7
100			75 2	87 3			79 4	73.5
125							82 4	80 8
150	159 9	61 0					43 9	74 1
200			87 0	71 3	107 7	110 5		
300	171 8	57 9						
350	132 2	95 2						
400	84 7	145 5	62 3	79 3	63 0	**207.7**		
450	73 2	**174.1**						
500	54 5	**205.3**	53.4	87 0	41 8	**325.3**		
550					27 1	**333.7**		
600	32 3	**188.2**	51 5	84 5	25 9	**357.4**		
650					15 4	**377.2**		
800			48 4	95 7				

* MMS 13 μg/ml; ** MMS 6.5 μg/ml; *** MCA 2 μg/ml; ****MCA 4 μg/ml

In conclusion, — was evaluated as negative for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells under non-activation conditions. The test article was positive for inducing forward mutations under activation conditions.

TDOC-0001890: *In vivo* micronucleus assay in CD-1 mice using — , a degradation product of AL-6515 (nepafenac). Vol. 23

Key findings: — was negative in the micronucleus assay under the experiment conditions in this study.

Study no.: TDOC-0001890
Protocol #: N-04-135
Conducting laboratory and location: —

—

Date of study initiation: 8/24/2004
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: — formulated in sterile water for injection), Lot#: ERM #:10195:033, purity = —

Methods

Strains/species/cell line: —.CD-1 (ICR) BR mice, 9 weeks old, 31.1-36.8 g, 6/group/time point (Only males were used in micronucleus assay.)

Doses used in definitive study: 12.5, 25, or 50 mg/kg, iv, tid for one day (at 1-hr intervals)

Basis of dose selection: A dose range finding study

Negative controls: Sterile water for injection

Positive controls: Cyclophosphamide (CP) 80 mg/kg, po (gavage), single dose

Incubation and sampling times: Animals (6/dose/time point) were terminated at 24 and 48 hr after the last dosing and bone marrow samples were prepared. Positive control animals were sacrificed at 24 hr after dosing. The number of micronucleated polychromatic erythrocyte (MPCE) then was determined for 2000 PCE per animal. The proportion of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was determined per 500 erythrocytes.

Dosing schedule

Treatment (mg/kg/tid)	Route	Dosing volume (ml/kg)	24 hr harvest	48 hr harvest
12.5	iv	20	6	
25	iv	20	6	
50	iv	20	6	6
Vehicle	iv	20	6	6
CP 80 mg/kg, po, single dose	Oral gavage	10	6	

Results

In the dose range-finding assay, animals (3/sex/dose) were treated by three consecutive intravenous injections (at 1-hr intervals) at 25, 50 or 100 mg/kg and were observed for 2 days for toxic signs and/or mortality. Mortality occurred one or two days after dosing in 3/3 males and 2/3 females at 100 mg/kg, and 1/3 female at 50 mg/kg. Clinical signs including hypoactivity, tremors, irregular respiration, and squinted eyes were noted in animals at 100 and 50 mg/kg groups. Based on these results, the maximum tolerated dose was estimated to be 50 mg/kg/tid and doses of 12.5, 25, and 50 mg/kg/tid were selected for the micronucleus assay.

In the micronucleus assay, convulsions and gasping were seen immediately after the third dosing in one animal treated at 12.5 mg/kg. No mortality or other abnormal clinical observations were seen. No statistically significant increase in the micronucleus frequency was observed in polychromatic erythrocytes (PCEs) from male mice treated with 12.5, 25, and 50 mg/kg/tid of — at either 24-hr or 48-hr bone marrow sampling time points. A statistically significant decrease in the PCE/NCE ratio was observed with 50 mg/kg/tid of — at the 48-hr sampling time point (0.30 ± 0.04 vs. control's 0.42 ± 0.04), but not the 24-hr sampling time point. The values were within the historical control range 0.16-1.00. The sponsor indicated that the decrease in the PCE:NCE ratio relative to the concurrent vehicle control was direct evidence of bone marrow exposure to the test article or its metabolite(s).

Study validity: The positive controls produced a significant increase in the number of MPCE. The number of MPCE from vehicle controls was within the historical control range. The study was valid.

In conclusion, — was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

Summary of special toxicity studies:

— a known degradation product of AL-6515, was evaluated in a battery of genotoxicity studies. The compound was negative in the Ames test and *in vivo* mouse bone marrow micronucleus assay. In an *in vitro* mouse lymphoma TK assay, — was positive for inducing forward mutations under activation conditions.

In an ocular toxicity study conducted in NZW rabbits, AL-6515 ophthalmic suspension (0.1%) containing the degradation product — , at concentrations up to — (tid for one month) showed no local and systemic toxicity.

2.6.6.9 Discussion and Conclusions

Nepafenac, an NSAID, is being developed for the — treatment of pain and inflammation associated with cataract surgery. Pharmacological studies showed that nepafenac rapidly penetrated the cornea and was converted to the active moiety amfenac by tissue hydrolases. The cyclooxygenase inhibitory activity of nepafenac was weaker than that of amfenac. Topical administration of nepafenac significantly inhibited trauma-induced prostaglandin production and leakage of the ocular vasculature.

AL-6515 was hydrolyzed to amfenac in all species with all routes tested. Plasma exposures to amfenac were higher than those to AL-6515. The plasma half-life for amfenac amide and amfenac was short. The plasma half-lives of radioactivity were long, suggesting other uncharacterized metabolites. Following topical ocular administration of ^{14}C-AL-6515 to rabbits, radioactivity was absorbed into the eye with high concentrations of radioactivity in the conjunctiva and cornea. ^{14}C-AL-6515 or its radioactive drug equivalents did not bind to melanin pigmented tissues. Oral administration of ^{14}C-AL-6515 to pregnant rats resulted in distribution of radioactivity to maternal tissues and placental transfer of radioactivity into the developing fetus. Radioactivity was also found in the milk of lactating rats. ^{14}C-AL-6515 bound moderately to plasma proteins of rat, monkey, and human *in vitro* (73% to 84%) in a concentration-independent manner over the concentration range of 10 to 1000 ng/ml. Incubation of ^{14}C-amfenac amide in precision-cut human liver slices produced 12 metabolites. The major metabolite was amfenac with the remaining metabolites being present in relatively low amounts. Drug-derived radioactivity was rapidly excreted after iv administration to rats. The major route of excretion was via urine. Biliary excretion was also an important elimination pathway.

Several acute and repeated-dose oral systemic toxicity studies were conducted in rats with the duration up to 6 months. In the 2-week study, jejunal serositis and mesenteric lymphoid hyperplasia were noted in rats of 25 mg/kg/day group. The sponsor indicated that these changes were considered to be secondary to intra-abdominal trauma, possibly associated with gavage procedures. However, distinct gavage trauma was not observed grossly or microscopically in abdominal tissues, and a relationship between drug treatment and these findings could not be entirely ruled out. In the 3-month toxicity study in SD rats,

histopathological examination showed renal papillary necrosis in two of ten females at 15 mg/kg only. GI and renal lesions were common findings in animals treated with high doses of NSAIDs. GI abnormalities including stomach or intestine distended with fluid or gas, abnormal mucoid contents in the stomach and small intestine, abnormal fluid, granular or gelatinous material in the abdominal cavity, abdominal adhesions, and perforated or eroded mucosa of the GI tract were noted in rats at $\geq$ 30 mg/kg doses in acute and reproductive studies, indicating that the GI tissues were the target organs of toxicity. TK evaluations showed that at 10 mg/kg/day dose (at which dose no GI toxicity was noted), systemic exposures to AL-6515 and AL-6295 were 500 and 1600 times human exposure under the proposed clinical dosage (see table below). Because of the great safety margin, GI toxicity is not a concern for this drug in this indication.

AUC (ng-hr/ml)	Rats (10 mg/kg)	Human (0.1%, tid x 4 days)	Animal/human
AL-6515	189±22	0 368±0 106	500
AL-6295	1550±106	0 976±0 284	1600
Cmax (ng/ml)			
AL-6515	49.5±21.9	0 310±0 104	160
AL-6295	388±99	0 422±0 121	900

In the 6-month toxicity study in F344 rats, higher incidences of corneal mineralization (5 of 25 in males vs. 0 in control animals) and uterus hydrometra (5 of 25 in females vs. control's 1 of 25) were seen at 10 mg/kg/day. Similar changes were not seen in other studies including 6-month ocular toxicity in which 1.0% AL-6515 ophthalmic suspension was used. Corneal and uterus abnormalities were not listed in the common adverse events seen in clinical studies. In addition, the systemic exposure to AL-6515 and AL-6295 at 10 mg/kg/day was much higher than that in humans. These findings might not be toxicologically significant.

Several repeated dose ocular toxicity studies were conducted with durations up to 3 months in monkeys (concentrations up to 1.0%, qid) and NZW rabbits (concentrations up to 1.0%, qid), and 6 months in pigmented rabbits (concentrations up to 1.5%, tid). The drug was well tolerated. No drug-induced systemic and ocular toxicity was observed. In all studies, minimal to moderate conjunctival congestion and transient and sporadic incidences of minimal conjunctival discharge were seen in the eye treated with vehicle and drugs. Because of the similar incidences and severity between control and treated eyes, these changes were not considered as drug-related. In a rabbit study in which nepafenac ophthalmic suspension (up to 1.0%) was administered prior and subsequent to a corneal incision, no significant ocular and systemic toxicity as well as postoperative ocular complications were noted.

AL-6515 was nonmutagenic in the Ames test and in L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay. The drug was also negative in *in vivo* micronucleus assay. AL-6515 was positive for the induction of structural chromosome aberrations in CHO cells.

In a fertility and early embryonic development study conducted in SD rats. Male animals of the 15 mg/kg group showed lower sperm motility and sperm concentrations compared to the control males. Histological examination in the 15 mg/kg group showed slightly decreased spermatozoa in the duct of the epididymis, and slightly more intraluminal single necrotic cells in the epididymis in two of three animals examined. In females, there were no toxicologically significant differences in copulation and fertility indices between control and treated groups. However, a decrease in the number of viable fetuses and an increase in the early resorption and post-implantation loss were noted in animals at 10 and 15 mg/kg. Oral administration of AL-6515 in rats at 3.0 mg/kg showed no developmental toxicity in this study.

In the embryo-fetal development study in pregnant rats, a slight decrease in fetal body weight (3.3 ± 0.5 g vs. control's 3.5 ± 0.2 g) was seen in HD (30 mg/kg) group. One HD animal had 9 dead fetuses, 6 resorptions, and no viable fetuses. The observed malformations were not considered treatment-related due to the low incidence and lack of dose-dependence. Regarding developmental variations, the incidences of unossified 5th and 6th sternebrae and 7th cervical ribs were significantly higher in the HD group than in the control group. Based on the study results, the dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

In the embryo-fetal development study in pregnant rabbits, abortion occurred in one MD (10 mg/kg) animal and one HD (30 mg/kg) animal. One HD animal had a premature delivery. HD animals showed a decrease in body weight gain and food consumption. Regarding reproductive evaluation, HD animals showed an increase in post-implantation loss which was mainly due to an increase in early resorptions. There was a statistically significant increase in the number of litters with skeletal malformations and in the number of litters with total malformations in the 30 mg/kg/day group when compared to the controls. Low incidences of malformations were seen in the MD and LD groups and were not considered drug-related because they occurred at a low incidence, were dissimilar in nature, and were not statistically different from the controls. Based on the study results, the dose of 3 mg/kg was considered a NOEL for maternal toxicity and a dose of 10 mg/kg was considered a NOEL for developmental toxicity in rats.

The dose of 10 mg/kg/day was the NOEL for both rat and rabbit segment 2 studies. The following table compares the plasma exposure to AL-6515 and AL-6295 between animals at 10 mg/kg/day and humans following multiple bilateral dosing of nepafenac ophthalmic suspension 0.1%.

AUC (ng-hr/ml)	Rats (10 mg/kg)	Rabbits	Human (0.1%, tid x 4 days)	Rat/human	Rabbit/human
AL-6515	97 0-207	28 4-62 5	0 368±0 106	260	77
AL-6295	2340-4190	663-3070	0 976±0 284	2400	680
Cmax (ng/ml)					
AL-6515	69 6-242	39 3-70 8	0 310±0 104	225	127
AL-6295	793-1710	666-2100	0 422±0 121	1900	1578

In the perinatal and postnatal study in pregnant F0 rats, AL-6515 produced dystocia and associated maternal mortality in F0 females at levels ≥ 3 mg/kg/day, and developmental toxicity in F1 offspring at levels ≥ 10 mg/kg/day. The developmental toxicity was characterized by decreased F 1 pup survival and decreased F1 pup body weights during lactation and growth phases. A no-observed-effect level (NOEL) for maternal effects in F0 females was not established in this study. The NOEL for developmental toxicity in F1 offspring was determined to be 3 mg/kg/day.

— , a known degradation product of AL-6515, was evaluated in a battery of genotoxicity studies. The compound was negative in the Ames test and *in vivo* mouse bone marrow micronucleus assay. In an *in vitro* mouse lymphoma TK assay, — was positive for inducing forward mutations under activation conditions. In an ocular toxicity study conducted in NZW rabbits, AL-6515 ophthalmic suspension (0.1%) containing the degradation product — . at concentrations up to — (tid for one month) showed no local and systemic toxicity. The reviewer has discussed with the chemistry team for this degradation product. Considering the positive finding in one *in vitro* genotoxicity study, and the level of the degradation product that was below the qualification threshold, the chemistry team will set a low acceptance criterion for this degradation product.

2.6.6 TOXICOLOGY TABULATED SUMMARY

Please see Volume 5, Module 2.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Nepafenac is a nonsteroidal anti-inflammatory agent. Nonclinical PK studies showed that following topical ocular administration of ^{14}C-AL-6515 to rabbits, radioactivity was absorbed into the eye with high concentrations of radioactivity in the conjunctiva and cornea. Nonclinical toxicity studies showed no unexpected toxicologically significant events.

Unresolved toxicology issues (if any): No

Recommendations:

This application is approvable from a nonclinical perspective with some minor modifications of labeling as revised in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

Suggested labeling:

Minor modifications of labeling are recommended in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

Original labeling:

Revised labeling:

Carcinogenesis, Mutagenesis, Impairment of Fertility:

Nepafenac has not been evaluated in long-term carcinogenicity studies.

Increased chromosomal aberrations were observed in Chinese hamster ovary cells exposed *in vitro* to nepafenac suspension. Nepafenac was not mutagenic in the Ames assay or in the mouse lymphoma forward mutation assay. Oral doses up to 5,000 mg/kg did not result in an increase in the formation of micronucleated polychromatic erythrocytes *in vivo* in the mouse micronucleus assay in the bone marrow of mice.

Nepafenac did not impair fertility when administered orally to male and female rats at 3 mg/kg (approximately 90 and 380 times the plasma exposure to the parent drug, nepafenac, and the active metabolite, amfenac, respectively, at the recommended human topical ophthalmic dose).

Pregnancy: Teratogenic Effects

Pregnancy Category C: Reproduction studies performed with nepafenac in rabbits and rats at oral doses up to 10 mg/kg/day have revealed no evidence of teratogenicity due to nepafenac, despite the induction of maternal toxicity. At this dose, the animal plasma exposure to nepafenac and amfenac was approximately 260 and 2400 times human plasma exposure at the recommended human topical ophthalmic dose (following multiple bilateral tid dosing of NEVANAC™ ophthalmic suspension) for rats and 80 and 680 times human plasma exposure for rabbits, respectively. In rats, maternally toxic doses

Nepafenac has been shown to cross the placental barrier in rats. There are no adequate and well-controlled studies in pregnant women.

Because animal reproduction studies are not always predictive of human response, NEVANAC™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Non-teratogenic Effects: Because of the known effects of prostaglandin biosynthesis inhibiting drugs on the fetal cardiovascular system (closure of the ductus arteriosus), the use of NEVANAC™ ophthalmic suspension during late pregnancy should be avoided.

Nursing Mothers: NEVANAC™ ophthalmic suspension is excreted in the milk of pregnant rats. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when NEVANAC™ ophthalmic suspension is administered to a nursing woman.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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

/s/

Zhou Chen
7/19/05 01:49:07 PM
PHARMACOLOGIST

Robert Osterberg
7/22/05 01:38:51 PM
PHARMACOLOGIST

Lillian Gavrilovich
7/25/05 04:49:44 PM
MEDICAL OFFICER

REVIEW NDA 21-862
Submission Date: February 25, 2005

To:	**Raphael Rodriguez**
From:	**Zhou Chen, Ph.D., DAIOP**
Through:	**Robert Osterberg, Ph.D., DAIOP**
Date:	**July 22, 2005**
Re:	**NDA 21-862**
	— a degradation product
Sponsor:	**Alcon, Inc.**

— is a degradation product of nepafenac. In an *in vitro* mouse lymphoma TK assay, — was positive under activation conditions. The sponsor proposed an acceptance criterion of — The chemistry team is considering lowering the limit to — based on the positive genotoxicity study.

Although the degradation product is positive in one *in vitro* genotoxicity study, an ocular toxicity study conducted in NZW rabbits with AL-39187 at up to 6.5% of nepafenac (tid for one month) showed no local and systemic toxicity. At the proposed limit of —, the total daily degradant ocular exposure will be — person — µg/kg/day). The proposed clinical duration of the drug (nepafenac ophthalmic solution) is only for 2 weeks. In addition, Amfenac sodium has been marketed in Japan since 1986 (as Fenazox) in an oral dosage form (50 mg qid) for the treatment of pain and inflammation associated with rheumatoid and osteoarthritis. Based on the nonclinical study results, very low ocular dose levels, very low systemic exposure via the ocular route and previous human experience, the reviewing pharmacologist states that — — is qualified and will present no safety concerns.

In conclusion, from the safety point of view, the limit of — for — is acceptable.

cc:
NDA 21-862/Division File
HFD-520/CSO/Rodriguez
HFD-520/TL Pharm/Osterberg
HFD-520/Pharm/ChenZ

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/s/

Zhou Chen
7/22/05 01:16:17 PM
PHARMACOLOGIST

Robert Osterberg
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