

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-257

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS: Travatan, travoprost ophthalmic solution 0.0015% and 0.004%, glaucoma, intraocular pressure, ocular hypertension, open angle glaucoma

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NDA 21-257

DRUG: Travatan™ (Travoprost) Ophthalmic Solution

OTHER CODE NAMES: CH4074, ALO6221, AL-6221 or AL-06221

SPONSOR: Alcon Universal Ltd. **Authorized US Agent**
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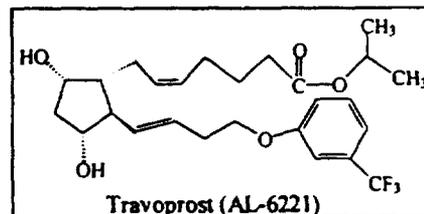
DRUG CATEGORY: PGF_{2α} Analogue
(FP prostanoid receptor agonist)

FORMULA: (5Z, 13E)-(9S, 11R, 15 S)-9, 11, 15-Trihydroxy-5, 13-prostadienoic acid, 1-methylethyl ester;
C₂₆H₃₅F₃O₆; MW=500.56

CAS REGISTRY N^o: 157283-68-6

INDICATION: Reduction of Intraocular Pressure (IOP) in Patients with Open Angle Glaucoma (OAG) or Ocular Hypertension (OHT)

DOSAGE FORM: 0.0015% and 0.004% Ophthalmic Solution



Ingredient	Travoprost Ophthalmic Solution, 0.004%		Travoprost Ophthalmic Solution, 0.0015%	
	% (w/v)	mg/ml	% (w/v)	mg/ml
Travoprost				
Polyoxyl 40 Hydrogenated Castor Oil (HCO-40)				
Tromethamine				
Boric Acid				
Mannitol				
Edetate Disodium				
Benzalkonium Chloride				
NaOH and/or HCl, Adjust				
Purified Water				

RELATED INDs/NDAs/DMFs:

PROPOSED DOSE: 1 drop (25 µl) per eye, once daily, used as either primary or adjunctive therapy (Total dose could be 2 µg/patient/day or 0.04 µg/kg for a 50 kg adult)

ROUTE OF ADMINISTRATION: Ocular, topical

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1. PHARMACOLOGY

1.1. OVERVIEW

Travoprost (AL-6221), an isopropyl ester derivative of the free acid AL-5848, is believed to be hydrolyzed to the free acid by ester hydrolase enzymes located in the cornea and to appear in the aqueous humor as the free acid. AL-5848 is a highly selective and potent agonist at the FP prostanoid receptor with a K_i for binding to the FP receptor of 52 nM and a functional potency (EC_{50}) at this receptor of 4 nM. The selectivity ratio of AL-5848 for binding to the FP receptor relative to other prostanoid receptors is in excess of 67-fold. Travoprost demonstrates both higher potency and higher selectivity for the FP receptor than the two prostaglandins that have been previously approved for therapeutic use to lower IOP [Xalatan (latanoprost) by Pharmacia Corporation and Rescula (isopropyl unoprostone) by Ueno in Japan] based on comparison of the active free acids of each compound.

1.2. PHARMACODYNAMICS-RELATED TO THERAPEUTIC EFFICACY

1.2.1.1 IOP Reduction in Lasered Hypertensive Cynomolgus Monkeys

In 2 studies, instillation of a single dose of 0.3 μ g travoprost into a lasered eye of a cynomolgus monkey produced a peak reduction in IOP of 19-26% (8.5-11 mm Hg) at 3 to 4 hr postdose. When continued in a bid dosing regimen, 0.3 μ g travoprost produced an IOP reduction of 18.5-30% (8.5-12 mm Hg) 16 hr after dose 4, and an acute peak reduction of 31-28.5-31% (12-13.5 mm Hg) at 2 and 4 hr after dose 4. A single dose of 0.1 μ g of travoprost did not significantly lower IOP. However, continued bid dosing resulted in significant lowering of IOP after dose 4 and dose 5.

In 2 other studies, once daily dosing at 5 pm with 0.3 or 1.0 μ g of travoprost over several days lowered IOP by 17.5-25% at 16 and 24 hr after dose 3 and subsequent doses. No additional IOP reduction was observed with an additional dose given 16 hr after dose 8, indicating that maximum effect was obtained with the daily dosing regimen. By 48 hr after the last dose, IOP returned to baseline.

1.2.1.2 Effects on Pupil Diameter in Cats

Published data indicated that the cat iris sphincter was stimulated to contract by FP prostanoid receptor agonists. In these studies, the sponsor used this pharmacological response to characterize the *in vivo* miotic activity of prostaglandins of this class, providing potency data for comparison of various compounds. In this experimental model, pupil diameter was determined before and at multiple times following topical ocular administration of travoprost at 0.01-1 μ g. Travoprost induced a dose-related miosis with ED_3 value of 0.014 μ g, which was less than those for latanoprost and PGF 2α isopropyl ester (0.13 and 0.03 μ g, respectively). In conclusion, travoprost was approximately 10-fold more potent than latanoprost and 2-fold more potent than PGF 2α isopropyl ester in the cat pupil diameter model.

1.2.1.3 IOP Reduction in Guinea Pigs

The effect of travoprost on IOP of guinea pigs was evaluated during three days of twice daily topical ocular instillation. Using a 0.003% solution of travoprost, a 0.3 μ g dose was instilled in the left eyes of ten guinea pigs after a baseline IOP measurement, with subsequent doses given approximately 8,

24, 32, and 48 hours later. Right eyes received vehicle on the same schedule. The results showed that travoprost reduced IOP below baseline by a maximum of 13.1% after the first dose and a maximum of 17.6% after the fifth dose. In this species, the IOP reduction did not persist through 16 hours after Dose 4.

1.2.1.4 Comparison of various esters of travoprost free acid

The purpose of these studies was to compare the free acid of travoprost (AL-5848) with travoprost (isopropyl ester) or the corresponding ethyl and neopentyl esters with respect to efficacy for IOP reduction in monkeys, efficacy for miosis in cats and the potential for conjunctival hyperemia in rabbits. In these studies, all esters were more potent for all three effects than the free acid. Travoprost and the ethyl ester reduced IOP by a similar amount in monkeys with 1 µg doses (-22% for travoprost and -24% for the ethyl ester), but the neopentyl ester and the acid were less effective (-13.5% at 1 µg and -17% at 3 µg, respectively). In the rabbit, the ethyl ester produced a significant degree of conjunctival hyperemia at 3 µg or greater doses, whereas travoprost was free of significant hyperemia with a 3 µg dose. Thus, the therapeutic index for travoprost was better than that for the free acid or the corresponding ethyl or neopentyl esters.

1.2.1.5 Increases in Optic Nerve Head Blood Flow in Dutch-Belted Rabbits

The purpose of this study was to investigate the effects of travoprost on optic nerve head blood flow (ONHBF) in Dutch-belted rabbits. ONHBF and systemic circulatory parameters were monitored using a crossover design with once daily treatment (1.2 µg travoprost or vehicle) for seven days. Baseline values for ONHBF, systemic blood pressure, heart rate and acid-base status did not differ when measured at different times. The ONHBF and systemic variables were not changed by the vehicle treatment. After one week topical ocular treatment with travoprost, ONHBF was significantly increased by 13.4% when compared to baseline or when compared to the vehicle group. IOP was significantly decreased by 5.5% when compared to baseline or to the vehicle treated group. In this study, there were no significant changes in systemic blood pressure or arterial blood gases or pH.

1.3. SAFETY PHARMACOLOGY

1.3.1.1 Prostanoid receptor selectivity

AL-5848, the free acid parent of travoprost, exhibits high affinity in a ligand binding assay for the FP receptor of bovine corpus luteum ($K_i = 52$ nM). In contrast, AL-5848 had low micromolar affinity for DP, EP3, EP4, IP and TP subtypes of prostanoid receptors (see table below). Furthermore, AL-5848 was also evaluated in ligand binding assays for 32 nonprostanoid receptors (including muscarinic, adrenergic and serotonin receptors), and showed no meaningful affinity for these receptors. Thus, based on ligand binding studies, travoprost, presumably acting by means of its free acid parent AL-5848, possesses high affinity and selectivity for the FP prostanoid receptor and a low affinity for nonprostanoid receptors.

AL-5848 receptor binding affinity (K_i)

Receptor	DP	EP3	EP4	FP	IP	TP
Affinity (nM)	46000	3500	12000	52	90000	120000

AL-5848 has also been evaluated in functional *in vitro* assays measuring intracellular second messenger responses in cultured cells. AL-5848 demonstrated high potency ($EC_{50} = 4$ nM) as a full

agonist on FP receptor coupled phosphoinositide turnover in Swiss 3T3 mouse fibroblast cells. In contrast, AL-5848 was inactive in an embryonic bovine tracheal (EBTr) cell line and in a human ciliary epithelial cell line which demonstrate adenylyl cyclase second messenger responses to DP and EP2 active compounds, respectively.

These data support the conclusion that AL-5848, derived from the isopropyl ester derivative travoprost, is a potent, high affinity and highly selective agonist for the FP receptor.

1.3.1.2 Conjunctival Hyperemia

The purpose of these studies was to evaluate the ocular irritation potential of travoprost with different formulations in New Zealand albino rabbits following ocular topical instillation (0.3, 1 or 3 µg). Conjunctival changes were graded using a slit-lamp before and 1, 2, 3 and 5 hr after instillation. The percent of eyes scoring +2 or greater at each time point was calculated. The table below presents results obtained with travoprost as overall incidence of hyperemia (percent of observations where hyperemia was present with four hourly observations in ten eyes). Travoprost, at doses up to 3 µg, did not induce a significant degree of ocular hyperemia in rabbits.

Conjunctival hyperemia in rabbits following travoprost treatment

Study number	15665	15680	15714
Compound	Incidence of hyperemia (µg dose)		
Travoprost	0% (0.3)a	18% (0.3)a	15% (3.0)a
	0% (0.3)b	0% (1.0)a	5% (3.0)c
Latanoprost		10% (3.0)a	3% (3.0)b
		8% (1.8)	0% (1.8)

1.3.1.3 Systemic Pharmacology

Safety pharmacology studies were conducted to evaluate travoprost in CNS, cardiovascular, renal, and gastrointestinal models with subcutaneous and/or intravenous routes of administration. In cardiovascular studies, travoprost produced a moderate increase in cardiac contractility at 10 µg/kg, equivalent to 250-fold the daily therapeutic dose. A small increase in GI propulsion was noted with a 30 µg/kg dose. As expected for a potent FP prostanoid agonist, AL-5848, the parent free acid of travoprost, induces contraction of estrogen primed rat uterus at concentrations above 3 nM. It appeared that the topical ocular administration of travoprost had a low potential for producing pharmacological effects other than known FP prostanoid receptor mediated actions.

CNS: Neuropharmacological profile was determined in 2 mouse studies. In the first study, male CD-1 mice were subcutaneously treated with travoprost at 1, 10 or 30 µg/kg and were observed for 24 hr. No apparent neuropharmacological signs were observed. Body temperature was not affected. Travoprost did not increase the barbiturate-induced sleep time.

In the second mouse study, animals were orally (gavage) treated with travoprost at 1, 10 or 30 µg/kg and were observed for neuropharmacological signs through 24 hr. Decreased activity and mild labored breathing were noted in 1 mouse at mid dose (2 hr after dosing, lasted for 1 hr) and 2 mice at high dose (1 hr after dosing, lasted for 2 hr). No other abnormal findings were noted.

Renal: The purpose of this study was to determine the potential effect of travoprost on urinary volume output, pH and electrolyte concentrations in rats. Following 17-18 hr fast, male Sprague-Dawley rats were hydrated with 0.9% saline at 25 ml/kg. Animals were intravenously treated with travoprost at 1 or 10 µg/kg. The rats were force urinated and urine was collected over 4 hr after dosing. The results showed that travoprost at 1 and 10 µg/kg iv produced no biologically relevant changes in urine volume output, pH and Na⁺, K⁺, and Cl⁻ concentrations.

Cardiovascular: In a study conducted in normotensive rats, intravenous injection of travoprost at 1 and 10 µg/kg did not produce biologically significant changes in blood pressure and heart rate.

In a dog study, 4 dose levels of travoprost (0.1, 0.3, 1.0 and 10 µg/kg) were evaluated using intravenous administration. Travoprost at 0.1, 0.3, and 1.0 µg/kg had no biologically meaningful effects on blood pressure (systolic, diastolic and mean), heart rate, cardiac output, left ventricular pressure, +dP/dt and left ventricular end diastolic pressure when administered in an ascending cumulative manner. Travoprost at 10 µg/kg increased all cardiovascular parameters, with the exception of heart rate and left ventricular end diastolic pressure.

In another dog study using AL-5848, the parent free acid of travoprost, the drug was given to 4 dogs using cumulative intravenous administration at 0.1, 0.3, 1.0 and 10 µg/kg. AL-5848 at 0.1, 0.3 and 1.0 µg/kg produced similar minimal, transient effects on some cardiovascular parameters (small increases in blood pressure, left ventricular pressure, +dP/dt and left ventricular end diastolic pressure). AL-5848 at 10 µg/kg increased blood pressure, heart rate, left ventricular pressure and +dP/dt. Decreases in cardiac output and left ventricular end diastolic pressure were noted. Both the magnitude and duration of the effects were greater than those observed following the lower doses of AL-5848.

In a dog study using subcutaneous administration, travoprost (10 and 30 µg/kg) was given to 8 dogs (4/group) and hemodynamic parameters were observed for 4 hr after dosing. The subcutaneous administration of travoprost at 10 µg/kg produced a steady increase in +dP/dt with maximum effect of 30% at 180 minutes following dose administration. Other cardiovascular parameters were unaffected. The 30 µg/kg dose of travoprost produced minimal increases (16%) in blood pressure, heart rate, cardiac output and left ventricular pressure. Contractility (+dP/dt) increased steadily to a maximum of 51% at 155 minutes.

In a study to determine the effects of travoprost on the cardiovascular response of the anesthetized dog to various autonomic agonist (epinephrine, norepinephrine, acetylcholine, histamine, and isoproterenol), travoprost at 1 or 10 µg/kg was subcutaneously injected to 4 dogs. The pharmacodynamic effects were determined by comparing responses to the iv injections of the autonomic agonists prior to and immediately after travoprost injection. Travoprost did not significantly potentiate or inhibit the cardiovascular response of the anesthetized dog to any autonomic agonist.

In an in vitro assay, three concentrations of AL-5848, the free acid of travoprost, (1, 10 and 100 nM) were evaluated using isolated canine cardiac Purkinje fibers with pacing stimulation frequencies of 0.5 and 1 Hz to determine the effects of travoprost on action potentials of dog Purkinje fibers. The action potential duration at 90% repolarization (APD90) was slightly (4.8%) greater when exposed to 1 nM AL-5848 than with vehicle, but similar magnitude effects at higher concentrations did not achieve statistical significance. In contrast, *d,l*-sotalolol, a K⁺ channel blocker known to increase action potential duration, prolonged APD90 by 77% and 55% at 0.5 and 1 Hz, respectively. Since the small prolongation of APD90 noted with AL-5848 was not concentration-related, did not demonstrate an inverse frequency

effect, and achieved statistical significance only at the lowest concentration of 1 nM, it was concluded that AL-5848 has no meaningful potential to prolong cardiac repolarization.

Respiratory: Two studies were conducted in guinea pigs with intravenous injections of travoprost at 1 and 10 µg/kg. The first study was to determine the effects of the drug on pulmonary parameters. Travoprost (iv) produced no significant effects on airway resistance and dynamic lung compliance. A small increase in respiratory rate following travoprost treatment was not considered to be biologically significant.

The second study was to determine the ability of travoprost (iv) to modulate a histamine-induced bronchoconstriction in the guinea pig. A submaximal concentration of histamine was intravenously administered prior to and 5 min following treatment with travoprost (1 and 10 µg/kg, iv) or vehicle. The results indicated that travoprost did not alter histamine-induced bronchospasm in guinea pigs.

Gastrointestinal: One study was conducted to determine the potential effect of travoprost on peristalsis in mice. Male CD-1 mice were administered travoprost at 1, 10 or 30 µg/kg via subcutaneous injection. Thirty min after injection, a 10% suspension of activated charcoal in 0.25% methylcellulose was administered orally at 10 ml/kg. The animals were terminated 30 min after receiving charcoal and the length of the intestine and the distance traveled by charcoal were evaluated. The results showed that travoprost at 1.0, 10 and 30 µg/kg produced a 14%, 23% and 41% increase, respectively, in gastric motility when compared to the control article treated group.

Reproductive: The sponsor conducted an in vitro study to determine the effect of AL-5848, the parent free acid of travoprost, on isolated rat uterus. AL-5848 was applied cumulatively starting at 1×10^{-10} M. The highest concentration tested was 1×10^{-6} M. Concentration-dependent contractions were observed between 3×10^{-9} M and 1×10^{-6} M. This effect is consistent with the known agonist activity of AL-5848 on FP prostanoid receptors.

General Pharmacology Conclusions:

- Travoprost, at doses up to 30 µg/kg, produces minimal or no effects on CNS, renal and respiratory systems.
- Travoprost and AL-5848, the parent free acid of travoprost, increase cardiac contractility with intravenous or subcutaneous doses of 10 µg/kg, but have minimal or no effect with lower doses.
- Based on evaluation of AL-5848 in canine Purkinje fibers, travoprost has minimal potential for effects on cardiac repolarization.
- AL-5848, contracts rat uterus as expected for an FP prostanoid receptor agonist.
- A 250-fold excess above the therapeutic clinical dose of travoprost (0.04 µg/kg; corresponding to one drop of 0.004% travoprost, once daily in each eye of a 50 kg person) is required to produce meaningful systemic effects in preclinical models.

1.4. SUMMARY OF PHARMACOLOGY

- Travoprost is a highly selective FP prostanoid receptor agonist.
- Travoprost solutions reduce IOP in ocular hypertensive lasered cynomolgus monkeys after topical ocular instillation of single or multiple doses.

- Travoprost produces a dose-related reduction of IOP in lasered eyes of monkeys, with an apparent maximal response produced by 0.3 to 1.0 μg doses.
- Once daily dosing with travoprost lowers IOP to a similar extent as BID dosing. The duration of response to once daily dosing with travoprost is expected to provide adequate IOP control for clinical anti-glaucoma therapy.
- After topical ocular instillation of multiple doses, travoprost increases optic nerve head blood flow with no significant changes in systemic blood pressure or arterial blood gases or pH.
- Conjunctival hyperemia may be produced by topical ocular administration of travoprost; however, if hyperemia is observed, it is expected to be relatively mild.
- No meaningful responses were noted in the systemic pharmacology studies at doses less than 250-fold excess above the therapeutic dose, suggesting that systemic untoward effects with the proposed clinical doses are unlikely to occur.

2. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

[Reviewer's Comments: Travoprost is an isopropyl-ester prodrug of AL-5848. In vivo and in vitro, it is rapidly de-esterified by esterase. As a result, nonclinical PK studies using non-radiolabeled travoprost were analyzed for concentrations of AL-5848.]

2.1. OCULAR ADME

2.1.1.1 Distribution of Radioactivity in Ocular Tissues Following a Single Topical Ocular Dose of 0.004% ^3H -AL-6221 to Male New Zealand Rabbits. Alcon Technical Report No.: 019:38570:0598 (Vol.45 Pg. 5 – 8240)

The purpose of this study was to determine the ocular distribution of travoprost in male New Zealand white rabbits following a single unilateral topical ocular administration of ^3H -AL-6221 (30 μl , 0.004%). Aqueous humor, conjunctiva, cornea, lens, iris-ciliary body, vitreous humor, retina and choroid were sampled from 4 rabbits per time point. Samples were collected at 0.5, 1, 2, 4, 6, 8, 10, 24, 48, and 72 hours after dosing. Radioactivity concentrations were determined using liquid scintillation counting. The results of this study (see table below) showed that ^3H -AL-6221 was absorbed into the eye with highest concentrations found in the anterior tissues. Low concentrations were found in the posterior tissues and plasma. Local distribution of drug to posterior tissues of the dosed eyes was observed. Radioactivity concentrations in the undosed eye were low and rapidly fell below detection limits. Except for cornea and lens, radioactivity was rapidly eliminated from the ocular tissues.

Distribution of radioactivity in ocular tissues of rabbits treated with ^3H -AL-6221

	Aqueous humor	Choroid	Conjunctiva	Cornea	Iris-ciliary body	Lens	Retina	Vitreous humor	plasma
C_{max} (ng-eq/g)	21.8	2.05	105	327	15.0	0.135	0.153	0.025	0.078
T_{max} (hr)	2.0	1.0	0.5	0.5	1.0	2.0	1.0	1.0	0.5
$T_{1/2}$ (hr)	1.5	1.1	1.4	13.8	1.5	28.6	0.5	0.4	2.6
$AUC_{0-\infty}$ (ng-eq hr/g)	27.2	4.0	168	766	49.3	3.41	0.266	0.055	0.175

2.1.1.2 AL-5848 Plasma Concentrations From Toxicology Study N-96-115: "Three-Month Topical Ocular Irritation and Systemic Toxicity Evaluation of AL-6221 Ophthalmic Solution in Rabbits." Alcon Technical Report 060:38570:1099 Vol. 46 Pg. 5-8523.

The purpose of this study was to determine the plasma concentrations of AL-5848 from New Zealand albino rabbits as part of a 3-month TID topical ocular toxicology study with 0.001, 0.003 and 0.01% AL-6221 ophthalmic solutions. Due to the rapid hydrolysis of AL-6221 in rabbit plasma, determination of parent drug was not feasible. Each animal received 1 drop (40 µl) of the appropriate test article to each eye three times a day for 90 days. Blood samples were collected from all AL-6221 treated animals 1 hr following the first daily dose on Days 1, 30, 59 and 90. Samples were analyzed using a validated liquid chromatography/mass spectrometry (LC/MS) method with a quantitation limit of 0.020 ng/ml.

The results are summarized in the table below. Systemic exposure to AL-5848 following topical administration of AL-6221 was demonstrated at the highest dose and in a small number of samples (5 out of 48) at the middle dose. All assays for the lowest dose were below the assay quantitation limit. No accumulation and sex-related differences were noted.

Plasma concentrations of AL-5848 in rabbits treated with travoprost ophthalmic solution (ng/ml)

Dose (%)	Day			
	1	30	59	90
0.001	BLQ	BLQ	BLQ	BLQ
0.003	BLQ	BLQ	BLQ	BLQ
0.01	0.060±0.014	0.042±0.013	0.046±0.014	0.038±0.009

BLQ: Below limit of quantitation (<0.020 ng/ml)

- 2.1.1.3 AL-5848 Plasma Concentrations From Toxicology Study N-96-194: "Six-Month Topical Ocular Irritation and Systemic Toxicity Evaluation of AL-6221 Ophthalmic Suspension in Rabbits." Alcon Technical Report 026:38570:1297 Vol. 46 Pg. 5- 8542.

The purpose of this study was to determine the plasma concentrations of AL-5848 from New Zealand albino rabbits as part of a 6-month BID topical ocular toxicology study with 0.001, 0.003 and 0.01% AL-6221 ophthalmic solutions. Each animal received 2 drops (70 µl) of the appropriate test article to each eye two times a day for 181 days. Blood samples were collected from all AL-6221 treated animals immediately prior to the first daily dose (trough) and 30 min following the second daily dose (peak) on Days 1, 86 and 181. Samples were analyzed using a validated liquid chromatography/mass spectrometry (LC/MS) method with a quantitation limit of 0.020 ng/ml.

The results are summarized in the table below. All pre-dose samples assayed except one (high dose male, Day 86, AL-5848 concentration: 0.026 ng/ml) were BLQ. Systemic exposure to AL-5848 was demonstrated in rabbits topically dosed BID with 0.001, 0.003 and 0.010% AL-6221 ophthalmic suspensions for 6 months. No significant accumulation over the treatment period was observed. No sex-related differences in plasma AL-5848 concentrations were demonstrated. Systemic exposure to the AL-6221 hydrolysis product increased in an approximately dose-proportional manner with increasing parent drug dose.

Peak plasma concentrations of AL-5848 in rabbits treated with travoprost ophthalmic solution (ng/ml)

Dose (%)	Day		
	1	86	181
0.001	BLQ	0.034±0.008	BLQ
0.003	0.067±0.032	0.085±0.026	0.034±0.009
0.01	0.188±0.056	0.357±0.131	0.164±0.075

BLQ: Below limit of quantitation (<0.020 ng/ml)

2.1.1.4. AL-5848 Plasma Concentrations From Toxicology Study N-96-193: "One-Year Topical Ocular Study of AL-6221 Ophthalmic Solution in Monkeys." Alcon Technical Report 001:33:0100 Vol. 46 Pg. 5- 8695.

The purpose of this study was to determine the plasma concentrations of AL-5848 as part of a one-year topical ocular toxicology study with 0.0015, 0.004 and 0.012% AL-6221 ophthalmic solutions administered BID to cynomolgus monkeys. Blood samples were collected from all AL-6221 treated animals on Day 1 and in Weeks 4, 13, 26 and 52 of treatment. On the designated sampling days, blood samples were collected immediately prior to the first daily dose (trough) and approximately 0.5 and 1.0 hr following the second daily dose. Samples were analyzed using a validated [redacted] method with a quantitation limit of 0.020 ng/ml for AL-5848.

The results are summarized in the table below. All trough plasma samples were below limit of quantitation (<0.020 ng/ml). Systemic exposure to AL-5848 was demonstrated in cynomolgus monkeys dosed topically BID with 0.0015, 0.004 or 0.012% AL-6221 ophthalmic solutions. Steady-state was achieved with no significant accumulation over the treatment period. While evidence of a sex-related pharmacokinetic difference at the high dose was seen in the 0.5 hr data (males>females), the magnitude of the difference was 2-fold or less. Systemic exposure to the AL-6221 hydrolysis product increased in an approximately dose-proportional manner with increasing parent drug dose. Comparison of high dose group samples assayed with and without esterase incubation produced relative differences of 1.76% and 4.96% for the 0.5 and 1.0 hr post-dose samples, suggesting that trace levels of intact AL-6221 might be present.

Plasma concentrations of AL-5848 in monkeys treated with travoprost ophthalmic solution (ng/ml)

Treatment		Males				
Dose (%)	Hr	Day 1	Week 4	Week 13	Week 26	Week 52
0.0015	0.5	0.017±0.008	0.020±0.012	0.024±0.010	0.026±0.011	0.018±0.010
	1.0	BLQ	BLQ	BLQ	BLQ	BLQ
0.004	0.5	0.025±0.010	0.030±0.010	0.059±0.024	0.057±0.028	0.039±0.024
	1.0	BLQ	BLQ	0.016±0.008	0.014±0.007	0.012±0.005
0.012	0.5	0.059±0.012	0.181±0.058	0.185±0.027	0.204±0.028	0.132±0.038
	1.0	0.018±0.009	0.049±0.009	0.054±0.004	0.050±0.008	0.027±0.014
Treatment		Females				
Dose (%)	Hr	Day 1	Week 4	Week 13	Week 26	Week 52
0.0015	0.5	BLQ	BLQ	0.024±0.003	0.017±0.008	BLQ
	1.0	BLQ	BLQ	BLQ	BLQ	BLQ
0.004	0.5	0.028±0.013	0.048±0.021	0.073±0.032	0.058±0.018	0.036±0.011
	1.0	BLQ	0.016±0.012	0.025±0.017	0.016±0.007	BLQ
0.012	0.5	0.069±0.030	0.092±0.030	0.110±0.049	0.099±0.041	0.059±0.010
	1.0	0.029±0.016	0.033±0.010	0.036±0.018	0.027±0.013	0.026±0.003
Treatment		Males and Females				
Dose (%)	Hr	Day 1	Week 4	Week 13	Week 26	Week 52
0.0015	0.5	0.014±0.007	0.015±0.010	0.024±0.007	0.022±0.010	0.014±0.008
	1.0	BLQ	BLQ	BLQ	BLQ	BLQ
0.004	0.5	0.027±0.011	0.039±0.018	0.066±0.027	0.058±0.022	0.038±0.017
	1.0	BLQ	0.013±0.008	0.021±0.013	0.015±0.007	0.011±0.004
0.012	0.5	0.064±0.022	0.137±0.064	0.147±0.054	0.152±0.065	0.096±0.047
	1.0	0.024±0.014	0.041±0.013	0.045±0.015	0.038±0.015	0.026±0.010

BLQ: Below limit of quantitation (<0.020 ng/ml)

2.2. SYSTEMIC ADME

2.2.1.1 Pharmacokinetics of AL-6221 and AL-5848 in Male Sprague Dawley Rats Following a Single 0.1 mg/kg Intravenous or Subcutaneous Doses AL-6221. Alcon Technical Report No.: 025:38570:0499 (Vol.45 Pg. 5 – 0001)

The purpose of this study is to determine the plasma pharmacokinetics of AL-5848 in male Sprague Dawley rats following single intravenous (0.1 mg/kg) and subcutaneous (0.01, 0.03 and 0.1 mg/kg) administration of travoprost. The study used four groups of animals, one intravenous and three subcutaneous, in a serial sacrifice design with 4 animals per time point. Plasma samples were collected 10, 20, 30, 40 min and 1, 2, 3, 4 and 6 hr after administration. AL-5848 concentrations were determined by an [redacted] method with a lower quantitation limit of 0.04 ng/ml. Travoprost is an isopropyl-ester prodrug of AL-5848. In vitro studies have shown that travoprost is rapidly deesterified in rat plasma with essentially quantitative conversion occurring in less than 5 minutes. As a result, analysis of travoprost plasma concentrations was not feasible. The results summarized in the table below indicated that following subcutaneous administration in rats, plasma exposure was dose-proportional, and AL-5848 was rapidly eliminated with t_{1/2} values of 20-30 min.

Plasma PK parameters in rats treated with travoprost

Treatment	Intravenous	Subcutaneous	Subcutaneous	Subcutaneous
Dose (mg/kg)	0.1	0.01	0.03	0.1
C _{max} (ng/ml)		1.79	5.14	18.4
T _{max} (min)		20	30	40
T _{1/2} (min)	15.6	31.0	23.5	28.5
AUC _{0-∞} (ng-min/ml)	876	138	484	1527

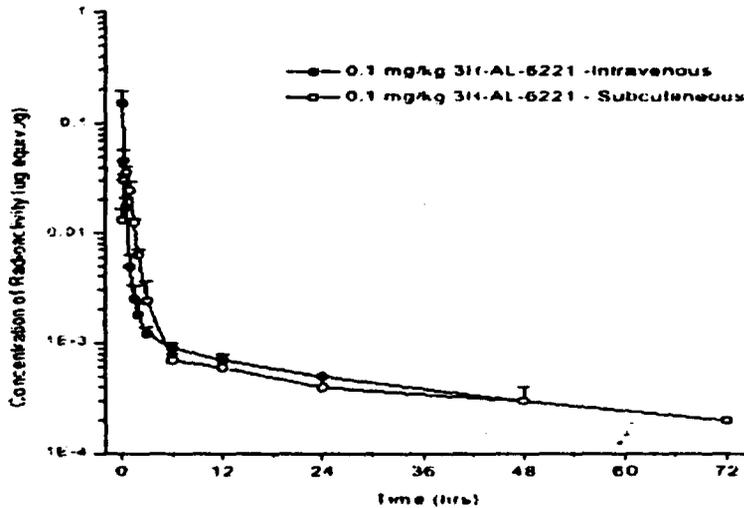
2.2.1.2 Pharmacokinetics of Radioactivity in Male Sprague Dawley Rats Following Single 0.1 mg/kg Intravenous or Subcutaneous Doses ³H-AL-6221. Alcon Technical Report No.: 005:38570:0398 (Vol.45 Pg. 5 – 0097)

The purpose of this study was to determine the plasma pharmacokinetics of total radioactivity in male Sprague-Dawley rats following a single 0.1 mg/kg intravenous or subcutaneous dose of ³H-AL-6221. Blood samples were collected at 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 6, 12, 24, 48 and 72 hr after dosing. Plasma radioactivity concentrations were determined by liquid scintillation counting. The results (see table and figure below) showed that ³H-AL-6221 was rapidly and well absorbed following subcutaneous dosing. For both intravenous and subcutaneous dose routes the elimination of radioactivity from rat plasma was biphasic with a terminal half-life of approximately 37 hours.

Plasma PK parameters of radioactivity in rats treated with ³H-AL-6221

Treatment	Intravenous	Subcutaneous
Dose (mg/kg)	0.1	0.1
C _{max} (ng-eq/ml)		35.5±4.7
T _{max} (min)		30
T _{1/2} α (min)	6.2±1.3	
T _{1/2} β (hr)	35.2±4.1	39.8±2.7
AUC _{0-∞} (ng-eq hr/ml)	92±13	87.8±9.4

**Mean Concentrations of Radioactivity in Plasma
Following Intravenous and Subcutaneous Doses of ³H-AL-6221**



2.2.1.3 Plasma Pharmacokinetics of Radioactivity in Male New Zealand White Rabbits Following a Single 0.1 mg/kg Intravenous Dose of ³H-AL-6221. Alcon Technical Report No.: 002:38570:0198 (Vol.45 Pg. 5 – 8194)

The purpose of this study was to determine the plasma pharmacokinetics of total radioactivity in five male Sprague-Dawley rats following a single 0.1 mg/kg intravenous dose of ³H-AL-6221. Blood samples were collected at 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 6, 12, 24, 48, 72 and 96 hr after dosing. Plasma radioactivity concentrations were determined by liquid scintillation counting. The results (see table below) showed that the elimination of radioactivity from rat plasma was biphasic with a terminal half-life of approximately 48 hours.

Plasma PK parameters of radioactivity following a single iv dose of ³H-AL-6221

Dose (mg/kg)	C ₀ (µg-eq/ml)	AUC _{0-∞} (µg-eq hr/ml)	T1/2 α (min)	T1/2 β (hr)	Vd (L/kg)
0.1	0.251±0.04	0.1645±0.016	8.4±2.8	48±0.01	41.7±5.1

2.2.1.4 Pharmacokinetics of Radioactivity in Plasma Following Intravenous and Topical Ocular Dosing of ³H-AL-6221 in Male Beagle Dogs. Alcon Technical Report No.: 047:38570:1298 (Vol.45 Pg. 5 – 8210)

The pharmacokinetics of radioactivity were determined in male beagle dogs following intravenous and topical ocular doses of ³H-AL-6221. Mean intravenous doses in Groups 1, 2 and 3 were 0.01, 0.1, and 0.9 µg/kg, respectively. Animals in Group 4 received a single bilateral topical ocular dose (30 µl) of a 0.004% ³H-AL-6221 ophthalmic solution. The mean topical ocular dose was 0.3 µg/kg. Three animals were used in each dose group. Plasma samples were collected at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 hours after dosing. Plasma concentrations of radioactivity were determined by liquid scintillation counting. The results of this study (see table and figure below) showed linear pharmacokinetics of radioactivity over a 100-fold intravenous dose range (0.01, 0.1, 0.9 µg/kg). Plasma

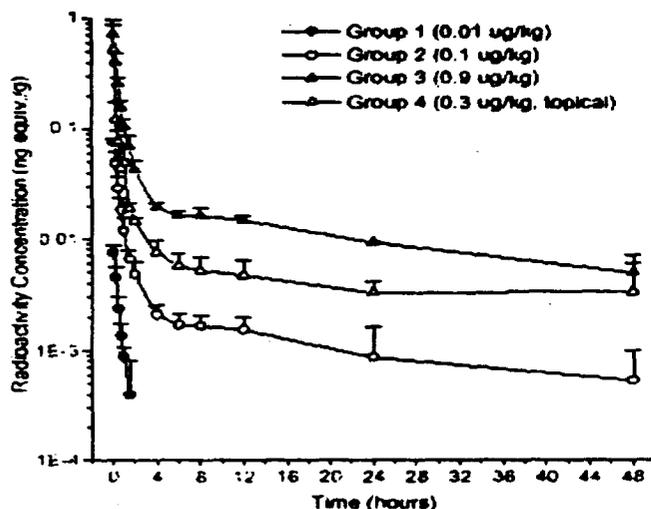
concentrations decline in a biphasic manner with a rapid initial distributive half-life of 25 min and a slower terminal half-life of 30 hr. Radioactivity was well absorbed following the topical ocular dose.

Plasma PK parameters of radioactivity in dogs treated with ³H-AL-6221

Treatment	Intravenous	Intravenous	Intravenous	Topical ocular
Dose (µg/kg)	0.01	0.1	0.9	0.3
C ₀ /C _{max} (ng-eq/ml)	0.0097±0.001	0.095±0.014	0.942±0.190	0.517±0.436
T1/2 (hr)		26.3±10.7	25.2±12.3	30.9±9.7
AUC _{0-∞} (ng-eq hr/L)	BLQ	0.1260±0.0385	1.1059±0.1992	0.4094±0.0280
Bioavailability				114-115%

BLQ: < 0.003ng-eq/ml

Main Concentrations of Radioactivity in Plasma Following Intravenous and Topical Ocular Doses of ³H-AL-6221



2.2.1.5 Distribution of Radioactivity in the Tissues, Blood and Plasma of Male Sprague-Dawley Rats Following Single and Multiple 0.1 mg/kg Subcutaneous Doses of ³H-AL-6221. Alcon Technical Report No.: 33:38570:1098 (Vol.45 Pg. 5 – 8261)

The purpose of this study was to determine the systemic distribution of travoprost in male Sprague-Dawley rats following single or multiple (qd x 14 days) subcutaneous administration of ³H-AL-6221 at 0.1 mg/kg. Three animals per time point were sacrificed at 0.5, 1, 3, 6, 12, 24, 48, 72, 120 and 168 hr after last dosing and blood and tissue samples were collected. Radioactivity concentrations were determined using liquid scintillation counting. The results are summarized in the table below. Following subcutaneous dosing, maximal tissue concentrations were reached within 0.5-1 hr. Highest concentrations of radioactivity were found in the liver, kidney, lung and plasma. The decline in radioactivity concentrations was biphasic with a large initial decline seen within the first 3 hr followed by a slower decline in the terminal phase. The disappearance of radioactivity from tissues and organs typically paralleled that seen in plasma. Accumulation of radioactivity in tissues was observed following the 14-day multiple dose regimen used in this study.

Maximal concentrations of radioactivity in tissues following single and multiple doses of ³H-AL-6221 (0.1 mg/kg)

Tissue	Single dose		Multiple dose		T1/2 (hr)
	Tmax (hr)	Cmax (µg-eq/g)	Tmax (hr)	Cmax (µg-eq/g)	
Adrenal glands	0.5	0.0094±0.0019	0.5	0.0148±0.0020	97.5
Blood	1	0.0204±0.0046	0.5	0.0291±0.0060	116

Tissue	Single dose		Multiple dose		T1/2 (hr)
	Tmax (hr)	Cmax (µg-cg/g)	Tmax (hr)	Cmax (µg-cg/g)	
Bone	0.5	0.0056±0.0021	0.5	0.0110±0.0024	111
Brain	1	0.0042±0.0013	0.5	0.0085±0.0020	91.6
Eyes (both)	1	0.0038±0.0015	0.5	0.0068±0.0008	97.4
Heart	0.5	0.0116±0.0028	0.5	0.0161±0.0028	73.6
Kidneys	1	0.345±0.058	0.5	0.374±0.037	52.4
Liver	1	0.182±0.018	0.5	0.240±0.023	35.4
Lungs	0.5	0.202±0.054	0.5	0.209±0.019	59.7
Lymph node (mesenteric)	1	0.0176±0.0047	0.5	0.0233±0.0003	97.2
Skeletal muscle	1	0.0033±0.0012	0.5	0.0006±0.0001	99.1
Plasma	0.5	0.0408±0.0146	0.5	0.0584±0.0117	71.5
Urinary bladder	1	0.0367±0.0214	1	0.0278±0.0107	72.9

2.2.1.6 Distribution of Radioactivity in Male and Female Rat Tissues Following a 14-Day 0.1 mg/kg Subcutaneous Dosing Regimen with ³H-AL-6221 by Whole Body Autoradiography. Alcon Technical Report No.: 037:38570:1098. Vol. 45 Pg. 5 - 8308.

The purpose of this study was to determine the systemic distribution of travoprost by whole body autoradiography in Crl:CD(SD)BR rats following multiple (qd x 14 days) subcutaneous administration of ³H-AL-6221 at 0.1 mg/kg. One animal of each sex was sacrificed at 1, 6, 12 and 24 hr after the last dose for analysis of radioactivity levels by autoradioluminographic methods. The results showed that highest levels of radioactivity were found at the dose site and in kidney, liver, lung and small intestinal contents at 1 hr postdose. By 6 hr postdose, radioactivity was limited to the gastrointestinal contents, dose site and urine whose levels declined further to low levels by the 24 hr time point. No evidence was found for site specific accumulation of radioactivity following the 14-day dosing regimen. Gender-related differences between male and female rats were not found.

2.2.1.7 Distribution of Radioactivity in Pregnant and Fetal Rat Tissues Following a Single 0.1 mg/kg Subcutaneous Dose of ³H-AL-6221 to Pregnant Female Sprague Dawley Rats. Alcon Technical Report No.: 035:38570:1098. Vol. 45 Pg. 5 - 8340.

The purpose of this study was to determine the distribution of radioactivity in pregnant and fetal rat tissues following a single 0.1 mg/kg subcutaneous dose of ³H-AL-6221 in pregnant Sprague Dawley rats. Tissues from 3 animals per time point were collected on Day 12 and Day 18 of gestation at 1, 6, 12 and 24 hr after dosing. Tissue and plasma samples were analyzed for total radioactivity by liquid scintillation counting. The results on Days 12 and 18 of gestation were similar. Following subcutaneous dosing, maximal tissue concentrations in the dams were reached within 1 hr with highest concentrations found in kidney, liver and lung tissues. In amniotic fluid and fetal tissues only low levels of radioactivity were reached which were typically 2-4% of those found in maternal plasma. Fetal tissues with the highest levels were liver and lung. Radioactivity levels in both tissues of the dam and fetus declined in a biphasic manner.

2.2.1.8 Excretion and Mass Balance of Radioactivity in Male Sprague Dawley Rats Following a Single 0.1 mg/kg Subcutaneous Dose of ³H-AL-6221. Alcon Technical Report No.: 34:38570:1098 Vol. 45 Pg. 5 - 8359.

The purpose of this study was to determine the excretion of radioactivity in five male Sprague Dawley rats following a single 0.1 mg/kg subcutaneous dose of ³H-AL-6221. Urine and feces samples

were collected at 24 hr intervals through 168 hr. Samples were analyzed for total radioactivity by liquid scintillation counting. The results are summarized in the table below. Approximately 95% of the dose was excreted in the first 24 hr. Radioactivity was excreted primarily in the feces. Mean cumulative percents of dose excreted in feces and urine were $74.0 \pm 4.5\%$ and $34.6 \pm 3.4\%$, respectively. Less than 0.15% of the dose was recovered in expired air. At sacrifice only 0.3% remained in the carcass. The results of this study demonstrated that radioactivity from $^3\text{H-AL-6221}$ was rapidly and completely excreted in urine and feces and that the major route of excretion was in the feces.

Percents of radioactivity recovered in rats treated with a single dose of $^3\text{H-AL-6221}$ (0.1 mg/kg)

Hr	Urine	Feces	Cage rinse	CO ₂ trap	carcass
0-24	33.6±2.68	61.2±14.0	0.51±0.24	0.02±0.01	
24-48	0.77±0.75	9.43±4.79	0.18±0.26	0.02±0.02	
48-72	0.14±0.10	3.04±5.83	0.05±0.07	0.03±0.02	
72-96	0.04±0.01	0.19±0.14	0.02±0.01	0.03±0.02	
144-168	0.02±0.00	0.02±0.02			0.28±0.14
Subtotal	34.6±3.41	74.0±4.52	0.77±0.46	0.11±0.04	0.28±0.14

2.2.1.9 Excretion of Radioactivity in Milk From Lactating Female Sprague Dawley Rats Following a Single 0.1 mg/kg Subcutaneous Dose of $^3\text{H-AL-6221}$. Alcon Technical Report No.:36:38570:1098 Vol.45 Pg. 5 - 8389.

The purpose of this study was to determine the excretion of radioactivity in milk from lactating female Sprague Dawley rats following a single 0.1 mg/kg subcutaneous dose of $^3\text{H-AL-6221}$. Three animals/time point were sacrificed at 1, 6, 12, and 24 hours post dose immediately following collection of milk. Blood samples were collected from all animals at the time of sacrifice. The results (see table below) showed that radioactivity from $^3\text{H-AL-6221}$ was excreted in the milk of lactating rats. The results of this study also indicated that the disappearance of radioactivity in the plasma of lactating female rat was similar to that observed previously in male rats (Report No.: 005:38570:0398).

Concentrations of radioactivity in blood, plasma and milk in rats treated with $^3\text{H-AL-6221}$

Collection time (hr)	$\mu\text{g-eq } ^3\text{H-AL-6221/g}$		
	BLOOD	PLASMA	MILK
1	0.0194±0.0042	0.0364±0.0085	0.0090±0.0020
6	0.0007±0.0002	0.0011±0.0002	0.0127±0.0034
12	0.0004±0.0001	0.0007±0.0001	0.0024±0.0010
24	0.0001±0.0001	0.0004±0.0000	0.0003±0.0001

2.2.1.10 Biliary Excretion of Radioactivity Following a Single Subcutaneous Dose of $^3\text{H-AL-6221}$ in Bile Duct Cannulated Sprague Dawley Rats. Alcon Technical Report No.: 09:33:0100. Vol. 45 Pg. 5 - 8415.

The purpose of this study was to determine the excretion of radioactivity in bile in 6 male Sprague Dawley rats following a single 0.1 mg/kg subcutaneous dose of $^3\text{H-AL-6221}$. Bile was collected via an exteriorized bile duct cannula. Bile samples were collected at time intervals of 0-4, 4-8, 8-12, 12-24, 24-48 and 48-72 hr after dosing. Radioactivity was determined by liquid scintillation counting. The results of this study showed that approximately 50% of a 0.1 mg/kg dose of $^3\text{H-AL-6221}$ was excreted in bile and that greater than 95% of total amount excreted in bile was recovered in the first 4 hr after dosing. The radioactivity in the bile was in the form of at least 12 metabolites with no unchanged AL-6221 or its deesterified free acid form, AL-5848, being present.

2.2.1.11 In Vitro Binding of [³H-Ph]AL-5848 to Human, Monkey and Rat Plasma Proteins. Alcon Technical Report No.: 014:33:0300 Vol. 45 Pg. 5 - 8431.

The purpose of this study was to determine the binding of [³H-Ph]AL-5848 to human, monkey and rat plasma proteins. In vivo, travoprost is rapidly hydrolyzed to the active carboxylic acid, AL-5848. In vitro studies in plasma have shown that travoprost is hydrolyzed in less than 5 min with essentially quantitative conversion. In this study, the percents of binding were determined by the [redacted] method at concentrations of 0.01, 0.1, 1.0 and 100 ng/ml. The results are summarized in the table below. Between species, the degree of protein binding was similar (approximately 80%). Over a 1000-fold concentration range of 0.01 to 100 ng/ml, the percent of bound drug for all species was independent of drug concentration.

Percent of [³H-Ph]AL-5848 bound to human, monkey and rat plasma proteins (%)

Concentration (ng/ml)	Human	Monkey	Rat
0.01	[redacted]	76.6±3.1	77.2±1.1
0.1	[redacted]	83.4±0.5	80.7±0.2
1.0	[redacted]	81.9±0.3	78.0±0.5
100	[redacted]	81.9±0.3	78.5±0.4
Overall	[redacted]	81.0±3.0	78.6±1.5

2.2.1.12 Metabolite Profiles in Urine, Feces, Plasma and Bile Following a Single 0.1 mg/kg Subcutaneous Dose of [³H-Ph]AL-6221 to Male Sprague-Dawley Rats. Alcon Technical Report No.: 020:38570:1097. Vol. 45 Pg. 5 - 8455.

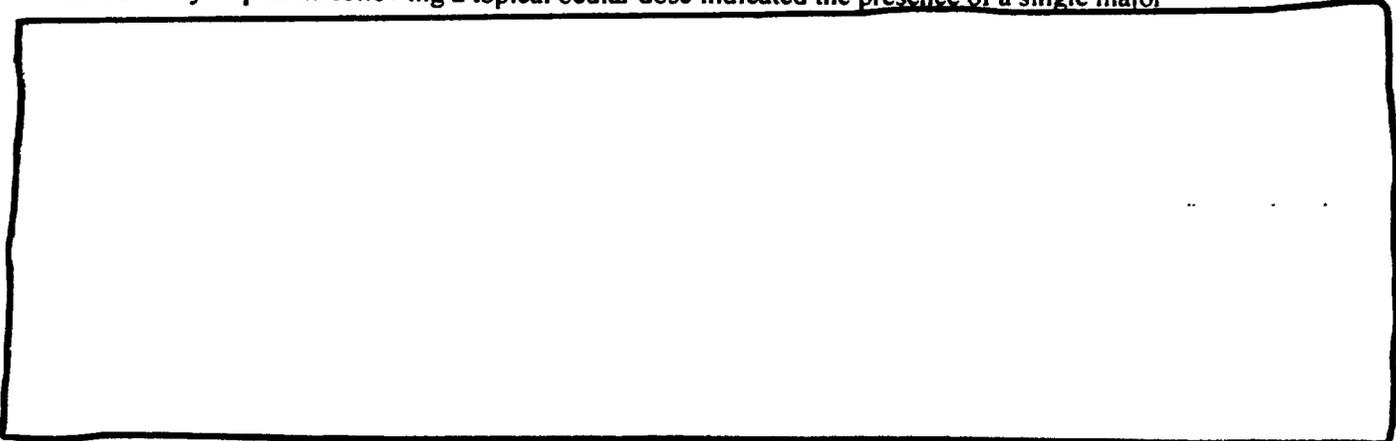
The purpose of this study was to determine the metabolite profiles of travoprost in plasma, urine, feces and bile in Sprague Dawley rats following a single 0.1 mg/kg subcutaneous dose of [³H-Ph]AL-6211. Urine, feces and bile samples were collected up to 72 hr and plasma samples at 1.0, 3.0 and 6.0 hr after dosing. Metabolite profiles were generated by [redacted]

[redacted] profiles indicated the presence of several metabolites in urine (5), feces (9), plasma (5) and bile (12). Profiles of the 1.0 hr plasma sample displayed two dominant peaks. The first peak, denoted as Mp1, eluted at 23.2 ± 0.2 min and was present at 5.5 ng-eq/g [redacted]. The second dominant and largest peak, designated Mp5, eluted at 27.3 ± 0.2 min, the same time as AL-5848, and was present at 14.4 ng-eq/g [redacted]. At the 3.0 and 6.0 hour time points, there were insufficient amounts of radioactivity to obtain meaningful profiles [redacted]. [redacted] of radioactivity in urine, feces and bile indicated that a single component (retention time: 23.0 – 23.7 min) represented approximately 90%, 50% and 40% of the mean relative peak area percents, respectively. This component was present in plasma as Mp1. Since the [redacted] profiles did not show radioactivity at short retention times (i.e., less than 18 min), it was concluded that the formation of polar conjugates, such as glucuronide or sulfate conjugates, did not occur in the metabolism of AL-6221.

2.2.1.13 Metabolite Profiles of Plasma Following a Single 0.9 µg/kg Intravenous and 0.3 µg/kg (1.2 µg/eye) Topical Ocular Dose of [³H-Ph]AL-6221 to Male Beagle Dogs. Alcon Technical Report No.: 008:33:100. Vol. 46 Pg. 5 - 8479.

The metabolite profiles of AL-6221 were determined in male beagle dogs following single, 0.9 µg/kg intravenous and 0.3 µg/kg (1.2 µg/eye) topical ocular doses of [³H-Ph]AL-6221. blood samples were collected at 5, 15, 30, 45 and 60 min after dosing. Metabolite profiles were generated by gradient [redacted]

The representative plasma profiles are shown in the figures below. The profiles of radioactivity in plasma following a topical ocular dose indicated the presence of a single major



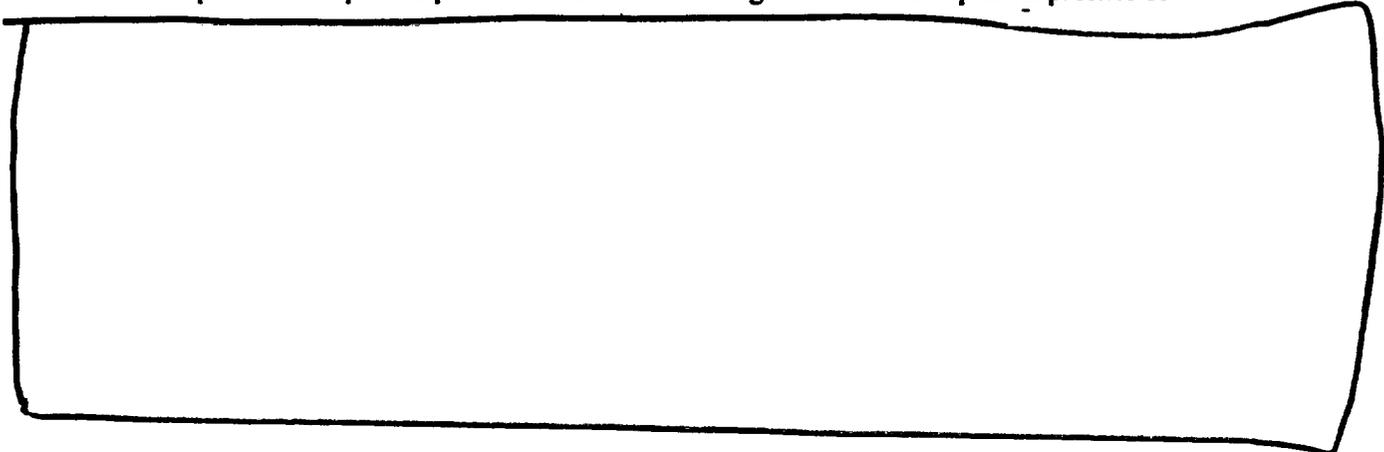
radioactive component (Mp3) up to 60 minutes after dosing. Metabolite Mp3 had a retention time of 25.0 ± 0.2 min, which was same as AL-5848. At the first sampling time point of 5 minutes, a minor radioactive peak Mp4 (R.T. 30.3 min) was observed at low concentrations and had a retention time which was the same as AL-6221. Profiles of radioactivity in plasma following an intravenous dose indicated the presence of at least four radioactive components. At 5 min after dosing, metabolites Mp3 (26.2 min) and Mp4 (31.2 min) were present at concentrations of 0.299 and 0.347 ng-eq/g, respectively. Mp3 and Mp4 represented approximately 42% and 49% of the peak area percents, respectively. At 60 minutes after dosing, Mp3 was present at a concentration of 0.0469 ng-eq/g, while Mp4 had decreased to a concentration of 0.0047 ng-eq/g.

In conclusion, the profiles of radioactivity in dog plasma indicated that travoprost was rapidly converted to its de-esterified form AL-5848 which was the major component up to 1 hr after dosing. The profiles also showed the presence of at least 2 minor metabolites. The lack of radioactivity components at short retention times indicated that travoprost was not metabolized to polar conjugates such as glucuronides.

2.2.1.14 Metabolite Profiles in Urine and Plasma Following a Single 0.1 mg/kg Intravenous Dose of [³H-Ph]AL-6221 to Cynomolgus Monkeys. Alcon Technical Report No.: 019:38570:0399. Vol. 46 Pg. 5 - 8492.

The purpose of this study was to determine the metabolite profiles of AL-6221 in monkey plasma and urine following a single 0.1 mg/kg intravenous dose of [³H-Ph]AL-6221. Blood samples were collected at 30, 60 and 120 min after dosing. Urine samples were collected from 0 to 6 hr after dosing. Metabolite profiles were generated by gradient with in-line radiochemical detection.

The representative plasma profiles are shown in the figures below. The plasma profiles of



radioactivity in this study indicated two major metabolites and the presence of as many as seven minor metabolites. One of these metabolites (Mp6) was retained at the same time as AL-5848, the de-esterified acid form of AL-6221. The profiles of radioactivity in urine indicated the presence of at least four metabolites. Of these, two were major metabolites (Mu5 and Mu8) representing approximately 29% and 28% of the peak area percents, respectively. AL-6221 and AL-5848 were not detected in the urine.

2.2.1.15 Identification of the Principal Metabolites of AL-6221 in Rat and Monkey Plasma and Urine. Alcon Technical Report No.: 018:33:0400. Vol. 46 Pg. 5 - 8507.

The purpose of this study was to determine the structures of travoprost (AL-6221) using liquid chromatography-mass spectrometry (LC-MS). Total ion chromatograms (TIC), Q1 scans and product ion scans were acquired in both positive-ion and negative-ion modes. Male cynomolgus monkeys and male Sprague Dawley rats were administered single intravenous (0.1 mg/kg) and subcutaneous (0.1 mg/kg) doses of [³H Ph]AL-6221, respectively. Plasma was collected at 0.25 and 0.50 hr after dosing monkeys and rats, respectively. Urine was collected at intervals of 0-6 and 0-8 hr after dosing monkeys and rats, respectively.

AL-6221 and AL-5848 were identified in monkey plasma. Two additional metabolites produced molecular ions at *m/z* 403 and *m/z* 429, respectively. These molecular ions corresponded to 1,2,3,4-tetranor-13,14-dihydro-15-oxo-AL-5848 and 1,2-dinor-13,14-dihydro-15-oxo-AL-5848, respectively. These metabolites were also identified in monkey urine. Travoprost and AL-5848 were not found in the urine. Other metabolites in monkey plasma and urine were not abundant enough to be identified using

Four metabolites were identified in rat urine. The major urine metabolite provided a molecular ion at *m/z* 401, which corresponded to 1,2,3,4-tetranor-15-oxo-AL-5848. Three minor metabolites produced molecular ions at *m/z* 427, *m/z* 403 and *m/z* 429, which corresponded to 1,2-dinor-15-oxo-AL-5848, 1,2,3,4-tetranor-13,14-dihydro-15-oxo-AL-5848 and 1,2-dinor-13,14-dihydro-15-oxo-AL-5848, respectively. In rat plasma, AL-5848 was identified. A second major metabolite at a shorter retention time was identified to be 1,2,3,4-tetranor-15-oxo-AL-5848.

In vivo, travoprost was rapidly hydrolyzed by esterases to its active carboxylic acid, AL-5848. Based on metabolite profiling data and the identification of the major metabolites in the present study, it appeared that subsequent metabolism followed from AL-5848.

Between rat and monkey, a species difference in the metabolism of travoprost was evident. In the rat, metabolites were formed with and without reduction of the double bond. Based on the mass spectrometry data, the ratio of unsaturated to saturated metabolites was approximately 2:1. In the monkey, the major metabolites identified were 13,14-dihydro metabolites.

2.2.1.16 AL-5848 Plasma Concentrations From Toxicology Study N-98-98: "Second Pilot Study for Effect of Prostaglandin on Embryo-Fetal Development in the Mouse." Alcon Technical Report 005:33:0100 Vol. 46 Pg. 5- 8629.

The purpose of this study was to determine the plasma concentrations of AL-5848 as part of a 16-day teratology study of AL-6221 in timed-pregnant female mice (CrI:CD-1(ICR)BR) administered 0.03, 0.1, 0.3 or 1.0 µg/kg/day of AL-6221 by subcutaneous injection. The dosing period was gestation days 6 through 16. On the last day of treatment, plasma samples were collected approximately 0.5 hr following

the dose. Due to the rapid hydrolysis of AL-6221 in mouse plasma, analysis of parent drug was unfeasible. Samples were analyzed using a validated [redacted]

The assay results demonstrated quantifiable exposure to AL-5848 in only 4 of 8 high dose study animals with the concentrations ranging from 0.104 to 0.126 ng/mL. All other assay results were below the limit of quantitation (0.10 ng/ml).

3. TOXICOLOGY

3.1 OCULAR TOXICITY

3.1.1.1 28-Day Topical Ocular Irritation and Systemic Toxicity Evaluation of AL-6221 Ophthalmic Solution in Rabbits, TR No. 057:38520:0496 (Vol. 19)

Report No: TR 057:38520:0496
 Study Aim: To determine the ocular irritation potential of AL-6221 ophthalmic solution following topical ocular administration to New Zealand white rabbits for 28 days
 Compound/Vehicle: 0.01% (Lot #: 95-15196-1) and 0.001% (Lot #: 95-15195-1) AL-6221 Ophthalmic Solution; the composition of AL-6221 and vehicle is listed in the following table.

Ingredient (% w/v)	Vehicle	0.001% AL-6221	0.01% AL-6221
AL-6221	-	0.001+5% excess	0.01+5% excess
[redacted]	0.1	0.1	0.1
Tromethamine, USP, AR	0.12	0.12	0.12
Boric Acid, NF	0.3	0.3	0.3
Mannitol, USP	4.6	4.6	4.6
[redacted]	0.1	0.1	0.1
[redacted]	0.01+5% excess	0.01+5% excess	0.01+5% excess
[redacted]	pH adjust	pH adjust	pH adjust
Purified H ₂ O	QS 100	QS 100	QS 100

Dose & Route: 1 drop (40 µl)/eye, both eyes, bid (Days 1→6) or tid (Days 7→28).
 Animals: New Zealand white rabbits, 4-month old, weighing 3.0-3.6 kg; 4/sex/group.
 Study Location: Toxicology Unit, Alcon Laboratory, Inc., 6201 South Freeway, Fort Worth, TX 76134

Compliance with GLP/QAU: Yes
 Study Date: 4/17-5/15/96
 Study Design: AL-6221 or vehicle was applied to both eyes of each rabbit bid during Days 1→6 and tid during Days 7→28 as shown in the following table.

Group	AL-6221 Treatment	N ^o of Animals	Treatment Volume (µl)/eye	Treatment Frequency		Dose (µg/day)	Dosing Duration
				Days 1→6	Days 6→28		
1	Non-treated Control	4/sex/group	-	bid	tid	-	28-day
2	0 (Vehicle Control)		40			0	
3	0.001%		40			2.4	
4	0.01%		40			24	

- The following parameters were monitored.
- Mortality and Morbidity - 2x/day
 - Biomicroscopic Examination - Days 0, 3, 7, 14, 21 & 28
 - Indirect Ophthalmoscopic Examination - Days 0 & 28

- Pachymetry - Days 0 & 27
- Body Weight - Weekly
- Clinical Pathology (Blood Chemistry & Hematology) - Day 27
- Necropsy (Organ Weights & Histopathology) - Day 29. The tissues and organs listed below were observed grossly. Organs denoted with * from all animals were weighed at the scheduled necropsy. The eyes, adnexa, and nasal lacrimal tissues from all animals were processed for microscopic examination.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	Cervical Lymph Nodes	Spinal Cord (3 Sections)
Bone (Long)	Rectum	Mammary Gland and Skin	Spleen*
Bone Marrow	Ovaries*	Nasal-Lacrimal Tissue	Tattoo
Brachial Plexus	Testes*	Oviduct	Thymus
Brain*	Heart*	Pancreas with Attached Duodenum	
Epididymis	Kidneys*	Peripheral Nerve (Sciatic)	Thyroid/Parathyroid
Eyes with Optic Nerve and Adnexa	Larynx/Tongue	Pituitary	Trachea
Esophagus	Liver*	Prostate	Urinary Bladder/Urethra
Stomach (Cardia, Fundus, Pylorus)	Gall Bladder	Ribs/Sternum	Ureters
Small Intestine (Ileum, Jejunum, Peyer's Patch)		Salivary Glands	Uterus/Cervix/Vagina
Sacculus Rotundus	Lungs	Seminal Vesicle	Any Gross Lesions

Results:

- Mortality and Clinical Signs - No death occurred and no remarkable clinical signs were noted.
- Slitlamp Biomicroscopy Examination - Minimal flare was seen once in one eye of 2 non-treated control animals, 1 vehicle control animal and 1 animal in 0.01% AL-6221 groups (both eyes). These changes were not considered to be treatment-related.
- Indirect Ophthalmoscopy (Fundus Examination) - Normal.
- Pachymetry - No biologically relevant findings were noted.
- Body Weight Gain - Normal.
- Blood Chemistry & Hematology - No toxicologically significant, treatment-related changes were noted.
- Organ Weights - There were no statistically significant differences in absolute & relative organ weights of adrenals, brain, gonads, heart, kidneys, liver, and spleen for both ♂ & ♀ rabbits.
- Gross Pathology - No treatment-related abnormalities were noted.
- Microscopic Examination of Eye Tissues - No remarkable changes were characterized.

In summary, New Zealand white rabbits were topically treated with 0.001% or 0.01% AL-6221 ophthalmic solution 2 times a day for 6 days and 3 times a day for 22 days. The drug was well tolerated. No toxicologically significant ocular or systemic findings were noted.

3.1.1.2 Three Month Topical Ocular Irritation and Systemic Toxicity Evaluation of AL-6221 Ophthalmic Solution in Rabbits, TR No. 119:38520:0896 (Vol. 20)

Report No.: TR No. 119:38520:0896
Study Aims: To determine the ocular irritation and systemic toxicity potential of AL-6221 ophthalmic solution following tid topical ocular administration to New Zealand white rabbits for 3 months.
Compound/Vehicle: 0.01% (Lot N^o 96-15488-1), 0.03% (Lot N^o 96-15487-1), and 0.001% (Lot N^o 96-15486-1) AL-6221 ophthalmic solution; the composition of AL-6221 and vehicle is listed in the following table.

Ingredient (% w/v)	Vehicle	0.001% AL-6221	0.003% AL-6221	0.01% AL-6221
AL-6221	-	0.001 + 5% excess	0.003 + 5% excess	0.01 + 5% excess
Polyoxyl 35 castor oil, NOC	0.1	0.1	0.1	0.1
Tromethamine, USP, AR	0.12	0.12	0.12	0.12
Boric Acid, NF	0.3	0.3	0.3	0.3
Mannitol, USP	4.6	4.6	4.6	4.6
	0.1	0.1	0.1	0.1
	0.01 + 5% excess	0.01 + 5% excess	0.01 + 5% excess	0.01 + 5% excess
	pH 7.4	pH 7.4	pH 7.4	pH 7.4
Purified H ₂ O	QS 100	QS 100	QS 100	QS 100

Dose & Route: 1 drop (40 µl)/eye, both eyes tid for 3 months.

Animals: New Zealand white rabbits, 3-month old, weighing 2.4-2.8 kg; 6/sex/group.

Study Location: Toxicology Unit, Alcon Laboratory, Inc., 6201 South Freeway, Fort Worth, TX 76134

Compliance with GLP/QAU: Yes

Study Date: 4/17-5/15/96

Study Design: AL-6221 or vehicle was applied to both eyes (1 drop/eye) of each rabbit tid for 3 months as shown in the following table.

Group	AL-6221 Treatment	Nº of Animals	Treatment Volume (µl)/eye	Treatment Frequency	Dose (µg/day)	Dosing Duration (Days)
1	Non-treated Control	6/sex/group	-	tid	-	91 (♂)/92 (♀)
2	0 (Vehicle Control)		0			
3	0.001%		40		2.4	
4	0.003%		7.2			
5	0.01%		24			

The following parameters were monitored.

- Mortality and Morbidity - 2x/day.
- Biomicroscopic Examination - Days 0, 7, 14, 21, 28, 42, 56, 70 and 91.
- Indirect Ophthalmoscopic Examination - Days 0 & 91.
- Pachymetry - Days 0 & 91.
- Body Weights - Days 0, 7, 14, 21, 28, 42, 56, 70, 84, 91, and immediately prior to necropsy.
- PK/TK - Days 1, 30, 59, and 90. Plasma samples were collected from all animals in Groups 3, 4, and 5 at one hr post the 1st daily dosing.
- Clinical Pathology (Blood Chemistry & Hematology) - Day 86 (♂)/87 (♀)
- Necropsy (Organ Weights & Histopathology) - Day 92 (♂)/93 (♀). The following tissues and organs were observed grossly. Organs denoted with * from all animals were weighed at the scheduled necropsy. The eyes, adnexa, and nasal lacrimal tissues from all animals, all tissues from Groups 1 and 5 animals, and gross lesions from Groups 2-4 were processed for microscopic examination.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	Cervical Lymph Nodes	Spinal Cord (3 Sections)
Bone (Long)	Rectum	Mammary Gland and Skin	Spleen*
Bone Marrow	Ovaries*	Nasal-Lacrimal Tissue	Tattoo
Brachial Plexus	Testes*	Oviduct	Thymus
Brain*	Heart*	Pancreas with Attached Duodenum	
Epididymis	Kidneys*	Peripheral Nerve (Sciatic)	Thyroid/Parathyroid
Eyes with Optic Nerve and Adnexa	Larynx/Tongue	Pituitary	Trachea
Esophagus	Liver*	Prostate	Urinary Bladder/Urethra
Stomach (Cardia, Fundus, Pylorus)	Gall Bladder	Ribs/Sternum	Ureters
Small Intestine (Ileum, Jejunum, Peyer's Patch)		Salivary Glands	Uterus/Cervix/Vagina
Sacculus Rotundus	Lungs	Seminal Vesicle	Any Gross Lesions

Results:

- Mortality and Clinical Signs – No death occurred during the treatment period. Ocular discharge was noted in one eye in one rabbit each in Groups 4 and 5. Since this sign was only observed in several occasions in 1 animal of the group, it could not be regarded as drug-related.
- Biomicroscopic Examination – No drug-related abnormal findings were noted.
- Indirect Ophthalmoscopic Examination – No abnormal findings were noted.
- Pachymetry – No toxicologically significant, drug-related differences were noted.
- Body Weight – No treatment-related differences were noted.
- PK/TK – See Section ADME
- Clinical Pathology – No treatment-related changes in serum chemistry and hematology were noted.
- Organ Weights - There were no statistically significant differences in absolute & relative organ weights of adrenals, brain, gonads, heart, kidneys, liver, and spleen for both ♂ & ♀ rabbits.
- Gross Pathology - No treatment-related abnormalities were noted.
- Microscopic Examination - No drug-related changes were characterized.

In summary, New Zealand white rabbits were topically treated with 0.001%, 0.003% or 0.01% AL-6221 ophthalmic solution 3 times a day for 3 months to determine the ocular irritation and systemic toxicity potential of travoprost. The drug was well tolerated. No toxicologically significant ocular or systemic findings were noted.

3.1.1.3 Six Month Topical Ocular Irritation and Systemic Toxicity Evaluation of AL-6221 Ophthalmic Solution in Rabbits, TR No. 029:38520:0497 (Vol. 21)

Report No: TR No. 029:38520:0497

Study Aim: To determine the ocular irritation and systemic toxicity potential of AL-6221 ophthalmic solution following bid topical ocular administration to New Zealand white rabbits for 6 months.

Compound/Vehicle: 0.01%, 0.03%, and 0.001% AL-6221 Ophthalmic Solution; the composition of AL-6221 and vehicle is listed in the following table. The second lots of dosing supplies were introduced into the study on Day 100.

Ingredient (% w/v)	Vehicle Lot N° 96-16049	0.001% AL-6221 Lot N° 96-16052	0.003% AL-6221 Lot N° 96-16053	0.01% AL-6221 Lot N° 96-16050				
AL-6221	-	0.001 + 5% excess	0.003 + 5% excess	0.01 + 5% excess				
Polyoxyl 35 Castor Oil, NOC								
Tromethamine, USP, AR								
Boric Acid, NF								
Mannitol, USP								
Disodium EDTA, USP								
Benzalkonium Cl Solution, NF								
NaOH, NF and/or HCl, NF								
Purified H ₂ O								
Ingredient (% w/v)					Vehicle Lot N° 96-17107	0.001% AL-6221 Lot N° 96-17108	0.003% AL-6221 Lot N° 96-17109	0.01% AL-6221 Lot N° 96-17075
AL-6221					-	0.001 + 5% excess	0.003 + 5% excess	0.01 + 5% excess
Tromethamine, USP, AR								
Boric Acid, NF								
Mannitol, USP								
Disodium EDTA, USP								
Benzalkonium Cl Solution, NF								
NaOH, NF and/or HCl, NF								
Purified H ₂ O								

Dose & Route: 1 drop (25 µl)/eye, both eyes, bid for 6 months.

Animals: New Zealand white rabbits, 2-4 months old, weighing 2.4-2.7 kg; 6/sex/group.
 Study Location: Toxicology Unit, Alcon Laboratory, Inc., 6201 South Freeway, Fort Worth, TX 76134

Compliance with GLP/QAU: Yes
 Study Date: 8/28/96 - 3/6/97
 Study Design: AL-6221 or vehicle was applied to both eyes (1 drop/25 μ l/eye) of each rabbit bid for 6 months as shown in the following table.

Group	AL-6221 Treatment	N ^o of Animals	Treatment Volume (μ l/day)	Treatment Frequency	Dose (μ g/day)	Dosing Duration (Days)
1	Non-treated Control	6/sex/group	-	bid	-	189 (♂)/190 (♀)
2	0 (Vehicle Control)		0			
3	0.001%		1			
4	0.003%		3			
5	0.01%		10			

The day of the first dosing was designated as day 0. The following parameters were monitored.

- Mortality and Clinical Observations - 2x/day
- Body Weights - Pretreatment (Day 0); weekly for the 1st 3-month and biweekly thereafter
- Biomicroscopic Examination - Days 0 (pretreatment), 7, 14, 21, 35, 63, 84, 112, 147, 168 and 189.
- Indirect Ophthalmoscopic Examination - Days 0 (pretreatment), 84 & 189.
- Pachymetry - Days 0 (pretreatment), 90 & 188.
- PK/TK - Days 1, 86, and 181. Plasma samples were collected from 4/sex animals in Groups 3-5 prior to the 1st daily dosing and at 30 min post the last dose.
- Clinical Pathology (Blood Chemistry & Hematology) - Months 3 and 6
- Necropsy (Organ Weights & Histopathology) - Day 191/192. The following tissues and organs were observed grossly. Organs denoted with * from all animals were weighed at the scheduled necropsy. All tissues from all animals and gross lesions were processed for microscopic examination.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	Cervical Lymph Nodes	Spinal Cord (3 Sections)
Bone (Long)	Rectum	Mammary Gland and Skin	Spleen*
Bone Marrow	Ovaries*	Nasal-Lacrimal Tissue	Tattoo
Brachial Plexus	Testes*	Oviduct	Thymus
Brain*	Heart*	Pancreas with Attached Duodenum	
Epididymis	Kidneys*	Peripheral Nerve (Sciatic)	Thyroid/Parathyroid
Eyes with Optic Nerve and Adnexa	Larynx/Tongue	Pituitary	Trachea
Esophagus	Liver*	Prostate	Urinary Bladder/Urethra
Stomach (Cardia, Fundus, Pylorus)	Gall Bladder	Ribs/Sternum	Ureters
Small Intestine (Ileum, Jejunum, Peyer's Patch)		Salivary Glands	Uterus/Cervix/Vagina
	Lungs	Seminal Vesicle	Any Gross Lesions

Results:

- Mortality and Clinical Observations – No death occurred during the treatment period. No remarkable clinical signs attributed to topical ocular treatment with AL-6221 were noted.
- Biomicroscopic Examination – No toxicologically significant, treatment-related changes were noted.
- Indirect Ophthalmoscopic Examination - Normal
- Pachymetry - No toxicologically significant, treatment-related changes were noted.
- Body Weights – No treatment-related differences were noted.
- PK/TK – Data are presented in Section "ADME".
- Clinical Pathology – No biologically relevant effects of AL-6221 on clinical chemistry and hematology were observed.

- Organ Weights - There were no statistically significant differences in absolute & relative organ weights of adrenals, brain, gonads, heart, kidneys, liver, and spleen between control and treated animals.
- Gross Pathology - No treatment-related abnormalities were noted.
- Microscopic Examination - No treatment-related findings were noted.

In summary, New Zealand white rabbits were topically treated with 0.001%, 0.003% or 0.01% AL-6221 ophthalmic solution 2 times a day for 6 months to determine the ocular irritation and systemic toxicity potential of travoprost. The drug did not elicit any signs of ocular or systemic toxicity.

3.1.1.4 One-Year Topical Ocular Irritation and Systemic Toxicity Evaluation of AL-6221 Ophthalmic Solution in Primates, TR No. 080:30:0400, [REDACTED] 1521-001 (Vol. 22-25)

Study No: [REDACTED]
 Report No: TR No. 080:30:0400
 Study Aim: To evaluate ocular and systemic toxicity potential of AL-6221 following topical application bid to monkeys for 1 year.
 Compound/Vehicle: AL-6221 - 0.0015% (Lot N^o 97-19332, ASE2999A), 0.004% (Lot N^o 97-19331, ASE2998A), 0.012% (Lot N^o 97-19342, 98-21974); Xalatan (0.005%) (Pharmacia Upjohn, Lot N^o YE 53036, ZB 53089, YE 53035); Vehicle (Lot N^o 97-19333, ASE2996A). The formulation of AL-6221 ophthalmic solution was the same as the proposed clinical formulation.
 Dose & Route: 1/drop/eye, one eye only, bid
 Dosing Duration: At least 52 weeks
 Animals: Cynomolgus monkeys, 3-5 years old, weighing 2.6-5.3 kg for ♂ and 2.2-4.4 kg for ♀, 4/sex/group
 Study Location: [REDACTED]
 Compliance with GLP/QAU: Yes
 Study Date: 11/27/1997 -11/27/1998

The following parameters were monitored during the study.

- Mortality and Clinical Observations - 2x/day
- Body Weights - Once pre-dose, weekly, and prior to necropsy
- Food Consumption - Daily
- Ophthalmoscopic Examinations (indirect ophthalmoscopy and slit-lamp biomicroscopy) - Prior to the initiation of the study, in Weeks 2, 4, 8, 13, 26, 39 and at the end of the study
- Eyelash Photograph - Weeks 2, 4, 8, 13, 26, 39 and 52
- Iris Color Examination - Pre-dose and every 4 weeks
- Bone Density Examination (left distal radius trabecular and cortical bone) - Once prior to the initiation the study, Weeks 27/28 and 51
- ECG and Blood Pressure (Bp) - Pre-dose, Weeks 25 and 51
- Iris Colorimetry - Prior to study initiation and monthly
- PK/TK - Day 1, and Weeks 4, 13, 26 and 52. Blood samples were collected from all animals prior to the 1st daily dosing and at 30 and 60 min post the last dose.
- Clinical Pathology - Pre-dose, Weeks 1, 13, 26, 39 and 52
- Necropsy (Including Organ Weights & Histopathology) - All tissues and organs were examined grossly. Organs denoted with * from all animals were weighed at the scheduled necropsy. All tissues

from all animals listed in the following table and gross lesions were processed for microscopic examination.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	submandibular Lymph Nodes	Spinal Cord
Bone (Long)	Rectum	Mammary Gland and Skin	Spleen*
Bone Marrow	Testes*	Ovaries*	Tongue
Brain*	Heart*	Pancreas	Thymus
Epididymis	Kidneys*	Peripheral Nerve (Sciatic)	Thyroid/Parathyroid*
Eyes with Optic Nerve and Adnexa		Pituitary*	Trachea
Esophagus	Liver*	Prostate	Urinary Bladder
Stomach	Gall Bladder	Stern	Uterus
Ileum	Jejunum	Salivary Glands	Any Gross Lesions
Duodenum	Lungs	Seminal Vesicle	

Results:

- Mortality and Clinical Observations – No treatment-related death occurred during the treatment period. The only clinical change associated with AL-6221 was the impression that the treated eye was enlarged. No remarkable clinical signs attributed to topical ocular treatment with AL-6221 were noted.
- Body Weights – A decrease in body weight gain was noted in male animals (see table below). No treatment-related differences in females were noted.

Body weight changes in male animals treated with travoprost (kg)

Group	1	2	3	4	5
Treatment	Vehicle	AL-6221(0.0015%)	AL-6221(0.004%)	AL-6221(0.012%)	Xalatan(0.005%)
Pre-dose	4.1±0.9	4.1±0.7	3.8±0.9	3.9±0.7	3.7±0.6
Week 52	5.9±1.4	5.3±1.1	4.9±1.3	4.8±1.0	5.1±0.5
% of control	100	90	83.1	81.4	86.4
Body weight gain	1.5±0.6	1.1±1.0	1.1±0.8	0.9±0.3	1.4±0.2
% of control	100	73.3	73.3	60	93.3

- Food Consumption – No treatment-related, toxicologically significant changes in food consumption was noted.
- Bone Density – No findings in cortical, trabecular or total bone density that could be associated with the treatment with AL-6221 were noted in the study.
- Ocular Examination – A retraction of upper or upper and lower eyelid of the treated (right) eye was noted in treated groups in Week 26 (see table below). Exophthalmos was also noted in these groups. From Week 26, increased palpebral fissures of the treated eyes were observed in all Groups 3 and 4 animals, and in 6 of 8 Group 2 animals. Increased palpebral fissures were also noted in Group 5 animals (positive control group) and was considered treatment-related. No toxicologically significant findings were noted in examinations using indirect ophthalmoscopy and slit lamp biomicroscopy.

AL-6221-induced ocular changes

Group	1	2	3	4	5
Treatment	Vehicle	AL-6221(0.0015%)	AL-6221(0.004%)	AL-6221(0.012%)	Xalatan(0.005%)
Retraction of upper or upper and lower eyelid	0	1♂, 3♀	2♂, 3♀	1♂, 2♀	0
Exophthalmos	0	1♂, 3♀	2♂, 3♀	4♀	0

- Iris Color Evaluation – Treatment-related iris color changes evidenced by the decrease of red, green, blue colors or overall intensity were noted in all treated groups. The incidence is summarized in the table below.

Incidence of iris color changes in monkeys treated with AL-6221

Group	1	2	3	4	5
Treatment	Vehicle	AL-6221(0.0015%)	AL-6221(0.004%)	AL-6221(0.012%)	Xalatan(0.005%)
Slight color changes	0	2♂, 1♀	1♀	2♂	1♂
Distinct changes	0	0	1♂, 1♀	1♂, 1♀	2♀

- Ocular irritation Grading – There were no drug-related, biologically relevant changes.
- Eyelash Observations – No treatment-related changes were noted during the study.
- ECG and Bp – There were no drug-related ECG and blood pressure changes during the study.
- Clinical Pathology – No biologically relevant effects of AL-6221 on clinical chemistry, hematology and urine analysis were observed.
- Organ Weights - There were no drug-related, toxicologically significant changes in absolute & relative organ weight examination.
- Gross Pathology – Ocular changes in the eye treated with AL-6221 characterized by dark iris, cornea with rough surface, and eye sunken, discolored or large were noted in Groups 2, 3 and 4 animals (see table below). Rough corneal surface was also noted in 1 animal each in the vehicle and xalatan control groups. The sponsor indicated that this phenomenon might be related to repeated exposure to the dose or dosing procedure rather than to the active ingredient. No other positive findings related to the drug effects were noted.

Incidence of necropsy findings in animals treated with AL-6221

Group	Males					Females				
	1	2	3	4	5	1	2	3	4	5
N	3	4	4	4	4	4	4	4	4	4
Iris dark	0	1	1	2	1	0	0	0	1	0
Eye sunken	0	1	2	3	0	0	2	2	4	0
Cornea, rough surface	0	0	0	1	1	1	2	1	2	0
Discolored	0	0	0	0	0	0	0	1	0	0
Large	0	1	0	0	0	0	0	0	0	0

- Microscopic Examination – Minimal irregularity of corneal epithelium was noted in the treated eyes in the Groups 2 (2♂ and 2♀) and 3 (2♀) animals. The superficial cells of the corneal epithelium appeared multifocal flattened. Similar changes were also seen in four Group 5 animals (1♂ and 3♀). No other positive findings related to the drug effects were noted.

In summary, cynomolgus monkeys were topically treated with 0.0015%, 0.004% or 0.012% AL-6221 ophthalmic solution 2 times a day for 1 year to determine the ocular irritation and systemic toxicity potential of travoprost. Clinical observations showed that the treated eye seemed enlarged. Body weight gain was relatively low in male treated groups. Retraction of upper or upper and lower eyelid, exophthalmos, increased palpebral fissure and iris color changes were noted in the treated animals. Post-mortem examinations showed dark iris, cornea with rough surface, eye sunken, discolored and large and minimal irregular surface of the corneal epithelium. The changes, also observed in xalatan-treated animals, were not clearly dose-related. No other treatment-related positive findings were noted. No NOAEL was determined in this study.

3.2 SYSTEMIC TOXICITY**3.2.1 ACUTE TOXICITY****3.2.1.1 Single Dose Intravenous Toxicity Study in Rats, TR No. 061:30:0300, MPI 298-041 (Vol. 26)**

Study No: 298-041
 Report No: 061:30:0300
 Study Aim: To determine potential toxicity of AL06221 following a single iv administration to rats.
 Compound: AL06221 (Lot#: AL06221-04, purity = 96.46%)
 Vehicle Control:
 Dose/Route/Duration: 0 and 10 mg/5 ml/kg iv single dose
 Animals: Sprague-Dawley rats, 9 weeks of age, weighing 264-292 g for ♂ and 192-223 g for ♀, 5/sex/group
 Study Location:
 GLP/QAU: Yes
 Study Date: 7/9-23/98
 Study Design: Rats (5/sex/group) were given a single dose of 10 mg/kg AL06221 or vehicle control via iv administration. Animals were observed for 14 days. The following parameters were conducted.

- Clinical Signs and Mortality - Day 1: at 1, 2, and 4 hr post dosing; Days 2-14: 2x/day.
- Body Weights - Days 1, 8, and 15.
- Necropsy - Day 15.

Results:

- Clinical Signs and Mortality - No deaths occurred. No remarkable clinical signs were attributable to the treatment.
- Body Weights - There were no differences in mean body weights recorded on Days 1, 8, or 15.
- Necropsy - No treatment-related gross changes were identified.

In summary, Sprague Dawley rats were intravenously treated with a single dose of travoprost at 10 mg/kg and were observed for 14 days. The drug was well tolerated. Clinical observations, body weight measurement and necropsy examination revealed no systemic toxicity.

3.2.2 SUBCHRONIC TOXICITY

3.2.2.1 28-Day Intravenous Toxicity Study in Mice with AL-6221, TR No. 093:38520:0797, MPI Study 298-018 (Vol. 27)

Study No: 298-018
 Report No: 093:38520:0797
 Study Aim: To determine potential toxicity of AL06221 in a 4-week iv study in mice
 Compound: AL06221 (Lot#: AL06221-04, purity = 96.46%)
 Vehicle Control:
 Dose/Route/Duration: 0, 100, 300 and 1000 µg/5 ml/kg, iv, qd x 28 days
 Animals: CD-1 (ICR)BR mice, 8 weeks of age, weighing 27-33 g for ♂ and 23-26 g for ♀, 10/sex/group
 Study Location:
 Compliance with GLP/QAU: Yes
 Study Date: 11/26/96-12/17/97
 Study Design: Mice (10/sex/group) were given 0.1, 0.3 or 1.0 mg/kg AL06221 or vehicle control via iv administration qd for 28 days. Toxicity was assessed as shown below.

- Clinical Signs and Mortality - Twice daily.
- Body Weights - Weekly

- Food Consumption – Weekly
- Ophthalmoscopic Examination – pretest and during the last week of study
- Clinical Pathology – Blood and urine samples were collected from all animals at study termination
- Necropsy (Including Organ Weights & Histopathology) – All animals received a complete necropsy examination. All tissues listed in the following table from the animals of control and high dose groups, and gross lesions from all animals were processed and microscopically examined. Organs denoted with * from all animals were weighed at the scheduled necropsy.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	Mandibular Lymph Nodes	Spinal Cord
Bone (sternum and femur)	Rectum	Mammary Gland and Skin	Spleen
Bone Marrow	Testes*	Ovaries*	Tissue Masses
Brain*	Heart*	Pancreas	Thymus
Epididymis	Kidneys*	Peripheral Nerve (Sciatic)	Thyroid/Parathyroid*
Eyes with Optic Nerve and Adnexa	Injection Site	Pituitary	Trachea
Esophagus	Liver*	Prostate	Urinary Bladder
Stomach	Gall Bladder		
Ileum	Jejunum	Salivary Glands	Any Gross Lesions
Duodenum	Lungs	Tibial Nerve	Vagina

Results:

- Clinical Signs and Mortality – One male mouse in high dose group died on day 24. The reason of the death was unknown. This animal appeared normal immediately after dosing, but was found dead at 15 min after dosing. During the study period, no remarkable clinical signs attributable to the treatment were noted.
- Body Weights – No treatment-related differences in body weights were noted.
- Food Consumption – Average food consumption was similar between control and treated animals.
- Ophthalmoscopic examination – No toxicologically significant findings were noted.
- Clinical Pathology – No drug-related changes in clinical chemistry, hematology and urinalysis were observed.
- Necropsy - No treatment-related gross changes were identified.
- Organ weights – No biologically relevant, toxicologically significant changes in organ weights were noted.
- Histopathology – There were no treatment-related positive findings in histopathological examination.

In summary, CD-1®(ICR)BR mice were treated (iv qd) with AL-6221 at 0.1, 0.3 and 1.0 mg/kg for 28 days. One male at 1.0 mg/kg died on day 24. The reason of the death was unknown. In the other animals, the drug was well tolerated. No treatment-related positive findings were noted in any of the evaluations performed.

3.2.2.2 28-Day Intravenous Toxicity Study in Rats with AL-6221, TR No. 092:38520:0797, [redacted] Study 298-019 (Vol. 28)

Study No: 298-019
 Report No: 092:38520:0797
 Study Aim: To determine potential systemic toxicity of AL06221 in a 4-week iv study in rats
 Compound: AL06221 (Lot#: AL06221-04, purity = 96.46%)
 Vehicle Control: [redacted]
 Dose/Route/Duration: 0, 100, 300 and 1000 µg/ml/kg, iv, qd x 28 days

Animals: Crl:CD®BR VAF/Plus rats, 6 weeks of age, weighing 182-209 g for ♂ and 144-175 g for ♀, 10/sex/group

Study Location: [Redacted]

Compliance with GLP/QAU: Yes

Study Date: 11/26/96-12/17/97

Study Design: Rats (10/sex/group) were given 0.1, 0.3 or 1.0 mg/kg AL06221 or vehicle control via iv administration qd for 28 days. Toxicity was assessed as shown below.

- Clinical Signs and Mortality – At least twice daily
- Body Weights – Weekly
- Food Consumption – Weekly
- Ophthalmoscopic Examination – pretest and during the last week of the study
- Clinical Pathology – Blood and urine samples were collected from all animals at study termination
- Necropsy (Including Organ Weights & Histopathology) – All rats received a complete postmortem examination. All tissues listed in the following table from the animals of control and high dose groups, and animals that died during the treatment period, and gross lesions from all animals were processed and microscopically examined. Organs denoted with * from all animals were weighed at the scheduled necropsy.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	Mandibular Lymph Nodes	Spinal Cord
Bone (sternum and femur)	Rectum	Mammary Gland	Spleen
Bone Marrow	Testes*	Ovaries*	Tissue Masses
Brain*	Heart*	Pancreas	Thymus
	Kidneys*	Sciatic Nerve	Thyroid/Parathyroid*
Eyes with Optic Nerve and Adnexa	Injection Site	Pituitary	Trachea
Esophagus	Liver*	Prostate	Urinary Bladder
Stomach		Skin	Uterus and cervix
Ileum	Jejunum	Salivary Glands	Any Gross Lesions
Duodenum	Lungs	Tibial Nerve	Vagina

Results:

- Mortality – Eight treated animals were found dead during the treatment period (see table below). These rats appeared normal immediately after dosing but 7 of the 8 rats were found dead 15 min later. The other rat was found dead 2 hr after dosing. Histopathological examination performed on the rats that died during the treatment period showed moderate edema of the lungs in 1 male at 1.0 mg/kg and mild hemorrhage of the cervical spinal cord in 1 male at 0.3 mg/kg. These lesions were possibly the causes of the deaths for these 2 rats. The reason of the deaths for the remaining 6 rats was unknown. Mortality did not occur in the control animals.

Incidence of mortality in rats treated with AL-6221

Dosage (mg/kg)	Vehicle	0.1	0.3	1.0
Males	0	2 (Days 7 and 18)	3 (Days 13, 13 and 24)	2 (Days 19 and 26)
Females	0	0	1 (Day 12)	0

- Clinical Signs - During the study period, no remarkable clinical signs attributable to the treatment were noted.
- Body Weights – No treatment-related differences in body weights were noted.
- Food Consumption – Average food consumption was comparable between control and treated animals.
- Ophthalmoscopic Examination – No toxicologically significant findings were noted.
- Clinical Pathology – No drug-related changes in clinical chemistry, hematology and urinalysis were observed.

- Necropsy - No treatment-related gross changes were identified.
- Organ Weights – No biologically relevant, toxicologically significant changes in organ weights were noted.
- Histopathology – There were no treatment-related microscopic findings in animals that either died or were terminally sacrificed after 28-day treatment. In the rats died during the treatment period, moderate edema of the lungs was noted in 1 male at 1.0 mg/kg and mild hemorrhage of the cervical spinal cord was seen in 1 male at 0.3 mg/kg.

In summary, Crl:CD®BR VAF/Plus rats were treated (iv qd) with AL-6221 at 0.1, 0.3 and 1.0 mg/kg for 28 days. Seven male and one female treated animals were found dead between day 7 and day 26. Histopathological examination performed on the rats that died during the treatment period showed moderate edema of the lungs in 1 male at 1.0 mg/kg and mild hemorrhage of the cervical spinal cord in 1 male at 0.3 mg/kg. The reason of the deaths for the remaining 6 rats was unknown. There was no dose relationship relative to the deaths. In the other animals, the drug was well tolerated. No treatment-related positive findings were noted in any of the evaluations performed.

3.2.2.3 13-Week Intravenous Toxicity Study in Rats with AL-6221, TR No. 048:30:0300, MPI 298-021 (Vol. 29-30)

Study No: 298-021
 Report No: 048:30:0300
 Study Aim: To determine the subchronic toxicity of AL06221 in a 13-week iv study in rats
 Compound: AL06221 (Lot#: AL06221-04, purity = 96.46%)
 Vehicle Control:
 Dose/Route/Duration: 0, 100, 300 and 1000 µg/ml/kg, iv, qd x 13 weeks
 Animals: Crl:CD®BR VAF/Plus rats, 6 weeks of age, weighing 154-174 g for ♂ and 119-143 g for ♀, 15/sex/group
 Study Location:
 Compliance with GLP/QAU: Yes
 Study Date: 11/26/96-12/3/98
 Study Design: Rats (15/sex/group) were given 0.1, 0.3 or 1.0 mg/kg AL06221 or vehicle control via iv administration qd for 13 weeks. Toxicity was assessed as shown below.

- Mortality – Twice daily
- Clinical Signs – 5 times daily
- Body Weights – Weekly
- Food Consumption – Weekly
- Ophthalmoscopic Examination – pretest and during the last week of study
- Clinical Pathology – Blood and urine samples were collected from 10 animals/sex/group at study termination
- Necropsy (Including Organ Weights & Histopathology) – All rats received a complete postmortem examination. All tissues listed in the following table from the animals of control and high dose groups, and animals that died during the treatment period, and gross lesions from all animals were processed and microscopically examined. In addition, the femur, sternum, liver and spleen from all animals were microscopically examined. Organs denoted with * from all animals were weighed at the scheduled necropsy.

Adrenal*	Cecum	Mesenteric Lymph Nodes	Skeletal Muscle
Aorta	Colon	Mandibular Lymph Nodes	Spinal Cord
Bone (sternum and femur)	Rectum	Mammary Gland	Spleen
Bone Marrow	Testes*	Ovaries*	Tissue masses
Brain*	Heart*	Pancreas	Thymus
Eyes with Optic Nerve and Adnexa	Kidneys*	Sciatic Nerve	Thyroid/Parathyroid*
Esophagus	Injection site	Pituitary	Trachea
Stomach	Liver*	Prostate	Urinary Bladder
Ileum	Epididymis	Skin	Uterus and cervix
Jejunum	Lungs	Salivary Glands	Any Gross Lesions
Duodenum			Vagina

Results:

- Mortality – Fifteen male rats and seven female rats died between Days 10 and 76 of the study (see table below). These rats appeared normal immediately after dosing but 15 of the 22 rats were found dead at 15 min after dosing, 6 rats were found dead 2 hr after dosing, and one rat was found dead at the end of the day. No dose-relationship to these mortalities was noted. The reason of the deaths was unknown. Mortality did not occur in the control animals.

Incidence of mortality in rats treated with AL-6221

Dosage (mg/kg)	Vehicle	0.1	0.3	1.0
Males	0	7 (Days 10, 21, 33, 37, 53, 68 and 72)	5 (Days 31, 32, 37, 45 and 46)	3 (Days 20, 40 and 76)
Females	0	2 (Days 11 and 40)	4 (Days 37, 44, 61 and 74)	1 (Day 68)

- Clinical Signs - During the study period, no remarkable clinical signs attributable to the treatment were noted.
- Body Weights – No treatment-related differences in body weights were noted.
- Food Consumption – Average food consumption was comparable between control and treated animals.
- Ophthalmoscopic Examination – No toxicologically significant findings were noted.
- Hematology – Slight decreases in RBC counts, platelet counts, HB and hematocrit levels were noted in treated animals (see table below). These changes might be related to the bone marrow cavity changes observed in the histopathological examination.

Hematology changes in rats treated with AL-6221

Dosage (mg/kg)	Males				Females			
	Vehicle	0.1	0.3	1.0	Vehicle	0.1	0.3	1.0
RBC ($10^6/\mu\text{l}$)	8.41±0.63	7.85±0.52	7.64±0.40	7.84±0.58	7.82±0.36	7.21±0.74	7.09±0.63	6.71±0.63
Hb (g/dl)	14.8±0.84	14.2±0.81	14.1±0.74	14.5±0.91	14.8±0.56	14.1±1.18	14.1±0.89	13.4±0.70
Hematocrit (%)	45.8±2.95	42.9±2.44	42.7±2.49	44.5±2.37	45.7±2.03	43.3±3.16	42.8±2.33	41.3±2.24
Platelet ($10^3/\mu\text{l}$)	1140±112.7	1058±252.8	896±132.1	860±106.5	1078±213.4	915±215.5	878±120.3	827±167.9

- Clinical Chemistry and Urinalysis – A decrease in serum potassium and urine pH was seen in male and female animals at 0.3 and 1.0 mg/kg (see table below). At the same doses in male rats, serum albumin and A/G ratios were slightly lower (3.4 g/dl and 1.0 vs. control's 3.7 g/dl and 1.2). Since the decrease was slight, and the same changes were not seen in females, the decrease in serum albumin and A/G ratios might not be toxicologically significant.

Changes in clinical chemistry and urinalysis in rats treated with AL-6221

Dosage (mg/kg)	Males				Females			
	Vehicle	0.1	0.3	1.0	Vehicle	0.1	0.3	1.0
K ⁺ (mEq/l)	7.1±1.6	6.6±1.4	5.6±0.4	6.0±1.0	7.8±1.3	7.5±1.1	7.4±1.4	6.4±1.5
Urine pH	7.1±0.5	6.8±0.4	6.7±0.3	6.3±0.4	6.7±0.9	6.0±0.6	6.0±0.7	5.9±0.5

- Necropsy - No treatment-related gross changes were identified.
- Organ weights – No biologically relevant, toxicologically significant changes in organ weights were noted.
- Histopathology – Treatment-related microscopic changes are summarized in the table below. The bones were considered as the primary target organs evidenced by multifocal endosteal fibrosis and hyperostosis. Endosteal fibrosis was characterized by laying down a thin layer of fibrous tissue to the endosteal surface. Hyperostosis was characterized by thickening of cancellus and compact bone by formation of new bone on the endosteal surface. These changes resulted in narrowing of the marrow cavity and reduction in bone marrow. Hematopoiesis seen in the spleen and liver was considered to be secondary to the narrowing of the bone marrow cavities. The bone marrow was histologically normal.

Microscopic examination in rats treated with AL-6221

Dosage (mg/kg)	Vehicle		0.1		0.3		1.0	
	♂	♀	♂	♀	♂	♀	♂	♀
N	15	15	15	15	15	15	15	15
Bone, femur: Fibrosis, endosteal	Total		5	10	10	12	8	12
	Trace		1	4	3	2		
	Mild		4	6	7	8	8	12
Moderate					2			
Bone, femur: Hyperostosis	Total		5	8	8	12	14	15
	Trace		4	4	6			
	Mild		1	4	2	11	9	9
Moderate					1	5	6	
Bone, sternum: Fibrosis, endosteal	Total		12	8	11	7	8	6
	Trace		6	5	2	3	2	1
	Mild		6	3	9	4	6	5
Bone, sternum: Hyperostosis	Total		14	15	10	15	15	15
	Trace		10	7	5	3		
	Mild		4	8	5	12	15	12
Moderate							3	
Liver: hematopoiesis, extramedullary	Trace		4	6	6	11	9	11
Spleen: hematopoiesis, extramedullary, increased	Trace		6	9	2	3	3	2
	Mild		1	8	6	13	10	12
							13	

In summary, Crl:CD®BR VAF/Plus rats were treated (iv qd) with AL-6221 at 0.1, 0.3 and 1.0 mg/kg for 13 weeks. Fifteen male rats and seven female rats died between day 10 and day 76 of the study. The reasons of the deaths were unknown. However, mortality did not occur in the control animals. It was possible that the deaths were treatment-related. A decrease in serum potassium and urine pH was noted in treated animals of mid and high dose groups. A slight decrease in RBC parameters was also noted in treated animals, which might be related to the histopathological changes. Histopathological examination showed that the bones were the primary target organ evidenced by endosteal fibrosis and hyperostosis. Hematopoiesis seen in the spleen and liver was considered to be secondary to the narrowing of the bone marrow cavities. No NOAEL was determined in this study.

3.2.2.4 13-Weeks Intravenous/Intraperitoneal Toxicity Study in Mice with AL-6221, TR No. 047:30:0300, MPI 298-020 (Vol. 31-32)

Study No: 298-020
 Report No: 047:30:0300
 Study Aim: To determine the subchronic toxicity of AL06221 in a 13-week iv study in mice
 Compound: AL06221 (Lot#: AL06221-04, purity = 96.46%)
 Vehicle Control: