by gavage for 13 weeks. The study also established dose levels for a chronic toxicity study.

Rats were observed once or twice daily for morbidity and mortality and weekly for clinical symptoms. Ophthalmological examinations were done prior to treatment initiation and after 12 weeks of treatment. Body weights were recorded prior to study initiation, weekly, and at sacrifice. Food consumption was measured weekly. Hematology, serum chemistry, and urinalysis were evaluated at necropsy. Gross pathology was performed on all surviving rats sacrificed at study termination. Organ weights were recorded for adrenals, brain, gonads, heart, kidneys, liver, spleen, and thymus. Histopathology was evaluated on 32 tissue types from Groups 1 and 4, and on lungs and kidneys only of Groups 2 and 3.

RESULTS

- mortality: none during the study-
- observed effects: ↑ redness around eyes and nose fur of ♂ G3 and G4-
  discolored inguinal fur in ♂ G3 and G4-
  rales in 1♂ G4-
  ♀ presented with similar signs and chromodacryorrhea in all groups at similar frequencies-
- food consumption: ↓ (*,**) ♂ (G2-4) W5, W6, W7-
  ↓ (*,**) ♀ (G3, G4) W6, W7, and W13-
- body weight: mean weight and weight gain ↓ at ≥3 mg/Kg W6 due to water deprivation-
- clinical chemistry: • Albumin/globulin ratio: ↓ ♀ [G2(*), G3(**), G4(**)]-
  • Cholesterol: ↑ ♀ [G2(*), G3(**), G4(**)]-
  • ALT: ↓ ♂ [G2(*), G3(**), G4(**)]- • AST: ↓ ♂ [G3(*), G4(**)]-
  • Ca: ↑ ♀ G4(**)- • Globulin: ↑ ♀ [G3(**), G4(**)]-
- hematology: • RBC: ↓ [♂ G4(*), ♀ G3(*)]- • Hb: ↓ ♀ [G3(**), G4(*)]-
  • Platelets: ↑ DR ♀ G3(*), G4(**)-
- urinalysis: DR ↓ ♂♀ Na(*), K(*), sp.gr.(*)- DR ↑ ♂♀ vol.(*)-
- organ weights: spleen-absolute: ♂ [DR ↓ G3(**), G4(**)]; ♀ DR ↓ G4(**)-
  relative: ♂ [↓ G2(*), G3(**), G4(**); ♀ ↓ G4(*)-
  kidney-absolute: ♀ ↑ G4(**)-
  relative: ♂ DR G4(**); ♀ DR G4(**)-
  liver-relative: ♀ ↑ G2(*), G3(*), G4(**)-
- gross pathology: lesions were scattered and incidental-
- histopathology: conducted by

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male(female)

| ORGAN | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Lymph node, mandibular: hyperplasia | 1(1/14) | | 1/1 | 3(3) |
| Trachea: acute inflammation<br>infiltrate, cellular | 0<br>(0) | | | 1<br>(1) |
| Esophagus: chronic inflammation | 1 | | | 0 |
| Heart: cardiomyopathy | 13(5) | | | 9(8) |
| Stomach: mucosa, glandular, mineralized | 0 | | | 3(1) |
| Liver: necrosis<br>hepatocyte, vacuo, cytoplasm | 0<br>(0) | | | 1<br>(1) |

| ORGAN | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Lung: alveolar histiocytosis | 0 | 1 | 0 | 0 |
| alveolus, epith, hyperplasia | 0 | 1 | 0 | 0 |
| alveolus, edema | (0) | (1) | (0) | (0) |
| artery, media, hypertrophy | 0 | 0 | 1 | 0 |
| inflammation, acute | 0(0) | 2(1) | 0(2) | 0(0) |
| inflammation, granulo, diffuse | 4(4) | 4(3) | 7(3) | 1(3) |
| inflammation, granulo, focal | 0(0) | 2(1) | 4(0) | 14(3) |
| inflammation, subacute | 0(1) | 4(2) | 0(1) | 0(3) |
| Kidney: nephrocalcinosis | 12(12) | 15(13) | 14(15) | 12(15) |
| nephropathy | 1(0) | 0(0) | 0(0) | 1(8) |
| renal tubule, epith, regeneration | 0 | 0 | 1 | 0 |
| cortex, cyst | (1) | (0) | (0) | (0) |
| cortex, infiltrate, cellular | (0) | (2) | (0) | (0) |
| Urinary Bladder: calculus | 3 | 1/1 | 1/1 | 1 |
| dilatation | 0 | 1/1 | 0/1 | 0 |
| Salivary Gland: infiltrate, cellular | (0) | | | (1) |
| Testis: atrophy | 1 | | | 0 |
| Bone Marrow: fibrosis | (2) | | | (2) |
| Harderian Gland: infiltrate, cellular | 1 | | | 0 |

There were 15 ♂ and 15 ♀ examined in Groups 1 and 4. Kidneys and lungs were examined in all groups (15/sex/group). Only one ♂ urinary bladder was examined in Groups 2 (1/1) and 3 (0/1).

The lung inflammation (diffuse granulomatous, focal granulomatous, acute, and subacute inflammation) was dose related in males and interpreted by the pathologist "as a direct effect, probably irritation, of the test article on terminal bronchioles after aspiration of gavaged material." Some lungs with diffuse granulomatous inflammation contained a pale blue-gray amorphous material at the level of terminal bronchioles or alveolar ducts, and was interpreted as the vehicle or the vehicle with mucus.

The kidney nephropathy was treatment related in females but not in males. The report states the "character of nephropathy in these animal was not suggestive of the mechanism of injury in the kidney." The nephropathy was indicated as of minimal severity. Other changes in the kidney and in other tissues appeared to be incidental. The NOAEL for this gavage study was 3 mg/Kg/day.

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**STUDY 15.**
**SIX-MONTH ORAL GAVAGE TOXICITY STUDY OF AL04862 IN RATS.**

Report Nº: TR 082:38520:0496 Vol. 1.27
Compound: AL04862-025 (ERM 5712:039)
Formulation: 1% suspension
Route: Oral, gavage at 10 mL/Kg
Diet: Purina Certified Rodent Chow 5002 *ad libitum*
Dose Levels: G1 0 vehicle control G2 1 mg/Kg G3 3 mg/Kg G4 8 mg/Kg
Strain: Fischer F344, body wt ♂ 84-102 g, ♀ 77-96 g, 5 weeks of age
Number: 25/sex/group
Control Treatment: 1% aqueous carboxymethylcellulose
Study Site:

Date: May 5, 1995 to August 20, 1996
GLP/QAU Statements: Both present and signed.

The purpose of this study was to evaluate the toxicity of 1% AL04862 administered by gavage to rats for six months as a suspension . The animals were observed twice (one on weekends) daily for morbidity and mortality, and adverse clinical symptoms were recorded weekly. Ophthalmological examinations were conducted prior to treatment initiation, at 3 months, and at study termination. Body weight and food consumption was recorded weekly during the first 13 weeks and every two weeks thereafter. Hematology, blood chemistry, and urinalysis were evaluated at 3 months and at treatment termination. Necropsies were done on all rats in the study. Gross and microscopic evaluations were done on all rats in G1 and G4. In addition, the kidneys and urinary bladder were evaluated in G2 and G3.

## RESULTS AND DISCUSSION

- mortality: 1G3♂ sacrificed moribund at ≈ 3 months - the animal had a congenital anomaly consisting of a diaphragmeted hernia-
- signs: (number of animals in which the sign observed, G1 - G4, respectively)
  chromodacryorrhea DR (2, 4, 5, 7); redness around eyes (4, 7, 9, 9); lacrimation (2, 4, 7, 6)-
  noisy breathing (0, 1, 1, 3); other signs were of random occurrence-
- ophthalmology: 1G2♂ with atrophic retina
  1G3♂ with dilated retinal vessels (moribund sacrificed rat)-
  1G2♂ with keratitis
  1G1♂, 3G2♂, 2G3♀, 1G4♂ with unilateral retinal atrophy-
- food consumption: ↓ significantly in ♂ G2 and G4 W16, 18, and 20-
  ↓ significantly in ♀ G4 W8, 9, 14, 18-
  ↑ significantly in ♀ G2 W22-
- body wt: ↓ significantly in ♂ G2 W15-W26-
  ↓ significantly in ♂ G4 W19-W26-
  ↓ significantly in ♀ G4 W7-W26 (except W25)-
- body wt gain: ↓ significantly in ♂ G2 W2, 6, 11, 12-
  ↓ significantly in ♂ G3 W2, 5-
  ↓ significantly in ♂ G4 W2, 5, 6, 19-
  ↓ significantly in ♀ G3 and G4 W4, 26-

The mean cumulative body wt gain in ♂ G2 and G4 and in ♀ G3 and G4 were significantly decreased over the study duration.

- 3-month clinical chemistry (significant changes)
  - chloride: ↑ ♂ (G2, G4), ♀(G3, G4)-
  - AST: ↓ ♀ G3 and G4-
  - BUN: ↑ ♂ G2, G3, G4-
  - albumin: ↓ ♀ G3 and 4-
  - albumin/globulin ratio: ↓ ♀ G3-
  - cholesterol: ↑ ♂ G4-

6-month clinical chemistry (significant changes)
- chloride: ↑ ♂ G4 and ♀ G3
- AST: ↓ ♀ G2 and G3-
- BUN: ↑ ♂ G4 and ♀ G2 and G4-
- globulin: ↑ ♀ G3 and G4-
- albumin/globulin ratio: ↓ ♀ G3 , G4-
- cholesterol: ↑ ♂ G4-
- creatine kinase: ↓ ♀ G2 and G4-
- ALT: ↓ ♀ G2 and G3-
- LDH: ↓ ♀ G4-
- creatinine: ↑ ♂ G4-
- glucose: ↓ ♀ G2 and G4-

- 3-month hematology (significant changes)
  - Hb: ↓ ♂ G2 and ↓ ♀ G4-
  - RBC: ↓ ♀ G4-
  - Hct: ↓ ♀ G4
- 3-month urinalysis (significant changes)
  - Na ↓ ♀ G2, G3, G4-
  - K: ↓ ♂ G4 and ♀ G3 and G4
  - pH ↓ ♂ G3-
  - specific gravity: ↓ ♂ G3 and G4-

6-month hematology (significant changes)
- mean % reticulocyte count: ↑ ♂ G3-
- absolute reticulocyte count: ↑ ♂ G3-

6-month urinalysis (significant changes)
- Na: ↓ ♀ G2, G3, G4-
- K: ↓ ♂ G4 and ♀ G2, G3, and G4-
- pH: ↑ ♀ G4
- specific gravity: ↓ ♂ G4 and ♀ G2-4-
- volume: ↑ ♀ G4-

- organ weights (significant changes):

absolute weight
- spleen: ↓ ♂ G2, G3, G4-
- kidney: ↑ ♀ G4 DR-

relative weight
- brain: ↑ ♂ G2 and G4-
- heart: ↑ ♂ G4; ♀ G3 and G4 DR-
- kidneys: ↑ ♂ G2, G3, and G4; ♀ G3 and G4
- liver: ↑ ♀ G4 DR
- testes: ↑ ♂ G4
- fasted body wt: ↓ ♂ G2 & G4; ♀ G3 and G4

- gross pathology:
  - spleen: enlarged in 2 ♀ G3-
  - liver: mass 1♀G4 (indicated as a hepatodiaphragmatic nodule)-
  - thymus: lesion 1♂G3; pigmentation 1♂G2, 1♂G4-
  - mandibular lymph nodes: pigmentation 2♂G4-
  - eye: 1♂G3-
  - testes: small 1G2, 1G3-
  - ovary: cyst 4G1, 5G2, 4G4; uterus: dilatation 4G1, 1G3, 2G4-
- histopathology:

| Tissue | Group 1 0 mg/Kg/day ♂ | ♀ | Group 2 1 mg/Kg/day ♂ | ♀ | Group 3 3 mg/Kg/day ♂ | ♀ | Group 4 8 mg/Kg/day ♂ | ♀ |
|---|---|---|---|---|---|---|---|---|
| Pancreas: atrophy, acinar | 1 | 1 | | | | | 4 | 1 |
| Lymph node, mandibular, hemorrhage | 2 | | | | | | 6 | |
| Harderian gland: inflammation, acute | 3 | | | | | | 5 | |
| Kidney: mineralization | 23 | 24 | 22 | 25 | 24 | 25 | 25 | 25 |
| nephropathy | 6 | 2 | 4 | 2 | 8 | 2 | 3 | 22 |
| fibrosis | | | | | | | | 5 |
| inflammation, chronic | | | 2 | | 2 | 1 | | 6 |
| Urinary bladder: crystalline material, lumen | | 0 | | 2 | | 3 | | 4 |
| Ovary: cyst | | 9 | | 5 | | 0 | | 14 |

The severity of the kidney mineralization increased dose proportionately in females and in males the severity increase only in the high dose. Mineralization in females of Groups 3 and 4 was considered to be biologically significant. Severity of the kidney nephropathy, chronic inflammation, and fibrosis in the high dose females was considered to be biologically significant. The kidney was the target organ in this study.

Crystalline material in the lumen of the urinary bladder was characterized by the presence of multiple round 5-50 micron diameter, lightly eosinophilic, homogeneous beads adhering to the mucosal surface. This

dose related increase was considered to be treatment related but of equivocal biological significance. The NOEL in this study appears to be 1 mg/Kg/day.

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**STUDY 16.**
**FERTILITY AND GENERAL REPRODUCTION STUDY IN RATS WITH AL04862.**

Report Nº: TR 089:38520:0994 Vols. 1.28 - 1.30
Compound: AL04862, lot AL-4862-HO-2-5,
Formulation:
Route: Oral, gavage
Diet: Provided *ad libitum*.
Strain: Sprague-Dawley Crl:CD®BR VAF/Plus®, age ♂ 8W, ♀ 11W, body wt. ♂ 214-274 g, ♀ 214-276 g.
Number: 40/sex/group
Dose Levels:

| Group: | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| mg/Kg/day: | 0 | 2 | 6 | 18 |
| mg/mL: | 0 | 0.40 | 1.20 | 3.60 |
| mL/Kg/day: | 5 | 5 | 5 | 5 |

Control Treatment: 0.5% carboxymethylcellulose in distilled water
Study Site:
Date: August 12, 1993 - June 7, 1996
GLP/QAU Statements: Both present and signed.

The purpose of this study was to evaluate the potential for producing reproductive toxicity of AL04862 in male and female rats. The survival and growth in the F2 generation was also evaluated.

The drug mixture was prepared at least once per week. Animals were dosed daily, with dosage based on the most recent body weight. F0 males were dosed 14 W prior to mating and females 2 W prior to mating. Dosing continued for each sex until euthanasia on lactation day 21 for females that delivered and following completion of parturition for males. In addition, twenty-three females per group were euthanized for cesarean section on gestation D20 for examination of F1 pups for external, skeletal, and visceral abnormalities. Selected F1 pups were subsequently mated and allowed to deliver and rear the F2 offspring up to lactation D21.

RESULTS AND DISCUSSION

F0 Generation

- mortality: 4♂ (2G1, 1G2, 1G4) euthanized in extremis due to physical injuries or severe clinical signs-
- clinical observations: dark material around eyes/nose/mouth in G3 and G4 and salivation in G4-
- body weight: ↓ in mean body weight (*,**) and weight gain (**) in ♂ G2, G3, G4 and ♀ G3 and G4--
- food consumption: significant ↓ in ♂ G2 (W17-18) and G4 (W1-3, 9-10, 13-15) and ♀ G2 (W3-4), ♀ G3 (W3-5) -G4 (W1 through gestation and lactation Days 7-21)-

Cesarean Section Observations

- mean fetal body wts ↓ significantly (**, DR) in G2, G3, and G4-
- number of corpora lutea, implants, viable fetuses, early and late resorptions and post-implant losses comparable over all groups-
- no dead fetuses-

Fetal Morphology

- no significant difference in number or type of malformations in treated vs control groups-
- no external findings - visceral findings (distended ureters) decreased significantly (*) in G4 - slight increase in number with unossified sternebrae 5 and 6 in G4-

Fertility - Gestation - Parturition - Lactation

- no difference in copulation or fertility index, in precoital interval, length of gestation, or pregnancy status-
- no change in lactation D6-

Gross Necropsy of Fo Males and Females

- testes weight was significantly low (**, 9.9%) in G2 and 5.6% low in G4 compared to G1-
- no significant difference in the mean number of uterine implantation scars-

F1 Generation

- lactation Day 0 showed no significant changes in number of dead or live fetuses, number of litters with live offspring, mean live litter size, or ♂♀ ratio-
- pup viability Day 0 (no. live, no. dead, no. litters with live offspring, mean live litter size, sex ratio) was not significantly different from control-
- pup viability during lactation (no pups alive Day 1 - Day 21) was not significantly different from control-
- no unusual external observations-
- mean pup weight during lactation was significantly (**, 11.4%-11.9%) reduced D14 and D21 in G4 after selection and showing DR decrease - G3 weight decreased by 3%-7% after selection through D21-
- no unusual gross necropsy in F1 pups found dead during lactation or culled at completion of weaning, but number with dilated kidney pelvis was increased in G4 (1, 1, 1, 4 in G1-G4, respectively)-
- no unusual developmental or functional testing results-

Postweaning clinical observations

- mean body weight ↓ in ♂ G4 - significant W11 (*, 7%) - mean body wt gain ↓ ♂ G4 at W5-6 and W8-9 and in ♀ G4 W5-6 and W7 through W8 - gestation body weight ↓ D0 through D20 (*, or **) - lactation body weight ↓ D1 (*) and D21 (**)-
- survival and clinical observations were unremarkable-
- reproductive parameters no different from control-
- gross necropsy findings for F1 animals were unremarkable - testes wt G4 ↓ (*, 6.5%)-

F2 Generation

- no differences in these pups compared to control pups-
- mean body weight was similar to controls-
- no unusual external observations or gross changes reported related to treatment-

There were no adverse effects on fertility or reproductive performance of F0 rats dosed at 2, 6, or 18 mg/Kg/day, but DR toxicity was seen in both sexes at all dose levels as decreased body weight, decreased body weight gain, and/or decreased food consumption. As a result, F1 pups showed a decrease in body weight at 6 and 18 mg/Kg/day. These significant decreases did not adversely affect F1 fertility or reproductive performance. The mean absolute testes weight of the high dose rats were significantly lower than the control. Microscopic findings in the testes were reported to be within normal limits. The NOEL was 18 mg/Kg/day for fertility and general reproductive performance and around 2 mg/Kg/day for maternal and fetal toxicity.

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**STUDY 17.**
**DEVELOPMENTAL TOXICOLOGY STUDY IN RATS WITH AL04862.**

Report Nº: TR 087:38530:0994 Vols. 1.31 - 1.32
Compound: AL04862 CAI, lot Nº AL04862-HO-2-5.
Formulation:
Route: Oral, gavage
Diet: Chow provided *ad libitum*.
Strain: Sprague-Dawley Crl:CD®BR VAF/Plus®
Number: 40 ♀/group

| Dose Levels: Group: | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| mg/Kg/day: | 0 | 2 | 6 | 18 |
| mg/mL: | 0 | 0.40 | 1.20 | 3.60 |
| mL/Kg/day: | 5 | 5 | 5 | 5 |

Control Treatment: 0.5% w/v CMC in distilled water.
Study Site:
Date: August 31, 1993 to June 7, 1996
GLP/QAU Statements: Both present and signed.

The purpose of this study was to evaluate the potential developmental effects of AL04862 in rats, and to assess survival, growth, development, behavior , and reproductive capacity of F1 offspring, through weaning of an F2 generation.. Animals were dosed from gestation Day 6 through gestation Day 17. On gestation Day 20, a total of 22 females from each group were euthanized for cesarean section and examined for external, visceral, and skeletal abnormalities. Other animals in the group were allowed to deliver and rear their pups. Selected F1 pups were mated and allowed to deliver their offspring until Day 21.

## RESULTS AND DISCUSSION

F0 Females

- 1 female in each group did not deliver and was euthanized D25-
- DR soft stools and fecal staining-
- gestation body wt ↓ G4 , beginning D9 and was DR - D18 (**, 5%), D20 (**, 5%)
- gestation body wt gain decreased (**, D6-9, D12-18, D6-18, and D0-20-
- food consumption reduced G3 (*-**) and G4 (**) during gestation-
- gross observations at necropsy indicated no significant difference in groups-

Cesarean Section Observations

- mean fetal body wt ↓ (**, 13.5%) G4-
- corpora lutea, implantation sites, pre-implantation loss, viable fetuses, dead fetuses, late or early resorptions, post-implantation loss, and ♂/♀ ratio were comparable in all groups-

Fetal Morphological Observations

- fetal malformations: G4 1 liter (1 fetus) with small kidney and undescended testis - 1G4 liter (1 fetus) with cleft palate-
- fetal variations: G3 and G4 slight increase in litters and fetuses with unossified #5 and/or #6 sternebra, reduced ossification of skull, and unossified hyoid - none significant-

Pregnancy - Gestation - Parturition - Lactation - Litter Retrieval

- pregnancy rate: G1 (100%), G2( 92.5%), G3( 95%), G4( 95%)-
  mean gestation length G1 21.9 days, G2-4 22.0 days-
  no prolonged delivery or dystocia-
  pups returning to nest lactation day 6 in G1 (100%), G2 (96%), G3 (100%), G4 (100%)-

F1 Generation

- no significant differences from control in terms of viability, external observations, body weight, necropsy observations, developmental landmarks/functional testing/open field testing, postweaning survival, clinical observations and body weights, fertility, gestation, parturition and lactation, or necropsy observations-
- sex ratio (♂/♀) G1 (137:130), G2 (130:135), G3 (134:127), G4 (112:143)-
- thin hair coat G4-
- in the 1 of 4 pups found dead during lactation in G4: omphalocele, domed head, atelectasis, sternal cleft, diaphragm hernia, and misshapen liver - the other pups presented with atelectasis-
- G4 body weight gain significantly (*, 9.4% - 12.4%) increased-
- G4 lactation body weight significantly (*,**, 8.8% - 11.5%) increased-

F2 Generation

- significant difference (*) in ♂/♀ ratio in G4 (167:119), G1 (133:138), G2(154:158), G3(135:132)-
- significant increase in pup weight G4 (*,**, 8% - 9.8%) at D4, D7, D14, and D21-

- in 1 of 6 pups found dead during lactation in G4: anophthalmia, microphthalmia, craniorachischisis, atelectasis, distended ureters, and macroglossia - all others were found with atelectasis-
- at scheduled euthanasia (pups/litter) G4 head depressed areas (1/1), light red discoloration in center of depressed area of head (1/1), dark red lungs (1/1), distended ureter (1/1) also G3 (2/2) - and dilated kidney pelvis G2 (1/1), G2 (3/3), G4 (2/2)-
- apparent umbilical hernia (occurrence/animals affected): G1 (0/0), G2 (2/2), G3 (1/1), G4 (6/5)-

Maternal toxicity was produced at 6 and 18 mg/Kg/day in the F0 rats and fetal toxicity was observed in the high dose (18 mg/Kg/day) in the F1 generation. No malformations were reported at 2 and 6 mg/Kg/day; at 18 mg/Kg/day, however, one fetus was found with cleft palate and one was observed with undescended testis and a small kidney. The number of litters with variations was slightly higher in the 6 and 18 mg/Kg/day groups. In the high group pups found dead during lactation, there were several findings that were not observed in the controls, and lung atelectasis was increased at 18 mg/K. In the F2 generation, the male-female sex ratio (167:119) at 18 mg/Kg differed significantly from the control and historical data.

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**STUDY 18.**
**ORAL TERATOLOGY STUDY IN RABBITS WITH AL04862.**

Report Nº TR 088:38520:0994 Vol. 1.33
Compound: AL04862 CAI, Lot Nº AL-4862-HO-2-5,
Formulation: Suspension
Route: Oral, gavage
Diet: *Ad libitum*
Strain: NZW, 5-7 months old, 3 to 5 Kg body weight
Number: 20 females/group

| Dose Levels: Group: | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| mg/Kg/day: | 0 | 1 | 3 | 6 |
| mL/Kg/day: | 1 | 1 | 1 | 1 |
| mg/mL: | 0 | 1.0 | 3.0 | 6.0 |

Control Treatment: 0.5% w/v CMC in distilled water
Study Site:
Date: September 21, 1993 - June 7, 1996
GLP/QAU: Both present and signed.

This study was done to evaluate the potential teratogenic effects of AL04862 in pregnant rabbits. Dosage was a single daily dose administered by gavage from gestation Day 6 through gestation Day 18. Suspensions were prepared fresh weekly and stored refrigerated. Animals were observed daily for clinical signs of toxicity and mortality. Body weights were recorded gestation D 0, 6, 9, 12, 15, 19, 24, and 28. Food consumption was measured 10 times over Days 0-29. All surviving animals were euthanized Day 29. Animals found dead or that aborted were necropsied. Each fetus was examined for external, visceral and skeletal examinations.

RESULTS AND DISCUSSION

mortality: 1 gravid G4 ♀ found dead D19-
clinical signs G4: 2 emaciated, 5 no feces, and 9 reduced feces-
abortions: 1G4 D17, and 1G4D26-
pregnancy rate: G1 (100%), G2 (80%), G3 (85%), G4 (85%)-
gestation body weight: G4 [D12 (*, 7.5%), D15 (**, 8.9%), D19 (*, 7.5%)]-

gestation body wt gain: G3 [D6-9 (**, -27 g)],
G4 [D6-9 (**, -158 g), D9-12 (**, -29 g), D6-19 (**, -10 g), D19-29 (**, + 337 g)]-
food consumption: ↓ G3 D6-9 (*, 15% ), ↓ G4 D619 (**, 41%)-
maternal necropsy observations: unremarkable-
cesarean section observations: no significant differences from control; however, the number of corpora lutea, implantation sites, pre-implantation loss, and viable fetuses were reduced in G4-
fetal variations: accessory skull bones significantly increased in the number of litters G1 (0%), G2 (25%), G3 (12.5%), G4 (30.7%); historical value (29.4%) - fetuses: G1 0/135 (0%), G2 4/108 (3.7%), G3 2/103 (1.9%), G4 5/80 (6.25%) - historical value (0 to 3.9%)- these historical values do not agree with the data from
fetal malformations: no significant external or soft tissue malformations-
skeletal malformations: vertebral anomaly with or without associated rib anomaly increased in the number of fetuses (1.5%, 1.8%, 6.7%, 5%) and in the number of litters involved 10%, 12.5%, 31%, 31%)-
total number with malformations: fetuses G1( 9/135), G2 (4/108), G3 (12/103), G4 (5/80)-
litters G1 (7/20), G2 (4/16), G3 (7/16), G4 (5/13)-

Maternal toxicity occurred in the high dose (6 mg/Kg/day) as reduced or no feces, emaciation, reduced body weight gain, abortions, and mortality. Maternal toxicity was also seen at 3 mg/Kg/day in the form of significant weight loss during the first third of the study. NOEL for maternal toxicity was, 1 mg/Kg/day. The study did show an increase in vertabral/rib anomalies at 3 and 6 mg/Kg and a significant increase in fetal variations in the form of accessory skull bones. Based on the vertebral/rib anomalies, AL04862 may be considered teratogenic in rabbits.

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**4. MUTAGENICITY STUDIES**

**STUDY 20.**

***E.COLI*** **MUTATION ASSAY WITH AL04862.**

Report Nº: TR 027:38520:0793 Vol. 1.36
Compound: AL04862-03, Lot Nº 4035-85-IIA,
Formulation:
Strains:

Vehicle Control:
Negative Control: 100 µL of appropriate tester strain
Positive Controls:

Study Site:
Date: October 1, 1992 - January 7, 1994
GLP/QAU Statement: Both present and signed.

The doses used in the mutagenic assays were based on the results of a range finding study. In the mutagenicity assay, tester strains were exposed to

Plating was done in triplicate. For an assay to be considered to have a positive response

RESULTS

Under the conditions in which these assays were done, positive increases were not produced in the number of

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**STUDY 21.**

**MOUSE LYMPHOMA FORWARD MUTATION ASSAY WITH A CONFIRMATORY ASSAY WITH AL04862.**

Report Nº: TR 124:38520:1294 Vol. 1.36
Compound: AL04862, Lot Nº BH01,
Formulation: Solution
Dose Levels: 200 to 3000 µg/mL
Strain: cells
Negative Control: 1% DMSO
Positive Control:
Positive Control:
Study Site:
Date: March 3, 1994 - January 18, 1995
GLP/QAU Statements: Both present and signed.

Two trials, each with six dose levels, were carried out each trial contained three culture dishes per dose level. Concentrations ranged from , in one trial and from in the second trial. The treatment and recovery times in both trials were 4 hours exposure, two days recovery, and 10-14 days incubation. A mutant frequency of greater than which is twice the average mutant frequency of the concurrent vehicle controls, was considered a positive response in the first nonactivation ( ) trial. In the second nonactivation trial, a frequency greater than was considered a positive response. Responses of in the first activation trial and in the second activation trial were the criteria for a positive response.

RESULTS

None of the mutant frequencies with AL04862 exceeded twice the vehicle control in the mutation assays in the absence of metabolic activation. However, dose related mutant frequencies exceeded the minimum criteria by -fold in the presence of metabolic activation in both assays . Moderate to high toxicity occurred in these activation assays, with growth relative to the control ranging in the first assay, and mutant frequencies exceeding the minimum criterion of at all concentrations tested (375-1990 µg/mL). In the second activation assay, relative growth ranged Mutant frequencies exceeded the minimum criterion of at all concentrations except 200 µg/mL. The two positive controls, exceeded vehicle control mutant frequencies by fold. AL04862 can be considered negative in the absence of metabolic

activation. In the presence of metabolic activation, positive results were obtained in both trials at concentrations ≥375 µg/mL AL04862, with high to very high toxicity occurring.

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**STUDY 22.**
***IN VIVO* SISTER CHROMATID EXCHANGE ASSAY.**

Report Nº: TR 095:38520:0696 Vol. 1.36
Compound: AL04862, Lot Nº AL-4862-HO-18/19/21/6,
Formulation:
Route: Oral, gavage
Dose Levels: ♂ (0, 500, 1000, 1333 mg/Kg), ♀ (0, 250, 500, 1000 mg/Kg)
Strain:
Number: 5/sex/group
Control Treatment:
Positive Control:
Study Site:
Date: August 31, 1995 - July 17, 1996
GLP/QAU Statements: Both present with signatures.

Dosage for the study was based on the results from a pilot study conducted with 0, 250, 500, 750, 1000, 1333 mg/Kg doses. The animals were implanted with a about one hour before dosing. Two hours prior to sacrifice, the animals were given an Vehicle and positive controls were sacrificed at approximately 24 hours and the AL04862 treated groups at approximately 30 hours. Twenty-five cells/animal were analyzed for chromatid exchange CE), with a total of 100 cells analyzed per animal.

RESULTS

All animals remained healthy during the study. There was no significant increase in the number of CE in males or females from AL04862.

Under the conditions of this study, AL04862 can be considered negative in the chromatic exchange assay.

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**STUDY 23.**
**MOUSE MICRONUCLEUS ASSAY WITH AL04862.**

Report Nº: ~~TR~~ 125:38520:~~1294~~ ~~Vol. 1.36~~
Compound: AL04862, Lot Nº BH01,
Formulation: Suspension
Route: Oral, gavage
Dose Levels: 250, 500, 100 mg/Kg
Strain:
Number: 5/sex
Vehicle Control:
Positive Control:
Study Site:
Date: March 3, 1994 - December 21, 1994
GLP/QAU Statements: Both present and signed.

Bone marrow was harvested at 24, 48, and 72 hours after AL04862 administration and after 24 hours for the vehicle and positive control administration. was collected for staining and slide preparation. The number of micronuclei counted and the

## RESULTS

Two females at 500 mg/Kg and three at 1000 mg/Kg died during the study. AL04862 did not produce a significant increase in the number of at any time in the study. produced significant increases ($p<0.05$) in in both sexes and a significant decrease in the male ratio at 24 hours. The ratio for the mid dose group was also significantly low for males at 24 hours. Based on the results, AL04862 was not considered positive in the mouse micronucleus test.

# THIS SECTION WAS DETERMINED **NOT** TO BE RELEASABLE

2 pages

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**STUDY 25.**
**CELL PROLIFERATION ASSAY WITH AL04862.**

Report Nº: TR 102:38520:0994 Vol. 1.36
Compound: AL04862-03, Lot Nº 4035-85-IIA
Formulation: Solution
Route: Oral, gavage at 10 mL/Kg
Dose Levels: 0, 20, 60, 180 mg/Kg/day for 25 consecutive days.
Strain:
Number: 12/group
Negative Control:
Positive Control:
Study Site:
Date: October 2, 1996
GLP/QAU Statements: Both present and signed.

Based on this study, AL04862 at doses did not show a potential for the induction of liver hepatocytes. The conclusion indicated that AL04862 "is likely not to induce liver tumor formation in a long term study."

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**STUDY 26.**
**SENSITIZATION ASSAY WITH AL04862**
Report Nº TA168:38520:0293 Vol. 1.37

AL04862 was considered negative in the sensitization assay.

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**STUDY 27.**
***IN VITRO* GLUTATHIONE REACTIVITY EXPERIMENTS WITH AL04862.**
Report Nº TR 004:38520:0292 Vol. 1.37

This study was done to evaluate the reactivity of AL04862 with reduced glutathione (GSH) in *in vitro* incubations in the presence and absence of glutathione S-transferase. Rat liver cytosol was the source of GSH transferase. AL04414A was used as a negative control, AL01545 was used as an enzyme-dependent positive control, and AL03129 was used as an enzyme-independent positive control. AL03129 was also used as a positive control to observe nonenzymatic carbonic anhydrase inhibitor-GSH reactivity.

AL04862 was said to compare well with the negative control, AL04414A. The positive control, 1 and 5 mM AL01545, decreased GSH concentrations by 20% and 56%, respectively. AL04862 is not expected to react with GSH.

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**STUDY 28.**
**ONE-MONTH TOPICAL OCULAR IRRITATION EVALUATION OF AL07118 OPHTHALMIC SUSPENSION IN RABBITS.**
Report Nº TR 098:38520:0696 Vol. 1.37

AL07118 is the of AL04862 found following accelerated stability studies. This study evaluated the potential for AL07118 to produce ocular irritation in the presence or absence of AL04862, following tid topical ocular administration to rabbits for one month. Irritation studies were evaluated at almost twice the concentration to be used in the marketed product. AL07118 was evaluated alone as a 0.2% suspension in the marketed formulation and as a 0.2% suspension in the presence of 0.8% AL04862 in the marketed formulation. The two suspensions were compared to the vehicle. AL07118 (lot Nº 6312-10A) The study was done by Alcon under GLPs. Histopathology was evaluated

Ocular discharge occurred in two animals treated with 0.2% AL07118 and two animals treated with the mixture. There was no significant difference in male or female body weights in the three groups. findings were not significant; pachymetry measurements, however, were increased mildly (13%-14%) in the drug treated groups. Gross and microscopic pathology revealed no significant findings. Blood samples showed peak and trough concentrations of 1.22±0.19 and 0.77±0.18 µg/mL, respectively, for AL07118 and 5.75±0.14 and 4.69±4.69±0.26 µg/mL, respectively, for AL07118/AL04862. Using detectable levels of AL07118 were not observed in animals dosed with the mixture. No interconversion of the was detectable in rabbits dosed with AL07118.

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**STUDY 29.**
***E. COLI*** **MUTATION ASSAY WITH AL07118.**
Report Nº TR 142:38520:1096 Vol. 1.37

This study evaluated the (AL07118) of AL04862 in the mutation assay

*E.coli* tester strain were used in the study. See the above study for the purity of AL07118. conducted the study under GLPs.

The results did not show a positive increase in the number of revertants per plate with any of the strains.

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**STUDY 30.**
***IN VIVO* MICRONUCLEUS ASSAY WITH AL07118.**
Report Nº TR-141:38520:1096 Vol. 1.37

AL07118 was evaluated with a single dose at 0, 450, 900, and 1800 mg/Kg in the micronucleus assay in nine week old mice (5/sex). Suspensions in were administered orally by gavage at a maximum possible concentration of 72 mg/mL. Water was used as the negative control - positive control. Bone marrow was evaluated at 24, 48, and 72 hours after dosing. The purity of AL07118 (lot Nº 6312-10A) was identical to that used in the above study. . conducted the study under GLPs.

The results indicated AL07118 did not significantly increase the number of micronuclei in bone marrow At 48 hours, however, females dosed at 900 mg/Kg showed a statistical increase (*, twice control) in the mean % . This result was within the historic control and can be considered an anomalous value, as no other dose was significant and the control value was unusually low at 48 hours.

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## PHARMACOKINETICS - DRUG METABOLISM

**STUDY 1.**
**DISTRIBUTION OF RADIOACTIVITY IN OCULAR TISSUES FOLLOWING A SINGLE TOPICAL OCULAR DOSE OF 1 % $^{14}$C-AL04862 OPHTHALMIC SUSPENSION TO MALE NEW ZEALAND WHITE RABBITS.**
Report Nº 011:38570:0496 Vol. 1.41

Ocular tissue uptake of $^{14}$C following a single topical ocular dose of 1 % $^{14}$C-AL04862 suspension was evaluated in this study. Alcon conducted the study. PK data is indicated in the following table.

| TISSUE | Cmax (µg equivalents/g) | Tmax | $t_{1/2}$ |
|---|---|---|---|
| Aqueous Humor | 0.259 ± 0.065 | 2 hr | 3.11 hr |
| Cornea | 6.40 ± 2.36 | 0.5 hr | 5.21 hr* |
| Iris-ciliary Body | 0.654 ± 0.216 | 1 hr | 33.6 days |
| Lens | 0.0976 ± 0.0117 | 20 days | 294 days |
| Conjunctiva | 16.3 ± 6.8 | 0.5 hr | ND |
| Vitreous Humor | < 0.0012 | 2 hr | ND |
| Retina | 0.330 ± 0.043 | 36 days | 95.9 days |
| Choroid | 0.297 ± 0.079 | 0.5 hr | 74.4 days |
| Blood | 1.07 ± 0.127 | 1 day | 38.9 days |
| Plasma | < 0.0007 | 1 hr | ND |

* Not interpreted as a terminal half-life. ND = not determined because of limited data

Elimination of radioactivity from tissues containing carbonic anhydrase, such as the eye (lens, cornea,

choroid, retina, and iris-ciliary body) and blood, was slow. The highest concentrations were found in the conjunctiva and cornea, then in decreasing order in the iris-ciliary body, retina, choroid, aqueous humor, lens, and vitreous humor. Mean concentrations of radioactivity in the lens and iris-ciliary body in the non-dosed eyes were due to systemic circulation and were similar to the dosed eyes.

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**STUDY 2.**
**DISTRIBUTION OF RADIOACTIVITY IN OCULAR TISSUES FOLLOWING A SINGLE TOPICAL OCULAR DOSE OF 1% $^{14}$C-AL04862 OPHTHALMIC SUSPENSION TO MALE DUTCH BELTED RABBITS.**
Report Nº 012:38570:0496 Vol. 1.41

This study followed the radioactivity distribution in blood and eyes of Dutch belted rabbits following a single 30 µL topical ocular dose (300 µg of $^{14}$C-AL04862, 11.0 µCi) to the right eye. In a Phase 1 study, four animals were sacrificed at 1, 2, 4, 8, 48, 72, 144, 240, and 384 hours. A Phase 2 study monitored concentrations up to 57 days. The results are compared with the NZW strain in the following table.

**PK Parameters of Radioactivity after Topical Administration of 1% $^{14}$C-AL04862 to Dutch Belted and New Zealand White Rabbits**
(From Vol. 1.41, p. 5C-0188)

| | Cmax (µg equivalents/g) | | Tmax | | $t_{1/2}$ | |
|---|---|---|---|---|---|---|
| TISSUE | Dutch Belted | NZW* | Dutch Belted | NZW | Dutch Belted | NZW |
| Aqueous Humor | 0.670±0.475 | 0.259±0.065 | 1 hr | 2 hr | 3.75 hr | 3.11 hr |
| Lens | 0.0641±0.0152 | 0.0976±0.0117 | 13 days | 20 days | 162 days | 294 days |
| ICB | 3.85±2.33 | 0.654±0.216 | 4 hr | 1 hr | 37.5 days | 33.6 days |
| Retina | 0.338±0.024 | 0.330±0.043 | 20 days | 36 days | NA | 95.9 days |
| Choroid | 0.651±0.260 | 0.297±0.079 | 4 hr | 0.5 hr | 50.4 days | 74.4 days |
| Blood | 1.36 to 1.63 | 0.988 to 1.07 | 1 - 6 days | 1 - 6 days | 32 days | 38.9 days |

* From Alcon technical Report 011:38570:0496
ICB = iris-ciliary body
NA - unable to calculate terminal half-life from available data

As in the above study (Report Nº 011:38570:0496), concentrations of radioactivity in the retina and choroid of dosed and untreated eyes were similar due to systemic circulation. Of note were the lower concentrations of $^{14}$C in blood, choroid, retina, ICB, and aqueous humor of NZW rabbits than were found in the pigmented Dutch Belted rabbits. Half-lives were somewhat longer in the NZW rabbits.

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**STUDY 3.**
**COMPARISON OF AL04862 SUSPENSION OCULAR BIOAVAILABILITY IN DIFFERENT FORMULATIONS; EFFECTS OF TIME, CONCENTRATION, AND VISCOSITY.**
Study Nº 030:38570:1292 Vol. 1.41

This study looked at several suspension concentrations of AL04862, the effect of time, and formulation viscosity on the ocular bioavailability of AL04862 in the aqueous humor and ICB following a single topical ocular dose of 30 µL on the cornea to both eyes.

The results of did not significantly affect the ocular bioavailability, and increasing the concentration from 0.5% to 2% in one suspension study or changing the viscosity of a 1% suspension did not increase the ocular bioavailability of AL04862. A 0.5% suspension of the formulation did not significantly improve the bioavailability of AL04862, but gave similar results in absorption as the formulation. When 0.05% dodecyl maltoside (DDM) was added to increasing corneal penetration, no significant increase in AUC was observed.

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STUDY 4.
**RELATIVE OCULAR BIOAVAILABILITY OF AL04862 IN THE RABBIT FOLLOWING TOPICAL OCULAR ADMINISTRATION OF 2.0% OPHTHALMIC SUSPENSIONS.**
Report Nº 008:39800:0592 Vol. 1.41

Ophthalmic suspensions of 2% AL04862 were compared for their effect on ocular bioavailability. Concentrations in aqueous humor, ICB, and blood were determined using a system. The study was carried out in NZW rabbits by Alcon.

No significant statistical differences were reported with the various suspensions. Plasma levels of AL04862 could not be determined, as they were below quantitation limits of Whole blood concentrations were 3-5 µg/mL at 6 hours following treatment.

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STUDY 5.
**OCULAR BIOAVAILABILITY OF 2% AL04862 FORMULATED IN OPHTHALMIC SUSPENSIONS CONTAINING EITHER CARBOMER**
Report Nº 001:38570:0193 Vol. 1.41

The ocular bioavailability of a 2% AL04862 ophthalmic suspension containing either carbomer or carbomer was investigated following a single topical ocular dose of 30 µL to the cornea of each eye of NZW rabbits. Concentrations in aqueous humor and ICB were measured using No significant difference was seen between the two formulations.

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STUDY 6.
**AL04862 PHARMACOKINETICS FOLLOWING A SINGLE TOPICAL OCULAR DOSE IN NZW RABBITS.**
Report Nº 014:38570:0493 Vol. 1.41

Whole blood samples were collected from New Zealand Albino rabbits for 18 weeks following treatment with a single topical ocular dose of 4% AL04862 (30 µL) in suspension. Maximum mean AL04862 concentrations of 4.15 µg/mL were reached in three rabbits (3.90, 4.24, and 4.30 µg/mL) at 3 hr, 24 hr, 24 hr, respectively, and at 1, 2, 3, 4, 6, 8, 10, 12, 15, 17, and 18 weeks. At 18 weeks, whole blood concentrations were measurable for the three rabbits. Whole blood PK data are indicated in the following table.

**WHOLE BLOOD AL04862 PHARMACOKINETIC PARAMETERS**
(from Table 2, Vol.1.41, p.5C-0338)

| PARAMETER | RABBIT NUMBER | | | MEAN ± SD |
|---|---|---|---|---|
| | 35802 | 35803 | 35804 | |
| $C_{max}$ (µg/mL) | 4.24 | 3.90 | 4.30 | 4.15 ± 0.22 |
| $T_{max}$ (hr) | 3 | 24 | 24 | 17 ± 12 |
| AUC (µg·hr/mL) | 3584 | 2534 | 4347 | 3488 ± 910 |
| $T_{½}$ (hr) | 1125 | 1018 | 1397 | 1180 ± 195 |

At 18 weeks the whole blood concentrations were 0.258, 0.151, and 0.335 µg/mL for rabbit 35802, 35803, and 35804, respectively, giving a mean concentration of 0.248 ± 0.092. The mean terminal half-life for AL04862 in whole blood was 49 ± 8 days.

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**STUDY 7.**
**SYSTEMIC PHARMACOKINETICS OF AL04862 FOLLOWING A SINGLE TOPICAL OPHTHALMIC DOSE OR A SINGLE INTRAVENOUS DOSE IN THE RABBIT.**
Report Nº 012:39800:0692 Vol. 1.41

PK data were determined in NZW rabbits following iv doses of 5 and 30 mg/Kg and topical ocular dosing with 30 µL of 1% suspension to both eyes. Blood samples were collected up to 96 hours in the iv treated rabbits and up to 72 hours in the ocular treated rabbits.

**MEAN PLASMA AND WHOLE BLOOD PK PARAMETERS**
(Tables 1 and 2, pp. 5C-0354 and 5C-0355)

| Route | Dose (mg/Kg) | Cmax (µg/mL) | Tmax[‡] (hr) | $t_{½}$ (hr) | $AUC_{0-\infty}$ (µg·hr/mL) | Cl (mL/min/Kg) | Vss (L/Kg) |
|---|---|---|---|---|---|---|---|
| **Mean Plasma Pharmacokinetic Parameters** | | | | | | | |
| Topical | 0.3 mg/eye | BQL | NA | NA | BQL | NA | NA |
| IV | 5 | 4.05 ± 0.88 | 0.083 | 1.1 ± 0.1* | 2.84 ± 0.76 | 30.9 ± 9.1 | 1.50 ± 0.12 |
| IV | 30 | 29.6 ± 6.2 | 0.083 | 0.98 ± 0.02* | 38.6 ± 2.8 | 13.0 ± 1.0 | 0.93 ± 0.14 |
| **Mean Whole Blood Pharmacokinetic Parameters** | | | | | | | |
| Topical | 0.3 mg/eye | 2.05 ± 0.23 | 3 - 24 | 132 ± 42 | 385 ± 41 | NA | NA |
| IV | 5 | 15.4 ± 2.7 | 0.083 | 99 ± 13 | 797 ± 118 | NA | NA |
| IV | 30 | 40.5 ± 2.6 | 0.083 | 106 ± 23 | 862 ± 180 | NA | NA |

‡ first sampling point for IV
* not considered an accurate assessment of the elimination half-life
BQL = below quantifiable limits
NA not applicable

Plasma levels were not quantifiable six hours following the iv administration.

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STUDY 8.
ORAL BIOAVAILABILITY AND DOSE PROPORTIONALITY OF AL04862 IN THE RAT.
Report Nº 011:39800:0692 Vol. 1.41

PK data for AL04862 were determined in male D rats (3/group) following a single iv bolus injection of 5 mg/Kg and single oral doses of 20, 60, and 180 mg/Kg AL04862. A jugular vein cannula was inserted in the rats 24 hours prior to dosing. IV drug administration was through the cannula; the oral dose was by gavage. Blood samples (0.1 mL) were withdrawn at 5, 15, 39, and 45 minutes, and 10 times over the period 1 - 144 hr.

**MEAN PLASMA AND WHOLE BLOOD PK PARAMETERS**
(Tables 1 and 2, pp. 5C-0391 and 5C-0392)

| Route | Dose (mg/Kg) | Cmax (µg/mL) | Tmax (hr) | t½ (hr) | $AUC_{\infty}$ (µg·hr/mL) | Cl (mL/min/Kg) | F(%) |
|---|---|---|---|---|---|---|---|
| **Mean Plasma Pharmacokinetic Parameters** | | | | | | | |
| iv | 5 | 1.14 ± 0.19 | 0.083 | 0.63 ± 0.06[a] | 0.49 ± 0.05 | 171 ± 17[**] | NA |
| po | 20 | 0.50 ± 0.10 | 0.5 | 6.37 ± 3.07[a] | 1.39 ± 0.25[a] | NA | 70 ± 13 |
| po | 60 | 1.61 ± 0.63 | 0.25 | 20.0 ± 5.5[a] | 3.67 ± 1.46[a] | NA | 61 ± 25 |
| po | 180 | 2.57 ± 1.63 | 0.25 | 36.7 ± 9.3 | 8.20 ± 4.35[a] | NA | 45 ± 24 |
| **Mean Whole Blood Pharmacokinetic Parameters** | | | | | | | |
| iv | 5 | 12.59 ± 3.62 | 0.083[b] | 140 ± 33 | 1247 ± 281 | NA | NA |
| po | 20 | 15.17 ± 1.24 | 0.25 | 137 ± 22 | 1249 ± 78 | NA | NA |
| po | 60 | 14.63 ± 0.67 | 0.25 | 196[c] | 1303 ± 90 | NA | NA |
| po | 180 | 15.18 ± 3.90 | 0.25 | 160 ± 18 | 1423 ± 77 | NA | NA |

[a] not considered to be elimination half-life because plasma concentrations could not be detected at later time points.
[b] $AUC_{0-8}$ [c] first sampling point [d] n=2
[**] may be overestimated due to detection only to 2 hr and saturable red cell binding

Linear dose proportionality occurred up to 60 mg/Kg in the plasma plot of AUC vs dose.

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STUDY 9.
**METABOLITE PROFILES AND IDENTIFICATION OF MAJOR METABOLITES IN WHOLE BLOOD AND URINE FOLLOWING 5 MG/Kg ORAL AND IV DOSES OF $^{14}$C-AL04862 TO MALE SPRAGUE-DAWLEY RATS.**
Report Nº 029:38570:0695 Vol. 1.41

Metabolites were determined following iv and oral gavage treatment of male Sprague Dawley rats with 5 mg/Kg $^{14}$C-AL04862. The following radioactive products were detected.

- AL04862 parent compound
- AL04930 O-desmethyl-AL04862
- AL06339 N-desethyl-AL04862
- AL05859 N-desmethoxypropyl-AL04862

Urinary excretion recovered 27% of the radioactivity over 48 hours, with a radioactive profile almost identical for the oral and iv administration. Cage washings recovered about 4% from both dosing routes

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**STUDY 10.**
**ENTEROHEPATIC CIRCULATION OF RADIOACTIVITY AND BILIARY METABOLITE PROFILES FOLLOWING ORAL ADMINISTRATION OF $^{14}$C-AL04862 TO MALE FISCHER 344 RATS.**
Study Nº TR 017:38570:0496 Vol. 1.42

This study looked at the enterohepatic circulation of $^{14}$C- AL04862. The dosing formulation was prepared as a solution . The specific activity was 79 µCi/mg (44.2 µCi/mL Six male bile duct-cannulated rats were dosed orally with approximately 1.25 mg/Kg (25.4 µCi/rat) of drug (Group 1). A second group of four bile duct-cannulated rats received labeled bile obtained from Group 1 that was infused into the duodenum (Group 2). Bile, urine, feces, blood, and plasma were evaluated at 96 hours. Cage rinses were collected for counting. Biliary metabolites were also determined.

Results from the excretion data indicated 96 % of the dose was absorbed. In Group 1, recovery was 20.1% in the bile, 20.6% in urine, 3.9% in feces, 0.9% from cage rinses, with an estimated total amount of 47.1% in blood. The mean amount of radioactivity in plasma at 96 hours post dose was 0.007%. indicated at least 6 radioactive peaks. The major metabolite, also the major urinary metabolite, was the N-propionic analog (62%-81%). N-desmethoxypropyl metabolite was about 9%.

In Group 2, enterohepatic circulation accounted for more than 20% of the radioactivity excreted in the bile and about 1% of it contributed to the amount in blood. The study was conducted by Alcon Laboratories, Inc.

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**STUDY 11.**
**TISSUE DISTRIBUTION OF RADOACTIVITY FOLLOWING A SINGLE AND MULTIPLE ORAL ADMINISTRATIONS OF $^{14}$C-AL04862 TO MALE RATS.**
Study Nº TR 013:38570:0496 Vol. 1.42

This study was done at the The distribution of 4-$^{14}$C labeled AL04862 was determined in blood, plasma, and in 25 different tissues. The pharmacokinetic parameters for radioactivity in blood and plasma are presented in the following table.

**MEAN PHARMACOKINETIC PARAMETERS IN MALE RATS**
(From Vol. 1.42, p. 5C-0525)

| Parameters | Single Dose (1 mg/Kg) | | Multiple Dose (1 mg/Kg/dayx21 days) | |
|---|---|---|---|---|
| | Plasma | Blood | Plasma | Blood |
| $C_{max}$ (µg equivalents/g | 0.0520 | 10.6 | 0.154 | 19.2 |
| $T_{max}$ (days) | 0.25 | 0.25 | 0.0208 | 0.0208 |
| $T_{\frac{1}{2}}$ (days) | 0.3[a] | 19.8 | 0.3[a] | 19.0 |
| $AUC_{(0-\infty)}$ (µg equiv·day/g) | 0.691[b] | 209 | 1.08[b] | 290 |

[a] Estimated initial half-life. [b] Likely an overestimate of the $AUC_{0-\infty}$ due to hemolysis in plasma samples.

The tissues having the highest level of radioactivity were the liver, stomach, small intestine, kidneys, spleen, lungs, and salivary glands, with half-lives ranging from days. High levels of carbonic anhydrase have been determined in the kidney, eyes (retina and lens), stomach (parietal cell), salivary glands, prostate, and colon.

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**STUDY 12.**
***IN VITRO* PROTEIN BINDING OF $^{14}$C-AL04862 IN HUMAN, RAT, AND MONKEY PLASMA.**
Study Nº TR 030:38570:0596 Vol. 1.42

Protein binding determined by ultrafiltration was determined using 4-$^{14}$C-AL04862 of specific activity 79 µCi/mg over a concentration range of 0.01 to 10.0 µg/g. A summary table is presented below for the *in vitro* to plasma from humans, rats, and monkeys. The study was done by Alcon Laboratories.

**Plasma Protein Binding of $^{14}$C-AL04862**
(From Vol. 1.42, p. 5C-0780)

| Mean Percent Bound (n=5) | | | | |
|---|---|---|---|---|
| Species | 0.01 µg/g | 0.1 µg/g | 1.0 µg/g | 10 µg/g |
| Human | 62.7±1.6 | 59.1±1.4 | 58.5±0.2 | 58.8±1.4 |
| Monkey | 95.9±0.9 | 79.9±0.3 | 75.4±0.2 | 74.8±0.3 |
| Rat | 40.3±0.9 | 28.5±1.1 | 24.3±0.2 | 23.8±0.4 |

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**STUDY 13.**
**WHOLE BODY AUTORADIOGRAPHY OF RADIOACTIVITY FOLLOWING A SINGLE ORAL ADMINISTRATION OF $^{14}$C-AL04862 TO MALE RATS.**
Study Nº TR 029:38570:0596 Vol. 1.42

A single oral administration of 1 mg/kg $^{14}$C-AL04862 to male CDF® (F-344) rats indicated that tissue concentrations peaked around 24 hours in most of the tissues that were looked at. The highest levels were found in the blood, the cecal mucosa, followed by the adrenal, pituitary, thyroid, salivary glands, stomach mucosa, intestinal content, bulbo-urethral gland, bone marrow, hair follicles, kidney, liver, lung, and spleen. Very low levels were found in the brain, pineal body, spinal cord, thymus, Harderian gland, lachrymal glands, epididymis, prostate, seminal vesicles, testis, muscle, lymph nodes, brown fat, eyes, pancreas, skin, esophageal mucosa, and the intestines. The study was carried out by

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**STUDY 14.**
**TISSUE DISTRIBUTION AND FETAL TRANSFER OF RADIOACTIVITY IN PREGNANT FEMALE RATS FOLLOWING A SINGLE ORAL DOSE OF $^{14}$C-AL04862.**
Study Nº TR 015:38570:0496 Vol. 1.43

The distribution of radioactivity in 3 groups of pregnant F344 rats and in fetuses following a single 1 mg/Kg oral dose of $^{14}$C-AL04862 was investigated in this study. Pregnancies were timed so that radioactivity could be evaluated D12 and D18 in fetuses. Blood and fetal tissues were collected and analyzed for radioactivity. The third group of animals were sacrificed at 0.5, 2, 6, and 24 hours postdose for whole body autoradiography.

The results indicated that radioactive transfer to fetuses was greater on D12 of gestation than on D18. There was also a high concentration of radioactivity remaining in the blood at 24 hours postdose.
indicated radioactivity was rapidly absorbed and distributed into tissues, with peak tissue

levels occurring in fetuses at 6 hours after dosing. The highest concentrations were in the kidney, lung, liver, heart, and placenta. The maximum concentrations seen in the placenta and amniotic fluid were 0.637 and 0.331 µg equivalents/g, respectively.

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**STUDY 15.**
**EXCRETION AND MASS BALANCE OF $^{14}$C-AL04862 FOLLOWING INTRAVENOUS ADMINISTRATION TO MALE RATS.**
Study Nº TR 014:38570:0496 Vol. 1.43

The excretion of radioactivity in the urine, feces, and expired air was determined following a single dose of 1 mg/Kg $^{14}$C-AL04862 administered by iv to male CDF® (F-344) rats. A second group of rats were evaluated for radioactivity in blood, kidneys, lungs, liver, and carcass at D1, D7, D14, D21, and D28 following dosing. The study was conducted by

The mean percent of the dose excreted was 32.1% in urine, 29.2% in feces, 13.5% in the carcass, and 2.8% in the cage washing. Radioactivity was not detected in expired air. The amount of radioactivity remaining in the blood, kidneys, liver, and lungs at D28 was 2.83, 1.23, 0.233, and 0.392 µg equivalents of $^{14}$C-AL04862. Radioactivity in blood, kidneys, liver, and lungs declined with approximately a 20 day half-life, as the elimination graph indicated similar slopes for the four tissues.

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**STUDY 16.**
**LACTAL SECRETION OF RADIOACTIVITY FOLLOWING A SINGLE ORAL DOSE OF $^{14}$C-AL04862 TO FEMALE RATS (CHW-6208-114).**
Study Nº TR 016:38570:0496 Vol. 1.43

The excretion of radioactivity from $^{14}$C-AL04862 into the milk of lactating CDF® rats was determined 12 days following parturition. Females were dosed approximately D12 postpartum with a mean dose of 1.16 mg/Kg $^{14}$C-AL04862.

Mean concentrations at 0.5, 2, 6, and 24 hours following dosing were 0.002, 0.005, 0.018, and 0.026 µg equivalents/g, respectively. At 6 hours, blood concentration (12 µg equivalents/g) and plasma concentration (0.149 µg equivalents/g) were maximum. Radioactivity is retained in the carbonic anhydrase of the red blood cells.

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**STUDY 17.**
**LACK OF EVIDENCE FOR *IN VIVO* CHIRAL INVERSION OF AL04862 IN TATS, CYNOMOLGUS MONKEYS, AND HUMANS.**
Study Nº TR 025:38570:0596 Vol. 1.43

The inversion of AL04862 to AL04862 was investigate with a method. Earlier studies indicated inversion can occur at elevated temperatures *in vitro*, and *in vivo* conversion could be possible with its long retention in blood. Whole blood from humans, rats, and monkeys and urine from rats was analyzed. The detection limit was in blood and in rat urine. Blood samples were from the 6-Month Oral Rat Toxicity Study, the one year topical ocular monkey study, and from the human oral Safety/PK Study C-95-76 and collected after parent drug steady state.

No detectable concentrations of the S-AL04862 (AL07118) were found in any of the urine or blood samples treated with AL04862

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**STUDY 18.**

**PROFILES OF RADIOACTIVITY IN BLOOD FOLLOWING ADMINISTRATION OF SINGLE AND MULTIPLE ORAL DOSES OF $^{14}$C-AL04862 TO MALE RATS..**

Study Nº TR 027:38570:0596 Vol. 1.43

Tissue distribution and blood metabolites were determined in rats following single or multiple oral dosed of 1 mg/Kg/day of AL04862 for 12, 14, 17, and 21 days. Blood samples were collected at 0.5 and 24 hours after the single dose and at 24 hours after multiple doses. The following metabolites were identified:

| Metabolite | Steady-state Concentration (12 Days) |
|---|---|
| • N-desmethoxypropyl (AL05859) | µg equivalents/g |
| • O-desmethyl (AL04930) | ug equivalents/g |
| • N-desethyl (AL08520) | µg equivalents/g |

The parent drug was the major radioactive component at all times. Trace levels of two unidentified metabolites were seen that were more polar than AL04862. Steady state concentrations for all radioactive compounds were reached after 12 days.

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**STUDY 19.**

**AL04862 PARENT DRUG AND METABOLITE WHOLE BLOOD AND PLASMA CONCENTRATIONS FROM TOXICOLOGY STUDY N-94-57, "ONE YEAR CHRONIC TOPICAL OCULAR IRRITATION AND SYSTEMIC TOXICITY EVALUATION OF AL04862 OPHTHALMIC SUSPENSION IN PRIMATES."**

Report Nº 031:38570:0696 Vol. 1.44

This study determined the concentration of AL04862 and its metabolites in whole blood and plasma during the one year topical ocular toxicity study in Cynomolgus monkeys. The study contained 4/sex/group in the following five groups: G1 (untreated control), G2 (vehicle control), G3 (1.0%), G4 (2.0%), G5 (4.0%) - dosage was tid. Blood was collected on Days 1, 90, 180, and 359. The quantitative limits for determining each analyte were , in whole blood and in plasma. A validated method was used to determine the analytes.

Parent drug, AL04930, AL05859, and AL08520 were detected in whole blood. The concentrations of AL05859, the N-desmethoxypropyl metabolite, were below , in all samples. Systemic exposure was observed, however, for parent drug and the two metabolites, AL04930 and AL08520 at all dose levels. At Day 1 the trough whole blood samples were below the quantitation limits. Statistically significant sex differences were observed at times for AL04862 and the two metabolites at the 1.0% dose only. Steady state was reached for parent and the two metabolites by Day 90 in whole blood. Parent drug and the two metabolites were quantifiable in plasma, but values varied due to hemolysis, which resulted in higher concentrations of analytes.

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**STUDY 20.**

**PLASMA AND WHOLE BLOOD CONCENTRATIONS OF AL04862 FROM THREE MONTH TOPICAL OCULAR IRRITATION AND SYSTEMIC TOXICITY EVALUATION IN RABBITS.**

Report Nº 015:38570:0692 Vol. 1.44

Whole blood and plasma were analyzed for AL04862 concentrations. The study contained four groups

of 7/sex/group - G1 (untreated control), G2 (vehicle), G3 (2.0% AL04862), and G4 (4.0% AL04862). Dosing was topical qid in one eye. Blood samples were collected Day 32 (6 samples) and Day 88 (8 samples). Results are indicated in the following table. Below quantitation limits (BQL) were for whole blood and for plasma.

**Mean AL04862 Concentrations (µg/mL ± SD) in Whole Blood and Plasma**

| WHOLE BLOOD | | |
|---|---|---|
| **GROUP** | **DAY 32** | **DAY 88** |
| G1 Untreated | 0.60 ± 0.12 (n=6) | 0.47 ± 0.10 (n=7) |
| G2 Vehicle | 0.76 ± 0.74 (n=6) | 0.32 ± 0.04 (n=8) |
| G3 2.0% AL04862 | 5.77 ± 0.54 (n=6) | 6.53 ± 0.46 (n=8) |
| G4 4.0% AL04862 | 6.12 ± 0.60 (n=6) | 7.17 ± 0.39 (n=7) |
| **PLASMA** | | |
| G1 Untreated | BQL (n=6) | ND (n=7) |
| G2 Vehicle | BQL (n=6) | BQL (n=8) |
| G3 2.0% AL04862 | BQL (n=5) | BQL (n=7) |
| G4 4.0% AL04862 | BQL (n=6) | 0.063 ± 0.018 (n=6) |

ND = not detected

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STUDY 21.
**PLASMA AND WHOLE BLOOD CONCENTRATIONS FROM SIX MONTH TOPICAL OCULAR IRRITATION AND SYSTEMIC TOXICITY EVALUATION OF AL04862 IN RABBITS.**
Report 026:38570:0693 Vol. 1.44

Blood and plasma AL04862 concentrations were determined in rabbits dosed qid at 8:30 am, 10:30 am, 1:00 pm, and 3:30 pm with 2.0% and 4.0% AL04862. Blood was collected after 1 and 2 weeks, and at 1, 3, and 6 months from the drug treated groups. Blood from the vehicle and untreated groups was collected after 1, 3, and 6 months.

Dose proportionality was not observed in the drug treated groups. Steady state occurred by one week. Plasma levels were below the quantitation limit of in all samples.

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STUDY 22.
**WHOLE BLOOD AND PLASMA AL04862 AND AL07118 CONCENTRATIONS FROM TOXICOLOGY STUDY N-96-099: "ONE-MONTH TOPICAL OCULAR IRRITATION EVALUATION OF AL07118 IN AN OPHTHALMIC SUSPENSION IN RABBITS."**
Report Nº 037:38570:0796 Vol. 1.44

Blood and plasma were analyzed Day 30 for AL04862 and AL07118, the S-enantiomer of AL04862. Rabbits were treated with 0.2% AL07118 or 0.2/0.8% AL07118/AL04862 suspensions. Dosing was with one drop (≈ 30 µL) tid of the test article in each eye. A method was used for the qualitative assay.

Systemic exposure occurred for the S-enantiomer dosed alone and for AL04862 in the mixture. No AL04862 was detected ( detection limit - in those rabbits treated with the S-enantiomer, It was demonstrated earlier that AL04862 does not interconvert to the S-enantiomer in rats, monkeys, or humans. Of interest is the finding that no S-enantiomer was seen in the rabbits treated with the 0.2/0.8% S/R mixture. It was suggested this could be due to displacement of the S-enantiomer from the carbonic anhydrase due to saturable competitive binding of the two enantiomers, or to higher clearance and/or lower volume of distribution of S relative to R.

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STUDY 23.
**PLASMA AND WHOLE BLOOD CONCENTRATIONS FROM TWO WEEK ORAL TOXICITY EVALUATION OF AL04862 IN RATS.**
Report Nº 014:38570:0692 Vol. 1.44

Plasma and blood concentrations were determined in rats administered single daily oral doses of 0, 20, 60, or 180 mg/Kg/day of AL04862 for 16 days. Blood was collected Day 15 in males and Day 16 in females 18 hours after the final dose.

The results indicated systemic exposure occurred but did not show dose proportionality. Concentrations in plasma were at least 10 to 100x lower than in blood. What was unusual about the data was the finding of a peak in the control groups that had the same retention time as AL04862. Blood and plasma concentrations may be found under Study 10, page 35

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.STUDY 24.
**WHOLE BLOOD CONCENTRATIONS AL04862 AND METABOLITES AL04930, AL05859, AND AL08520 FROM STUDY N-95-77 "SIX-MONTH ORAL (GAVAGE) TOXICITY STUDY OF AL04862 IN RATS."**
Report Nº 004:38570:0296 Vol. 1.44

The analysis of blood indicated systemic exposure to AL04862, AL05859, AL04930, and AL08520, with blood concentrations of AL04862 at , in both sexes and in the three drug treated groups. AL04930 blood concentrations were around , in all groups. AL05859 concentrations varied and AL08520 concentrations were at (

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**LABELING:**

1) Under Carcinogenesis, Mutagenesis, Impairment of Fertility, the following paragraphs should be removed, as they do not apply under this section.

2) The paragraph relating to the mutagenic studies should be changed to read:

The following tests for mutagenic potential were negative: (1) *in vivo* mouse micronucleus assay: (2) *in vivo* sister chromatid exchange assay; (3) Ames *E. coli* assay. The *in vitro* mouse lymphoma forward mutation assay was negative in the absence of activation but positive in the presence of microsomal activation, with high to very high toxicity.

3) Under Teratogenic Effects. Pregnancy Category B:

The pregnancy category should be changed to C with the following paragraph: Developmental toxicity studies with Brinzolamide in rabbits at oral doses of 1, 3, and 6 mg/Kg/day (125 times the recommended human ophthalmic dose) produced maternal toxicity at 6 mg/Kg/day and a significant increase in the number of fetal variations as accessory skull bones which was only slightly higher than the historic value at 1 and 6 mg/Kg. In rats, statistically decreased body weights of fetuses from dams receiving oral doses of 18 mg/Kg/day (375 times the recommended human ophthalmic dose) during gestation were proportional to the reduced maternal weight gain, with no statistically significant effects on organ or tissue development. Increases in unossified sternebra, reduced ossification of the skull, and unossified hyoid that occurred at 6 and 18 mg/Kg were not statistically significant. No treatment-related malformations were seen. Following oral administration of $^{14}$C-brinzolamide to pregnant rats, radioactivity was found to cross the placenta and was present in the fetal tissues and blood. There are no adequate and well-controlled studies in pregnant women. AZOPT™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

SUMMARY AND EVALUATION:

AZOPT (brinzolamide) Ophthalmic Suspension 1% was developed as a carbonic anhydrase II (CA II) inhibitor in the treatment of IOP in patients with ocular hypertension or open-angle glaucoma. Carbonic anhydrase II is present in tubular renal cells, GI epithelia, erythrocytes, pancreas, eyes, and other tissues. The animal studies showed that Brinzolamide decreased the secretion of aqueous humor and produced decreases in IOP. The advantage of using a topical ocular drug for controlling IOP by inhibiting CA and reducing aqueous humor production is evident. Acetazolamide (Diamox), dichlorphenamide (Daranide), and methazolamide (Neptazane) are approved oral CA inhibitors for lowering IOP; however, these systemic drugs have adverse side effects.

General Pharmacology of AL04862:

Oral administration of 0.3, 1, or 5 mg/Kg AL04862 to male rats produced significant changes in urine volume, increases in $Na^+$ and $K^+$ concentrations, and decreases in $Cl^-$ concentration at 1 and 5 mg/Kg, as would be expected for a CA inhibitor. No changes were seen at 0.3 mg/Kg. These parameters were also changed significantly in this study with 5 mg/Kg acetazolamide. No signs of neuropharmacological or potential anticonvulsant activity were observed in rats 24 hours after iv administration of 1, 10, or 30 mg/Kg AL04862, and mice administered 1, 10, or 30 mg/Kg AL04862 by gavage showed no change in rotarod performance at 30 or 60 minutes after dosing. The GI propulsion time of a charcoal suspension was decreased significantly in mice with 30 mg/Kg AL04862 gavage. Pentobarbital sodium sleep time was prolonged in mice with 1, 10, or 30 mg/Kg AL04862, but the results were not dose related and not biologically significant. IV infused 1 or 10 mg/Kg AL04862 to anesthetized dogs produced biologically significant increases in cardiac output,+dP/dt, contractile force, and circulatory parameters at 10 mg/Kg but no changes in EKG lead II. No significant

changes occurred in blood $pCO_2$, $pO_2$, or pH in dogs or in conscious rats dosed with AL04862 at 0.3, 1, or 3 mg/Kg. AL04862 was negative in the sensitization assay.

Effect of AL04862 on IOP:

Significant ($p<0.05$) reduction in the IOP of treated eyes occurred in rabbits following 1 mg (25 µL) instillation. The reduction was significant up to 6 hours. IOP was also significantly ($p<0.001$) reduced over 6 hours in monkeys treated with 0.6 mg AL04862 (30 µL) instilled in eyes made hypertensive with and in rabbits treated with a single topical ocular instillation of 1 mg. In addition to these studies, other studies were carried out in monkeys or rabbits which evaluated the effect of the vehicle, the formulation, viscosity effects, drop size, and multiple administrations on the IOP response.

Topical Ocular Toxicity Studies With AL04862:

One day, three-month, and six month studies were carried out in New Zealand rabbits to evaluate the topical ocular toxicity of Al04862 suspensions. The longest study for this purpose was a one year chronic topical ocular irritation and systemic toxicity evaluation of AL04862 in monkeys.

In the one day study, 2% AL04862 was administered every 30 minutes for a total of ten doses. Only minimal to moderate conjunctival irritation was reported with the formulation. The three month study looked at 2% and 4% suspensions. Significant ($p<0.05$) changes in BUN (elevated), platelets (decreased), and RBC (elevated) were seen in both drug groups. Pachymetry measurements indicated an increase in corneal thickness. Only minimal ocular irritation was produced in the study. Plasma levels of the drug were at 0.063 µg/mL.

In the 6-month study (2% and 4% suspensions q.i.d. in OD), significant changes were seen in MCHC, BUN, creatine, globulin, A/G ratio, CPK, P, and K. There were no significant changes in conjunctival congestion, swelling, discharge, corneal cloudiness, fluorescein staining, lens changes, or in neovascularization. The optic nerve head and retinal and choroidal vessels were within normal limits. Pachymetry measurements were significantly larger in the vehicle and drug treated eyes when compared to the untreated controls. Relative spleen weight was significantly elevated in the 4% drug group. Whole blood levels were similar in the drug groups and did not vary significantly from week one to the 6-month measurement. Plasma levels were below the quantitation limit of

Suspensions of 1%, 2%, and 4% were administered t.i.d. in the one year monkey ocular irritation and systemic toxicity study. The biomicroscopic and indirect ophthalmic examinations were within normal limits. Corneal pachymetry results showed no significant difference between vehicle and drug treated eyes; the thickness was increased in the vehicle control as well as in the treated groups during the study. Significant changes were seen in the following clinical chemistry and hematology parameters: increase in BUN, BUN/creatinine ratio, sodium, lymphocytes, decreases in total protein, hematocrit, WBC, and hemoglobin. Systemic blood exposures were obtained for AL04862, and the O- and N-desmethyl metabolites. By Day 90, blood steady state exposure was obtained for AL04862 and the two metabolites. Blood concentrations of AL04862 varied between for all drug groups over Day 90 to Day 359. No lesions were considered by the pathologist to be associated with the test article, nor did any lesions appear to be dose-related; however, there were numerous lesions that appeared in the drug treated groups that were not seen in the vehicle control and may be related to the treatment.

In a one month topical ocular irritation evaluation of a 0.2% suspension of the S-enantiomer of AL04862, the ocular discharge that occurred was no greater than that produced by a mixture of 0.2%R- and 0.8% S-enantiomer. Using no interconversion of the S- to the R-enantiomer was detectable in rabbits dosed with the S-enantiomer. There was also lack of evidence for *in vivo* inversion of the R- to the S-

enantiomer in rats, monkeys, and humans following oral administration of AL04862.

Systemic Toxicity Studies with AL04862:

Systemic toxicity studies were carried out in mice (acute, 4-week oral, 13-week oral) and rats (acute, 2-week oral, 4-week oral, 13-week oral, and 6-month oral).

Acute toxicity in rats produced tremors, decreased defecation and activity, labored breathing, red discolored urine, and impaired or loss of righting reflex. Toxic signs in mice were similar, but red discolored urine was not reported. Red discoloration of the brain was reported in one of two female mice dosed at 1000 mg/Kg, but not at higher doses. Mortality occurred in the low dose (1000 mg/kg) in both mice and rats..

The four-week oral study in mice was a range-finding study with dose levels of 0, 10, 30, 100, 200, and 300 mg/Kg/day. Mortality was dose related (0, 1, 0, 2, 4, 10). Significant reduction in body weight occurred at ≥100 mg/Kg. These animals developed decreased defecation and activity, hunched posture, labored breathing, tremors, and eye discoloration.

In the 13-week oral toxicity study in mice (0, 5, 10, 20, 40, 80 mg/Kg/day), no drug related mortality or body weight changes occurred. Food consumption was decreased at ≥40 mg/Kg. Clinical signs were decreased defecation at 80 mg/Kg. Histopathology revealed occasional kidney mineralization, hydronephrosis, and increased incidence of chronic nephritis at ≥10 mg/Kg. Moderate kidney necrosis was reported in one 20 mg/Kg female. Glandular mineralization, dilatation, and hyperplasia were seen in the stomach at 40 and/or 80 mg/Kg. Urinary bladder lesions were characterized by renal tubular basophilia, hyperplasia, lymphocytic infiltration, inflammation, and an increase incidence in chronic nephritis at ≥10 mg/Kg These kidney and urinary bladder lesions were said to be common effects of carbonic anhydrase inhibitors in rodents.

In the two-week oral toxicity study in rats (0, 20, 60, 180 mg/Kg/day), the high dose resulted in one moribund animal. Body weight decreases occurred at ≥60 mg/Kg. Significant changes in hematology and serum chemistry parameters were limited to ≥60 mg/Kg. At 20 mg/Kg, AlP, Ca, Na, urea nitrogen, and Hot/Hb/RBC differed significantly from controls. Urinalysis changes included increases in pH and volume and a decrease in Na and K. Drug related lesions were centered in the stomach (inflammation, squamous epithelial hyperplasia, squamous papillomas), eye (posterior synechia), kidney (increased mineralization), lungs (focal hemorrhage, inflammation), and slight thinning of the zone of proliferating cartilage of the epiphyseal plate of the long bone.

The four-week oral study in rats was also a range-finding study carried out at 0, 3, 10, 30, 100, and 300 mg/Kg/day. Mortality was limited to the high dose, with all animals dying or sacrificed moribund. At necropsy, these animals had red brains due to hyper vascularization and/or blood clots, dark material in the stomach mucosa, congestion and necrosis in the stomach, inflammation of forestomach, and dark red material in the cecum. Significant decreases occurred in body weight and food consumption at ≥ 100 mg/Kg. Hematologic changes were observed at 100 mg/Kg in RBCs(↓), Hot(↓), Hb(↓), MCH(↑), and MCV(↑), and serum electrolytes were elevated at 10-100 mg/Kg. Other changes involved serum cholesterol, phosphorus, Alk P, BUN, creatinine, and the A/G ratio. Thymus and spleen weights were reduced and kidney, heart, brain, and adrenal weights were elevated in the higher dosed groups. At 100 mg/Kg, the histopathology lesions were limited to the thymus, stomach, and kidney. Stomach lesions consisted of suppurative inflammation, congestion, and necrosis. Acute hemorrhage was observed in the thymus, and acute hemorrhage and nephropathy were seen in the kidney of all groups but increased in the drug treated groups.

Thirteen weeks of oral treatment in rats at 0, 1, 3, and 10 mg/Kg/day produced no mortality. Significant food consumption was reduced. Mean body weight and body weight gain were reduced at week 6 due to inadvertent water deprivation. Significant changes were seen in some of the clinical chemistry and

hematology parameters (increases in Ca, globulin, cholesterol, and decreases in ALT, AST, A/G ratio, decrease in RBC and hemoglobin and increase in platelets). Urine volume was increased and Na, K, and specific gravity decreased. Increases in spleen, kidney and liver weights occurred. Microscopic findings that were drug related were kidney nephropathy and perhaps mineralization in the stomach mucosa. One high dose male developed liver necrosis, and focal inflammation occurred in the lungs at 10 mg/Kg. The NOAEL was 3 mg/Kg/day.

In the 6-month oral toxicity study in rats (0, 1, 3, 8 mg/Kg), no drug related mortality occurred. Clinical signs, such as chromodacryorrhea, redness around the eyes, and lacrimation, were dose related and reported in all groups, including the control. Body weight and food consumption were significantly reduced at 1 and 8 mg/Kg. Body weight gain was also reduced in all drug groups on several occasions. There were many significant changes that occurred in clinical chemistry parameters at 3 and 6 months. Notably were the increases in chloride, BUN, cholesterol, globulin, and creatinine and decreases in AST, albumin. A/G ratio, ALT, LDH. Hematology changes that were significant were increases in Hb and decreases in RBC and Hct. At 6 months, the reticulocyte count was increased at 3 mg/Kg. At gross pathology, a liver mass (hepatodiaphragmatic nodule) was reported in one female at 8 mg/Kg, enlarged spleens occurred at 3 mg/Kg, and mandibular lymph node pigmentation at 8 mg/Kg. Drug related microscopic lesions occurred in the kidney (fibrosis, chronic inflammation, mineralization nephropathy), urinary bladder (crystalline material in the lumen), and an increase in ovarian cysts in the high dose.

Reproductive and Developmental Studies

Rats dosed with 0, 2, 6, 18 mg/Kg/day showed no adverse effects on fertility or reproductive performance. However, dose related maternal toxicity occurred at ≥6 mg/Kg, and testes weight for the high dose males were significantly low. F1 pups in the 6 and 18 mg/Kg groups had low body weights. Fetal morphology was no different than seen in controls. F2 pups did not differ from control pups. The NOEL was 18 mg/Kg/day for fertility and general reproductive performance and around 2 mg/Kg/day for maternal and fetal toxicity.

Developmental toxicology studies were conducted in rats at 0, 2, 6, and 18 mg/Kg/day and in rabbits at 0, 1, 3, and 6 mg/Kg/day during the major period of organogenesis. Maternal toxicity was produced in F0 rats at 6 and 18 mg/Kg/day and in rabbits at 6 mg/Kg/day. Fetal toxicity appeared at 18 mg/Kg in rats as decreased fetal body weight and reduced ossification of skull bones and increases in litters and fetuses with unossified number 5 and 6 sternebra. Fetal malformations in rats indicated one fetus from the high dose with a small kidney and undescended testis and one fetus with cleft palate. In rats the number of F1 litters with variations was higher in the mid and high dose, and the male/female pup ratio from the F2 generation differed significantly from the other groups. In rabbits, accessory skull bones were significantly increased at 1 and 6 mg/Kg groups. The rat study did not show evidence for a teratogenic effect; the rabbit study, however, produced evidence of a teratogenic potential.

In the perinatal and postnatal study in rats (0, 1, 5, 15 mg/Kg/day), maternal toxicity was present at 15 mg/Kg/day as a significant decrease in body weight gain and food consumption. Food consumption was also significantly reduced during lactation. One pup was found dead at 15 mg/Kg/day with mottled lungs and one with cleft palate. The NOEL in this study was about 5 mg/Kg/day.

Mutagenicity Studies

Mutagenicity studies were carried out with AL04862 in the following assays: *E. coli* mutation assay, mouse lymphoma forward mutation assay, *in vivo* sister chromatid exchange, and the mouse micronucleus assay. AL04862 was positive in the presence in the mouse lymphoma forward mutation assay. All other assays were negative. A cell proliferation assay was also negative at doses of 0, 20, 60, and 180 mg/Kg/day AL04862 for 25 consecutive days. The S-

enantiomer was evaluated in the -*E.coli* mutation assay and the micronucleus assay - both were negative. The S enantiomer of brinzolamide was evaluated in the *E.coli* mutation assay and in the in vivo micronucleus assay - both were negative.

ADME Studies

Radioactive studies with a 1% AL04862 suspension administered to NZW rabbits have shown that the drug is absorbed and distributed in ocular tissues, blood, and plasma following a single topical ocular dose. Elimination from ocular tissues was slow. The $t_{1/2}$ in the lens was 294 days, iris-ciliary body 34 days, retina 96 days, choroid 74 days, and blood 39 days. Half-lives in Dutch belted rabbits were generally shorter.

AL04862 urinary metabolites determined in rats following iv and oral treatment include the O-desmethyl, the N-desethyl, and the N-desmethoxypropyl AL04862, with the parent drug the major component. Three other unidentified minor radioactive compounds were also observed. Twenty-seven percent of the radioactivity was excreted in the urine over 48 hours. Approximately 21% of an oral dose of labeled AL04862 was eliminated in the bile, 21% in the urine, 4% in the feces, and an estimated 47% remained in the blood. Following an iv dose, 32% was excreted in the urine, 29% in feces, and 14% remained in the carcass. Mean plasma protein binding from 10 µg/g in an ultrafiltration study was 24% in rats, 75% in monkeys, and 59% in humans. Tissue distribution and fetal transfer of radioactivity in pregnant rats following a single oral dose of labeled AL04862 showed a rapid absorption and distribution in tissues and fetuses. Peak levels occurred in fetuses at 6 hours. The highest concentrations were detected in the kidney, lungs, liver, heart, and placenta.

**RECOMMENDATION:**

There are no objections to the approval of AL04862. The decision to grant a waiver of the carcinogenicity studies must be made. From the data presented in this submission, there does not appear to be a cause for concern regarding the carcinogenicity potential of brinzolamide, and I recommend the carcinogenicity studies be waived.
Several changes are recommended for the labeling and are noted in that section of this review (pp. 68-69).

/S/

Almon W. Coulter, Ph.D.

/S/ 6-25-97

Team Leader: Conrad Chen, Ph.D.

cc:
NDA 20-816
HFD-550/Division File
/LLoBianco
/CYaciw
/ACoulter
/ELudwig
HFD-345
F/T by AWC 6/20/97

JUL 28 1997

# THE DIVISION OF ANTI-INFLAMMATORY, ANALGESIC, AND OPHTHALMIC DRUG PRODUCTS

## PHARMACOLOGY/TOXICOLOGY REVIEW

## REVIEW OF AMENDMENT TO PENDING APPLICATION

**NDA 20-816**

**SPONSOR:** Alcon Laboratories, Inc.
Post Office Box 6600
Fort Worth, Texas 76134-2099

**DRUG:** AZOPT™ (Brinzolamide Ophthalmic Suspension) 1%

**SUBMISSION:** June 12, 1997 **DATE RECEIVED:** June 16, 1997

**REVIEW COMPLETED:** July 14, 1997

**REVIEWER:** Almon W. Coulter, Ph.D.

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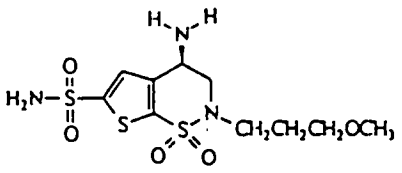
This NDA amendment contains a four-month safety update to the pending NDA application. The report contains safety information from clinical studies and preclinical pharmacology studies on AL-8520B, the desethyl metabolite of brinzolamide. AL-8520B was designated AL06339 in the original submission. These additional pharmacology studies with AL-8520B include the following:

1) Neuropharmacological Profile of AL-8520B in Rats.
2) Effect of AL-8520B on Urine Electrolyte Concentration and Volume Diuresis in Rats.
3) Neurotoxicity Assay of AL-8520B
4) Pharmacodynamic Assay of AL-8520B
5) Gastrointestinal Assay of AL-8520B
6) Cardiovascular (Hemodynamic) Studies of AL-8520B in Dogs.
7) Barbiturate Sleep Time Potentiation of AL-8520B in Mice.
8) The Effect of AL-8520B on Blood Gases in Conscious Rats.



AL06339

N-desethyl-brinzolamide

ABBREVIATIONS USED IN THE REVIEW

| | | |
|---|---|---|
| DR = dose related | * = p≤0.05 | ** = p≤0.0125 |
| ↑ = increase | ↓ = decrease | G = group(s) |

**PHARMACOLOGY:**

## 1) Neuropharmacological Profile of AL-8520B in Rats.

Report Nº: 001:39730:0297, pp. 9-031 to 9-040
Compound: AL-8520B, PRI reference Nº 3635, purity not indicated.
Formulation: Solution
Route: IV - one administration at 3 mL/Kg.
Diet: *Ad libitum*
Strain: Sprague-Dawley/Crl:CD®(SD)BR
Number: 10 ♂/group, 47 days old, body weight 197-228 g
Dose Levels: Group 1 2 3 4
0 1.0 10 30 mg/Kg AL-8520B (free base)
Control Treatment:
Study Site:
Date: December 5, 1996 - February 7, 1997
GLP/QAU Statements: Not present.

This study evaluated the neuropharmacological potential of the metabolite when administered by the intravenous route. The rats were observed for neuropharmacological signs at 15, 30, and 45 minutes, and at 1, 2, 3, 4, and 24 hours following treatment. Body temperature was also taken at 60 minutes following AL-8520B administration.

RESULTS

All results were presented in the following table. No other data were present.

(from p. 9-040)

| Group | Number | Treatment | Dose (mg/Kg) | Mean Body Temperature | Signs Observed |
|---|---|---|---|---|---|
| 1 | 10 | 10% | 0 | 37.15 | 0-24 hr: no signs |
| 2 | 10 | AL-8520B | 1 | 36.58 | 0-24 hr: no signs |
| 3 | 10 | AL-8520B | 10 | 35.93 | 0-24 hr: no signs |
| 4 | 10 | AL-8520B | 30 | 35.73 | 0-24 hr: no signs |

No additional signs were reported over the observation period.

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## 2) Effect of AL-8520B on Urine Electrolyte Concentration and Volume Diuresis in Rats.

Report Nº: 002:39730:0297
Compound: AL-8520B, PRI reference Nº 3635, purity not indicated.
Formulation:
Route: IV at 10 mL/Kg, one administration at 10 mL/Kg.
Diet: *Ad libitum*
Strain: Sprague Dawley - Crl:CD®(SD)BR, 49 days old, 157-189 g body weight.

Dose Levels:

| Group 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 0 | 0.3 | 1.0 | 3.0 mg/Kg AL-8520B | 5.0 mg/Kg acetazolamide |

Number: 10 ♂/group
Control Treatment:
Study Site:
Date: December 11, 1996 - April 11, 1997
GLP/QAU Statements: Not present.

The purpose of this study was to evaluate the potential effect of AL-8520B upon urinary volume output, pH, and urinary electrolyte concentrations in rats. The rats were hydrated with following the injection of AL-8520B or acetazolamide. Urine was collected over a 4 hour period, and the volume, pH, and the electrolyte ($Na^+$, $K^+$, $Cl^-$) concentrations determined.

RESULTS

- DR ↑ in urine volume G3(*), G4(*), G5(*)-
- DR ↑ pH G2(**), G3(**), G4(**)-
- DR ↓ $Cl^-$ mEq/L G3(*), G4(*), G5(*)-
- DR ↑ in G3(*), G4(*), G5 $Na^+$ and $K^+$ when expressed as µEq/100 g of weight-

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## 3. Neurotoxicity Assay of AL-8520B

Report Nº: 003:39730:0297
Compound: AL-8520B,
Formulation:
Route:
Diet:
Dose Levels:

Strain:
Number:
Control Treatment:
Study Site:
Date: December 9, 1996 - February 6, 1997
GLP/QAU Statements:

This study evaluated the potential of AL-8520B

RESULTS

Based on the this test, no neurological deficit was indicated.

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## 4. Pharmacodynamic Assay of AL-8520B

Report Nº: 004:39730:0297
Compound: AL-8520B,
Formulation: Solution

Route:
Diet:
Dose Levels:
Strain:
Number:
Control Treatment:
Study Site:
Date: December 12, 1996 - April 11, 1997
GLP/QAU Statements: Not present:

The study was done to determine the potential effects of AL-8520B administration on the cardiovascular response to various agents were compared to prior to vehicle or test article infusion. Gross abnormalities were also noted. The following agonists were used in the study:

RESULTS

Administration of vehicle or AL-8520B produced no significant change in the mean arterial pressure or on heart rate. Of the five agonists, was the only one that produced a statistical (*) significant increase in the mean arterial blood pressure. showed no gross changes

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**5. Gastrointestinal Assay of AL-8520B**

Report Nº: 005:39730:0297
Compound: AL-8520B,
Formulation: Solution
Route:
Diet:
Dose Levels: Group

Strain:
Number:
Control Treatment:
Study Site:
Date: November 27, 1996 - February 6, 1997
GLP/QAU Statements: Not present.

This assay was done to determine the potential ability of AL-8520B to affect

RESULTS

There was a DR decrease in the mean gastric ratio, becoming

significant AL-8520B

## 6. Cardiovascular (Hemodynamic) Studies of AL-8520B in Dogs.

Report Nº: 006:39730:0297
Compound: AL-8520B, PRI reference Nº 3635
Formulation: Solution
Route: IV 15 minute infusion at 1 mL/Kg.
Diet: *Ad libitum*
Dose Levels: Group 1 2 3
0 1.0 10 mg/Kg AL-8520B
Strain: Beagle, 6-7 months of age, 8.4-13.0 Kg
Number: 2♂, 2♀
Control Treatment:
Study Site:
Date: December 5, 1996 - April 11, 1997
GLP/QAU Statements: Not present.

This study was conducted to determine the potential acute effects of AL-8520B on the cardiac and circulatory functions in acutely prepared, open-chest anesthetized dogs. Systolic and diastolic arterial pressure, mean arterial pressure, heart rate, left ventricular pressure, left ventricular end diastolic pressure, +dP/dt at 40 mm Hg intraventricular pressure, cardiac output, and lead II EKG were evaluated.

RESULTS

Table 1 was inadvertently missing. The report stated no biologically relevant changes occurred in arterial blood pressure (systolic, diastolic and mean), heart rate, cardiac output, left ventricular pressure, left ventricular end diastolic pressure, or +dP/dt.

## 7. Barbiturate Sleep Time Potentiation of AL-8520B in Mice.

Report Nº: 007:39730:0297
Compound: AL-8520B, PRI reference Nº 3635, purity not indicated.
Formulation: Solution
Route: Oral, gavage at 20 mL/Kg - single administration
Diet: *Ad libitum*
Dose Levels: Group 1 2 3 4
0 1.0 10 30 mg/Kg AL-8520B
Strain: Crl:CD-1®(ICR)BR, 37 day old, 22-28 g body weight.
Number: 10♂/group
Control Treatment:
Study Site:
Date: December 6, 1996 - February 6, 1997
GLP/QAU Statements: Not present.

The study determined the ability of oral AL-8520B to potentiate the sleep time induced by sodium pentobarbital. Animals were administered the control or AL-8520B one hour before receiving an intraperitoneal injection of 50 mg/Kg sodium pentobarbital (Nembutal® Sodium) at 10 mL/kg.

RESULTS

Sleep time was increased 11% with 1.0 mg/Kg, 12% with 10 mg/Kg, and 5% with 30 mg/Kg, but these increases did not differ significantly from the vehicle control.

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**8. The Effect of AL-8520B on Blood Gases in Conscious Rats.**

Report Nº: 008:39730:0297
Compound: AL-8520B, PRI reference Nº 3635, purity not stated.

Formulation: Solution
Route: IV at 10 mL/Kg
Diet: *Ad libitum*
Dose Levels:

| Group | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| | 0 | 0.3 | 1.0 | 3.0 mg/Kg AL-8520B | 5.0 mg/Kg acetazolamide |

Strain: Sprague Dawley - Crl:CD®(SD)BR, 56-96 days old, 221-396 g body weight.
Number: 10♂/group
Control Treatment:
Study Site:
Date: December 17, 1996 - April 11, 1997
GLP/QAU Statement: Not present.

The study evaluated the potential effect of AL-8520B on blood gas values (pH, $pCO_2$, and $pO_2$). Rats were fitted with an indwelling cannula in the abdominal aorta. Blood gas values were determined at 2, 5, 15, 30, 60, 90, and 120 minutes following IV administration.

RESULTS

There were no statistical significant changes in blood pH, $pCO_2$, or $pO_2$ in conscious rats at 0.3 ard 1.0 mg/Kg AL-8520B. A significant decrease occurred in the pH and $pCO_2$ at 120 minutes with 3.0 mg/Kg AL-8520B. The blood pH was significantly increased at 90 and 120 min with 5.0 mg/Kg

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**SUMMARY AND EVALUATION:**

The above eight general pharmacology studies were conducted with AL-8520, the N-desethyl metabolite of brinzolamide. These studies were also carried out earlier with brinzolamide. Both compounds produced similar pharmacological actions.

AL-8520B showed no outward observable neurological signs or body temperature changes following intravenous administration of up to 30 mg/Kg in rats, and mice lost no ability to remain on the rotarod following oral administration of up to 30 mg/Kg. These two studies gave some indication that AL-8520B lacks outward neurotoxicity.

With 0.3 and 1.0 mg/Kg AL-8520B, blood pH, $pCO_2$, and $pO_2$ were not changed in rats; however, at 30 mg/Kg, statistically significant decreases occurred in pH and $pCO_2$ values.

In rats, statistically significant and dose related increases in urinary volume, pH, and increased urinary excretion of $Na^+$ and $K^+$ were produced by intravenous administration of 1.0 and 3.0 mg/Kg AL-8520B, but not at 0.3 mg/Kg. No change occurred in $Cl^-$ excretion - at 5.0 mg/Kg produced statistically significant increases in urine volume and $Na^+$ and $K^+$ excretion - $Cl^-$ and pH changes were not observed.

In the assay of AL-8520B with five different increased the mean arterial blood pressure by 42% ($p \leq 0.05$) at 10 mg/Kg when compared to vehicle.

At 10 and 30 mg/Kg AL-8520B, a significant (*) decrease was produced in the mean gastric through the mouse GI tract. The results were dose related. Sodium pentobarbital sleep time in mice was increased slightly with up to 30 mg/Kg AL-8520B, but these results did not seem to be of any biological significance.

The cardiovascular (hemodynamic) study in dogs indicated no biologically relevant changes occurred in arterial BP, heart rate, cardiac output, left ventricular pressure, left ventricular end diastolic pressure, or +dP/dt, following IV administration of 1.0 or 10 mg/Kg AL-8520B. Table 1 of this study was missing and should be requested.

There were no issues that altered the safety profile or would require a change in the preclinical approvable status of brinzolamide, based on data submitted in this amendment.

**RECOMMENDATIONS:**

Table 1 from report Nº 006:39730:0297 [Cardiovascular (Hemodynamic) Studies of AL-8520B] was missing and should be submitted.

/S/

Almon W. Coulter, Ph.D.

/S/ 7-28-97

Team Leader: Conrad Chen, Ph.D.

cc:
NDA 20-816
HFD-550/Division File
/LLoBianco
/CYaciw
/ACoulter
/ELudwig
HFD-345
F/T By AWC 7/14/97