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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-727

Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

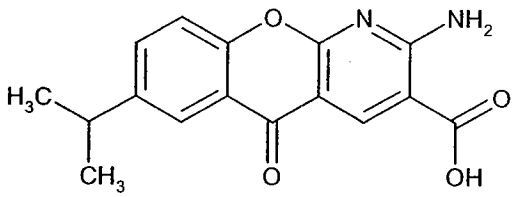
NDA number: 21-727
Review number: 1
Sequence number/date/type of submission: N-000/04-DEC-2003

Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Access Pharmaceuticals, Inc.
2600 Stemmons Freeway, Suite 176
Dallas, TX 75207
Manufacturer for drug substance:

Reviewer name: Norman A. See, Ph.D.
Division name: Division of Dermatologic and Dental Drug Products
HFD #: 540
Review completion date: 14-JUN-2004

Drug:

Trade name: Amlexanox 2 mg Mucoadhesive Patch
Generic names (list alphabetically): Amlexanox
Code name: AA-673; CHX 3673
Chemical names: 2-amino-7-isopropyl-5-oxo-5H-[1] benzopyrano [2,3-b] pyridine-3-carboxylic acid
CAS registry number:
Mole file numbers: NA
Molecular formula/molecular weights/structures: $C_{16}H_{14}N_2O_4$/298.3

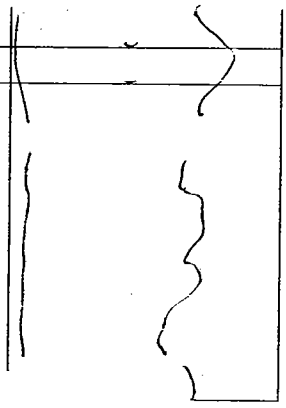


Relevant INDs/NDAs/DMFs: IND [redacted] IND [redacted] IND [redacted] IND 59,524; IND 59,949; IND [redacted] NDA 20-511

Drug class: Anti-inflammatory agent
Indication: Treatment of aphthous ulcers

Clinical formulation (per dosage unit):

Component	Amount
Amlexanox	2.00 mg
Ethylcellulose	[redacted]
Hypromellose (Hydroxypropyl methylcellulose)	[redacted]

Hypromellose film	
Povidone	
Hydroxyethylcellulose Carboxymethylcellulose sodium Polycarbophil Propylene glycol Sodium benzoate Purified water Red food color	

Route of administration: Topical to oral mucosa (and eventually swallowed)

Proposed use: The proposed use of the product involves placement of dosage units directly upon aphthous ulcers in the oral mucosa. One dosage unit is applied to each aphthous ulcer four times daily. The Dental Teamleader has estimated that a maximum of 20 units might be used per day in an individual with multiple ulcers (as a worst-case exposure estimate). A course of therapy would be expected to last approximately 7 days. Therefore, a course of therapy with the product would be expected to entail exposure to up to 40 mg amlexanox per day (0.67 mg/kg/day in a 60 kg patient) for 7 days. Development of aphthous ulcers is a recurring condition, and it is likely that a given individual would undergo numerous courses of therapy in a lifetime, resulting in chronic exposure to the product.

Introduction and drug history: Amlexanox oral paste 5% (Aphthasol) was approved under NDA 20-511 for treatment of aphthous ulcers on 17-DEC-1996. The label for Aphthasol provides for application of approximately 60 mg of paste to each aphthous ulcer four times daily for approximately 10 days. This equates to approximately 12 mg amlexanox per ulcer per day, applied to the oral tissues.

Amlexanox 50 mg tablets are approved in Japan (but not the U.S.) for treatment of asthma; a dosage of approximately 150 mg per day is typical.

Studies reviewed within this submission: The submission contained no new nonclinical data. The application references NDA 20-511. NDA 20-511 contains the following nonclinical studies (please see the attached review of NDA 20-511 for detailed review of the data; only the more relevant studies are listed):

1. Acute toxicology:

1.1. Acute toxicity of AA-673 in mice and rats, study report No. A-16-145, study No. 110/AC.

1.2 Acute oral toxicity study with rats, study report No. 70903807.

1.3 Acute dermal toxicity study in rabbits, study report No. 70903808.

2. Repeat dose toxicology.

2.1 Five-week oral toxicity study of AA-673 in rats, study report No. A-16-146, study No. 99/SU.

2.2 Twenty-six-week oral toxicity study of AA-673 in rats, study report No. A-16-185, study No. 143/CH.

2.3 Five-week oral toxicity study of AA-673 in beagle dogs, study report No. A-16-136, study No. 115/SU.

2.4 Five-week oral toxicity study of AA-673 in beagle dogs followed by 5- and 10-week recovery periods, study report No. A-16-485, study No. 304/SU.

2.5 Twenty-six-week oral toxicity study of AA-673 in beagle dogs, study report No. A-16-187, study No. 144/CH.

3. Genetic toxicology

3.1 Mutagenicity tests on amlexanox sodium salt (1): Rec-assay and reversion test in bacteria, study report No. A-16-541.

3.2 Micronucleus test on amlexanox (AA-673) in mice, study report No. A-16-476.

3.3 Mouse lymphoma mutation assay, study report No. 762164.

4. Carcinogenicity

4.1 18 month dietary oncogenicity study in mice with AA-673, study report No. A-16-498, study No. 295-060.

4.2 Two year dietary oncogenicity study in rats with AA-673, study report No. A-16-506, study No. 295-058.

5. Reproductive toxicology.

5.1 Effect of amlexanox (AA-673) on fertility and general reproductive performance of the rat, study report A-16-473.

5.2 Teratological study of amlexanox (AA-673) in the rat, study report No. A-16-472.

5.3 Teratological study of amlexanox (AA-673) in the rabbit, study report No. A-16-471.

5.4 Effect of amlexanox (AA-673) on peri- and post-natal development of the rat, study report No. A-16-474.

6. Special toxicology.

6.1 Nasal cavity irritation study of AA-673 nasal solution after forced deterioration in rats, study report No. A-16-527.

6.2 Nasal mucosal irritation study of AA-673 nasal solution after forced deterioration in rats, study report No. A-16-585.

6.3 Five-week toxicity study of AA-673 delivered into the nasal cavity in rats, study report A-16-274.

6.4 Ocular irritation study of AA-673 ophthalmic solution in frequent instillation in rabbits, study report No. AA-673/S-TX02.

6.5 The external ocular toxicity study of aged 0.25% AA-673 ophthalmic solution by 4-week repeated instillation in rabbits, study report No. AA-673/S-TX03.

6.6 Four-week ocular toxicity study of 0.5% AA-673 ophthalmic solution in rabbits, study report No. AA-673/S-TX01.

Studies not reviewed within this submission: The submission contained a number of photocopies of journal articles that were not specifically summarized in this review because they were judged to add no useful information to the database that was captured in the review.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability: This NDA is approvable with respect to pharmacologic and toxicologic concerns.

B. Recommendation for Nonclinical Studies: No additional nonclinical studies are recommended at this time.

C. Recommendations on Labeling: The following changes in the draft labeling are recommended:

1. Carcinogenesis, Mutagenesis, Impairment of Fertility: The text in this section should be stricken and replaced with:

"Amlexanox was not carcinogenic when administered to mice for 18 months at dosages up to 100 mg/kg/day (approximately 12 times the maximum human dose when comparing on the basis of body surface area estimates) or to rats for 24 months at dosages up to 250 mg/kg/day (approximately 60 times the maximum human dose). Amlexanox was negative in bacterial mutation assays in Salmonella, E. coli, and B. subtilis, in a mouse lymphoma assay, and in a micronucleus assay conducted in mice.

Amlexanox did not affect reproductive performance (fertility) or ability of rats to deliver and rear pups (perinatal development) when administered at dosages up to 300 mg/kg/day (approximately 70 times the maximum human dose).

2. Pregnancy. The text in this section should be stricken and replaced with:

"Pregnancy category B. Reproduction studies have been performed in rats and rabbits at doses up to 300 mg/kg/day (approximately 70 and 145 times the maximum human dose in rats and rabbits, respectively, when comparing on the basis of body surface area estimates) and have revealed no evidence of impaired fertility or harm to the fetus due to amlexanox. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed."

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: Little potential for toxicity was observed in a battery of toxicology studies conducted with amlexanox that included acute, subchronic, chronic, carcinogenicity, genetic, and reproductive studies. No-effect-levels (NOELs) in these studies were substantial multiples of the proposed human exposure (please see the "Detailed Conclusions and Recommendations" section of this review, and the attached Pharmacology review of NDA 20-511, for additional information). No toxicity that appeared relevant to the proposed clinical use was observed.

B. Pharmacologic Activity: Amlexanox acts through an unknown mechanism that may involve inhibition of various mediators of inflammation and/or protease enzymes.

C. Nonclinical Safety Issues Relevant to Clinical Use: None

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the pharmacology studies that support NDA 21-727.

Primary pharmacodynamics: The mechanism by which amlexanox acts is unknown. Amlexanox inhibits tissue necrosis factor-alpha, and this may be involved in increasing the rate of healing of aphthous ulcers. In vitro data suggest amlexanox may inhibit release of various mediators and enzymes, including IL-1β, IL-5, and inhibition of protease enzymes.

Mechanism of action: Unknown, although it has been suggested that amlexanox has anti-inflammatory properties.

Drug activity related to proposed indication: Unknown.

Secondary pharmacodynamics: NA

Pharmacology summary: Amlexanox acts through an unknown mechanism that may involve inhibition of various mediators of inflammation and/or protease enzymes.

Pharmacology conclusions: The mechanism of action of amlexanox is unknown.

II. SAFETY PHARMACOLOGY:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the safety pharmacology studies that support NDA 21-727.

III. PHARMACOKINETICS/TOXICOKINETICS:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the pharmacokinetics studies that support NDA 21-727.

IV. GENERAL TOXICOLOGY:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the toxicology studies that support NDA 21-727.

V. GENETIC TOXICOLOGY:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the studies, "mutagenicity tests on amlexanox sodium salt (1): Rec-assay and reversion test in bacteria" (study report No. A-16-541) and "micronucleus test on amlexanox (AA-673) in mice" (study report No. A-16-476). In addition, the sponsor has performed the following genetic toxicology study since NDA 20-511 was approved (the report of this study was submitted to IND 59,949):

Study Title: Mouse lymphoma mutation assay
Study No: 762164
Study Type: In vitro point mutation assay
Amendment #, Volume # and Page #: 001, 1, 078 (of IND 59,949)
Conducting Laboratory: [ ]
Date of Study Initiation/completion: In-life 28-APR-1998-11-JUN-1998; report dated 18-AUG-1998
GLP Compliance: Yes
QA- Reports Yes (X) No ( ):
Drug Lot Number: 1006-7113
Study Endpoint: Growth in medium containing trifluorothymidine (TFT), indicating mutation from tk^+tk^- to tk^-tk^-

Methodology:

- Strains/Species/Cell line: tk^+tk^- 3.7.2.C mouse lymphoma L5178Y cells
- Dose Selection Criteria: Cytotoxicity
  - Basis of dose selection: Cytotoxicity in range-finding studies
  - Range finding studies: Examined concentrations of amlexanox in culture medium ranging from 0.1 to 1000µg/mL, with and without S9
- Test Agent Stability: Chemical analysis of the test material formulations used in this study were not performed. However, data from previous studies with amlexanox suggest it was adequately stabile throughout the experimental period
- Metabolic Activation System: Aroclor 1254-induced S9 (supernatant of the post-mitochondrial 9000 g fraction from adult male Fischer rats)
- Controls:
  - Vehicle: DMSO in culture medium
  - Negative Controls: Vehicle
  - Positive Controls: Ethyl methanesulphonate and methyl methanesulphonate in absence of S9; 3-methylcholanthrene in presence of S9
  - Comments: Controls were adequate
- Exposure Conditions:
  - Incubation times: 4 hour exposure with and without S9; negative results in absence of S9 were repeated in a 24 hour exposure

- Doses used in definitive study: 10μg/mL-240μg/mL
- Study design: Following the exposure period, the cells were washed and grown for 9 to 12 days with and without TFT

- Analysis:
  - No. slides/plates/replicates/animals analyzed: 192 wells per concentration per assay
  - Counting method: Dissecting microscope
  - Cytotoxic endpoints: Reduced cell count in absence of TFT
  - Genetic toxicity endpoints: Increased numbers of cells that grew in presence of TFT

Results:

- Study Validity: Acceptable
- Study Outcome: Amlexanox did not increase the incidence of cell survival (colony formation) in medium that contained TFT in either the presence or absence of S9. Appropriate results were obtained with the controls.

Summary of individual study findings: Amlexanox was negative in a mouse lymphoma assay.

Genetic toxicology summary: Amlexanox was negative in a rec assay, an Ames assay, a mouse lymphoma assay, and a micronucleus assay.

Genetic toxicology conclusions: These data suggest that amlexanox is not genotoxic.

Labeling recommendations: See labeling portion of recommendations and conclusion, below.

VI. CARCINOGENICITY:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the toxicology studies that support NDA 21-727.

Carcinogenicity summary: Amlexanox was negative in carcinogenicity studies conducted in both mice and rats.

Carcinogenicity conclusions: These data suggest that amlexanox is not carcinogenic.

Labeling Recommendations: See labeling portion of recommendations and conclusion, below.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the toxicology studies that support NDA 21-727.

Reproductive and developmental toxicology summary: Amlexanox was evaluated for potential to induce reproductive toxicity in a series of studies that included a fertility study in rats, teratology studies in rats and rabbits, and a perinatal development study in rats. No evidence of toxicity was observed.

Reproductive and developmental toxicology conclusions: These data suggest that amlexanox is not a reproductive toxicant.

Labeling recommendations: See labeling portion of recommendations and conclusion, below.

VIII. SPECIAL TOXICOLOGY STUDIES:

Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the toxicology studies that support NDA 21-727.

Special toxicology summary: Amlexanox was evaluated for potential to induce local irritation in studies that involved instillation of drug solutions into the nasal cavity and eye in rats and rabbits. The materials were judged to be essentially non-irritating.

Special toxicology conclusions: Drug solutions that contained amlexanox were judged to be essentially non-irritating.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Amlexanox was evaluated in a series of toxicology studies that included acute, subchronic, chronic, carcinogenicity, genetic, and reproductive toxicology studies. Please see the attached Pharmacology review No. 1 of NDA 20-511 for details of the toxicology studies that support NDA 21-727. Briefly summarizing the pivotal studies:

Study Type	Study Summary
26 Week Rat	NOEL was 100 mg/kg/day (24 times the maximum clinical dose*); very little toxicity at 300 mg/kg/day (the highest dose used)
26 Week Dog	NOEL was 30 mg/kg/day (the highest dose used; 24 times the maximum clinical dose)
18 Month Mouse (Carcinogenicity)	NOEL was 30 mg/kg/day (4 times the maximum clinical dose); 100 mg/kg/day (the highest dose used) induced slight toxicity (small reduction in BW of males, reduced RBC parameters, and nephrosis, but no effect on survival)
24 Month Rat (Carcinogenicity	NOEL was 80 mg/kg/day (19 times the maximum clinical dose); 250 mg/kg/day (the highest dose used) induced minor liver toxicity, but no effect

)	on survival
Fertility, Rat (males dosed for 9 weeks prior to mating, females dosed starting two weeks prior to mating and then for remainder of study)	NOEL was 300 mg/kg/day (the highest dose used; approximately 70 times the maximum clinical dose)
Teratology, Rat	NOEL was 300 mg/kg/day (the highest dose used; approximately 70 times the maximum clinical dose)
Teratology, Rabbit	NOEL was 300 mg/kg/day (the highest dose used; approximately 145 times the maximum clinical dose)
Perinatal Development, Rat	NOEL was 300 mg/kg/day (the highest dose used; approximately 70 times the maximum clinical dose)

*Dose multiples are based upon body surface area estimates.

In addition, negative results were obtained when amlexanox was tested for genetic toxicity and carcinogenicity. Note that the NOEL (no effect level) values and dose-multiples offer a very conservative estimate of the safety margin, because: 1) they are from long-term studies, while a course of therapy with the product would be expected to entail exposure for only 7 days; and 2) the dose-multiples are based upon a worst-case scenario exposure to the drug product of 40 mg amlexanox per day (0.67 mg/kg/day in a 60 kg patient). The actual exposure of a given patient would probably be substantially less than this. Given the small magnitudes of the level and duration of the proposed exposure to amlexanox, and the relative lack of toxicity observed in nonclinical studies conducted with amlexanox (even at much higher exposure levels), the proposed exposure to amlexanox should be acceptably safe. Additional evidence of safety comes from the marketing history of amlexanox oral paste (NDA 20-511), which involves an essentially identical exposure to amlexanox. No serious adverse events have been reported during the approximately seven year marketing history of that product. All excipients in the proposed new product have been used in previously approved oral products, and are safe for the proposed new use.

Unresolved toxicology Issues (if any): NA

Recommendations: This NDA is approvable with respect to pharmacologic and toxicologic concerns. It is recommended that the labeling be revised as indicated below.

Labeling recommendations: The following changes in the draft labeling are recommended:

1. Carcinogenesis, Mutagenesis, Impairment of Fertility: The text in this section should be stricken and replaced with:

"Amlexanox was not carcinogenic when administered to mice for 18 months at dosages up to 100 mg/kg/day (approximately 12 times the maximum human dose when comparing on the basis of body surface area estimates) or to rats for 24 months at dosages up to 250 mg/kg/day (approximately 60 times the maximum human dose). Amlexanox was negative in bacterial mutation assays in Salmonella, E. coli, and B. subtilis, in a mouse lymphoma assay, and in a micronucleus assay conducted in mice.

Amlexanox did not affect reproductive performance (fertility) or ability of rats to deliver and rear pups (perinatal development) when administered at dosages up to 300 mg/kg/day (approximately 70 times the maximum human dose).

2. Pregnancy. The text in this section should be stricken and replaced with:

"Pregnancy category B. Reproduction studies have been performed in rats and rabbits at doses up to 300 mg/kg/day (approximately 70 and 145 times the maximum human dose in rats and rabbits, respectively, when comparing on the basis of body surface area estimates) and have revealed no evidence of impaired fertility or harm to the fetus due to amlexanox. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed."

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

X. APPENDIX/ATTACHMENTS:

Addendum to review: NA

Other relevant materials (Studies not reviewed, appended consults, etc.): Pharmacology review 1 of NDA 20-511 is attached, beginning on the next page.

Any compliance issues: NA

cc: list:
NDA 21-727
HFD-540
HFD-540/DivDirector/Wilkin
HFD-540/Deputy DivDirector/Kukich
HFD-540/SupPharm/Brown
HFD-540/Pharm/See
HFD-540/DO/Hyman
HFD-540/CMC/Pappas
HFD-540/PMS/Smith

Note: The following review of NDA 20-511 was written by John Wedig, Ph.D.

Evaluation of Pharmacology and Toxicology Data
Division of Topical Drug Products, HFD-540

NDA: # 20-511 (Resubmission Dated April 19, 1995)

Date Submitted: April 17, 1995

Date CDER Received: April 19, 1995

Assigned Date: April 21, 1995

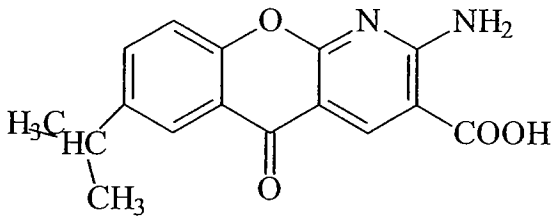
Date Review Completed:

Date Review Accepted By Supervisor:

Name of Drug: Amlexanox Oral Paste, 5% **Code Name:** AA-673; CHX 3673

Chemical Name: 2-amino-7-isopropyl-5-oxo-5H-[1] benzopyrano [2,3-b] pyridine-3-carboxylic acid

Structure:



Molecular Formula: $C_{16}H_{14}N_2O_4$

Molecular Weight: 298.30

Pharmacological Category: Antiallergic and anti-inflammatory; the mechanism of action for accelerating the healing of aphthous ulcers is unknown

Sponsor: Chemex Pharmaceuticals, Inc
Fort Lee Executive Park 1

Martha R. Charney, Ph.D.
Vice President, Regulatory Affairs

One Executive Drive
Ft. Lee, NJ 07024
Phone (201) 944-1449

Proposed Indication: Treatment of aphthous ulcers on the oral mucosal lining

Formulation:	Ingredient	Composition (% w/w)
	Amlexanox	5.0
	Mineral oil, USP	
Gelatin, NF		
	Pectin, NF	
	Carboxymethylcellulose sodium, USP	
	Carboxymethylcellulose sodium, USP	
	Glycerol monostearate, NF	
	White petrolatum, USP	
	Benzyl alcohol, NF	

Related Submissions: IND [redacted]
IND [redacted]
NDA 89-066 Stiefel Research
NDA 19-940 Actinex-Chemex
DMF [redacted]

Dosage Form and Route of Administration: The 5% oral paste (formulation noted above) is to be dabbed on the ulcer four times a day, preferably following oral hygiene after breakfast, lunch, dinner and at bedtime. The projected maximum human dose would be approximately 1mg/kg/day.

The pharmacology and pharmacokinetic studies have been previously summarized by Dr. Browder in the original review of IND 31,079 and amendment # 001. The following studies were reviewed under IND 34,787 by Dr. Morseth (see attached):

1) Acute Exposure Oral Toxicity Study With 5% CHX 3673 Cream (PH 402-CX-001-88; GLP).

2) Acute Exposure Dermal Toxicity Study In Rabbits With 5% CHX 3673 Cream (PH 22-CX-001-88; GLP).

3) Primary Dermal Irritation Study With 5% CHX 3673 Cream (PH 420-CX-001-88; GLP).

4) Delayed Contact Hypersensitivity Study In Guinea Pigs With CHX 3673 Cream (PH 424-CX-001-88; GLP).

5) Hamster Cheek Pouch Irritation Study (Multiple Dose) With CHX 3673 (PH 418-CX-001-90; GLP).

6) 8-Day Dermal Toxicity Study In Rabbits With CHX 3673 Cream (PH 430-CX-001-88)

Review Objectives: To assist in the safety evaluation of a 5% oral paste preparation for the treatment of aphthous ulcers by the evaluation of nonclinical laboratory studies for clinical studies.

Index Of Preclinical Studies:

Acute Evaluations
- Oral, dermal, skin and sensitization

Subacute Evaluations
- 5 Week Oral Toxicity Study In Rats
- 26 Week Oral Toxicity Study In Rats
- 5 Week Oral Toxicity Study In Beagle Dogs
- 5 Week Oral Toxicity Study In Beagle Dogs Followed By 5 And 10 Week Recovery Periods
- 26 Week Oral Toxicity Study In Beagle Dogs

Chronic Studies
- 18 Month Dietary Oncogenicity Study In Mice
- 2 Year Dietary Oncogenicity Study In Rats

Special Toxicity Studies
- Nasal Mucosal Irritation Study In Rats
- 5 Week Toxicity Study Of AA-673 Into The Nasal Cavity In Rats
- Ocular Irritation From Repeated Instillation
- Ocular Toxicity of Aged AA-673 Ophthalmic Solution-4 Weeks Of Instillation
- Four Week Ocular Toxicity of AA-673 Ophthalmic Solution In Rabbits

Reproductive Studies
- Segment I In Rats
- Segment II In Rats and Rabbits
- Segment III In The Rats

Mutagenicity Studies
- Ames Test
- Micronucleus Test-Mouse

Absorption And Kinetic Studies

Protein Binding And Erythrocyte Distribution

Tissue Distribution And Accumulation Studies

Enzyme Induction

Metabolism

Excretion

Nasal Administration

Intraocular Penetration

Acute Studies

1) Acute Toxicity Of AA-673 In Mice And Rats (Report # A-16-145, GLP)

Laboratory: [redacted]
Number of Animals: 10/sex/group
Animal Strain: Mice-Ta:ICR, Rats-Jcl:Wistar
The test material was suspended in 5% gum arabic. The animals were observed for 7 days after treatment and then necropsied. The LD50 (95% confidence limits) was found to be:

Mouse- mg/kg	Male	Female
Subcutaneous injection	3310(2960-3680)	3760(3370-4200)
Intraperitoneal injection	480(440-520)	450(410-490)
Oral gavage	2370(2160-2540)	2320(2120-2540)
RAT-mg/kg	Male	Female
Subcutaneous injection	1560(1320-1820)	1400(1180-1620)
Intraperitoneal injection	520(470-560)	500(460-540)
Oral gavage	ca 10000	ca 10000

A difference in LD50 values was noted between rats and mice. The major clinical signs noted after treatment were decreased activity and respiratory depression. The study is acceptable for its intended purpose.

2) Acute Oral Toxicity Study In Rats (Report # 70903807; GLP)

Laboratory: [redacted]

Number Of Animals: 5/sex/group

Animal Strain: Sprague Dawley, Charles Rivers

Study Design: The test material was suspended in 0.5% hydroxypropyl methylcellulose. The rats were observed for 14 days following dosing and then necropsied.

Results: The LD50(mg/kg) and 95% confidence limits were found to be : Male-5000(3346-7473) female-2828(1964-4073). Combined values were 3810 mg/kg. The major clinical sign noted after dosing was hypoactivity. The study is acceptable for its intended purpose.

3) Acute Dermal Toxicity Study In Rabbits (solution of Amlexanox; Report # 70903808 GLP)

Laboratory: [redacted]

Number Of Animals: 5 males and 5 females

Animal Strain: New Zealand Albino

Study Design: The test material was dissolved in trolamine and water to yield a 10% solution which was applied at 2 gm/kg. One-half of the animals had abraded skin sites. A pilot study using two animals per sex indicated no mortality.

Results: The study using 10 animals indicated no mortality at 2 gm/kg. This study is acceptable for its intended purpose.

4) Publication- Hairya, et al, Allergenicity and tolerogenicity of amlexanox in the guinea pig, Contact Dermatitis, 1994; 31: 31-36. Oral administration of amlexanox prior to sensitization resulted in complete non-responsiveness. It is proposed that a substantial reduction in the risk of sensitization from the use of an ophthalmic solution containing amlexanox may be achieved by the prior oral administration of tablets containing this drug.

Subacute Evaluations

1) Five Week Oral Toxicity Study of AA-673 In Rats (Report A-16-146; GLP)

Laboratory: [ ]

Number Of Animals: 10 males and 10 females per group

Animal Strain: Ta:Wistar [ ]

Dose Levels: 0, 40, 200 and 1000 mg/kg/day

Formulation: The compound was mixed with gum arabic and suspended in distilled water at concentrations of 0, 0.8, 4 and 10% (w/v) to correspond to the 0, 40, 200 and 1000 mg/kg doses-i.e. 10, 5, 5 and 10 ml/kg/ day respectively.

Route: Oral gavage once a day.

Study Design: The rats were dosed 7 days a week for 5 weeks. The water intake and 24 hour urine volume were determined for 5/sex/group at the beginning and end of the study. Body weight and food consumption was determined weekly. A urinalysis was performed on 5/sex/group toward the end of the treatment period. Hematology and serum chemistry was evaluated on all animals (fasted) at the termination of treatment. A piece of liver was taken at necropsy from 5/sex/group for determination of enzymatic activity. At necropsy 16 organs/animal were weighed from 10/sex/group and 21 tissues/animal were processed for histology from 5/sex/group. Kidney and liver tissue from one male in the control group and two males in the 1000 mg/kg group was examined with an electron microscope.

RESULTS

Mortality: One male in the 200 mg/kg group died during the course of the evaluation due to a technical dosing error-i.e. not treatment related .

Clinical Observations, Body weight, Food Consumption, Urinalysis, Urine Chemistry, Water Intake, Urine Volume, Hematology, Hepatic Drug Metabolizing Activity: No treatment related findings.

Organ And Organ-to-Body Weights: A significant increase in the mean absolute and relative to body organ weight was noted for the cecum and stomach in the animals treated with 1000 mg/kg. This was considered to be treatment related.

Serum Chemistry: The alkaline phosphatase levels were significantly increased in the males and females given 1000 mg/kg as compared to the controls. This was a treatment related effect not noted in other groups.

Gross Necropsy: A treatment related white-yellowish mucous was observed on the surface of the gastric mucosa of almost all females and one male in the 1000 mg/kg group. This was not noted in the other groups.

Histopathology: Treatment related findings included the following in the 1000 mg/kg group:

Glandular stomach-

6 animals- thickening of mucosa with hypersecretion
5 animals- dilation of glandular lumen

Forestomach-

2 animals- hyperplasia of mucosa

Cecum-

4 animals- hypertrophy and desquamation of epithelium

Electron Microscopy: A slight dilation of the bile cuniculi in the liver was seen at a dose of 1000 mg/kg.

Summary: The no adverse affect level of AA-673 from this evaluation is 200 mg/kg. The target organs appear to be the cecum and the glandular stomach at a dose of 1000 mg/kg-i.e. pathological changes and weight increases. Electron microscopic changes were noted in the liver and a significant elevation in serum alkaline phosphatase was noted at this dose level. All of these changes were minimal in nature. The study is acceptable for its intended purpose.

2) 26 Week Oral Toxicity Study Of AA-673 In Rats (Report # A-16-185; GLP)

Laboratory: [ ]

Number Of Animals: 12 males and 12 females per group

Animal Strain: Jcl:Wistar Rats

Dose Levels: 0, 30, 100 and 300 mg/kg/day

Formulation: Dietary admix. Test diets were made up weekly.

Route: Oral

Study Design: Animals were fed diets containing the drug for 26 weeks. Clinical signs were monitored daily, food consumption 2 X week and body weight weekly. Five males and 5 females had a urinalysis done pretest and during weeks 6, 14 and 26. Hematology and serum chemistry evaluations were done on fasted animals at necropsy. All animals were necropsied and organ weights were obtained. Histopathological evaluation was done on 5 males and 5 females from each group. Liver from the control and the 100 and 300 mg/kg groups was examined under an electron microscope.

RESULTS

Mortality: No treatment related mortality occurred. There were two incidental deaths.

Diet Analysis: Concentrations of AA-673 were analyzed during weeks 5, 10, 15, 20 and 25 and found to be within 88 to 113% of theoretical. AA-673 was stable in the [ ] rat chow for 2 weeks at room temperature. No homogeneity data were given.

Dietary Intake: The group mean dietary intakes were close to theoretical. Some of the ranges were outside of 10%.

Clinical Observations, Urinalysis, Hematology, Body Weight, Gross Necropsy Observations and Histopathological Analysis:
No treatment related effects were noted on any of these parameters.

Food Consumption: Males in the 300 mg/kg group consumed significantly more food than the control animals for most weekly periods up through 15 weeks. Females receiving the same dose did not.

Organ Weights: An increase in the cecum weight was noted only in the males receiving 100 and 300 mg/kg. No histopathological change was seen in the cecum or the other parts of the gastrointestinal tract indicating this effect was not treatment related.

Serum Chemistry: A significant increase was noted in the mean alkaline phosphatase levels only in the males given 300 mg/kg.

Electron Microscopy: A slight dilatation of the bile canaliculi in the centrolobular hepatocytes was seen in one male given 300 mg/kg and was considered to be treatment related.

Summary: The no effect level of AA-673 appears to be 100 mg/kg due to the elevated serum alkaline phosphatase and the dilated bile cuniculi in the males given 300 mg/kg. The study is acceptable for its intended purpose.

3) Five Week Oral Toxicity Study Of AA-673 In Beagle Dogs (Report A-16-136; GLP)

Laboratory: [ ]

Number Of Animals: 3 males and 3 females per group

Animal Strain: Canine, beagle; [ ]

Dose Levels: 0, 10, 30 and 100 mg/kg/day

Route: Orally in the morning by gelatin capsule containing the pure drug

Study Design: The dogs were dosed 7 days a week for 5 weeks. Food consumption was determined daily and body weight 2 x weekly. Clinical observations were done pre dose and 1 and 6 hours post dosing. Physicals, ophthalmic examinations (internal and external), hematology evaluations including clotting times, urinalysis and water intake were done pretest, during the midpoint and at the end of the study. Serum chemistry was done pretest and weekly. Blood for plasma drug levels was taken 2, 10 and 24 hours post dosing on drug day 36. Liver tissue from all dogs was assayed for drug metabolism (hydroxylase and N-demethylase). Organ weights were obtained at necropsy from all animals and 25 tissues/animal were prepared for histological examination. Selected liver samples were silver stained and selected liver and kidney tissues were prepared for enzyme histochemistry.

RESULTS

Mortality: No treatment related deaths occurred.

Body Weight, Clinical Signs, Food Consumption, Physical Examinations, Ophthalmological Examinations, Hematology and Prothrombin Times, Urinalysis, Water Intake, Hepatic Drug Metabolism, Organ Weights, Hepatic Silver Stains and Enzyme Histochemistry of Kidney:
No consistent or distinct treatment related effects were noted.

Serum Chemistry: Ornithine carbamyl transferase, alkaline phosphatase and glutamic pyruvic transaminase were increased in the 100 mg/kg group. This was treatment related.

Plasma Levels Of AA-673: Peak plasma concentrations were reached about 2 hours post dosing. The drug blood concentrations indicated that the increase in plasma levels was greater than the increase in dose.

Gross Necropsy: A slight discoloration of the liver in two males and two females given 100 mg/kg was noted.

Histopathology: Treatment related finding in the 100 mg/kg group included- Proliferation of the bile ducts accompanied by fibroplasia in the peripheral zone of the liver lobule; atrophy and degeneration of the hepatocytes in close proximity to this lesion; hypertrophy of the epithelium of the gallbladder.

Enzyme Histochemistry: An increase in alkaline phosphatase activity of the proliferated bile ducts was noted in animals given 100 mg/kg.

Summary: Hepatotoxicity was noted at the 100 mg/kg dose. The no effect level appears to be 30 mg/kg. This study is acceptable for its intended purpose.

4) Five Week Oral Toxicity Study Of AA-673 In Beagle Dogs Followed By 5 And 10 Week Recovery Periods (Report # A-16-486; GLP)

Laboratory: [redacted]

Number Of Animals: 6 females in the control group and 9 females in the treatment group

Animal Strain: Canine, beagle [redacted]

Dose Level: 0 and 100 mg/kg

Route: Orally in the morning by gelatin capsule containing the pure drug

Study Design: The dogs were dosed 7 days a week for 5 weeks followed by a recovery period of 5 and 10 weeks. Food consumption and clinical observations were done daily. Serum chemistry was done pretest and at the end of the dosing and recovery periods. Two control and three treated animals were necropsied at the end of treatment and after 5 and 10 weeks of no dosing. Organ weights were obtained at the end of the AA-673 dosing period and the 5 week recovery period. Liver and gallbladder tissue were prepared for histological examination. Liver tissue was prepared for enzyme histochemistry and electron microscopic examination.

RESULTS

Mortality: No treatment related mortality occurred.

Clinical Signs: Most of the AA-673 dosed animals occasionally vomited undigested food throughout the treatment period.

Body Weight: Some animals showed a slight decrease during the dosing period which returned to expected values during the recovery period.

Serum Chemistry: Ornithine carbamyl transferase, alkaline phosphatase and glutamic pyruvic transaminase were increased in the treated animals at the end of the dosing period. The values were in the expected range 5 weeks after cessation of dosing.

Gross Necropsy: A slight discoloration of the liver surface was noted in 2 of the treated dogs after 5 weeks of dosing. This was not noted in any of the recovery dogs.

Histopathology: Hypertrophy of the bile duct epithelium, proliferation of peri-bile duct connective tissue and atrophy of hepatocytes around interlobular connective tissue was noted in all of the treated animals. After 5 weeks of recovery the only finding was a slight increase in the interlobular connective tissue in one dog. This change was not observed after 10 weeks of recovery.

Enzyme Histochemistry: A marked increase of alkaline phosphatase activity was noted in the bile cuniculi of the 3 treated dogs. This activity returned to expected values after the 5 week recovery period.

Electron Microscopy: A protrusion of hepatocytes into the bile cuniculi noted at the end of the dosing period was absent in the dogs after 5 weeks of recovery.

Summary: Hepatotoxicity noted after treatment with 100 mg/kg for 5 weeks was absent 10 weeks after no dosing, indicating complete recovery. The study is acceptable for its intended purpose.

5) 26 Week Oral Toxicity Study In Beagle Dogs (Report # A-16-187; GLP)

Laboratory: [redacted]

Number Of Animals: 3/ sex/group

Animal Strain: Canine, beagle, [redacted]

Dose Level: 0, 3, 10 and 30 mg/kg/day

Route: Orally in the morning by gelatin capsule containing the pure drug

Study Design: The dogs were dosed 7 days a week for 26 weeks. Food consumption was determined daily and body weight approximately weekly. Clinical observations were done pre dose and 1 and 6 hours post dosing. Physicals, ophthalmic examinations (internal and external), hematology, prothrombin times, serum chemistry, urinalysis, 24-hour water intake and urine volume were done pretest and during weeks 5, 13 and 26. All animals were subjected to a complete necropsy and their organs were weighed. Tissues from all animals were examined histologically. Enzyme histochemistry was done on liver tissue from all treatment groups. Liver tissue from the control and 30 mg/kg group was examined with an electron microscope.

RESULTS

Mortality, Body Weight, Food Consumption, Clinical Signs, Physical Examinations, Ophthalmological Examinations, Hematology, Prothrombin Times, Serum Chemistry, Urinalysis, 24-Hour Water Intake and Urine Volume, Gross Necropsy, Organ Weight, Histopathology and Electron Microscopy:

No consistent or distinct treatment related changes were noted.

Enzyme Histochemistry: A slight increase in alkaline phosphatase in the bile cuniculi of the central part of the liver lobule of one of two males in the 30 mg/kg group was noted.

Summary: The maximum non-toxic dose level in this evaluation was 30 mg/kg. This study is acceptable for its intended purpose.

CHRONIC STUDIES

1) 18 Month Dietary Oncogenicity Study In Mice With AA-673 (Report # 295-060; GLP)

Laboratory: [redacted]

Number Of Animals: 50/sex/group; 6 weeks old at study initiation

Animal Strain: mouse, $B_6C_3F_1$, [redacted]

Dose Levels: 0, 3, 10, 30 and 100 mg/kg

Formulation: Dietary admix. Test diets were made up weekly. Homogeneity studies indicated a 20 minute mix resulted in preparations that assayed plus or minus 10% of theory for AA-673 consistently. Stability studies indicated the AA-673 was stable(plus or minus 5% of theory) in [redacted] Chow [redacted] under laboratory conditions over a period of

10 days. The two lots of AA-673 used for mixing the diets were assayed at the beginning of each use span and found to be 99.9% pure. The sponsor provided analytical data indicating that AA-673 was stable at room temperature for at least two years.

Pilot Study: A 17 week dietary dose range finding study in this strain of mouse was conducted at [ ] using dose levels of 0, 25, 50, 100, 200, 500 and 1500 mg/kg (the latter two dosage levels from study week 14, and representing a change in the 25 and 50 mg/kg/day dose levels). A treatment related toxic nephrosis was noted beginning at a dose of 100 mg/kg . This effect increased in incidence and severity with increasing dose. No other treatment related effects were seen.

Study Design: Animals were fed the diets for 78 weeks. Food consumption and bodyweight were determined pretest, weekly during the first 14 weeks and thereafter every 2 weeks. Food efficiency was determined for the first 14 weeks. Clinical observations were done daily. Hematology evaluations were done at term and if possible on animals in extremis. All animals were subjected to a complete necropsy. A complete set of tissues was prepared for histopathological evaluation from the control and 100 mg/kg dose group, all animals that died or were sacrificed in extremis, plus all tissue masses with regional lymph nodes, gross lesions and the kidneys from the 3, 10 and 30 mg/kg groups.

RESULTS

Compound Consumption and diet analysis: The mean weekly compound consumption of all the AA-673 treated groups was within 10% of theory except for four instances during the 78 week treatment period. Diet assays every four weeks for AA-673 concentration in all groups indicated only six diet mixes that were greater or less than 10% of theory.

Mortality, Clinical Signs and Food Consumption: No treatment related effects were noted on these parameters.

Body Weight: No consistent treatment related effect was noted. In the males given 100 mg/kg there was a decrease in body weight in the last 6 months of treatment.

Hematology: A significant decrease in erythrocytes, hemoglobin and hematocrit were noted in the males given 100 mg/kg. This was not noted in the corresponding female group.

Gross Necropsy Observations: Males in the 100 mg/kg group had an incidence of 35/50 with granular kidneys. This treatment related effect was not noted in the females.

Histology: Toxic nephrosis of the kidney was noted in 50/50 males in the 100 mg/kg group.

Summary: The test material, AA-673, was determined to have no tumorigenic effect. The no effect level for toxicity to the kidney was 30 mg/kg. This study is acceptable for its intended purpose.

2) Two Year Dietary Oncogenicity Study In Rats With AA-673 (Report # 295-058; GLP)

Laboratory: [ ]

Number Of Animals: 50/sex/group; 5 weeks old at study initiation

Animal Strain: [ ] Fisher 344 rats [ ]

Dose Levels: 0, 25, 80 and 250 mg/kg/day

Formulation: Dietary admix. Test diets were made up weekly. Homogeneity studies indicated a 10 minute mix resulted in preparations that assayed plus or minus 10% of theory for AA-673 consistently. Stability studies indicated the AA-673 was stable (plus or minus 5% of theory) in [ ] Chow [ ] under laboratory conditions over a period of 10 days. The three lots of AA-673 used for mixing the diets was assayed at the beginning of each treatment span and found to be 99.9% pure. The sponsor provided analytical data indicating that AA-673 was stable at room temperature for at least two years.

Pilot Study: A 13 week dietary ranging finding study in Fisher 344 rats was conducted at [ ] using dose levels of 0, 125, 250, 500 and 1000 mg/kg. Body weight was decreased at 1000 mg/kg. Serum levels of alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were increased in the males given 500 mg/kg and in both sexes at 1000 mg/kg. Histopathological evaluation of the liver indicated dilation of the extrahepatic and common bile ducts, bile duct hyperplasia, cholangitis, necrosis and pericholangitis. These were seen in both sexes at 1000 mg/kg and in the males at 500 mg/kg. Females at 500 mg/kg indicated only one trace instance of pericholangitis as did the males at 250 mg/kg. The dose of 125 mg/kg did not appear to produce any toxic effects.

Study Design: Animals were fed the diets for 104 weeks. Food consumption and body weight were determined pretest, weekly during the first 14 weeks and thereafter every 2 weeks. Food efficiency was determined for the first 14 weeks. Clinical observations were done daily. The animals were palpated for masses weekly. Hematology evaluations were performed on animals at term and on ones that were sacrificed in extremis. All animals were subjected to a complete necropsy. A complete set of tissues was prepared for histological evaluation from the control and 250 mg/kg dose group and all animals that died during the course of the study or were sacrificed in extremis. All tissue masses with

regional lymph nodes, all gross lesions, liver and adrenals from all animals were also prepared for histopathological examination.

RESULTS

Compound Consumption And Diet Analysis: The mean compound consumption of all the AA-673 treated groups was within 10% of theory except for three 2 week periods when it exceeded the 10% over the 104 weeks period. Diet assays every four weeks for AA-673 concentration in all groups indicated 14 values which were less than 10% of theory-i.e. 11 in the 80's and 3 in the high 70's.

Mortality, Hematology, Clinical Signs , Food Consumption And Food Efficiency: No treatment related effects were noted on these parameters.

Body Weight: There was a frequent significant decrease in body weight of the males given 250 mg/kg the second half of the study. The actual difference was small, 6%. This was occasionally noted in the high dose females.

Gross Necropsy Observations: Dilatation of the extrahepatic bile duct was noted in males given 250 mg/kg as well as an increase in eye lens discoloration.

Histology: Prominent biliary changes were noted in the males from the 250 mg/kg group. They included cystic dilatation, calculus formation and inflammation of the extrahepatic bile duct. Cholangitis and pericholangitis was noted in the liver. This effect was limited to a slight increase in pericholangitis in the females given 250 mg/kg.

Summary: The test material AA-673 was determined not to be carcinogenic. The no effect level for toxicity was determined to be 80 mg/kg. This study is acceptable for its intended purpose. See attached CAC forms for the rat and mouse.

SPECIAL TOXICITY STUDIES

1) Nasal Cavity Irritation Study Of AA-673 Nasal Solution After Forced Deterioration (Report # A-16-527). Only a summary report was available. The irritation potential of a deteriorated sample of AA-673 introduced into the nasal cavity of Jcl:Wistar rats 4 X/day for 14 days was evaluated. It was concluded that no irritation was produced by the deteriorated AA-673 applied to the nasal mucosa of rats under the test conditions.

2) Nasal Mucosal Irritation Study Of AA-673 Nasal Solution After Forced Deterioration In Rats (Report # A-16-585;GLP)

Laboratory: [ ]

Number Of Animals: 110/group

Animal Strain: Jcl:Sprague Dawley Rats
Duration Of Dosing: every 15 minutes for a total of nine times in one group
every 2 hours daily for 14 consecutive days

Dose Levels: 25 ul instilled in the left nostril per dose-AA-673 nasal solution or saline

Study Design: The animals were dosed and observed for clinical signs twice daily during the treastment period and once daily during the following observation period. They were weighed weekly. One and 7 days after the last instillation, 5 animals/group were sacrificed. The nasal area was prepared for histological examination.

RESULTS

No abnormalities were noted in clinical signs or at autopsy in either group of treated rats. Histopathological examination of the nasal tissues indicated that AA-673 did not cause irritation.

Summary: A deteriorated AA-673 nasal solution does not cause irritation to the nasal tissues. The study is acceptable for its intended purpose.

3) Five Week Toxicity Study Of AA-673 Delivered Into The Nasal Cavity In Rats (Report # A- 16-274; GLP)

Laboratory: [ ]

Number Of Animals: 5/sex/group

Animal Strain: Jcl:Sprague Dawley Rats [ ]

Duration Of Dosing: 5 Weeks, 7 days a week, 4 times a day. Each dose volume was 0.025 mL

Dose Levels: Saline control, 0.1 mL/rat/day
vehicle control, 0.1 mL/rat/day
AA-673 0.1 mg/rat/day; 0.1 mL/rat/day
AA-673 0.25 mg/rat/day; 0.1 mL/rat/day

Route: The solution was delivered 4 times a day to the left nasal cavity by means of a micropipette through the nostril.

Study Design: Animals were treated 4 times a day for 5 weeks. Clinical observations were noted daily. Body weights were taken on the 0, 1st, 3rd and 7th day and then twice weekly. A complete necropsy was conduced on each animal and the organs were weighed. The upper respiratory tract of each animal was prepared for histology and stained with three stains.

RESULTS

Mortality, Body Weight, Clinical Observations, Organ Weights and Gross Necropsy Observations:
No treatment related effects were note.

Histopathology: A very slight increase in the number of goblet cells in the respiratory region of the nose was noted in the animals treated with 0.25 mg/rat/day. However, there was no dose response relationship and this effect was also seen in the vehicle and saline controls. There were no changes indicative of degeneration of the cells.

Summary: The local irritative effect of AA-673 solution is very slight. The study is acceptable for its intended purpose.

4) Ocular Irritation Study Of AA-673 Ophthalmic Solution In Frequent Instillation In Rabbits (Report # AA-673/S-TX02)

Laboratory: [redacted]

Number Of Animals: 9

Animal Strain: Japanese white aboriginal rabbits

Dose : several drops of the 1.0% AA-673 ophthalmic solution

Route: instillation in the conjunctival sac of the right eye

Study Design:

Group 1- 3 rabbits- 32 topical installations in the eye at 15 minute intervals for a day

Group 2- 3 rabbits- 16 topical installations in the eye at 30 minute intervals for a day

Group 3- 3 rabbits- not used

The eyes were examined before treatment and 30 minutes after the last treatment. The cornea was stained with fluorescein dye and examined at these times. The animals behavior was also monitored.

RESULTS

Chemosis and redness of the conjunctivae and discharge were noted. No lesions were produced. The irritation cleared up 24 hours after the last instillation. The study is acceptable for its intended purpose

5) The External Ocular Toxicity Study Of Aged 0.25% AA-673 Ophthalmic Solution By 4 Week Repeated Instillation In Rabbits(Report # AA-673/S-TX03)

Laboratory: [ ]

Number Of Animals: 5 males

Animal Strain: Japanese white rabbits

Dose : Two drops of an aged (5 days) 0.25% AA-673 solution or physiological saline

Route: Instillation in the eye

Study Design: Animals had AA-673 (right eye) or saline (left eye) instilled onto the eye 9 times daily at 1 hour intervals for 28 days. The eyes were scored with the Draize procedure pretest and 30 minutes after the last instillation on days 1, 3, 7, 14, 21 and 28. Slit lamp examination with fluorescein staining followed the same schedule. Body weights were taken pretest and weekly and clinical observations were done daily.

RESULTS

The aged AA-673 0.25% solution had no effect on the rabbit eye or other parameters measured. This study is acceptable for its intended purpose.

6) Four Week Ocular Toxicity Study Of 0.5% AA-673 Ophthalmic Solution In Rabbits (Report # AA-673/S-TX01)

Laboratory: [ ]

Number Of Animals: 10

Animal Strain: Japanese white aboriginal rabbits

Dose Levels: 2 drops/dose (about 0.1 mL) ; 5 rabbits received AA-673 and 5 received saline

Formulation: 0.5% AA-673 ophthalmic solution or physiological saline

Route: conjunctival; AA-673 or physiological saline was put in the right eye; left eye was untreated

Study Design: The animals had either the drug or saline instilled onto the conjunctivae 9 times a day at 1 hour intervals for 29 days. The eye was scored using the Draize procedure and the cornea was examined using fluorescein and a slit lamp pretest and 1, 3, 7, 14, 21 and 28 days after study initiation. The pupil size and intraocular pressure was measured 2, 4 and 7 days prior to study termination Body weight and general condition were noted pretest and weekly thereafter.

RESULTS

No treatment related effects were noted on any of the parameters measures during the 29 day study. The study is acceptable for its intended purpose.

Reproductive Studies

1) Effect Of Amlexanox (AA-673) On Fertility And General Reproductive Performance Of The Rat (Report # A-16-473; GLP)

Laboratory: [ ]

Number Of Animals: 26 males and 26 females per group

Animal Strain: Jcl:Wistar [ ]

Dose Level: 0, 30, 100 and 300 mg/kg

Route: Oral intubation

Formulation: The drug was suspended in 5% gum arabic solution at a concentration of 6%. It was further diluted with 5% gum arabic to make 2 and 0.6% (w/v) suspensions. The controls received a 5% gum arabic solution. The dose volume to each group was 5 ml/kg. The doses were made up fresh daily. The dosing solutions were assayed pretreatment and 3 X during the study. All assays were well within plus or minus 10% of theory. Homogeneity and stability for 24 hours were determined and found to be within plus or minus 10% of theory.

Study Design: The males were treated daily for 9 weeks prior to mating. The females were treated daily for 2 weeks before mating and during the mating period. Dosing continued throughout the remainder of the study. Approximately one-half of the females were killed on day 13 of pregnancy, the remainder were allowed to rear their litters to day 22 after delivery. Food consumption, body weight, estrous cycle, copulation rate, conception rate, fertility index and various other reproductive indices were monitored.

RESULTS

Mortality, Body Weight, Food Consumption, Estrous Cycle, Conception Rate, Pre-Implantation Loss, Post- Implantation Loss, Number Of Corpora Lutea, Number Of Live Embryos, Morphological Observations, Development Of Maturational Landmarks, Gestation Period, Parturition, Suckling, Litter Size, Pup Mortality and Body Weight

No treatment related effects were noted on any of these parameters- reproductive performance or pre and post natal development of the pups. The study is acceptable for its intended purpose.

2) Teratological Study of Amlexanox (AA-673) In The Rat (Report # A-16-472; GLP)

Laboratory: [ ]

Number Of Animals: Approximately 49 pregnant females per group

Animal Strain: Jcl:Wistar Rat, [ ]

Dose Levels: 0, 30, 100 and 300 mg/kg

Route: Oral intubation

Formulation: The drug was suspended in 5% gum arabic solution at a concentration of 6%. It was further diluted with 5% gum arabic to make 2 and 0.6% (w/v) suspensions. The controls received a 5% gum arabic solution. The dose volume to each group was 5 ml/kg. The doses were made up fresh daily. The dosing solutions were assayed

pretreatment and 1 X during the study. Assays were well within plus or minus 10% of theory. Homogeneity and stability for 24 hours were determined and found to be within plus or minus 10% of theory.

Study Design: The animals were mated at [ ] The rats were treated on days 6-17 of pregnancy. Twenty-one to 23 per group were necropsied on day 20 of gestation. Two-thirds of the fetuses were stained for skeletal examination. The remaining one-third were examined for visceral abnormalities using the freehand sectioning technique of Wilson. Various reproductive indices, food consumption, body weight, behavior and mortality were calculated. The remaining 12 to 13 animals in each group were allowed to deliver. All dams were necropsied on day 22 or 23 postpartum- the number of implantation sites was counted and the main organs were examined histologically. The pups were sexed, weighed and their development assessed morphologically-pinna detatchment, incisor eruption and eye opening. Two males and two females from each litter in all dose groups were necropsied and examined for internal and skeletal (x-ray) abnormalities. One male and 1 female were examined microscopically for evidence of brain abnormalities. The remaining pups were reserved for behavioral and reproductive studies. The behavioral studies included- an open field test, water T-maze test and a wheel rotation activity test. The reproductive performance test involved - mating non-litter mates, allowing them to deliver. The pups were sacrificed on days 9 to 11. The main organs were examined histologically. An assessment of internal and skeletal development was made as well as a histological examination of the brain. The reproductive organs were examined thoroughly.

RESULTS

Mortality, Skeletal Development, Development Of The Internal Organs, Brain Development, Body Weight, Food Consumption, Litter Size, Pup Weight, Morphological Development, Number Of Implants, Number Of Resorptions, Maturational Landmarks and Behavior

No consistent or distinct treatment related effects were noted. The study is acceptable for its intended purpose.

3) Teratological Study Of Amlexanox (AA-673) In The Rabbit (Report # A-16-471; GLP)

Laboratory: [ ]

Number Of Animals: Approximately 12 to 14 pregnant females per group

Animal Strain: KBL:JW rabbit [ ]

Dose Levels: 0, 30, 100 and 300 mg/kg

Pilot Study: A two week oral intubation in females of this strain of rabbit was conducted. All of the animals given 1000 mg/kg died. Two of 5 animals in the 300 mg/kg group showed a decrease in food consumption. On this basis the above doses were selected.

Route: Oral intubation

Formulation: The drug was suspended in 5% gum arabic solution at a concentration of 3%. It was further diluted with 5% gum arabic to make 1 and 0.3% (w/v) suspensions. The controls received a 5% gum arabic solution. The dose volume to each group was 10 ml/kg. The doses were made up fresh daily. The dosing solutions were assayed pretreatment and 2 X during the study. Assays were well within plus or minus 10% of theory. Homogeneity and stability of 0.6 and 6.0% (w/v) suspensions for 24 hours were determined previously and found to be within plus or minus 10% of theory.

Study Design: The animals were mated at [ ] They were treated from day 6 through day 18 of pregnancy. Food consumption and body weights were obtained on days 0, 6, 13, 19, 23 and 28 of gestation. All animals were observed for signs of toxicity daily. The dams were necropsied on day 28 of gestation. Various reproductive indices were noted. The placenta, amnion and amniotic fluid were examined microscopically. The fetuses were examined for external and visceral abnormalities and variations. The heart and kidneys were freehand sectioned with a razor blade and examined for abnormalities. The fetuses were then stained for skeletal examination of potential abnormalities and variations. Prior to preparing the fetus for skeletal staining the head was freehand sectioned with a razor blade and the brain was examined for abnormalities.

RESULTS

Mortality, Skeletal Development, Development Of The Internal Organs, Brain Development, Body Weight, Food Consumption, Litter Size, Pup Weight, Number Of Implants, Number Of Resorptions And Histological Examination Of Organs

No consistent or distinct treatment related teratogenic or embryolethal effects were noted. A slight decrease in body weight gain and suppression of food consumption were noted in a few of the dams in the 300 mg/kg group the latter half of the treatment period. The study is acceptable for its intended purpose.

4) Effect Of Amlexanox (AA-673) On Peri- And Post-Natal Development Of The Rat (Report # A-16-474; GLP)

Laboratory: [ ]

Number Of Animals: 23 to 24 pregnant females per dose group

Animal Strain: Jcl:Wistar rat, [ ]

Dose Levels: 0, 30, 100 and 300 mg/kg/day

Pilot Studies: A 5 week oral toxicity study in rats indicated a no effect level of 200 mg/kg. An adverse effect was noted at 300 mg/kg in a 26 week oral rat study.

Route: Oral intubation

Formulation: The drug was suspended in 5% gum arabic solution at a concentration of 6%. It was further diluted with 5% gum arabic to make 2 and 0.6% (w/v) suspensions. The controls received a 5% gum arabic solution. The dose volume for each group was 5 ml/kg. The doses were made up fresh daily. The dosing solutions were assayed pretreatment and 2 x during the study. Assays were well within plus or minus 10% of theory. Homogeneity and stability of 0.6 and 6.0% (w/v) suspensions for 24 hours were determined previously and found to be within plus or minus 10% of theory.

Study Design: The pregnant rats were dosed from day 15 of pregnancy through suckling to day 21 postpartum. All animals were allowed to deliver and the F1 pups were examined for morphological development and assessed in behavioral tests- negative geotaxis and grip strength.. The dams were necropsied on day 22-23 postpartum and the number of implantation sites counted. Two males and two females were necropsied at the same time and examined for external and internal abnormalities, skeletal and brain abnormalities. The remaining F1 pups after weaning were assessed for testes descent and vaginal opening and then a select few from each litter were used for behavioral and reproductive performance studies. Behavioral studies included pupillary reflex, pain response, rotarod performance, open field test, preyer's reflex, running wheel activity test and the water T-maze test. All F2 pups were necropsied on days 7 to 9 postpartum . Selected animals were examined for skeletal abnormalities and variations and brain abnormalities. The presence or absence of sperm in the epidimides and follicles and luteinization in the ovaries was determined.

RESULTS

Mortality, Motor Coordination, Grip Strength, Numbers Of Newborn per Litter, Number Of Implantation Sites, Number Of Resorptions, Sex Ratio, Reflexes, Pain Response, Auditory Response, Rotarod Performance, Clinical Signs, Body Weight, Copulation Rate, Gestation Period, Delivery, Nursing, Conception Rate, Skeletal Or Visceral Abnormalities and Brain Abnormalities

Summary: No treatment related changes were noted on any of the above mentioned parameters. This study is acceptable for its intended purpose.

Mutagenicity Studies

1) Mutagenicity Tests On Amlexanox Sodium Salt (1): Rec-assay And Reversion Test In Bacteria (report # A-16-541)

Laboratory: [redacted]

Study Design: Two bacterial mutagenic assays were used to assess the drug- a repair test (modified rec assay) and a reverse mutation test (Ames test). Nine positive control agents were used and demonstrated to be active. The test strains for the repair test were B subtilis H17(rec+) and M45(rec-) and for the reverse mutation test were E. coli WP2uvrA and S. typhimurium TA100, TA98 and TA1537.

RESULTS

Negative results were obtained in the rec-assay at dosages of 125 and 1250 ug/disk. In the reverse mutation assay at dosages ranging from 100 to 5000 ug/plate negative results were obtained with and without metabolic activation (S9 fraction). It was concluded that the drug is not mutagenic or DNA damaging. The study is acceptable for its intended purpose.

2) Micronucleus Test On Amlexanox (AA-673) In Mice (Report # A-16-476; GLP)

Laboratory: [redacted]

Number Of Animals: 5 males/group

Animal Strain: SPF (C3HxSWV)F1, [redacted]

Dose Level: Single oral dose 0, 125, 500 and 2000 mg/kg
Single dose daily for four days 0 and 500 mg/kg

Formulation: Amlexanox was suspended in %5 gum arabic solution at 1.25, 5 and 20 %(w/v) such that all animals were given 10 mL/kg. Homogeneity and stability studies over 24 hours for this concentration range were acceptable-i.e. plus or minus 10% of theory.

Study Design: The drug was administered orally in a single dose at 0, 125, 500 and 2000 mg/kg or 0 and 500 mg/kg daily doses for 4 consecutive days. Mitomycin C, the positive control, was injected once intrapertioneally at a dose of 2 mg/5 mL/ kg. The animals were killed 30 hours after treatment and bone marrow was removed from the femur and

processed into slides. The frequency of polychromatic erythrocytes and reticulocytes was determined.

RESULTS

No evidence of an increased frequency of bone marrow micronucleated erythrocytes in the drug treated groups was noted. This suggests that the compound is not mutagenic. This study is acceptable for its intended purpose.

Absorption, Distribution, Metabolism And Excretion Studies

1)This information was translated from the article published in Japanese, Metabolic Fate of Amlexanox (AA-673), A New Antiallergic Agent, In Rats, Mice, Guinea-Pigs And Dogs, Japanese Pharmacology & Therapeutics 13: 4933-4954.

Laboratory: [ ]

Animal Strain: male and female Jcl:Wistar rats
male Jcl:ICR mice
male Crj:Hartley guinea-pigs
male beagle dogs [ ]

Formulation: The drug was labelled with ^{14}C in the pyridine ring and had a radiochemical purity of greater than 99%. The 14C-AA-673 was appropriately diluted with nonlabelled drug and was suspended in 5% gum arabic solution for oral administration or was dissolved in a minimum volume of 1N NaOH and diluted with phosphate buffered saline for intravenous injection. The animals were dosed at the rate of 10 mg/kg.

Absorption and Kinetics

The ratio of radioactivity in urine was calculated following oral gavage and intravenous dosing to rats, mice, guinea-pigs and dogs (fasted or fed). Bioavailability was estimated to be 46, 61, 76 and 47% in rats, mice, guinea-pigs and dogs, respectively. The site of absorption was studied in pyloric-ligated rats after intragastric or intraduodenal administration of the drug. The plasma concentration was significantly higher after intraduodenal administration suggesting the drug was absorbed mainly from the small intestine. Further studies using a jejunal loop indicated absorption was mainly by the portal route in this area. The use of thoracic duct fistulated rats given the drug orally indicated absorption was unlikely by the lymphatic route.

The absorption of the drug after oral gavage was rapid in the rat, mouse and dog. It was delayed in the guinea-pig probably due to absorption from a wide range of the intestine.

The level of ^{14}C AA-673 and its metabolites in plasma were studied for at least 24 hours following oral gavage in rats, mice, guinea-pigs and dogs. The plasma concentration of the labelled drug and its metabolites were about equal in mice, guinea-pigs and dogs suggesting the metabolic characteristics are about the same. The rat had a substantial quantity of metabolite in the plasma which was identified as a conjugate that was not noted in the other species. The composition of the metabolites from the plasma of man resembles that found in mice, guinea-pigs and dogs but not rats.

In man a single oral application of 5mg from 5% paste resulted in an area under the curve(AUC, 0 to 24 hours) of 0.36 ug.hr/ml. Ten mg/kg given intraduodenally to the rat resulted in an AUC (0 to infinity) of 4.23 ug.hr/ml. Ten mg/kg oral doses to the mouse and dog gave AUC (0 to infinity) values of 9.67 and 8.56 ug.hr/ml respectively.

Protein Binding And Erythrocyte Distribution

In vitro studies indicated radiolabelled drug was bound to plasma protein to the extent of 96 to 99% in mice, rats, guinea-pigs and dogs. The three concentrations of drug tested (0.5, 5.0 and 50 ug/ml were in the concentration range found in plasma from the oral gavage studies) indicated no dependence of binding on concentration. The binding was further studied and found to be reversible.

The percentage of drug bound or stuck to erythrocytes from these four species varied from 6 to 23% using the same drug concentration in another in vitro experiment. There did not appear to be a dependence of binding upon concentration.

Tissue Distribution And Accumulation Studies

Rats were dosed by oral gavage 1 x day for up to five days and their tissues examined for accumulation of radioactivity. No tissue accumulation of radioactivity was noted except in the organs responsible for the excretion of the drug and its metabolites. Rats were given the labelled drug intradudonally and killed at varying times up to 24 hours post dosing. Whole body autoradiography, also did not indicate any tissue accumulation other than those involved in the excretion of the drug over the 24 hour study period. These results agreed with those of the tissue distribution studies.

On day 20 of gestation rats were orally dosed with ^{14}C AA-673. Fetuses were removed from 15 minutes to 8 hours post dosing for analysis. Radioactivity was detected in the fetus and amniotic fluid indicating transfer or drug/metabolites across the placenta. There did not appear to be concentration of the drug or metabolites in the fetus since the concentration at each of the sampling times was lower than the concentration in the maternal plasma. Lacteal secretion was examined at the same times in females dosed orally with labelled drug on day 14/15 after

parturition. Radioactivity was secreted in the milk. The predominant component was unchanged drug. The concentration in milk was higher than that in plasma as time progressed.

Enzyme Induction

The ability of AA-673 to cause enzyme induction was studied. Rats were orally dosed with 0, 10, 30 or 100 mg/kg/day for a total of 7 days and the activity of hepatic microsomal enzymes was studied 24 hours after the last dose. There was no increase in liver weight, microsomal protein per gram of liver, enzymatic activity per mg protein, and microsomal content of cytochromes p450 and b5 were the same for the AA-673 treated animals vs the controls. The positive control material, phenobarbital, caused significant increases in weight of the liver, microsomal protein, all of the enzymatic activities and the microsomal content of both cytochromes. AA-673 did not cause hepatic microsomal enzyme induction in rats.

Metabolism

The metabolites in the urine and feces were identified after oral administration of the radiolabelled drug to rats, mice, guinea-pigs and dogs. In the plasma and excreta of all four species the drug was metabolized by hydroxylation and oxidation of the isopropyl moiety. The drug was metabolized by conjugation with glucuronic acid only in the rat (major) and guinea-pig (minor). Amlexanox (major fecal component) and the hydroxylated derivative (major urine metabolite) were present in the urine and feces from all four species. Unchanged amlexanox and the hydroxylated derivative have been found in the serum and urine of man after oral administration of the unlabelled drug. The urinary metabolic profiles were qualitatively similar for all species.

An in vitro study with rat tissue slices of brain, heart, lung, liver, kidney and duodenum was conducted with labelled drug to investigate the metabolism. It was determined that the conjugation was carried out mainly in the intestinal mucosa and the hydroxylation and oxidation of the isopropyl moiety were in the liver and kidney. Glucuronidation was only carried out in the rat.

Excretion

After oral administration of the labelled drug, almost all of the radioactivity was eliminated within 48 hours in rats, mice and dogs and within 120 hours in guinea-pigs. The bulk of the radioactivity appeared in the feces (75 to 91%) rather than the urine (5 to 23%).

Rats were given an oral dose of labelled drug 1 x day for 5 days and various pharmacokinetic parameters were determined. The results of this multiple dose study indicated no accumulation of either the parent drug or its metabolites during the five day study.

Summary: The drug is well absorbed from the intestine of rats, mice, guinea-pigs and dogs. It is distributed widely in tissues with no accumulation and is metabolized. The drug and its metabolites are preferentially eliminated from the body by fecal excretion and secondarily by the urinary route. AA-673 does not cause hepatic enzyme induction. These studies are acceptable for their intended purpose.

2) Pharmacokinetics And Metabolism of Amlexanox (AA-673), A New Antiallergic Agent, After Nasal Administration To Rats (Report # A-16-525; a two page report was provided)

Laboratory: [ ]

Study Design: Rats were given a single 0.25 mg/kg nasal dose of ^{14}C-AA-673 and sequential blood samples were obtained as well as feces and urine over the 24 hour study period. Animals were subjected to whole body autoradiography.

RESULTS

The ^{14}C-AA-673 was rapidly absorbed with a Tmax of 5 minutes followed by a biphasic decline. Whole body autoradiography indicated the radioactivity to be widely distributed in tissues. Excretion patterns indicated rapid elimination within 48 hours with 36 and 67% of the dose appearing in the urine and feces respectively. Analysis of the metabolites indicated that glucuronidation and oxidation of the isopropyl group occurred. This metabolic pattern is similar to the one after oral administration.

Summary: Absorption after nasal dosing is rapid. The drug does not appear to accumulate in tissues and is rapidly eliminated in the feces and urine. This study is acceptable for its intended use.

3) Intraocular Penetration of AA-673 Ophthalmic Solution, An Antiallergic Agent (Report # AA-673/S-DK02)

Laboratory: [ ]

Number of animals: total of 39 used in groups of 3 to 6

Animal Strain: Japan White Rabbit; males

Dose Level: 50 ul of a 0.25% ophthalmic solution of drug was instilled into both eyes

Route: Instillation into the conjunctival sac of the eye

Study Design: The animals were dosed and approximately 4 mL of blood was taken at the following times- 20 and 40 minutes and 1, 2, 4, 6, 8, 24 and 48 hours after instillation. Immediately after the collection of blood the animal was sacrificed. The eyeball together with the conjunctivae and extraocular muscle was removed. The conjunctivae was removed and a sample of anterior chamber aqueous was collected. The eyeball was quick frozen and cut into anterior and posterior segments. The lens, vitreous body, retina, choroid and iris and ciliary body were removed. All the tissues including blood were assayed using high pressure liquid chromatography after preparation.

RESULTS

The maximum concentration in the blood was reached in 20 minutes and then it declined thereafter. The concentration time course in each ocular tissue showed that after reaching their respective peaks, the concentrations declined exponentially and then slowly after 24 hours in the cornea and after 8 hours in the conjunctivae and anterior sclera. Only a low concentration was found in the retina and choroid up to 2 hours post instillation. After 8 hours the concentration was below the limit of detection in these tissues.

Summary: AA-673 penetrates into the cornea and conjunctivae rapidly after instillation and then disappears slowly. The drug would be expected to show sustained efficacy toward diseases of the external segment of the eye.

Summary:

Amlexanox was not a sensitizer and did not cause irritation of the mucous membrane of the mouth in a 7 day hamster cheek pouch irritatioin study. In a 6 month oral rat and dog evaluation the no effect level was 100 and 30 mg/kg respectively for hepatotoxicity which was considered to be the target organ. This was shown to be reversible in the dog in a recovery study. Life time studies giving the drug by the dietary route in the rat and mouse indicated the drug was not carcinogenic. This is indicated on the label. The no effect level in the mouse study was 30 mg/kg for toxic nephrosis and in the rat study was 80 mg/kg for biliary changes- cystic dilation, calculus formation, inflamation of the extrahepatic bile duct, cholangitis and pericholangitis. No adverse effect was noted in fertility and general reproductive performance studies in the rat, teratology studies in the rat and rabbit and peri and post-natal studies in the rat up to a 300 mg/kg dose given orally. Amlexanox was not mutagenic in the Ames or mouse micronucleus test.

The mean mg of Amlexanox per patient per day is approximately 0.2 mg/kg/day for a 60 kg person (see attachment from Chemex dated June 14, 1995). No adverse effect was noted on general reproductive performance and fertility in rat and rabbit studies up to 300 mg/kg amlexanox. This would give a no effect level of approximately 1500 times the projected human dose, which is indicated on the label.

Absorption studies in the rat, mouse, guinea-pig and dog indicated the oral bioavailability to be about 50%. The intestine was the major site of absorption. The metabolic characteristics of the drug in plasma were about the same in the rat, mouse, guinea-pig and dog as they were in man following an oral dose. The rat was the only species that conjugated the material. The drug was highly bound to plasma proteins and there was no dependence of binding on the drug concentration. ^{14}C studies demonstrated no specific tissue accumulation (following a single or multiple doses) except in the organs responsible for excretion of the compound and its metabolites. The drug crossed the placental barrier and resided in the milk of lactating dams. Amlexanox was not a hepatic enzyme inducer. In the rat, mouse, guinea-pig, dog and man after oral dosing amlexanox was present in the feces (major component) and the urine(hydroxylated metabolite, minor component). After oral administration of the radiolabelled drug almost all of it was eliminated within 120 hours in rats, mice, guinea-pigs and dogs.

Conclusion:

The use of amlexanox for the treatment of aphthous ulcers on the oral mucosa as proposed would appear to be safe with respect to the results of the preclinical animals studies.

RECOMMENDATIONS

The question of projected human daily dose and the addition of wording to the package insert to instruct the patient as to what constitutes a dab-i.e. appropriate dose/ulcer was answered on June 14, 1995 by Dr. M. Charney. This NDA is approvable from the preclinical standpoint.

John Wedig, Ph.D.
Toxicologist

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