

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
22368Orig1s000

PHARMACOLOGY REVIEW(S)

INTEROFFICE MEMO

TO: NDA 22-368
Sequence number/date/type of submission: S000/February 26, 2009/original

FROM: Jean Q Wu, M.D., Ph.D.
Acting Pharmacology/Toxicology Supervisor
Division of Pulmonary and Allergy Products

DATE: November 17, 2009

I concur with primary pharmacology/toxicology reviewer's (Dr. Luqi Pei) recommendation that the toxicity profile of Aridol (inhaled Mannitol) has been adequately evaluated and the drug product should be approved with suggested labeling changes from a nonclinical perspective.

Mannitol has been used as a nutrient and/or dietary supplement and as an active/inactive ingredient in many drug products. Inhalation of D-mannitol provokes bronchoconstriction through inducing histamine release from mast cells. Aridol (inhaled Mannitol) delivered into the airways is responsible for increasing the osmolarity in the airways. It has been developed as a diagnostic agent for bronchial hyper-responsiveness. The intended use of Aridol is a single dose use up to 635 mg/patient under the supervision of healthcare providers.

The mannitol toxicology profile by non-inhalation use has been well established. Based on the previous communication with the Agency, the applicant completed the toxicology program of Aridol focusing on the effects of inhalation of mannitol on respiratory tract. The nonclinical inhalation toxicology studies were conducted in rats up to 3 months and in dogs up to 6 months. The target organ of toxicity for inhaled mannitol was identified as the respiratory system, i.e. increased incidences of alveolitis (high dose male only) and macrophage aggregation in the 3-month rat study, and cough, laryngeal ulceration and sinus histiocytosis (with ¼ incidence, minimal severity, and reversibility) in the 6-month dog study. The treatment related findings of the nasal cavity in rats were not considered relevant to human.

Mannitol was considered non-carcinogenic based on the 2-year dietary carcinogenicity studies of D-mannitol in F344/ N rats and B6C3F1 mice conducted under National Toxicology Program. In these studies, no evidence of carcinogenicity was found in either rats or mice of each sex, which were fed diets containing 0%, 2.5% or 5% D-mannitol (corresponding to doses of 0, 3,750, 7,500-mg/kg/day) for 103 weeks.

Mannitol was considered non-genotoxic based on the negative results in the studies conducted under National Toxicology Program. These studies included bacterial gene mutation assays, an *in vitro* mouse lymphoma assay, an *in vivo* mouse micronucleus assay, a dominant lethal assay in rats, an *in vivo* rat bone marrow study and an *in vitro* study using WI-38 human cells.

Mannitol was not considered teratogenic. According to the Joint FAO/WHO Expert Committee on Food Additives Monograph on Mannitol, "Mannitol was tested for teratogenic effects in mice,

rats, and hamsters. Pregnant mice and rats given oral doses of mannitol up to 1.6 g per kg for 10 consecutive days and hamsters up to 1.2 g per kg for 5 consecutive days showed no effects on maternal or fetal survival.” Since the hamster study covered only small fraction of the organogenesis period which was not considered adequate, the information from the hamster study was not recommended to be included in the suggested labeling.

The proposed specifications of impurities, extractables and leacheables in Aridol product were evaluated and considered acceptable by Dr. Pei in a separate Chemistry Consultation review dated August 6, 2009.

For the suggested labeling changes, refer to the labeling review by Dr. Pei dated November 13, 2009.

Jean Q Wu, M.D., Ph.D.
Acting Pharmacology/Toxicology Supervisor

Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-22368

ORIG-1

PHARMAXIS LTD

ARIDOL POWDER FOR
INHALATION

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/s/

JEAN Q WU
11/17/2009



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-368
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: February 26, 2009
PRODUCT: Aridol (Mannitol)
INTENDED CLINICAL POPULATION: Test kit for bronchial hyper-responsiveness in patients 6 years of age and older
SPONSOR: Pharmaxis Ltd.
DOCUMENTS REVIEWED: Not applicable
REVIEW DIVISION: Division of Pulmonary and Allergy Products
PHARM/TOX REVIEWER: Luqi Pei, Ph.D.
PHARM/TOX SUPERVISOR (acting): Jean Wu, M.D., Ph.D.
DIVISION DIRECTOR: Badrul Chowdhury, M.D., Ph.D.
PROJECT MANAGER: Miranda Raggio

Date of review submission to (DARRTS): November 13, 2009

LABELING REVIEW

Edits to nonclinical sections of the proposed labeling were recommended. The edits were made to ensure that the labeling will conform to the Draft Guidance for Industry “Labeling for Human Prescription Drug and Biological Products – Implementing the New Content and Format Requirements” (January 2006). The edits were limited to Sections 8.1 (Pregnancy) and 13 (Nonclinical Toxicology).

Aridol is a diagnostic agent. The review considered the best labeling format to describe the available nonclinical information of mannitol. Considerations were prompted by the uniqueness of the Aridol indication: one-time only and under the supervision of health care provider. It was felt initially that a full description of the available nonclinical data may not be applicable to the product because of its limited use. During the deliberation, the review compared the nonclinical information described in Aridol that is currently marketed in the United Kingdom and in Provocholine (methacholine) that is currently marketed in the United States (Table 2, page 6). The review concluded the full compliance with the PLR format will not provide excessive nonclinical information.

All nonclinical studies in support of the Aridol labeling were from the literature. The studies were completed by the governmental agencies such as the National Toxicology Program and the FDA. Results of the studies have been briefly described in Section 2.6.6.1 (Overall Toxicology Summary) of the original review completed on October 30, 2009. The review discusses the necessity of the information being included in the labeling.

The edits to the proposed labeling are reflected in three aspects: changing contents of Section 13.2, adding animal-to-human dose ratios to Sections 8.1 and 13, and editorial changes. No edits were recommended for Section 10 because it contained no nonclinical information.

CONTENT:

Sections 8.1 (Pregnancy):

(b) (4)

(b) (4)

5 pages of draft labeling has been withheld in full as B(4) CCI/TS immediately following this page

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22368	ORIG-1	PHARMAXIS LTD	ARIDOL POWDER FOR INHALATION

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/s/

LUQI PEI
11/13/2009

JEAN Q WU
11/13/2009



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

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DIVISION DIRECTOR: Badrul Chowdhury, M.D., Ph.D.
PROJECT MANAGER: Miranda Raggio

Date of review submission to DARRTS: October 30, 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Approval of Aridol is recommended from the nonclinical perspective. The applicant has completed a bridging toxicology program evaluating the toxicity profile of inhaled mannitol. The program consisted of inhalation toxicity studies up to 3 and 6 months in rats and dogs, respectively. The studies identified the respiratory system as the target organs of toxicity for inhaled mannitol. The organs did not show any neoplastic or pre-neoplastic findings. This toxicology program has satisfied the nonclinical prerequisite for the approval of Aridol.

B. Recommendation for nonclinical studies

None.

C. Recommendations on labeling

Labeling review will be completed at a later time after the review team decides what labeling format will be used for the product.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The target organs of toxicity of inhaled mannitol are the respiratory system. Inhalation toxicity studies up to 3 and 6 months in rats and dogs, respectively, were conducted. Increased incidences of microphage aggregation and alveolitis were observed in a 3-month study in rats. Coughing, laryngeal ulceration and sinus histiocytosis were observed in a 6-month study in dogs. There were no any neoplastic or pre-neoplastic findings in the respiratory tract. The available nonclinical data in the literature show that mannitol was non-carcinogenic, non-genotoxic and non-teratogenic.

B. Pharmacologic activity

Aridol inhalation may provoke bronchoconstriction in some patients. D-mannitol inhalation results in hyper-osmosis in the airways. The hyper-osmosis induces histamine release from mast cells. Histamine in turn provokes bronchoconstriction.

C. Nonclinical safety issues relevant to clinical use

None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

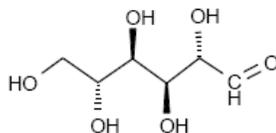
NDA Number: 22-368
Review Number : 1
Sequence number/date/submission type: 000/27-FEB-2009/Original NDA
Information to the Sponsor: Yes (), No (x)
Sponsor/or Agent: Pharmaxis Ltd, 1840 Gateway Dr., San Mateo, CA 94404

Manufacturer of the Drug Substance: (b) (4)

Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Drug Products
Review Completion Date: October 30, 2009

Drug:

Trade Name: Aridol
Generic Name: D-Mannitol
Code Name: None
Chemical Name: 1,2,3,4,5,6-hexanehexol, or cordycepic acid
Structure:



CAS Register Number: 69-65-8
Mole File Number: Not available
Molecular Form and Weight: C₆H₁₄O₆/182.2

Relevant IND/NDAs: MDF# (b) (4) IND 70,277

Drug Class: Diagnostic (Broncho-provocation) agent

Intended clinical population: Asthmatic patients 6 years of age and older

Route of Administration: Inhalation

Clinical Formulations: Capsules filled with 5, 10, 20 and 40 mg of D-mannitol powder. Mannitol will be delivered by a dry powder inhaler.

Proposed Clinical Dose: up to 635 mg/patient, single time use.

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

Studies reviewed within this submission: None.

Studies not reviewed within this submission:



(b) (4)

(b) (4)

Drug History:

This NDA application was developed under IND 70,277 under which the original application was filed on November 22, 2004. As the Sponsor of the IND, Pharmaxis is developing mannitol dry powder (b) (4) (b) (4) are Aridol (b) (4)



The Aridol NDA is currently under review (b) (4)

Pharmaxis and DPAP have held a number of meetings to discuss the development of mannitol programs. Four meetings dealt with nonclinical issues of Aridol development: the 19-NOV-04 Pre-IND meeting, the 16-JUN-05 guidance meeting, the 15-FEB-06 (b) (4)



Minutes of the meetings are available in DARRTs.

Through these meetings, Pharmaxis and the Division agreed on the following regarding the nonclinical requirement of mannitol inhalation (b) (4)

1. Two 14-day inhalation toxicity studies of mannitol in two animal species (one in each species) are needed for the registration of Aridol



(b) (4)

3. No studies of carcinogenicity, genetic toxicity, reproductive and developmental toxicity are needed for (b) (4) Aridol (b) (4) (b) (4)

Pharmaxis has completed mannitol inhalation toxicity studies up to 3 and 6 months in rats and dogs, respectively. These studies have been previously submitted and reviewed under IND 70,277. See Pharmacology and Toxicology Reviews (Appendices) completed by Dr. Luqi Pei on November 29, 2007 (Review# 4), July 21, 2006 (Review# 3), and March 18, 2005 (Review# 1).

DPAP requested in the pre-NDA meeting that Pharmaxis address the safety qualifications of impurities, leachables and extractables in the Aridol NDA. The current review will not address these issues because they were addressed separately through Chemistry Consultation Requests and Reviews. A Chemistry Consultation Request was filed by Dr. Deepika Arora on June 4, 2009 and a Pharmacology and toxicology Review of the Request was completed by Dr. Luqi Pei on August 5, 2009. Refer to the appropriate documents for additional information.

This NDA was submitted on February 26, 2009 (letter date) and accepted by the Agency on March 1, 2009. DPAP held a filing meeting on April 13, 2009. Dr. Luqi Pei completed a nonclinical fileability review on April 15, 2009.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Inhalation of D-mannitol provokes bronchoconstriction through inducing histamine release from mast cells. No pharmacology studies were performed under IND 70,277 or the current NDA. A literature review was performed as requested in the pre-NDA meeting. The review indicated that mannitol could induce the release of histamine from cultured human lung mast cells and blood basophils. The histamine release was apparently attributed to the hyperosmosis (2 – 3x normal) associated with mannitol. Mannitol treatment also enhances histamine release from mast cells induced by IgE. In the current application, inhaled mannitol delivered into the airways is responsible for inducing an osmotic gradient into airways.

2.6.2.2 Primary pharmacodynamics

Not applicable because no data was submitted.

2.6.2.3 Secondary pharmacodynamics

Not applicable because no data was submitted.

2.6.2.4 Safety pharmacology

Not applicable because no data was submitted.

2.6.2.5 Pharmacodynamic drug interactions

Not applicable because no data was submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable because no data was submitted.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No separate pharmacokinetic studies of inhaled mannitol were conducted in animals. Mannitol levels in the plasma and bronchoalveolar lavage fluid (BALF) were measured in some inhalation toxicity studies. Mannitol does-concentration relationship was seen in the plasma/serum but not in BALF. Figure 1 (below) shows the time course of mannitol plasma/serum concentration in dogs (Report No. 667108).

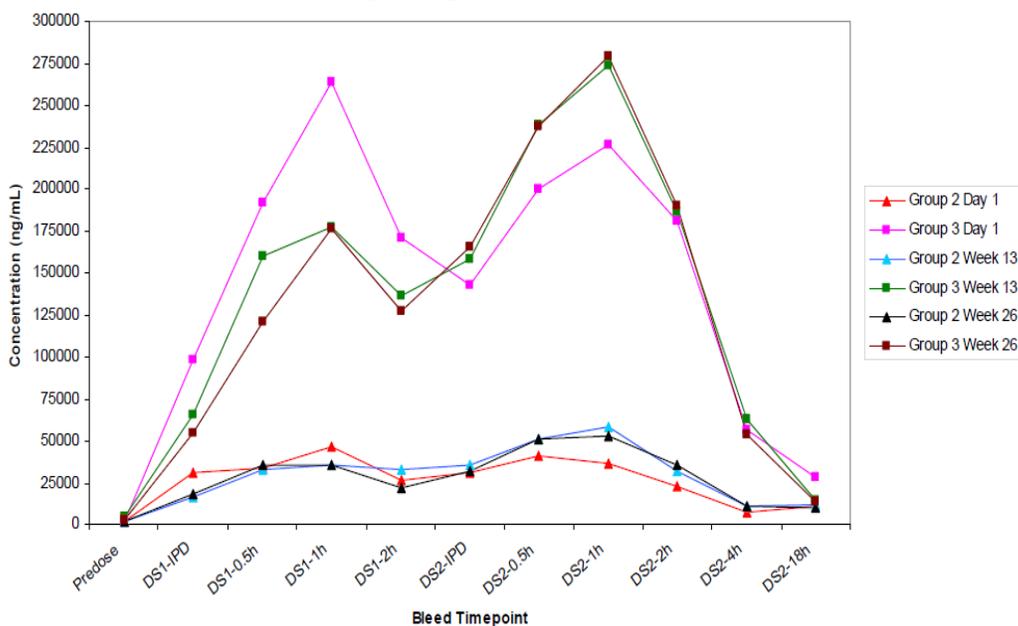


Figure 1 Serum mannitol concentrations after inhalation exposures in dogs. The estimated pulmonary deposits were 43 and 178 mg/kg/day for Groups 2 and 3, respectively. Each daily dosing consisted of two episodes (60 minutes each) of exposures with an interval of at least 2 hours between them. DS1 and DS2 indicate first and second episodes of the day, respectively (Source: P/T review #4, page 7).

Mannitol concentrations in BALF were determined in both rats and dogs. In a 13-week inhalation study in rats (Study XIS 005/0413185), the mean mannitol level in BALF was 0, 3.8 and 3.2 $\mu\text{g/ml}$ in the control, LD (pulmonary deposit dose, 12.4 mg/kg/day) and HD (pulmonary deposit dose, 21.0 mg/kg/day) groups, respectively. In the 26-week dog study

(Report No. 667108), BLAF mannitol concentrations were below the limit of quantitation (0.1 $\mu\text{mol/L}$) for both low (43 mg/kg/day) and high dose (178 mg/kg/day) groups.

2.6.4.2 Methods of Analysis

Mannitol levels were analyzed by liquid chromatography-tandem mass spectrometry using TurboIonSpray in positive ion mode. The data were quantified by comparing peak area ratios (test item to internal standard) of the samples to the appropriate calibration lines using weighted (1/x²) least squares regression. The assay lower limit of quantification (LLOQ) for Mannitol in dog serum was 100 ng/mL. The method was found to give linear calibration lines for Mannitol in dog serum and lung lavage wash samples over the range *ca* 100-100000 ng/mL.

2.6.4.3 Absorption

Not applicable because no data was submitted.

2.6.4.4 Distribution

Not applicable because no data was submitted.

2.6.4.5 Metabolism

Not applicable because no data was submitted.

2.6.4.6 Excretion

Not applicable because no data was submitted.

2.6.4.7 Pharmacokinetic drug interactions

Not applicable because no data was submitted.

2.6.4.8 Other Pharmacokinetic Studies

Not applicable because no data was submitted.

2.6.4.9 Discussion and Conclusions

Not applicable because no data was submitted.

2.6.4.10 Tables and figures to include comparative TK summary

Not applicable because no data was submitted.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable because new data was submitted.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Mannitol is non-carcinogenic, non-mutagenic and non-teratogenic. Comprehensive summaries of D-mannitol toxicology are available. See the National Toxicology Program Technical Report No. 236 (1982) at http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr236.pdf and the Joint FAO/WHO Expert Committee on Food Additives Monograph on Mannitol at <http://www.inchem.org/documents/jecfa/jecmono/v21je10.htm>.

Mannitol is used as a nutrient and/or dietary supplement and an ingredient in numerous drug products. As a dietary supplement, mannitol is considered generally recognized as safe [GRAS, 21CFR§582.5470]. Medically, mannitol is used as active and inactive ingredients. As an active ingredient, mannitol is a laxative and diuretic. As an inactive ingredient, mannitol is an excipient in numerous products. The routes of administration of these products included oral, parenteral (e.g., IV, and IP), topical and inhalation. Exubera® (an insulin product, NDA 21-868, approved on January 27, 2006), however, is an only inhalation product that uses a small amount of mannitol as an inactive ingredient. The toxicology program of Aridol and Bronchitol focused on effects of inhaled mannitol on the respiratory system due to the extensive nonclinical data available on mannitol.

General Toxicology:

The mannitol toxicology program consists of inhalation toxicity studies up to 3 and 6 months in rats and dogs, respectively. Table 1 (below) presents an overview of these toxicity studies. The studies identified the respiratory system as the target organ of toxicity for inhaled mannitol. Increased incidences of microphage aggregation and alveolitis were observed in a 3-month study in rats. Coughing, laryngeal ulceration and sinus histiocytosis were observed in a 6-month study in dogs. The NOAEL in the 6-month inhalation study in dogs was 43 mg/kg/day (pulmonary deposits).

Table 1 Overview of Inhalation Toxicity Studies of Mannitol

Study #	Species	Duration	Mannitol (mg/kg/day) ^a	NOAEL
26482/666958 ^b	Rat	7 days	57.3, 97.9	None
XIS 002/033951	Rat	2 weeks	0, 0.9, 2.5, & 6.9	6.9
XIS 005/0413185	Rat	13 weeks	0, 12.4, 21.0	None ^c
26050/666386	Dog	2 weeks	0, 25, 100, 197	None ^d
26966/667108	Dog	26 weeks	0, 43, 178	43 ^e

a. Estimated pulmonary deposits. The pulmonary deposit was considered 10% (rat) and 25% (dog) of the inhaled dose (reported).

b. A non-GLP compliant investigative dose-ranging study that did not examine the lung tissue microscopically.

c. The review is in agreement with the study report regarding the NOAEL determination.

d. The report states that the 197 mg/kg/day dose is “well-tolerated.”

- e. The study report and the DPAP disagree on the NOAEL. DPAP considers the NOAEL the low dose (43 mg/kg/day, pulmonary deposition) but the study report deemed the high dose (710 mg/kg/day, inhaled dose) as the NOAEL.

Rats:

Three inhalation toxicity studies of mannitol were completed in rats. The treatment duration of these studies was 1, 2 and 13 weeks, respectively. In a 3 month study, rats at ≥ 12.4 -mg/kg/day (pulmonary dose) mannitol showed increases in the incidence of macrophage aggregation in the lung and eosinophilic inclusion in olfactory epithelium in nasal cavity. Rats receiving 21.0-mg/kg/day mannitol also showed an increase in the incidence of alveolitis in the lung.

In a 7-day non-GLP dose-ranging study, Sprague-Dawley rats (5/sex/dose) were exposed nose-only to approximately 5 or 9 mg of mannitol/L of air for 120 to 240 minutes/day for 7 days. The rats were sacrificed immediately after the last exposure. The amount of mannitol delivered to the lung was determined by measuring mannitol concentrations in the bronchoalveolar fluid (3/sex/dose). The respective estimated achieved dose of mannitol was 573 and 979 mg/kg/day for the low-dose and high-dose groups, respectively. The respective mean mannitol concentration in BALF for the low and high dose groups was 36.7 and 42 $\mu\text{g/ml}$ in males and 43.6 and 33.4 $\mu\text{g/ml}$ in females. Necropsy did not reveal any treatment-related effect. Microscopic examination was not done.

In a 2-week study (XIS 002/033951), CD-1 rats (10/sex/dose) were given via nose-only inhalation pulmonary deposited doses of 0, 0.9, 2.5 and 6.9 mg/kg of mannitol for 14-days. Histological evaluations of the respiratory system were done in every group. The remaining organs were examined in the control and high-dose groups only. No significant, treatment-related effects were observed. The NOAEL was 6.9 mg/kg/day.

In a 13-week study (26050/666386), Sprague-Dawley rats (10/sex/group) were given via nose-only inhalation air (C), 12.4 (LD), or 21.0 (HD) mg/kg/day (pulmonary deposition) for 13 weeks. Additional rats (5/sex) were included in the control (RC) and high dose (RHD) groups to evaluate reversibility of lesions after a recovery period of 4 weeks. The duration of exposure was 180 minutes/day. D-mannitol concentrations were 0, 1.83 or 2.89 mg/L for the control, LD and HD groups, respectively. The estimated pulmonary deposition of D-mannitol was 0, 12.4 and 21.0 mg/kg/day for the control, LD and HD groups, respectively. D-mannitol contents in the bronchoalveolar fluid were approximately 0, 3.8 and 3.2 $\mu\text{g/ml}$ for the control, LD and HD groups, respectively. Both the LD and HD females showed statistically significant decreases in body weight gain (approximately 20%). Clinical pathology examinations revealed minimal decreases (approximately 50% or less) in white blood cell numbers and increases (12-30%) in serum phosphorus in the HD group. Microscopic examinations revealed increases in the incidence of alveolar macrophage aggregation and alveolitis. The respective incidence of alveolar macrophage aggregation in the C, LD, HD, RC and RHD was 3/10, 6/10, 9/10, 0/2 and 1/2 in females and 4/10, 5/10, 3/10, 1/1 and 3/4 in males. The increase in the incidence of alveolitis was observed in the high-dose male only (incidence: 0/10-C, 0-/10-MD, 1/10-HD, 0/1-RC, and 2/4-RHD, respectively). The most significant, treatment-related effect, but of no safety concern for the intended use of mannitol, was seen in the nasal cavity. Both the low and high-dose rats showed increases in the incidence of eosinophilic inclusion in olfactory epithelium of the

nasal cavity (Incidence: 3/20-Air, 15/20-LD, and 13/20-HD). This finding is not considered relevant to humans because minimal nasal exposure is expected from the intended clinical use. The study failed to establish NOAEL.

Dogs:

Two inhalation toxicity studies of mannitol were completed in dogs. The treatment duration of these studies was 2 and 26 weeks, respectively. Dogs receiving 178-mg/kg/day (pulmonary dose) for 26 weeks mannitol showed increases in the incidence of minimal laryngeal ulceration and sinus histiocytosis in mediastinal lymph node.

In a 2-week study (XIS 005/0413185), beagle dogs (3/sex/group) were exposed by inhalation via a face mask to air (C), 25 (LD), 100 (MD) or 197 (HD) mg/kg/day of D-mannitol (pulmonary deposition) for 14 days. Coughing occurred during and immediately after dosing in all treated groups (Incidence for M + F: 0/6-C, 1/6-LD, 4/6-MD and 4/6-HD). Spongy (4/6) and froth-filled lung (3/6) were reported in the HD group during necropsy. Microscopic examination revealed the following: lung congestion/hemorrhage (2/6-HD), and pigment in submandibular lymph node (3/6-HD); bronchoalveolitis (2/3 apiece for MD and HD males); peribronchiolar infiltration (Incidence: 0/3-C, and 3/3 apiece in LD, MD and HD in males; and 1/3-C, 1/3-LD, 2/3-HD and 0/3-HD in females); foamy alveolar macrophages in all treated females and HD males (respective incidence in control, LD, MD and HD: 1/3, 0/3, 0/3/ and 2/3 in males and 0/3, 2/3, 2/3 and 1/3 in females) and inflammatory foci and focal hyperplasia in trachea carina (1/3-HD female). The high dose males and females also showed increases in lung weight. The study did not establish a NOAEL. The findings, however, were absent in the 26-week study at a dose (178 mg/kg/day, see below) similar to the high dose of the 2-week study.

The 26-week study (CRL Study #667108) was conducted to evaluate the toxicity of inhaled mannitol. Beagle dogs (4/sex/dose) were exposed via a face mask to air, 43 or 178 mg/kg/day of D-mannitol for 26 weeks. The control and HD groups also included two additional dogs per sex to study reversibility of any lesions after a recovery period of 4 weeks. In addition to the routine toxicological evaluations, the study measured respiratory parameters, EKG, chest auscultation, and the cell numbers in the bronchoalveolar lavage fluid. Coughing occurred in both LD and HD groups while the histological changes occurred in the HD group only. Histological changes in the HD group included laryngeal ulceration and sinus histiocytosis in the mediastinal lymph node; but incidences were low (1/4 in each sex) and severity was minimal. Both lesions were reversible after a recovery period of 4 weeks. The review NOAEL was 43 mg/kg/day.

Genetic toxicology:

Mannitol is non-genotoxic. Studies conducted by the National Toxicology Program showed that D-mannitol tested negative in bacterial gene mutation assays, an *in vitro* mouse lymphoma assay, and an *in vivo* mouse micronucleus assay. Mannitol also tested negative in a dominant lethal assay in rats, an *in vivo* rat bone marrow study and an *in vitro* study using WI-38 human cells.

Carcinogenicity:

Mannitol is non-carcinogenic. The National Toxicology Program conducted 2-year dietary carcinogenicity studies of D-mannitol in F344/ N rats and B6C3F1 mice. Groups of 50 rats and 50 mice of each sex were fed diets containing 0%, 2.5% or 5% D-mannitol for 103 weeks. These concentrations correspond to nominal doses of 0, 3,750, 7,500-mg/kg/day. No evidence of carcinogenicity was found in either rats or mice of either sex.

Reproductive Toxicity:

Mannitol is non-teratogenic. According to the Joint FAO/WHO Expert Committee on Food Additives Monograph on Mannitol, “Mannitol was tested for teratogenic effects in mice, rats, and hamsters. Pregnant mice and rats given oral doses of mannitol up to 1.6 g per kg for 10 consecutive days and hamsters up to 1.2 g per kg for 5 consecutive days showed no effects on maternal or fetal survival.” <http://www.inchem.org/documents/jecfa/jecmono/v21je10.htm>.

Local Tolerance:

Mannitol tested negative in an eye irritation study in rabbits (Report No. X1S 003/134081) and an *in vitro* corneal opacity and permeability study in bovine eyes (Report No. X1S 003/034088).

2.6.6.2 Single-dose toxicity

Not applicable because no new data were submitted.

2.6.6.3 Repeat-dose toxicity

The application contained inhalation toxicity studies of mannitol up to 3 and 6 months in rats and dogs, respectively. These studies have been reviewed previously by Dr. Luqi Pei under IND 70,277. See Pharmacology and Toxicology Reviews 1, 2 and 4 (Appendix).

2.6.6.4 Genetic toxicology

The applicant submitted 3 reports of genetic toxicity tests of mannitol. These tests were conducted by the National Toxicology Program. The tests included a bacterial reverse gene mutation assay (NTP 821315 and others), an *in vitro* mouse lymphoma assay (NTP 315204), and an *in vivo* mouse micronucleus assay (NTP 90264). Each report concluded that mannitol tested negative in the assay. No detailed review of the reports is necessary based on the following considerations. It appeared that at least the bacterial gene mutation had been considered previously during the assessment of D-mannitol carcinogenicity. The National Toxicology Program Technical Report 236 (1982) which concludes that mannitol is non-carcinogenic states:

“D-Mannitol was not mutagenic for *Salmonella typhimurium* G-46 or TA 1530 or for *Saccharomyces cerevisiae* D-3 when tested without metabolic activation (Green, 1977). Mutagenesis testing results of the National Toxicology Program at three different laboratories showed that D-mannitol was not mutagenic for *Salmonella typhimurium* TA 98, 100, 1535, and 1537 (NTP Tech. Bull., 1981). Results of a dominant lethal assay in rats at doses of 20, 200,

2,000, and 5,000 mg/ kg of D-mannitol by gavage were negative. No increases in chromosome aberrations were observed in an *in vivo* rat bone marrow study or in an *in vitro* study using WI-38 human cells (FDA, 1974). (http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr236.pdf, page 15)

It also appears that the mouse lymphoma cell assay and the *in-vivo mouse* micronucleus assay (NTP 90264) were done recently to reflect the ICH standard test battery of genotoxicity testing. Each report concluded that D-mannitol tested negative in the assay. The following information is provided for the purpose of documentation.

D-Mannitol Bacterial Reverse Mutagenesis Test [NTP Studies 315204 (1981), 79044 (1981), 4632 (1979), and 27050 (1981)]

The Four histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and 1537) were used to evaluate the mutagenic potential of the test item D-mannitol both in the presence and absence of metabolic activation using the preincubation method. No statistically or biologically significant increases in the number of revertants were noted in any strain of the bacterium, either with or without metabolic activation (S9). D-mannitol was considered negative under the experimental conditions.

D-Mannitol Mouse Lymphoma TK Assay (NTP Study 851315)

Mannitol at concentrations of up to 5000 µg/mL failed to consistently increase the mutation frequencies in mouse lymphoma L5178Y cells in the presence or absence of metabolic activation. D-mannitol was considered negative in the mouse lymphoma/TK+/- assay under the testing conditions.

D-Mannitol *In-vivo* Micronucleus study (NTP Study 90264)

Male Balb/C mice were given D-mannitol up to 3000 mg/kg/day (ip) for 3 consecutive days did not cause any significant increase in structural chromosome aberrations in bone marrow or circulating polychromatic erythrocytes in male mice. D-mannitol was considered negative in the mouse micronucleus assay under the testing conditions.

2.6.6.5 Carcinogenicity

No new data were submitted. The applicant submitted reports of 2-year carcinogenicity studies of D-mannitol in F344/ N rats and B6C3F1 mice completed by the National Toxicology Program (Technical Report 236, 1982). Groups of 50 rats and 50 mice of each sex were fed diets containing 0%, 2.5% or 5% D-mannitol for 103 weeks. No evidence of carcinogenicity was found in either rats or mice of either sex. It was concluded that D-mannitol is non-carcinogenic based on the NTP report.

2.6.6.6 Reproductive and developmental toxicology

No data was submitted. Information in Section 2.6.6.1 was based on the Joint FAO/WHO Expert Committee on Food Additives Monograph on Mannitol (<http://www.inchem.org/documents/jecfa/jecmono/v21je10.htm>).

2.6.6.7 Local tolerance

The application contained an eye irritation study in rabbits (Report No. X1S 003/134081) and an *in vitro* corneal opacity and permeability study in bovine eyes (Report No. X1S 003/034088). Dr. Luqi Pei reviewed both studies previously in a pharmacology and toxicology review completed on March 18, 2005 under IND 70,277. See Appendix 1 for the review.

2.6.6.8 Special toxicology studies

Not applicable because no data was submitted.

2.6.6.9 Discussions and Conclusion

The application has adequately evaluated the toxicity profile of inhaled mannitol. The Division previously determined that a 6-month inhalation toxicity study of the compound in the most appropriate species is adequate to support registrations of (b) (4) Aridol (b) (4) (b) (4). This determination was based on the extensive use of D-mannitol as an excipient in non-inhalation drug products. Pharmaxis has agreed to the non-clinical prerequisite (ref.: IND 70,277, minutes of meetings on June 16, 2005, and February 15, 2006, a telephone conference on October 11, 2006; and the letter on July 26, 2006). Specifically, the agreements were:

- No additional or new studies of genetic toxicity, carcinogenicity, reproductive and developmental toxicity of mannitol are needed.
- (b) (4)
- Additional studies could be needed if these studies reveal safety concerns.

The above testing strategy was formed after considering the available information of D-mannitol. As being alluded to earlier, mannitol is a nutrient and/or dietary supplement and an ingredient in numerous drug products. As a dietary supplement, mannitol is GRAS compound. Medically, mannitol is used as active and inactive ingredients. As an active ingredient, mannitol is a laxative and diurectic. As an inactive ingredient, mannitol is an excipient in numerous products. The routes of administration included oral, parenteral, topical, as well as inhalation administration. Consequently, comprehensive summaries of D-mannitol toxicology are available.

Pharmaxis has submitted inhalation toxicity studies of mannitol up to 3 and 6 months in rats and dogs, respectively. These studies have been summarized in Section 2.6.6.1 (Overall Toxicology Summary, page 8). The studies identified the respiratory tract as the target organs of toxicity of inhaled mannitol. They did not reveal any neoplastic or pre-neoplastic findings in the respiratory system. The completed toxicology program has adequately evaluated the toxicity profile of inhaled mannitol and no additional toxicity studies are needed. The following discussions focus on the information relevant to the labeling review of Aridol.

There are currently numerous mannitol drug products approved and currently marketed in the US. These products are exclusively for the intravenous route of administration. Their labels do not describe the carcinogenic, genotoxic and reproductive toxicity potential of mannitol due to historic reasons: nonclinical studies were conducted after the approval of the listing reference product. Aridol is for the inhalation route of administration. It is reasonable to include the available nonclinical information in its labeling.

Carcinogenicity: Dietary mannitol is non-carcinogenic in laboratory animals. The National Toxicology Program conducted 2-year dietary carcinogenicity studies of D-mannitol in F344/N rats and B6C3F1 mice. Groups of 50 rats and 50 mice of each sex were fed diets containing 0%, 2.5% or 5% D-mannitol for 103 weeks. These concentrations correspond to nominal doses of 0, 3,750, 7,500-mg/kg/day mannitol. No evidence of carcinogenicity was found in either rats or mice of either sex. See the NTP Technical Report No. 236 (1982) at http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr236.pdf. The nominal dose was provided by the Joint FAO/WHO Expert Committee on Food Additives Monograph on Mannitol at <http://www.inchem.org/documents/jecfa/jecmono/v21je10.htm>.

Additional studies have been completed recently using alternative animal models. An example is a 9-month dietary study in transgenic DNA repair-deficit Xpa^{-/-} mice and double transgenic Xpa^{-/-} p53^{+/-} mice by Lina et al (*Toxicol Pathol*, 2004;32:192-201). Mannitol doses were 2%, 5% and 10% of diet. The study did not reveal any carcinogenic potential of mannitol. The study contained other 3 compounds: haloperidol, reserpine and phenacetin. The study assumed that D-mannitol was non-carcinogenic and used mannitol as a negative control.

A shortcoming of the available data is that the carcinogenicity studies may not reflect the effect of inhaled mannitol on the respiratory system. This concern has been alleviated by the finding that inhalation toxicity studies up to 6 months in treatment duration submitted did not reveal any evidence of pre-neoplastic or neoplastic change. The carcinogenicity evaluation of the inhaled mannitol is now considered adequate.

It is recommended that the Aridol labeling includes the NTP studies only. This recommendation is consistent with the applicant's proposal. The Lina study should not be used because it considered D-mannitol non-carcinogenic in the study design.

Mutagenicity: Assays assessing mutagenic potential and their results were summarized in Section 2.6.6.4 (page 11). All assays were conducted by the National Toxicology Program and FDA. The tests included a bacterial reverse gene mutation assay, an *in vitro* mouse lymphoma assay, and an *in vivo* mouse micronucleus assay, a dominant lethal assay in rats, an *in vivo* chromosomal aberrations assay in rat bone marrow and an *in vitro* test using WI-38 human cells. The applicant proposed to include the following 3 assays: a bacterial reverse gene mutation assay, an *in vitro* mouse lymphoma assay and an *in vivo* mouse micronucleus assay. The remaining assays should also be mentioned in the labeling.

Developmental Toxicology: This application did not conduct or submit any developmental toxicity studies. This is considered acceptable because D-mannitol is a GRAS compound. The Joint FAO/WHO Expert Committee on Food Additives Monograph on Mannitol states:

“Mannitol was tested for teratogenic effects in mice, rats, and hamsters. Pregnant mice and rats given oral doses of mannitol up to 1.6 g per kg for 10 consecutive days and

hamsters up to 1.2 g per kg for 5 consecutive days showed no effects on maternal or fetal survival.” See <http://www.inchem.org/documents/jecfa/jecmono/v21je10.htm>.

The applicant in Section 2.4.5.5 also provided the following summary information from literature review:

“As part of an embryotoxicity study, rats were administered mannitol intravenously at a dose rate of approximately 150 mg/kg once a day from days 6 to 15 of gestation. Mannitol was administered in combination with tartaric acid (0.06 mg/mL) and acted as the vehicle control. All pregnant females were euthanized on gestation day 20 and a complete uterine, placental and foetal examination was carried out. No significant compound-related effects were detected in the dams or in embryonic development.

A single dose of mannitol (550 mg/kg) was administered subcutaneously to pregnant rabbits on gestation day 12. On gestation day 29 the pregnant rabbits were euthanized and fetuses examined. No gestational and developmental toxicity as a result of treatment was seen. It can therefore be concluded that mannitol had no effect on embryonic development in rabbits after a single 550 mg/kg dose of mannitol”.

Mannitol developmental toxic potential was evaluated in a chick embryo neural retina cell assay. Mannitol did not affect *in vitro* cell aggregation, growth or differentiation at concentrations up to 40 mM. It was therefore concluded that mannitol did not exhibit developmental toxic potential and did not have any effect on embryonic cell development.”

The applicant proposed to include in the Aridol labeling oral teratogenicity studies in mice, rats ^{(b) (4)} [redacted]. The review agrees with the proposal to include the mouse and rat study although the treatment duration was slightly shorter than the currently acceptable standards.

(b) (4)

(b) (4)

2.6.6.10 Tables and Figures

Not applicable because no data was submitted.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable because no data was submitted.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The application has adequately evaluated the toxicity profile of inhaled mannitol. The applicant has submitted inhalation toxicity studies of mannitol up to 3 and 6 months in rats and dogs, respectively. The studies identified the respiratory system as the target organs of toxicity. The study did not reveal any neoplastic or pre-neoplastic findings.

The completed studies are considered adequate to support the registration of Aridol. The approval of Aridol is recommended from the nonclinical perspective.

Mannitol is used as a nutrient and/or dietary supplement and an ingredient in numerous drug products. As a dietary supplement, mannitol is considered generally recognized as safe [GRAS, 21CFR§582.5470]. Medically, mannitol has been used as active and inactive ingredients. As an active ingredient, mannitol is a laxative and diuretic. As an inactive ingredient, mannitol is an excipient in numerous products. The routes of administration included oral, parenteral (e.g., IV, and IP), and topical administration. Mannitol was also present as an inactive ingredient of an inhaled insulin product, Exubera® (NDA 21-868, approved on January 27, 2006).

The mannitol toxicology by non-inhalation use is well understood. Mannitol is non-mutagenic, non-carcinogenic and non-teratogenic. The National Toxicology Program evaluated carcinogenicity and mutagenicity of D-mannitol. It concluded that F344/N rats and B6C3F1 mice fed with up to 5% D-mannitol in diet for 103 weeks did not reveal any evidence of tumorigenicity. Mannitol was non-genotoxic in a bacterial mutation assay, an *in vitro* mouse lymphoma cell assay, an *in vivo* mouse micronucleus assay and other assays. The Joint FAO/WHO Expert Committee on Food Additives Monograph on Mannitol considered D-mannitol non-teratogenic.

Due to the extensive clinical and nonclinical data available on mannitol, the toxicology program of the current application focused on effects of inhaled mannitol, particularly its effect on the respiratory system. The Division determined in the 19-JUL-2004 pre-IND meeting that 14-day inhalation toxicity studies in 2 species (one in each species) were needed to support the registration of Aridol.

Pharmaxis has submitted inhalation toxicity studies of mannitol up to 3 and 6 months in rats and dogs, respectively. These studies were reviewed previously and summarized in detail in Section 2.6.6.1. Briefly, the studies identified the respiratory tract as the target organs of toxicity of inhaled mannitol. The studies did not reveal any neoplastic or pre-neoplastic findings in the respiratory system. The completed toxicology program has adequately evaluated the toxicity profile of inhaled mannitol and no additional toxicity studies are needed.

Unresolved toxicology issues (if any): None.

Recommendations: Approval of Aridol is recommended pending labeling review from the nonclinical perspective.

Luqi Pei, Ph.D.
Senior Pharmacologist

Appendix:

1. Pharmacology review No. 4
2. Pharmacology review No. 2
3. Pharmacology review No. 1

2.6 PHARMACOLOGY AND TOXICOLOGY REVIEW

CHEMISTRY CONSULT

Safety Evaluations of Impurity, Leachables and Extractables

NDA No.: 22-368
Drug Name: Aridol™ (mannitol dry powder) capsules
Sponsor: Pharmaxis Ltd,
Submission date: February 27, 2009
Consultation request date: June 4, 2009
Reviewer: Luqi Pei, Ph.D., Senior Pharmacologist
Review completion date: August 6, 2009

This review evaluates nonclinically the safety of impurities, extractables and leachables in Aridol™ (mannitol) powder capsules (NDA 22-368). There are no nonclinical safety concerns about any extractables, leachables or impurities in the drug product. (b) (4) level in drug substance, however, should be lowered to (b) (4) or to a level reflective of chemical stability data. Alternatively, the proposed specification of (b) (4) in the mannitol drug substance may be acceptable if the Agency can be assured that the drug substance (b) (4) will be in full compliance with the above recommendation.

This nonclinical safety evaluation of impurities, extractables and leachables in Aridol was generated in response to a Chemistry Consult Request issued by Dr. Deepika Arora *et al.*, on June 4, 2009. Supplemental information to the request was provided by Dr. Arora via two electronic messages on June 25, 2009. One of the 25-JUN-09 emails provided the sponsor's rationales in support of the proposed specifications of (b) (4). The other provided the proposed specifications of extractables and leachables in Aridol.

Impurities in Drug Substance and Drug Product

The Sponsor proposed a (b) (4) specification in both drug substance and drug product of Aridol™. This specification is greater than the respective ICH qualification standards of 0.15% and 0.2% drug substance and in drug products, respectively.¹ Table 1 (next page) presents estimates of (b) (4) exposures based on the ICH standards and proposed specifications under the intended use of mannitol. A (b) (4) concentration corresponds to a daily exposure (b) (4). This exposure level is approximately (b) (4) the ICH qualification thresholds for drug substance (b) (4) and drug products (b) (4) respectively.

¹ ICH Q3A states that the qualification thresholds of impurities in drug substances with the maximum daily dose of ≤ 2,000 mg/day are 0.15% or 1.0 mg/day whichever is lower. ICH Q3B states that the qualification thresholds for drug products with the maximum daily dose of 10 - 2,000 mg/day are 0.2% or 2.0 mg/day whichever is lower. (b) (4)

Table 1 ICH Standards and Proposed Sorbitol Exposures

(b) (4)



(b) (4)



Nonclinical qualifications of the proposed specifications are necessary because the expected (b) (4) exposure levels from the Aridol (b) (4) (b) (4) are greater than the ICH qualification thresholds. Pharmaxis provided their justifications to support the proposed (b) (4) specifications (Appendix 1). Briefly, Pharmaxis argues that:

1. (b) (4) level in European monographed mannitol is (b) (4)
2. (b) (4) (b) (4) was present in the 26-week inhalation toxicity study of mannitol in dogs (Study Report 667108). The NOAEL of the study provides adequate safety margins.
3. Aridol (mannitol BCT) is a single-use diagnostic product.

The above response appears sufficient to support the safety of (b) (4) specification in the Aridol product but insufficient to qualify the impurity level in mannitol drug substance, based on the following considerations:

1. (b) (4) is a manufacturing impurity of Aridol™ (mannitol). As a nutritional and dietary supplement, (b) (4) is considered as generally regarded as safe (GRAS, 21CFR§184.1835). There are, however, no available governmental environmental (inhalation) exposure standards of (b) (4)
2. There is no monographed mannitol for inhalation use in the US. It is unclear whether the European monographed mannitol is for inhalation use. If mannitol is monographed for oral route of administration, a difference in routes of administration between oral and inhalation would make such information little relevance to the current application.
3. The estimated (b) (4) exposure at the NOAEL dose was approximately 0.31 mg/kg/day in dogs. Dr. Luqi Pei reviewed previously the study (Report 667108) in a Pharmacology and Toxicology Review completed on November 27, 2007 in IND 70277. Beagle dogs (4/sex/dose) were exposed via a face mask to air, 43 or 178

mg/kg/day of D-mannitol (pulmonary deposits) for 26 weeks. Coughing occurred in both LD and HD groups while the histological changes occurred in the HD group only. Histological changes in the HD group included laryngeal ulceration and sinus histiocytosis in the mediastinal lymph node although their incidences were low (1/4 in each sex) and severity was minimal. That review finds a mannitol NOAEL of 43 mg/kg (low dose). With a mean (b) (4) concentration of 0.73% in mannitol tested, (b) (4) exposure (at the NOAEL) was 0.31 mg/kg/day (43 mg/kg/day x 0.73% = 0.31 mg/kg/day).

4. The safety margins between mannitol exposure in dogs and humans are smaller the sponsor argues. As discussed in Item 3 (above), the (b) (4) NOAEL in the 26-week dog study was 0.31 mg/kg/day. The expected human exposure of (b) (4) at the proposed specification (b) (4) and expected use mannitol (635 – (b) (4) (b) (4)) would be (b) (4) mg/kg/day. The dog NOAEL value of (b) (4) is approximately equal to the expected human exposure levels of (b) (4) mg/kg/day for Aridol (b) (4). In other words, (b) (4) safety margin would be approximately (b) (4). This safety margin is significantly smaller than what (b) (4) the sponsor had been arguing for.

Note that there are significant differences in safety (b) (4) margins. Specifically, safety margins for clinical doses were approximately (b) (4) by the review and the sponsor, respectively. The reason was that DPAP and the Sponsor used different parameters to derive the safety margins. The most prominent differences were the dog NOAEL value and the human body weight. The NOAEL in the 26-week dog study was considered to be 43 and 713 mg/kg/day by DPAP and the sponsor, respectively. Patient body weight was 50-kg and 60-kg in calculations by DPAP and Sponsor, respectively. Another difference was the clinical dose, the sponsor used 635 mg dose only while the review used both 635- (b) (4) g doses (Aridol (b) (4)).

Two major factors account for the difference in NOAELs: interpretation of study results and estimates of dog doses. The study had two mannitol treatment groups and one air control. The study report stated that inhaled mannitol doses in the low- and high-dose groups were 170 and 713 mg/kg/day, respectively. Due to difference in data interpretation, the Sponsor considered the HD the NOAEL while DPAP considered the LD the NOAEL. See the Pharmacology and Toxicology Review completed by Dr. Luqi Pei on November 27, 2007 in IND 70277 for discussions of the NOAEL. Further, the Sponsor used inhaled doses as the actual exposure while DPAP uses the pulmonary deposits (i.e., 25% of the inhaled dose). These two factors accounted for a 16.6-fold difference in NOAEL values in dogs.

(b) (4)

² The clinical doses of mannitol appear 635 (b) (4) mg/patient/day for Aridol (b) (4). These doses correspond to daily doses of 12.7- (b) (4) mg/kg mannitol, respectively, for a 50-kg patient. A (b) (4) concentration in (b) (4) mannitol yield daily (b) (4) exposure of (b) (4) and (b) (4) respectively.

5. There was no remarkable damage to the respiratory system at a higher dose of mannitol (178 mg/kg/day) in the 26-week study in dogs. See Item 3.
6. Aridol™ is indicated for single dose use. (b) (4) exposure under the recommended use of Aridol will be approximately (b) (4). There is no significant nonclinical safety concern for this one time exposure based on the available nonclinical information. Thus, (b) (4) of (b) (4) in Aridol may be acceptable due to the lack of safety concerns.
7. The argument of single dose use of Aridol, however, may not be applicable to the drug substance of mannitol. (b) (4). Thus, single use argument is not sufficient to justify the proposed level of (b) (4) in drug substance.
8. There are sufficient safety margins for the (b) (4) specification in mannitol. As discussed in Item 3, (b) (4) NOAEL in the 6-month dog inhalation toxicity study was 0.31 mg/kg/day. (b) (4) exposures at (b) (4) in the proposed mannitol of 635 – (b) (4) would be approximately (b) (4) mg/kg/day. The NOAEL would provide safety margins of approximately (b) (4). Such safety margins were considered acceptable for (b) (4) of mannitol in Dr. Pei's review dated November 27, 2007. The same safety margin is also considered acceptable for (b) (4). Hence, the safety margin is considered adequate to qualify the (b) (4) concentration at (b) (4) in mannitol.

Overall, there is no nonclinical safety concern about the proposed specification of (b) (4) in Aridol™ product. The available nonclinical data is sufficient to support the safety of (b) (4) in the drug substance of inhaled mannitol. The data is, however, insufficient to support the safety of (b) (4) in mannitol drug substance (b) (4). It is recommended that (b) (4) level in drug substance of inhaled mannitol be lowered to (b) (4) or to a level reflective of chemical stability data. Alternatively, the specification of (b) (4) in the mannitol drug substance is acceptable (b) (4) will be in full compliance with the above recommendation.

Leachables

There are no nonclinical safety concerns about any of the leachables identified in Aridol. Table 2 (next page) provides leachables and their estimated daily intakes of Aridol. The leachables include (b) (4). The estimated exposures of these compounds are generally low. Specifically, the daily

exposures of (b) (4) are (b) (4) or less except for the (b) (4) that has a daily exposure of (b) (4). The daily exposure of the (b) (4) and (b) (4) are below (b) (4). The exposure levels of the leachables are of no safety concern from the nonclinical perspective.

Table 2 Leachables and Their Estimated Intake from Aridol^a

(b) (4)



Conclusion

The available information indicates that there is no nonclinical safety about the proposed specifications of impurities, extractables and leachables in Aridol™ product. There are sufficient data to support the safety of (b) (4) in the drug substance of inhaled mannitol. The data is, however, insufficient to support the safety of (b) (4) in mannitol drug substance (b) (4).

It is recommended that (b) (4) level in drug substance of inhaled mannitol be lowered to (b) (4) or to a level reflective of chemical stability data if the (b) (4) level cannot be achieved. Additionally, the specification of (b) (4) in the mannitol drug substance is acceptable (b) (4).

(b) (4) will be in full compliance with the above recommendation.

Luqi Pei,
Senior Pharmacologist

Appendix 1

From: Arora, Deepika P.
Sent: Thursday, June 25, 2009 3:11 PM
To: Pei, Luqi
Subject: RE: NDA 22-368

Luqi,

Please see the complete response of the sponsor for qualifying (b) (4) (b) (4). They have roughly estimated the (b) (4) exposures during the study. Please let me know if you need more details to comment. Thanks!

(b) (4)

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22368	ORIG 1		ARIDOL POWDER FOR INHALATION

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LUQI PEI
08/06/2009

JEAN Q WU
08/06/2009

2.6 PHARMACOLOGY / TOXICOLOGY REVIEW

NDA Pharmacology Fileability Check List

Reviewer: Luqi Pei, Ph.D.

NDA No: 22-368
Drug Name: TRADENAME, Aridol, Mannitol dry powder
Date of submission: February 26, 2006 (stamp date)
Date of 45-day file-ability meeting: April 13, 2009
Information to the Sponsor: The sponsor should be asked to submit safety evaluation of or justifications for drug impurities and degradants and extractables. See Item 11 for additional information.
Date of check list: April 15, 2009

- (1) On its face, is the pharmacology/toxicology section of the NDA organized in a manner to allow substantive review? Yes.
- (2) On its face, is the pharmacology/toxicology section of the NDA legible for review? Yes.
- (3) Are final reports of all required and requested preclinical studies submitted in this NDA? Final reports of all toxicology study reports are submitted.

	Yes	No	NA
Pharmacology	()	()	(x)
ADME	()	()	(x)
Toxicology (duration, route of administration and species specified)			
acute	()	()	(x)
subchronic and chronic studies	()	(x)	()
reproductive studies	()	()	(x)
carcinogenicity studies	()	()	(x)
mutagenicity studies	()	()	(x)
special studies	()	()	(x)
others	()	()	(x)

- (4) If the formulation to be marketed is different from the formulation used in the toxicology studies, are repeating or bridging the studies necessary? No.

If no, state why not: The to-be-marketed formulation and the formulation used in toxicity studies are identical (mannitol dry powder). Bridging toxicity studies, therefore, are not necessary.

If yes, has the applicant made an appropriate effort to repeat the studies using the 'to

be marketed' product, to bridge the studies or to explain why such repetition or bridging should not be required?

- (5) Are the proposed preclinical labeling sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) appropriate (including human dose multiples expressed in either mg/m² or comparative systemic exposure levels) and in accordance with 201.57?

No. The proposed label attempted to follow the new product labeling recommendations (PLR). It, however, did not provide any dose ratios between animals and humans in preclinical sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage). Descriptions of nonclinical studies and findings were minimal. Significant revisions of the proposed labeling may be needed.

- (6) Has the applicant submitted all special studies/data requested by the Division prior to the submission including but not limited to pre-NDA discussion? Yes.
- (7) On its face, does the route of administration used in the pivotal toxicity studies appear to be the same as the intended clinical route? Yes.
- If not, has the applicant submitted a rationale to justify the alternative route? Yes/No
- (8) Has the applicant submitted a statement(s) that all of the toxicity studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? Yes.
- (9) Has the applicant submitted any studies or data to address any impurity or extractable issues (if any)? No, there appear no discussions about drug impurities and degradants, and extractables. This information was requested in the 12-MAR-08 pre-NDA meeting. The safety evaluation of impurities, degradants and extractables will be requested as indicated in Item 11.
- (10) Are there any outstanding preclinical issues? Yes.

If yes, the sponsor did not address at all the drug impurities and degradants, and extractables.

- (11) From a preclinical perspective, is this NDA fileable? Yes

If no, state below why it is not.

If yes, should any additional information/data be requested? Yes, the sponsor needs to address the safety qualification of drug impurities and degradants as well as extractables as requested in the 12-MAR-2008 pre-NDA meeting. The meeting minutes in the Additional Comments/Pharmacology and Toxicology section (p 10) states:

1. Address the safety qualification of drug impurities and degradation products according to the ICH Guidances Q3A and Q3B.
2. Address the safety qualification of any extractable/leachables from the device.

If yes, identify those below. Please convey this following information to the sponsor.

(See the above for rationales for the information request.)

Submit the following information as requested in the 12-MAR-2008 pre-NDA meeting:

1. The safety qualification of drug impurities and degradation products according to the ICH Guidances Q3A and Q3B.
2. The safety qualification of any extractable/leachables from the device.

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Luqi Pei
4/15/2009 10:21:31 AM
PHARMACOLOGIST

Molly Shea
4/15/2009 11:44:03 AM
PHARMACOLOGIST
I concur.

2.6 PHARMACOLOGY / TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND Number: 70,277
Review Number : 4
Sequence number/date/submission type: 035/ 31-JUL-07/ IT
036/ 1-AUG-07/ SM
037/ 6-AUG-07/ SM

Information to the Sponsor: Yes (), No (x)
Sponsor/or Agent: Pharmaxis Ltd, 1840 Gateway Dr., San Mateo, CA
94404

Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Products
Review Completion Date: November 29, 2007

Drug:
Trade Name: (b) (4) Aridol®
Generic Name: D-Mannitol
CAS Register Number: 69-65-8

Drug Class: Sugar

Intended clinical population: (b) (4)

Route of Administration: Inhalation (DPI)

Clinical Formulations: (b) (4)

(b) (4)

(b) (4)

Drug History:

(b) (4)

Studies Submitted and Reviewed in the Review:

Mannitol 26 weeks inhalation toxicity study in beagle dogs with a 4-week recovery period (CRL report# 26966 and Study# 667108). Submitted on 31-JUL-2007 (Serial No. 035), electronic submission.

Studies Submitted but Not Reviewed in this Review:

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

2.6.6.1 Overall Toxicology Summary

Repeat-Dose Toxicology:

A 6-month inhalation toxicity study in dogs (CRL Study #667108, Serial No. 035) was conducted to evaluate the toxicity inhaled mannitol. Beagle dogs (4/sex/dose) were exposed via a face mask to air, 43 or 178 mg/kg/day of D-mannitol for 26 weeks. The control and HD groups also included two additional dogs per sex to study reversibility of any lesions after a recovery period was 4 weeks. In addition to the routine toxicological evaluations, the study measured respiratory parameters, EKG, chest auscultation, and the cell numbers in the bronchoalveolar lavage fluid. Coughing occurred in both LD and HD groups while the histological changes occurred in the HD group only. Histological changes in the HD group included laryngeal ulceration and sinus histiocytosis in the mediastinal lymph node although their incidences were low (1/4 in each sex) and severity was minimal. Both lesions were reversible after a recovery period of 4 weeks. The review NOAEL was 43 mg/kg/day.

2.6.6.3 Repeat-Dose Toxicity

Study Title: Mannitol 26 weeks inhalation toxicity study in beagle dogs with 4 weeks recovery period. Submitted on 31-JUL-2007 (Serial No. 035), electronic submission.

Key findings:

- Beagle dogs (4/sex/dose) were exposed by inhalation to 0, 43 and 178 mg/kg/day (pulmonary deposit) of mannitol for 26 weeks.

- The HD dogs coughed throughout the study and showed low incidence (1/4 in each sex) of minimal ulceration in the larynx and sinus histiocytosis in the mediastinal lymph node.
- All findings were reversible after a recovery period of 4 weeks.
- The NOAEL was 43 mg/kg/day.

Study number: CRL Study #667108 and Report #26966
 Volume #, and page #: Not available for electronic submission (31-JUL-07, Serial 035)
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: May 18, 2006
 Study complete date: April 11, 2007
 Report date: N/A
 GLP compliance: Yes, with an unsigned GLP statement
 QA reports: Yes, with an unsigned GLP statement
 Drug lot # & purity: Batches # 3M021, 3M24, 3M28, EXP001 - 5, EXP007; purity: 98- 102%
 Formulation/vehicle: Mannitol dry powder

Method:

Beagle dogs (4/sex/dose) were exposed by inhalation to 0, 43 and 178 mg/kg/day (pulmonary deposit) of mannitol for 26 weeks. The control and HD groups also included two additional dogs per sex to study reversibility of any lesions after a recovery period was 4 weeks. The exposure was achieved via a face mask through inhaled air containing 0, 0.20 and 8.7 mg/L of mannitol particles for the control, low and high dose groups for 120 minutes/day.¹ The daily exposure was divided into two episodes of 60 minutes, with an interval of at least 2 hours between the episodes. The mean mass median aerodynamic diameter (MMAD) was 3.2 and 3.3 μm in LD and HD groups, respectively. Pulmonary and achieved doses were calculated from measured minute volumes, chamber mannitol concentrations, body weights and MMADs and applicable theoretic deposition factors. [See footnote a in Table 2] In addition to the routine toxicological evaluations, the study also measured respiratory parameters, EKG, chest auscultation, and the cell numbers in the bronchoalveolar lavage fluid. A complete list of organs and tissues were examined microscopically in both the main section and recovery dogs.

Species/strain: Dogs, Beagle
 #/sex/group: 4
 Age: Approximately 5.5 - 6 months
 Weight: M: 7.0 – 10.9 kg; F: 6.3 – 9.6 kg

(b) (4)

Doses in administered units: 0, 43, or 178 mg/kg/day (pulmonary deposits)
 Route, form: Inhalation via a face mask, dry powder, 120 minutes/day
 (60 minute/episode, 2 episodes/day, \geq 120 minutes between episodes)

Observations and times:

Clinical signs: Daily
Body weights: Weekly
Food consumption: Daily
Ophthalmoscopy: Pretreatment and Weeks 7, 13, 26 and end of recovery
Respiratory system: Respiratory rate, tide volume and minute volume at pretreatment and weeks 1, 7, 13 and 26
ECG: Immediately after dosing at pretreatment and weeks 7, 13 and 26
Chest Auscultation: Biweekly
Hematology: Pretreatment and weeks 7, 13 and 26
Clinical chemistry: Pretreatment and weeks 7, 13 and 26
Urinalysis: Pretreatment and weeks 7, 13 and 26
Bone marrow smear: Not evaluated although samples were collected at necropsy
Bronchoalveolar lavage: Right lobe at necropsy
Gross pathology: Sacrifice time
Sacrifice method: Pentobarbitone (IP)
Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, pituitary, prostate, salivary gland, spleen, testes, thymus, thyroid lobes (with parathyroids) and uterus
Histopathology: A complete panel – all animals in all groups were examined.
 Adequate Battery: yes (x), no ()
 Peer review: yes (x), no ()
Toxicokinetics: Day 1 and weeks 13 and 26, at hours 0.5, 1, and 2 after 1st and 2nd dose; and hours 4 and 18 (2nd dose only)

Results

Dose estimates: Table 3 (below) presents the dose estimates of the study. The estimated pulmonary deposition was 43 and 178 mg/kg/day for the low and high dose groups, respectively.

Table 2 Estimated Pulmonary Deposits in the 26-Week IH Study in Dogs

Treatment	Dose	Aerosol			Exposure (mg/kg/day)	
		MMAD (μm) ^a	GSD	Drug (mg/L)	Achieved Dose ^a	Pulmonary Deposit ^b
Air	0	-	-	-	-	-
Mannitol	LD	3.22	2.17	2.0	171.2	43
	HD	3.29	2.18	8.7	712.8	178

a. Achieved dose in the study report. Achieved delivered dose levels were estimated using the formula: Dose (mg/kg/day) = (MV x T x CC)/BW; where MV = Minute volume (overall group mean value from actual recorded results in the study), T = Duration of exposure (minutes), CC = Gravimetric chamber concentration of Mannitol = mg/L, and BW = Mid-week individual body weight in kg.

- b. Converted from the achieved dose using a deposition factor (0.25). Pulmonary deposits (mg/kg/day) = Achieved dose (mg/kg/day) x 0.25 (pulmonary deposition factor). For example, the pulmonary deposition for the HD = 99 x 0.25 = 25 mg/kg/day.

Mortality: None.

Clinical Signs: Cough mostly occurred in the HD dogs. Table 3 (below) presents the incidence of coughing at major milestones of the study. Coughing occurred during and post dosing in the HD group throughout the study, but only in the first week in the LD group

Table 3 Coughing In the 26-Week Inhalation Toxicity Study in Dogs

Time	Incidence of Coughing (M & F combined) ^a							
	43 mg/kg/day Mannitol (n = 56)				178 mg/kg/day Mannitol (n = 84)			
	Minimal	Moderate	Marked	Total	Minimal	Moderate	Marked	Total
Week 1 ^b				10				50
Week 4	0	0	0	0	2	8	3	13
Week 9	0	0	0	0	0	22	1	23
Week 13	1	0	0	1	18	7	1	26
Week 17	0	0	0	0	26	6	1	33
Week 22	0	0	0	0	14	5	3	22
Week 26	2	0	0	2	11	11	0	11

a. The control group is not listed because no coughing was observed in either sex at any time.

b. The severity of cough was not graded in Week 1.

Body Weight: Figure 1 presents the body weight-time course of the study in both sexes. Both LD and HD male dogs showed decreases in body weight throughout the study. The females, however, did not show significant changes in body weight from the control group. At the end of the study, the decrease in the mean body weight of the male LD and HD groups was 9.9% and 7.8%, respectively. [The actual body weight was 14.1, 12.7 and 13.0 kg for the C, LD and HD groups, respectively].

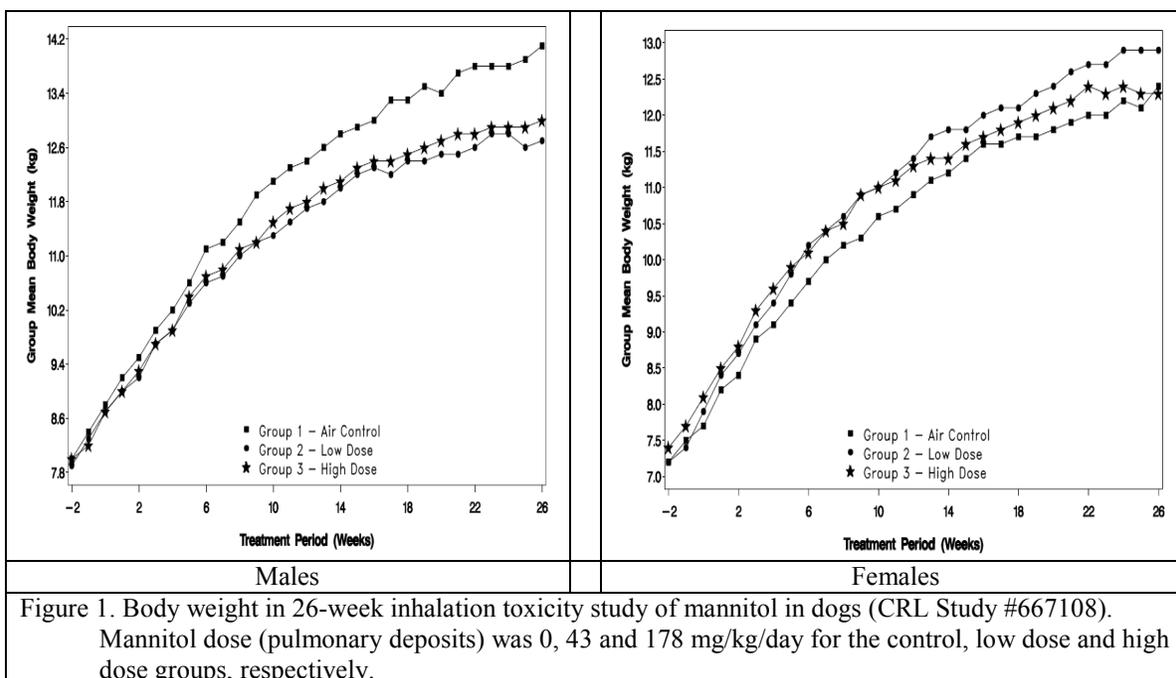


Figure 1. Body weight in 26-week inhalation toxicity study of mannitol in dogs (CRL Study #667108). Mannitol dose (pulmonary deposits) was 0, 43 and 178 mg/kg/day for the control, low dose and high dose groups, respectively.

Food Consumption: No treatment-related effect was observed.

Ophthalmoscopy: No treatment-related effect was observed.

Respiratory parameters: No treatment-related effect was observed in respiratory rate, minute volume and tidal volume.

ECG: No treatment-related effect was observed.

Hematology: No treatment-related effect was observed.

Clinical Chemistry: No treatment-related effect was observed.

Urinalysis: No treatment-related effect was observed.

Organ weight: No treatment-related effect was observed.

Gross pathology: No treatment-related effect was observed.

Histopathology: Microscopic changes were limited to the respiratory system in the HD group. Minimal laryngeal ulceration and sinus histiocytosis in the mediastinal lymph node (1/4 each in both sexes) were observed. No abnormalities were observed in the recovery dogs.

Blood mannitol concentrations: Mannitol was detected in the blood of both LD and HD dogs (Figure 2). The mannitol concentrations appear to dose-related. There is no significant change in the blood mannitol concentrations with increased dosing duration.

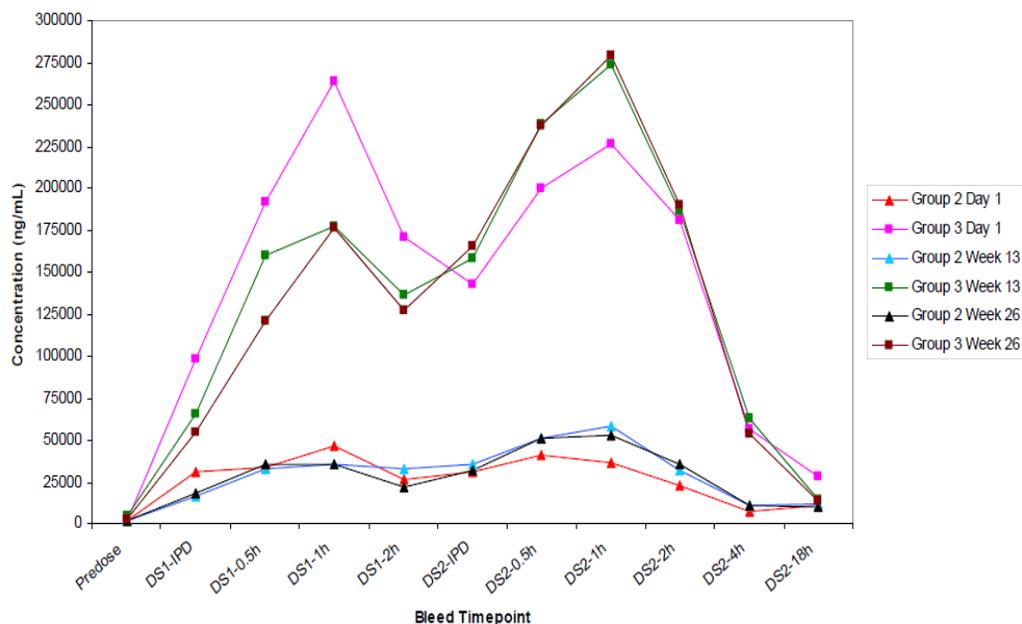


Figure 2. Plasma mannitol concentrations after inhalation exposures in dogs. The estimated pulmonary deposits were 43 and 178 mg/kg/day for Groups 2 and 3, respectively. DS1 and DS2 indicate first and second dosing episodes of the day, respectively. Each episode lasted 60 minutes.

2.6.6.9 Discussions and conclusions

(b) (4)



(b) (4)

OVERALL EVALUATION AND RECOMMENDATION

Summary

(b) (4)

(b) (4) The Division considers this toxicology program to be adequate. The NOAEL in the 6-month inhalation toxicity of mannitol in dogs is 43 mg/kg/day. The NOAEL provides safety margins of 2.7 – 3.4 on a mg/kg basis. These margins are smaller than the generally accepted safety margin of 6, but are considered acceptable since the dose-response curve of inhaled mannitol is rather shallow in dogs. At a high dose (178 mg/kg/day) that is four times the NOAEL, a low incidence (1/4 in each sex) and minimal in severity of laryngeal ulceration and histiocytosis in mediastinal lymph node was observed. These findings are completely reversible after a recovery period of 4 weeks. Laryngeal ulceration is also considered a clinically monitorable response. Histiocytosis in mediastinal lymph node is considered a defense (clearance) mechanism of the inhaled particles. The histological changes associated with the mannitol inhalation in dogs are not considered significant safety concerns regarding the proposed use of the drug.

Finally, clinical experience at the proposed doses and in the disease populations is available to evaluate the safety of the proposed of the drug. (b) (4)

(b) (4) Aridol (single-dose broncho-provoking diagnostic agent) is currently marketed in Australia (the March 28, 2006 submission, Serial No. 017). (b) (4)

In addition to the 6-month dog study, the sponsor conducted inhalation toxicity studies up to 13 weeks in rats and dogs. Table 5 (below) presents an overview of these toxicity studies, including the 6-month study. These studies identified the respiratory system as the target organ of toxicity for inhaled mannitol.

Table 5 Overview of Inhalation Toxicity Studies of Mannitol

Study #	Species	Duration	Mannitol (mg/kg/day) ^a	NOAEL
26482/666958 ^b	Rat	7 days	57.3, 97.9	None
XIS 002/033951	Rat	2 weeks	0, 0.9, 2.5, & 6.9	6.9
XIS 005/0413185	Rat	13 weeks	0, 12.4, 21.0	None ^c
26050/666386	Dog	2 weeks	0, 25, 100, 197	None ^d
26966/667108	Dog	26 weeks	0, 43, 178	43 ^e

a. Estimated pulmonary deposits.

b. A non-GLP compliant investigative dose-ranging study. The study did not show the dose-related increase in the concentration of mannitol in the bronchoalveolar fluid. Neither did it examine the lung tissue microscopically.

c. The review is in agreement with the study report in determination of NOAEL.

d. The report states that the 197 mg/kg/day dose is “well-tolerated.”

e. The report considers the NOAEL as 178 mg/kg/day.

A 7-day non-GLP dose-ranging inhalation study was conducted to investigate the achievement of pulmonary delivery of mannitol to the lung in Sprague-Dawley rats. Rats (5/sex/dose) were exposed nose-only to approximately 5 or 9 mg of mannitol/L of air for 120 to 240 minutes/day for 7 days. The rats were sacrificed immediately after the last exposure. The amount of mannitol delivered to the lung was determined by measuring mannitol concentrations in the bronchoalveolar fluid (3/sex/dose). The respective estimated achieved dose of mannitol was 573 and 979 mg/kg/day for the low-dose and high-dose groups, respectively. The respective mean mannitol concentration in BALF for the low and high dose groups was 36.7 and 42 µg/ml in males and 43.6 and 33.4 µg/ml in females. Necropsy did not reveal any treatment-related effect. Microscopic examination was not done.

In a 2-week rat study (XIS 002/033951), CD-1 rats (10/sex/dose) were given via nose-only inhalation pulmonary deposited doses of 0, 0.9, 2.5 and 6.9 mg/kg of mannitol for 14-days. Histological evaluations of the respiratory system were done in every group. The remaining organs were examined in the control and high-dose groups only. No significant, treatment-related effects were observed. The NOAEL was 6.9 mg/kg/day.

In a 2-week dog study (XIS 005/0413185), beagle dogs (3/sex/group) were exposed by inhalation via a face mask to air (C), 25 (LD), 100 (MD) or 197 (HD) mg/kg/day of D-mannitol (pulmonary deposition) for 14 days. Coughing occurred during and immediately after dosing in all treated groups (Incidence for M + F: 0/6-C, 1/6-LD, 4/6-MD and 4/6-HD). Spongy (4/6) and froth-filled lung (3/6) were reported in the HD group during necropsy. Microscopic examination revealed the following: lung congestion/hemorrhage (2/6-HD), and pigment in submandibular lymph node (3/6-HD); bronchoalveolitis (2/3 apiece for MD and HD males); peribronchiolar infiltration (Incidence: 0/3-C, and 3/3 apiece in LD, MD and HD in males; and 1/3-C, 1/3-LD, 2/3-HD and 0/3-HD in females); foamy alveolar macrophages

in all treated females and HD males (respective incidence in control, LD, MD and HD: 1/3, 0/3, 0/3 and 2/3 in males and 0/3, 2/3, 2/3 and 1/3 in females) and inflammatory foci and focal hyperplasia in trachea carina (1/3-HD female). The high dose males and females also showed increases in lung weight. The study did not establish a NOAEL.

In a 13-week rat study (26050/666386), Sprague-Dawley rats (10/sex/group) were given via nose-only inhalation air (C), 12.4 (LD), or 21.0 (HD) mg/kg/day (pulmonary deposition) for 13 weeks. Additional rats (5/sex) were included in the control (RC) and high dose (RHD) groups to evaluate reversibility of lesions after a recovery period of 4 weeks. The duration of exposure was 180 minutes/day. D-mannitol concentrations were 0, 1.83 or 2.89 mg/L for the control, LD and HD groups, respectively. The estimated pulmonary deposition of D-mannitol was 0, 12.4 and 21.0 mg/kg/day for the control, LD and HD groups, respectively. D-mannitol contents in the bronchoalveolar fluid were approximately 0, 3.8 and 3.2 µg/ml for the control, LD and HD groups, respectively. Both the LD and HD females showed statistically significant decreases in body weight gain (approximately 20%). Clinical pathology examinations revealed minimal decreases (approximately 50% or less) in white blood cell numbers and increases (12-30%) in serum phosphorus in the HD group. Microscopic examinations revealed increases in the incidence of alveolar macrophage aggregation and alveolitis. The respective incidence of alveolar macrophage aggregation in the C, LD, HD, RC and RHD was 3/10, 6/10, 9/10, 0/2 and 1/2 in females and 4/10, 5/10, 3/10, 1/1 and 3/4 in males. The increase in the incidence of alveolitis was observed in the high-dose male only (incidence: 0/10-C, 0-/10-MD, 1/10-HD, 0/1-RC, and 2/4-RHD, respectively). The most significant, treatment-related effect, but of no safety concern for the intended use of mannitol, was seen in the nasal cavity. Both the low and high-dose rats showed increases in the incidence of eosinophilic inclusion in olfactory epithelium of the nasal cavity (Incidence: 3/20-Air, 15/20-LD, and 13/20-HD). This finding is not considered relevant to humans because minimal nasal exposure is expected from the intended clinical use. The study failed to establish NOAEL.

(b) (4)

Recommendations:***Internal recommendations:***

(b) (4)

(b) (4)

External recommendations: None.

Luqi Pei, Ph.D.
Senior Pharmacologist/toxicologist

Linked Applications

Sponsor Name

Drug Name

IND 70277

PHARMAXIS LIMITED

ARIDOL

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LUQI PEI

11/29/2007

Non-Clinical Reviewer

TIMOTHY J MCGOVERN

11/29/2007

Non-Clinical Reviewer

I concur.

2.6 PHARMACOLOGY / TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND Number: 70,277
Review Number : 2
Sequence number/date/submission type: 008/ 13-JAN-06/ IN
009/ 17-JAN-06/ IT
012/ 06-MAR-06/ IT
016/ 30-MAR-06/ IT
017/ 28-APR-06/ IT

Information to the Sponsor: Yes (), No ()
Sponsor/or Agent: Pharmaxis Ltd, 1840 Gateway Dr., San Mateo, CA 94404

Manufacturer of the Drug Substance: (b) (4)

Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Products
Review Completion Date: July 21, 2006

Drug:
Trade Name: Aridol[®], (b) (4) 1[®]
Generic Name: D-Mannitol
Code Name: None
Chemical Name: 1,2,3,4,5,6-hexanehexol, or cordycepic acid
CAS Register Number: 69-65-8
Mole File Number: Not available
Molecular Form and Weight: C₆H₁₄O₆/182.2

Relevant IND/NDAs: DMF# (b) (4)

Drug Class: Sugar

Intended clinical population: (b) (4)

Route of Administration: inhalation

Clinical Formulations: Capsules filled with 5, 10, 20 and 40 mg of D-mannitol powder. Mannitol will be delivered by a dry powder inhaler.

Proposed Clinical Protocol: None.

Previous Human Experience:

(b) (4)

Studies Submitted and Reviewed in the Review

Mannitol toxicity study by inhalation administration to CD rats for 13 weeks followed by 4 week withdrawal period, Study No. XIS 005 / 043185, submitted on January 17, 2006.

2-Week inhalation toxicity study of mannitol in dogs, Study No. 26050, submitted on March 6, 2006.

Mannitol: Investigative dose inhalation toxicity study in rats. Report Nos. 26482 & 666958, submitted on April 28, 2006.

Studies Submitted but Not Reviewed in this Review: None.

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

Drug History:

Pharmaxis is developing D-mannitol powder (b) (4)

(b) (4) . Aridol[®] is a diagnostic agent for provoking bronchoconstriction in asthmatics (b) (4). (b) (4) The Aridol[®] program is currently in Phase-1 clinical development stage (b) (4).

The IND was opened on November 19, 2004. The original submission proposed to study efficacy of D-mannitol as a provoking agent (Aridol[®]) for eliciting bronchoconstriction. The submission proposed to give approximately 130 asthmatic subjects 6 – 50 years of age, in a dose-raising schedule, up to 635 mg of mannitol to provoke bronchoconstriction. The proposal was allowed to proceed based on available clinical experience with the inhaled mannitol.

The Division has met Pharmaxis three times in the past two years to discuss the nonclinical development plan of D-mannitol. These meetings were held on July 19, 2004, June 16, 2005, and February 16, 2006, respectively. The first meeting discussed nonclinical studies needed to support the development and registration of Aridol[®]. (b) (4)

Minutes of these meetings are available.

Through these meetings, Pharmaxis and the Division agreed on the following: 1) 14-day inhalation toxicity studies in two animal species are needed for the registration of Aridol[®], 2) no studies of carcinogenicity, genetic toxicity, reproductive and developmental toxicity are needed for (b) (4) Aridol[®] (b) (4), 3) (b) (4)

4) additional studies could be needed if these studies reveal safety concerns.

(b) (4)



(b) (4)

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2.6.6 TOXICOLOGY

2.6.6.1 Overall Toxicology Summary

Repeat-Dose Toxicology:

Repeat-dose Inhalation toxicity of D-mannitol was evaluated in rats and dogs for the treatment duration of up to 13 weeks. Table 1 (below) presents an overview of these toxicity studies. The studies identified the respiratory system as target organs of toxicity for inhaled mannitol.

Table 1. Overview of Inhalation Toxicity Studies of Mannitol

Study #	Species	Duration	Mannitol (mg/kg/day) ^a	NOAEL
26482/666958 ^b	Rat	7 days	57.3, 97.9	None
XIS 002/033951	Rat	2 weeks	0, 0.9, 2.5, & 6.9	6.9
XIS 005/0413185	Rat	13 weeks	0, 12.4, 21.0	None ^c
26050/666386	Dog	2 weeks	0, 25, 100, 197	None ^d

a. Estimated pulmonary deposits.

b. A non-GLP compliant investigative dose-ranging study. The study did not show the dose-related increase in the concentration of mannitol in the bronchoalveolar fluid. Neither did it examine the lung tissue microscopically.

c. The review is in agreement with the study report in determination of NOAEL.

d. The report states that the 197 mg/kg/day dose is “well-tolerated.”

A 7-day non-GLP dose-ranging inhalation study was conducted to investigate the achievement of pulmonary delivery of mannitol to the lung in Sprague-Dawley rats. Rats (5/sex/dose) were exposed nose-only to approximately 5 or 9 mg of mannitol/L of air for 120 to 240 minutes/day for 7 days. The rats were sacrificed immediately after the last exposure. The amount of mannitol delivered to the lung was determined by measuring mannitol concentrations in the bronchoalveolar fluid (3/sex/dose). The respective estimated achieved dose of mannitol was 573 and 979 mg/kg/day for the low-dose and high-dose groups, respectively. The respective mean mannitol concentration in BALF for the low and high dose groups was 36.7 and 42 µg/ml in males and 43.6 and 33.4 µg/ml in females. Necropsy did not reveal any treatment-related effect. Microscopic examination was not done.

In a previously reviewed 2-week rat study (XIS 002/033951), CD-1 rats (10/sex/dose) were given via nose-only inhalation pulmonary deposited doses of 0, 0.9, 2.5 and 6.9 mg/kg of mannitol for 14-days. Histological evaluations of the respiratory system were done in every group. The remaining organs were examined in the control and high-dose groups only. No significant, treatment-related effects were observed. The NOAEL was 6.9 mg/kg/day.

In the 2-week dog study (XIS 005/0413185), beagle dogs (3/sex/group) were exposed by inhalation via a face mask to air (C), 25 (LD), 100 (MD) or 197 (HD) mg/kg/day of D-mannitol (pulmonary deposition) for 14 days. Coughing occurred during and immediately

after dosing in all treated groups (Incidence for M + F: 0/6-C, 1/6-LD, 4/6-MD and 4/6-HD). Spongy (4/6) and froth-filled lung (3/6) were reported in the HD group during necropsy. Microscopic examination revealed the following: lung congestion/hemorrhage (2/6-HD), and pigment in submandibular lymph node (3/6-HD); bronchoalveolitis (2/3 apiece for MD and HD males); peribronchiolar infiltration (Incidence: 0/3-C, and 3/3 apiece in LD, MD and HD in males; and 1/3-C, 1/3-LD, 2/3-HD and 0/3-HD in females); foamy alveolar macrophages in all treated females and HD males (respective incidence in control, LD, MD and HD: 1/3, 0/3/, 0/3/ and 2/3 in males and 0/3, 2/3, 2/3 and 1/3 in females) and inflammatory foci and focal hyperplasia in trachea carina (1/3-HD female). The high dose males and females also showed increases in lung weight. The study did not establish a NOAEL.

In the 13-week rat study (26050/666386), Sprague-Dawley rats (10/sex/group) were given via nose-only inhalation air (C), 12.4 (LD), or 21.0 (HD) mg/kg/day (pulmonary deposition) for 13 weeks. Additional rats (5/sex) were included in the control (RC) and high dose (RHD) groups to evaluate reversibility of lesions after a recovery period of 4 weeks. The duration of exposure was 180 minutes/day. D-mannitol concentrations were 0, 1.83 or 2.89 mg/L for the control, LD and HD groups, respectively. The estimated pulmonary deposition of D-mannitol was 0, 12.4 and 21.0 mg/kg/day for the control, LD and HD groups, respectively. D-mannitol contents in the bronchoalveolar fluid were approximately 0, 3.8 and 3.2 µg/ml for the control, LD and HD groups, respectively. Both the LD and HD females showed statistically significant decreases in body weight gain (approximately 20%). Clinical pathology examinations revealed minimal decreases (approximately 50% or less) in white blood cell numbers and increases (12-30%) in serum phosphorus in the HD group. Microscopic examinations revealed increases in the incidence of alveolar macrophage aggregation and alveolitis. The respective incidence of alveolar macrophage aggregation in the C, LD, HD, RC and RHD was 3/10, 6/10, 9/10, 0/2 and 1/2 in females and 4/10, 5/10, 3/10, 1/1 and 3/4 in males. The increase in the incidence of alveolitis was observed in the high-dose male only (incidence: 0/10-C, 0-/10-MD, 1/10-HD, 0/1-RC, and 2/4-RHD, respectively). The most significant, treatment-related effect, but of no safety concern for the intended use of mannitol, was seen in the nasal cavity. Both the low and high-dose rats showed increases in the incidence of eosinophilic inclusion in olfactory epithelium of the nasal cavity (Incidence: 3/20-Air, 15/20-LD, and 13/20-HD). This finding is not considered relevant to humans because minimal nasal exposure is expected from the intended clinical use. The study failed to establish NOAEL.

2.6.6.3 Repeat-Dose Toxicity

Study Title: Mannitol Investigative Dose Inhalation Toxicity Study in Rats (draft)

Key findings: Rats (5/sex/dose) exposed to approximately 5 or 9 mg of mannitol/L of air for 120 to 240 minutes/day for 7 days showed detectable amounts of mannitol in the bronchoalveolar fluid. The mannitol concentration in the BALF was, however, variable and no dose-concentration relationship was observed. The respective mean mannitol concentration for the low and high dose groups was 36.7 and 42 µg/ml in males and 43.6 and

33.4 µg/ml in females. No treatment related effect was identified on the limited parameters evaluated. The study did not establish a NOAEL because microscopic examinations were not done in any groups.

Study number: 26482 and 666958
 Volume #, and page #: Volume 13.1, Page 3 - 68
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: January 31, 2006
 Study complete date: February 27, 2006
 Report date: N/A
 GLP compliance: No.
 QA reports: No.
 Formulation/vehicle: Mannitol dry powder

Methods:

Young adult Sprague-Dawley rats (5/sex/group) were exposed by nose-only inhalation to air containing 5 or 9 mg/L mannitol for 120 to 240 minutes/day for 7 days (Table 2). The amount of mannitol delivered to the respiratory tract (3 rats/sex/dose) was determined. Specifically, rats were sacrificed immediately after the last treatment. The lung was removed, weighed, and washed with 5 ml saline twice. Mannitol concentrations in the first wash BALF fluid were determined. Method of analysis was not given. The report states samples “were analysed using appropriate methodology developed for lung lavage washes from dogs.” Other parameters measured included clinical signs (daily), body weight (every 3 days), hematology (day 7), lung weight and necropsy.

Table 2. Duration of Daily Exposure in Rats

Time (Day)	Duration of Exposure (min.) ^a	
	Low-dose Group	High-Dose Group 2
1 - 5	240	240
6	240	120
7	120	120

a. The exposure duration for the last two days was reduced out of concerns about insufficient supplies of the testing material.

Results:

Exposure: The estimated achieved dose of mannitol was 573 and 979 mg/kg/day for the LD and HD groups, respectively. The respective estimated achieved doses ranged 565 – 674 and 619 – 738 mg/kg/day for low-dose males and females and 829 – 1272 and 926 – 1421 mg/kg/day for high-dose males and females (Table 3).

Table 3. Estimated Achieved Doses of Mannitol in Males and Female Rats

Days	Estimated achieved doses for both males and females (mg/kg/day) ^a								Mean
	1	2	3	4	5	6	7	8	
LD Group	595	592	642	623	665	706	365	392	572.5
HD Group	878	1069	1308	1347	1017	575	659	-	979

a. Source: page 29 of submission S017.

The estimated pulmonary deposited dose of mannitol was 57.3 and 97.9 mg/kg/day for the LD and HD groups, respectively, based on deposition fraction of 0.1. The calculation was based on the fraction (11.3 – 78.7%) of aerosol particles with aerodynamic diameters smaller than $5.0 \mu\text{m}$ (b) (4). The MMAD ranged 2.85 ± 3.19 (range: 1.44 ± 4.05 to 4.20 ± 2.39) and 3.62 ± 3.2 (range: 3.31 ± 4.2 to 4.00 ± 1.76) μm for Groups 1 and 2 respectively.

Clinical signs: Rat nostrils were occasionally “caked up” in both groups.

Body weight: No treatments-related effects were observed.

Hematology: No treatments-related effects were observed.

Lung weight: No treatment-related effects were observed.

BALF mannitol concentration: Mannitol was detected in every rat in the LD and HD group, but no dose-response relationship was observed (Table 4).

Table 4. Mannitol Concentrations in BALF in Rats

Group	Sex	Mannitol (mcg/ml)			Mean
		Rat A	Rat B	Rat C	
1 (Low Dose)	Male	12.6	70.4	27.2	36.7
	Female	50.2	60.1	20.5	43.6
2 (High Dose)	Male	10.4	58.9	57	42.1
	Female	11.7	50.6	37.9	33.4

Necropsy: No treatment-related effects were observed.

Study Title: Mannitol toxicity study by inhalation administration to CD rats for 13 weeks followed by 4 week withdrawal period.

Key findings:

- Rats were exposed to D-mannitol at estimated pulmonary deposition of 0, 12.4 and 21.0 mg/kg/day for 13 weeks.
- D-mannitol levels in bronchoalveolar fluid were approximately 0, 3.8 and 3.2 $\mu\text{g/ml}$ for the control, LD and HD groups, respectively.
- Both the LD and HD females showed statistically significant decreases in body weight gain (approximately 20%).
- Microscopic examinations revealed increases in the incidence of alveolar macrophage aggregation and alveolitis. The respective incidence of alveolar macrophage aggregation in the control, low-dose, high dose, recovery control and recovery high-dose groups was 3/10, 6/10, 9/10, 0/2 and 1/2 in females and 4/10, 5/10, 3/10, 1/1 and 3/4 in males. The increase in the incidence of alveolitis was observed in the high-

- dose male only (incidence: 0/10-C, 0-/10-MD, 1/10-HD, 0/1-RC, and 2/4-RHD, respectively).
- The most significant and treatment related effects were seen in the nasal cavity. Both the low and high-dose rats showed increases in the incidence of eosinophilic inclusion in olfactory epithelium of the nasal cavity (Incidence: 3/20-Air, 15/20-LD, and 13/20-HD).
 - The study did not establish a NOAEL.

Study number: XIS 005/0413185
Volume #, and page #: Volume 7.1, Page 1 - 666

Conducting laboratory and location:

(b) (4)

Date of study initiation: August 16, 2004
Study complete date: April 6, 2005
Report date: September 2, 2005
GLP compliance: Yes, with a signed GLP statement
QA reports: Yes, with a signed GLP statement
Drug lot # & purity: 3M08, 3M09, 3M10, 3M11; purity: 98- 102%
Formulation/vehicle: Mannitol dry powder

Methods:

Sprague-Dawley rats (10/sex/group) were exposed via nose-only inhalation to air, 12.4 or 21 mg/kg/day of D-mannitol powder (pulmonary deposition) for 13 weeks. The duration of exposure was 180 minutes/day. The MMAD was 3.9 and 4.4 μm for the LD and HD groups, respectively. Additional male rats (5/group) were included for analysis of bronchoalveolar lavage fluid for the presence of inflammatory cells and mannitol concentration (Table 5). More rats (5/sex/group) were included in the control and high dose group to evaluate reversibility of lesions after a recovery period of 4 weeks. Rats of the main section of the study were sacrificed 24 hours after the last dosing. The recovery rats were sacrifice 4 weeks after the last dosing. Rats in both the main study section and the recovery arm underwent pathological evaluations.

Table 5. Design of the completed 13-week inhalation toxicity study of mannitol in rats

Group	Treatment	Mannitol ^a (mg/kg/day)	Rat Distribution (n/sex)				
			Main ^b Study	Recovery Section	Lung Lavage Section ^c		
					Day 1	Week 7	Week 13
1	Air	0	10	5	5 M	5 M	5 M
2	Mannitol	12.4	10	-	5 M	5 M	5 M
3	“	21.0	10	5	5 M	5 M	5 M

a. Estimated pulmonary deposition. (See Table 6 for details.)

- b. Each group also included 2 additional and reserve rats/sex/group.
c. Additional rats for bronchoalveolar lavage study only.

Doses: 0, 12.4 and 21.0 mg/kg/day (Table 3, below)

Table 6. Estimated Pulmonary Deposits in the 13 Week IH Study in Rats

Treatment	Dose	Aerosol			Exposure (mg/kg/day)	
		MMAD (μm) ^a	GSD	Drug conc. (mg/L)	Achieved Dose ^b	Pulmonary Deposit ^c
Air	0	-	-	-	-	-
Mannitol	LD	3.9	2.19	1.83	124	12.4
	HD	4.4	2.37	2.89	210	21.0

- a. The report states that the MMAD was slightly larger than the ideal range of 1 - 3 μm because it used the formulation as supplied without any modification, (b) (4).
- b. Achieved dose (mean of males and females) reported by the sponsor. The estimated achieved dose assumed 100% deposit of inhalable particle (particles with diameters < 7 μm). The percentage of inhalable particles was 77% and 70% for the low and high dose groups, respectively. For example, the dose in female low dose group was obtained as: [1.53 (mg/L, mean aerosol mannitol concentration) x 160 (ml/min, RMV) x 180 (min, exposure duration/day) x 0.77 (fraction of particles < 7 μm in diameter)]/ 248 (g, mean body weight) = 137 mg/kg/day (report page 435).
- c. Converted from the achieved dose. Pulmonary deposits (mg/kg/day) = Achieved dose (mg/kg/day) x 0.1 (pulmonary deposition factor). For example, the pulmonary deposition for the HD = 124 x 0.1 = 12.4 mg/kg/day.

Species/strain: Rats (CrI:CD[®] (SD) IGS BR),
 #/sex/group: 10
 Satellite groups: 5/sex in control and high dose group, 15 males/group for lung lavage analysis on weeks 1, 4 and 13
 Age: 6 - 7 weeks
 Weight: M: 273 - 343g; F: 191 - 241 g
 Doses in administered units: 0, 12.4 and 21.0 mg/kg/day (See Table 5 (above) for dose estimates)
 Route, form: Nose-only IH, dry powder, 180 minutes/day

Observations and times:

Clinical signs: Daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Pretreatment and Week 13
EKG: Not assessed
Hematology: Weeks 6 and 13
Clinical chemistry: Weeks 6 and 13
Urinalysis: Weeks 6 and 13
Bone marrow: Week 13 and end of recovery period for RBC morphology analysis
Lavage: Weeks 1, 7 and 13 (5 males/time point). Each lung was weighed. The lung was then washed with 8.0 ml of saline 3 times. WBC count,

	differential WBC count and mannitol content in each washout fluid were measured.
<i>Gross pathology:</i>	Sacrifice
<i>Sacrifice method:</i>	Pentobarbitone (IP)
<i>Organs weighed:</i>	Adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid lobes (with parathyroids) and uterus
<i>Histopathology:</i>	Adrenals, brain, femur, heart, kidneys, liver, lungs, ovaries, spleen, spinal cord, sternum, stomach, testes, thyroid (with parathyroids) and uterus in control and high dose groups + respiratory tract of all animals of the main study section; tissues with gross abnormalities in the LD and all recovery rats. The design is considered acceptable because the toxicity of mannitol by non-inhalation routes of administration is a well understood compound. The interest of the currently application is primarily the respiratory system.
	Adequate Battery: yes (x), no ()
	Peer review: yes (), no (x)
<i>Toxicokinetics:</i>	None
<i>Other:</i>	Aerosol concentration and particle size distribution were measured in each exposure period from representative animal exposure positions. Particles were generated by a scraper from a compressed powder and a streamed air flowing over the scraped dust. Particle sizes were determined with an Anderson cascade impactor.

Results

Mortality: None.

Clinical Signs: No treatment-related effects were observed.

Body Weights: Females showed statistically significant decreases in body weight gain (Table 7). The decrease was no longer apparent after a recovery period of 4 weeks. There was no apparent treatment effect in absolute body weight.

Table 7. Body Weight and Weight Gains in Rats

Parameter	Time	D-mannitol (mg/kg/day)					
		Male			Female		
		0	12.4	21.0	0	12.4	21.0
N		17	12	17	17	12	17
Body weight (g)	Week 0	312 ± 18	312 ± 13	310 ± 16	210 ± 13	213 ± 6	217 ± 12
	Week 13	499 ± 37	471 ± 44	497 ± 37	280 ± 21	268 ± 11	275 ± 17
Weight gain (g) as % of control	Weeks 0 - 13	191 ± 23	159 ± 38	187 ± 30	70 ± 13	55 ± 9**	58 ± 15**
	Weeks 0 - 13	-	83	98	-	79	83
as % of control	Rec. period	-	-	99	-	-	105

** , p < 0.01.

Hematology: No remarkable findings were noticed. The HD group showed statistically significant but toxicologically unremarkable increases (< 50% increase) in white blood cell

count (Table 8). The increase was primarily attributed to increases in lymphocyte numbers. The increase was reversible; the recovery groups showed no difference. Other changes were not only small in magnitude but also observed only in one sex. The hematological changes are, therefore, considered unremarkable.

Table 8. Notable Hematological Changes in the 13-Week Rat Study

Sex		Male			Female		
D-mannitol (mg/kg/day)	Week	0	12.4	21.0	0	12.4	21.0
WBC (x 10 ⁹ /L)	6	11.1	12.2	16.0**	7.9	8.9	10.4**
	13	10.7	11.7	14.2*	6.7	8.1	8.4
Lymphocytes (x 10 ⁹ /L)	6	7.5	9.1	11.1**	6.4	7.4	9.0**
	13	7.9	8.6	10.2*	5.3	6.8	6.9
Monocytes (x 10 ⁹ /L)	6	0.22	0.23	0.32**	0.14	0.11	0.15
	13	0.21	0.23	0.29*	0.14	0.11	0.15
Platelets (x 10 ⁹ /L)	6	1017	1068	872*	1039	1023	1096

*, P < 0.05; **, p < 0.01.

Clinical chemistry: The high dose group showed statistically significant increases in serum phosphorus levels (Table 9). The increase was reversible. Potassium level was increased in the high dose males during week 6, but it is not considered toxicologically significant. The increase was absent in other time points of the treatment in males and any time point in the females.

Table 9. Notable Clinical Chemistry Changes in the 13-Week Rat Study

Sex		Male			Female		
D-mannitol (mg/kg/day)		0	12.4	21.0	0	12.4	21.0
K (mmol/L)	Week 6	3.8	3.8	4.1*	3.3	3.6	3.4
Phosphorus (mmol/L)	Week 6	2.20	2.17	2.47*	1.77	1.93	2.22**
	Week 13	2.17	2.12	2.28	1.55	1.87	2.02**

*, P < 0.05; **, p < 0.01.

Urinalysis: Not remarkable. The females showed dose-related decrease in urinary chloride concentration (Table 10), but the results were variable and appear to be within background levels. The recovery control group showed a mean of 34 mmol/L while the HD group showed values of 33 – 42 mmol/L during the exposure period.

Table 10. Urinary Chloride Levels during the Study

Mannitol (mg/kg/day)	Urinary Cl (mmol/L)						
		Male			Female		
		0	12.4	21.0	0	12.4	21.0
Week 6	45.4	58.7	44.7	60.2	46.7	42.4*	
Week 13	45.3	44.6	37.1	70.4	48.5*	32.6**	
Recovery	41.0	-	36.0	34.0	-	56.7	

Bone marrow smear: No treatment-related effects were observed.

Bronchoalveolar lavage Fluid:

White blood cell count: No treatment-related effects were observed.

Differential white blood cell count: No treatment-related effects were observed.

Mannitol content: Mannitol was detected in the bronchoalveolar lavage fluid of treated groups, but it lacked a dose-concentration relationship. Table 11 (below) presents individual and mean mannitol concentrations in week 7. This time point was selected because it had the most consistent values between individual and groups. Data from other time points (day 1 and week 13) were more variable although similar trends existed. The highest mannitol concentration was found in the first wash. The mannitol concentration declined as more washes were carried out. The mean mannitol concentration in the low and high dose groups was 3.8 and 3.2 µg/ml. One control sample showed a detectable amount of mannitol (3 µg/ml) on one occasion.

Table 11. Mannitol Concentration in Week 7

Group	Mannitol (µg/ml)									
	LD					HD				
Rat No	A	B	C	D	E	A	B	C	D	E
Wash 1	6	4	8	8	4	8	3	7	3	4
Wash 2	3	4	4	3	2	4	BLQ ^a	4	3	2
Wash 3	2	2	3	2	2	2	2	4	0	0
Individual mean	3.7	3.3	5.0	4.3	2.7	4.7	2.5	5.0	2.0	2.0
Group Mean	3.8					3.2				

a., BLQ, below the limit of quantitation (2 µg/ml). Data were extracted from vol. 2, p 586.

Organ weights: No treatment-related effects were observed in either pathologically examined or lung lavaged rats.

Gross pathology: Congested lymph nodes were observed in the treated groups (Table 12). The other findings listed, though slightly increased, are not considered to be definitively treatment-related.

Table 12. Noticeable Gross Pathology findings of the 13-Week inhalation Study of Mannitol

Sex Study Section Group N	Male					Female				
	Main Study			Recovery		Main Study			Recovery	
	Cont.	LD	HD	Cont.	HD	Cont.	LD	HD	Cont.	HD
Lung and bronchi: pale areas	4	6	2	1	3	2	3	7	2	2
Congested	0	1	2	0	1	0	0	0	0	0
Lymph node/mandibular: Enlarged	4	2	7	1	2	2	0	0	0	0
Congested	1	1	3	0	2	0	2	3	0	3
Uterus: fluid distension	-	-	-	0	0	2	1	4	3	3

Histopathology:

Increases in the incidence of alveolar macrophage aggregation and alveolitis (Table 13) were observed. The respective incidence of alveolar macrophage aggregation in the control, low-dose, high dose, recovery control and recovery high-dose was 3/10, 6/10, 9/10, 0/2 and 1/2 in females and 4/10, 5/10, 3/10, 1/1 and 3/4 in males. The increase in the incidence of alveolitis was observed in the high-dose male only (incidence: 0/10-C, 0-/10-MD, 1/10-HD, 0/1-RC, and 2/4-RHD, respectively). The most significant and treatment related effects were seen in

the nasal cavity. Both the low and high-dose rats showed increases in the incidence of eosinophilic inclusion in olfactory epithelium of the nasal cavity (Incidence: 3/20-Air, 15/20-LD, and 13/20-HD) that were reversible. This finding, however, is not considered relevant to humans because minimal nasal exposure is expected from the intended clinical use. Increases in the incidence of inflammation cells in the kidney and cysts in the thyroid and pituitary glands were seen in the high dose group, but the significance of these findings is unknown given the lack of systemic toxicity of mannitol from non-inhalation route of administration.

Table 13. Noticeable Microscopic Pathology findings

Sex	Male					Female				
	Main Study			Recovery		Main Study			Recovery	
	Study Section	Group	Group	Group	Group	Group	Group	Group	Group	Group
	Ctrl	LD	HD	Ctrl	HD	Ctrl	LD	HD	Ctrl	HD
N	10	10	10	5	5	10	10	10	5	5
Lung: Alveolar MΦ aggregation	4	5	3	1/1	3/4	3	6	9	0/2	1/2
Alveolitis	0	0	1	0/1	2/4	0	0	0	0/2	0/2
Alveolar hemorrhage	0	1	1	0/1	0/4	0	0	0	0/2	0/2
Lymph node/Mandibular: plasmacytosis	5	7	8	1/1	2/3	4	8	4	-	1/3
Hemorrhage	5	4	7	0/1	2/3	2	2	4	-	2/3
Apoptosis	1	0	2	0/1	0/3	0	0	0	-	0/3
Mediastinal: paracortex cellularity	0/9	4	3	-	-	0	2/9	1/7	-	-
Nasal turbinate: Olfactory epithelial	1	8	6	-	-	2	7	7	-	-
↑ esinophilic inclusion										
Trachea: sub-mucosal inflame. cell	3	4	4	0/1	0/4	1	1	3	-	-
Kidney: Inflammation	2	1/2	6	0/1	- ^a	2	-	1	-	-
Pituitary: developmental cyst(s)	0	-	2	-	-	0	-	1	-	-
Thyroid: prominent ultimobranchial cyst	2	-	7	-	-	4	-	3	-	-

a. -, not examined. For the recovery groups, the review does not consider organs without reported findings microscopically examined because the protocol calls for microscopic examinations of tissues with gross findings only.

Study Title: Two-Week Inhalation Toxicity Study of Mannitol in Dogs (Draft).

Key findings: Beagle dogs (3/sex/group) exposed to ≥ 25 mg/kg/day (pulmonary deposition) of D-mannitol via inhalation for 14 days showed dose-related pathological changes in the respiratory system. The changes included spongy lung and froth-filled trachea; lung congestion or hemorrhage, peribronchiolar infiltration, alveolitis, alveolar foamy macrophages; and focal hyperplasia of trachea carina.

- D-mannitol doses were air, 25, 100 or 197 $\mu\text{g}/\text{kg}/\text{day}$ (pulmonary deposition).
- Spongy (4/6)¹, froth-filled lung (3/6), lung and congestion/hemorrhage (2/6), and pigment in submandibular lymph node (3/6) were observed in HD group.
- Bronchoalveolitis was observed in the MD (2/3) and HD (2/3) males.
- Peribronchiolar infiltration was observed in all treated males. (The respective incidence in control, LD, MD and HD was 0/3, 3/3/, 3/3 and 3/3 in males, and 1/3, 1/3, 2/3 and 0/3 in females.)

¹ Numbers in the parenthesis following changes indicate incidence.

- Foamy alveolar macrophages were observed in all treated females and HD males. (The respective incidence in control, LD, MD and HD groups was 1/3, 0/3/, 0/3/ and 2/3 in males and 0/3, 2/3, 2/3 and 1/3 in females).
- Inflammatory foci and focal hyperplasia was observed in one HD female.
- The lung weight was increased in both HD males and females. It is unclear whether the increase in lung weight was a result of inflammation or mannitol accumulation or both.
- The study did not establish the NOAEL.

Study number: 26050 and 666387 (histopathology)

Volume #, and page #: Volume 13.1, Page 7 - 278

Conducting laboratory and location:

[REDACTED] (b) (4)

Date of study initiation: August 17, 2005

Study complete date: Not available

Report date: N/A

GLP compliance: Yes, with a unsigned GLP statement

QA reports: Yes, with a unsigned GLP statement

Drug lot # & purity: 3M15, 3M16; purity: 98- 102%

Formulation/vehicle: Mannitol dry powder

Methods:

Beagle dogs (3/sex/group) were exposed by inhalation via a face mask to air, 25, 100 or 197 mg/kg/day of D-mannitol for 14 days. Each daily dose was divided into two exposure sessions. Each session was approximately 60 minutes. The interval between two exposure sessions in one day was at least 120 minutes long. Standard batteries of clinical observation, clinical pathology and histological pathology examinations were carried out during and at the end of study.

Species/strain: Dogs, Beagle

#/sex/group: 3

Age: Approximately 5 months

Weight: M: 8.1 – 11.1 kg; F: 6.8 – 10.9 kg

Doses in administered units: 0, 25, 100, or 197 mg/kg/day

Route, form: Inhalation via a face mask, dry powder, 120 minutes/day (60 minute/episode, 2 episodes/day, ≥ 120 minutes between episodes)

Observations and times:

Clinical signs: Daily

Body weights: Twice weekly

Food consumption: Daily

Ophthalmoscopy: Pretreatment and Day 14

<i>Respiratory system:</i>	Respiratory rate, tide volume and minute volume at pre-treatment and days 7 and 14.
<i>ECG:</i>	Days 1 and 14 (within 15 minute post second dosing)
<i>Hematology:</i>	Pre-dosing and day 14
<i>Clinical chemistry:</i>	Pre-dosing and day 14
<i>Urinalysis:</i>	Pre-dosing and day 14
<i>Bone marrow smear:</i>	Day 14 at necropsy
<i>Bronchoalveolar lavage:</i>	Right lobe at necropsy
<i>Gross pathology:</i>	Sacrifice time
<i>Sacrifice method:</i>	Pentobarbitone (IP)
<i>Organs weighed:</i>	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, pituitary, prostate, salivary gland, spleen, testes, thymus, thyroid lobes (with parathyroids) and uterus
<i>Histopathology:</i>	A complete panel – all animals in all groups were examined. Adequate Battery: yes (x), no () Peer review: yes (), no (x)
<i>Toxicokinetics:</i>	Day 1 and 14 at hours 0.5, 1, and 2, 4 (2 nd dose only) and 18 (2 nd dose only) post dosing

Results

Dose estimates: Table 14 (below) presents the dose estimates of the study. The estimated pulmonary deposition was 25, 100 and 197 mg/kg/day for the low, mid and high dose groups, respectively.

Table 14. Estimated Pulmonary Deposits in the 2-Week IH Study in Dogs

Treatment	Dose	Aerosol			Exposure (mg/kg/day)	
		MMAD (μm) ^a	GSD	Drug (mg/L)	Achieved Dose ^a	Pulmonary Deposit ^b
Air	0	-	-	-	-	-
Mannitol	LD	2.6	2.2	1.05	99	25
	MD	2.6	2.4	3.15	251	100
	HD	0.9	2.5	9.22	789	197

- a. Achieved dose used by the study report. The estimated achieved dose assumed 100% deposit of inhalable particle (< 7.3 μm). The inhalable particle was approximately 92%, 91% and 85% of the test aerosols for the low, mid and high dose groups, respectively. The achieved dose was calculated by the formula: $D = (MV \times T \times CC)/BW$. Where, D is in mg/kg/day, MV = minute volume (overall group mean value per sex based on actual recorded results from the study), T = the duration of exposure in minutes, CC = gravimetric chamber concentration in mg/L, and BW = mid-period individual body weight in kg. Extracted from pages 53 and 54 of the report.
- b. Converted from the achieved dose using a deposition factor (0.25). Pulmonary deposits (mg/kg/day) = Achieved dose (mg/kg/day) x 0.25 (pulmonary deposition factor). For example, the pulmonary deposition for the HD = 99 x 0.25 = 25 mg/kg/day.

Mortality: None.

Clinical Signs: Coughing occurred during and immediately post dosing throughout the study. The combined incidence of males and females that coughed was 0/6, 1/6, 4/6 and 4/6 for the control, low, mid and high dose groups, respectively.

Body Weight: No treatment-related effect was observed.

Food Consumption: No treatment-related effect was observed.

Ophthalmoscopy: No treatment-related effect was observed.

Respiratory parameters: No treatment-related effect was observed in respiratory rate, minute volume and tidal volume.

ECG: No treatment-related effect was observed.

Hematology: No treatment-related effect was observed.

Clinical Chemistry: No treatment-related effect was observed.

Urinalysis: No treatment-related effect was observed.

Organ weight: The high-dose group showed increases in lung weight (Table 15). The increase was apparent in both absolute and relative lung weight. Only was the increase in lung weight relative to body weight, however, reached statistical significance in the males. The small sample size (n = 3) probably accounts for the lack of statistical significance.

Table 15. Absolute and Relative Lung weight in Dogs

	Male				Female			
	Cont'l	LD	MD	HD	Cont'l	LD	MD	HD
Body weight (kg)	11.0	11.1	11.6	11.1	9.9	9.3	9.4	10.0
Lung weight (g)	108.7	118.2	112.3	132.0	94.5	89.7	97.1	104.9
Lung weight relative to body (%)	0.99	1.02	1.05	1.19*	0.97	0.97	1.03	1.05

Gross pathology: The high-dose group showed spongy lung and froth-filled trachea (Table 16, below). The respective gender-combined incidence in the control, low, mid and high dose groups was 0/6, 0/6, 0/6 and 4/6 for spongy lung and 0/6, 0/6, 0/6 and 3/6 for froth-filled trachea.

Table 16. Notable Necropsy Findings in Dogs

	Male				Female			
	Cont'l	LD	MD	HD	Cont'l	LD	MD	HD
Lung: dark focus	0	0	2	0	0	0	0	0
Spongy	0	0	0	2	0	0	0	2
Trachea: froth-filled	0	0	0	2	0	0	0	1

Histopathology: Microscopic changes were limited to the respiratory system. The changes included congestion or hemorrhage, peribronchiolar infiltration, alveolitis, alveolar foamy macrophages and focal hyperplasia of trachea carina (Table 17). Congestion/hemorrhage (MD and HD), pigmentation and hyperplasia in the lymph node (HD), foamy alveolar macrophages, and inflammation/focal hyperplasia in trachea carina (HDF) were observed in one or both sexes in a dose-dependent manner (i.e., mid and/or HD groups). Peri-bronchiolar infiltration was observed in all treated males while the incidence of alveolitis was increased

in all treated females. The gender-combined incidence for the control, low, mid and high-dose groups was 0/6, 0/6, 1/6 and 2/6 for congestion/hemorrhage; 1/6, 4/6, 5/6 and 3/6 for peribronchiolar infiltration; 1/6, 2/6, 2/6 and 3/6 for foamy alveolar macrophages; and 1/6, 5/6, 2/6 and 3/6 for alveolitis.

Table 17. Notable Microscopic Findings in Dogs ^a

	Male				Female			
	Cont'l	LD	MD	HD	Cont'l	LD	MD	HD
Lung: Agonal congestion/hemorrhage	0	0	1	1	0	0	0	1
Alveolitis, minimal focal	0	2	0	0	1	3	2	3
Broncho-alveolitis	0	0	2	2	1	0	0	0
Perivascular infiltrates	3	3	3	3	3	3	3	3
Peri-bronchiolar infiltration	0	3	3	3	1	1	2	0
Foamy alveolar mΦ	1	0	0	2	0	2	2	1
Lymph node: submandibular/pigment / lymph hyperplasia	0	0	0	2	0	0	0	1
Retropharyngeal/ lymph hyperplasia	0	0	0	1	0	0	0	0
Trachea: inflammatory foci	0	0	0	0	0	0	0	1
- carina: focal hyperplasia	0	0	0	0	0	0	0	1

a. n = 3/group.

Toxicokinetics: Results not available yet.

2.6.6.9 Discussion and Conclusions

(b) (4)

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7/21/2006 02:10:49 PM
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7/21/2006 04:00:58 PM
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I concur.

2.6 PHARMACOLOGY / TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND Number: 70,277
Review Number : 1
Sequence number/date/submission type: 000/19-NOV-04; Stamp date: 22-NOV-04;
Original submission
Information to the Sponsor: Yes (), No (x)
Sponsor/or Agent: Pharmaxis Ltd, 1840 Gateway Dr., San Mateo, CA
94404
Manufacturer of the Drug Substance: (b) (4)
Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Drug Products
HFD #: HFD-570
Review Completion Date: March 18, 2005

Drug:
Trade Name: Aridol
Generic Name: D-Mannitol
Code Name: None
Chemical Name: 1,2,3,4,5,6-hexanehexol, or cordycepic acid
CAS Register Number: 69-65-8
Mole File Number: Not available
Molecular Form and Weight: C₆H₁₄O₆/182.2

Relevant IND/NDAs: MDF# (b) (4)
Drug Class: Diagnostic (Broncho-provocation) agent
Intended clinical population: Asthmatic patients
Route of Administration: inhalation
Clinical Formulations: Capsules filled with 5, 10, 20 and 40 mg of D-mannitol powder.
Mannitol will be delivered by a dry powder inhaler.

Proposed Clinical Protocol: Each of 130 asthmatic subjects 6 – 50 years of age will receive up to 635 mg of mannitol to provoke bronchoconstriction. A rising dose schedule will be employed and a total dose of mannitol per patient will be 5, 15, 35, 75, 155, 315, 475 and 635 mg. Dose-escalating will stopped when bronchoconstriction occurs.

Previous Human Experience:

The sponsor states that approximately 1240 asthmatic adults and 160 asthmatic children have been given up to 635 mg of mannitol dry powder by inhalation (p 1.068).

Studies Submitted and Reviewed in the Review

Study #	Description	Vol. #	Page #
	Summary of nonclinical information	2	1
XIS 001/033434	Single-dose inhalation toxicity of mannitol in rats	2	007
XIS 002/033951	14-day inhalation toxicity study of mannitol in rats	2	112
004/034088	Mannitol eye irritation study in rabbits	3	001
003/034081	Effect of mannitol on bovine corneal opacity and permeability in vitro	3	020

Studies Submitted but Not Reviewed in this Review: None.

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

Drug History:

A pre-IND meeting was held on July 19, 2004 to discuss the development program of mannitol indicated as a bronchial provocation agent in asthmatics. Nonclinical information available then included a literature review of mannitol toxicology and summaries of sponsor-completed studies. These studies included inhalation toxicity studies up to 14 days in rats, an ocular irritation study in rabbits, a (cow) cornea irritation study in vitro (planned). The Division informed the sponsor of the following:

- 1) The available data is adequate to open an IND.
- 2) Inhalation toxicity studies up to 14 days in a second animal species are also needed for the NDA filing.
- 3) Additional toxicity study(ies) in rats with higher mannitol doses may be needed, pending the review of completed studies.

Mannitol is used as a food additive, a drug and an excipient in drug products. As a food additive, mannitol is considered Generally-Regarded-As-Safe (GRAS). Medically, mannitol has been used as a laxative, diuretic and excipient. As an excipient, mannitol is present in many oral, parenteral (e.g., IV, and IP), and topical products. The sponsor states that inhaled mannitol at doses up to 635 mg/patient has been given to approximately 1,400 asthmatics and normal volunteers. No significant adverse effects associated with the use of mannitol were observed. Mannitol is non-carcinogenic and non-mutagenic.

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2.6.2 PHARMACOLOGY

Inhalation of hyper-osmotic D-mannitol may provoke bronchoconstriction by stimulating the release of histamine from mast cells. The submission contains no individual pharmacology study reports. It does contain a brief literature review. According to the review, hyper-osmotic mannitol (2 – 3x normal) induced the release of histamine from cultured human lung mast cells and blood basophils. Mannitol treatment also enhances histamine release from mast cells induced by IgE.

2.6.3. PHARMACOKINETICS AND TOXICOKINETICS

No data on pharmacokinetic and toxicokinetic data of inhaled mannitol in animals are provided.

2.6.6 TOXICOLOGY

2.6.6.1 Overall Toxicology Summary

General toxicology:

Inhalation toxicity studies of 1 and 14 days were conducted via a nose-only exposure system in rats. Respective D-mannitol doses (pulmonary deposition) in the low, mid and high dose groups were 2, 8 and 10 mg/kg/day in the single-dose study and 0.9, 2.5 and 6.9 mg/kg/day in the 14-day study. Table 1 (next page) presents an overview of the two toxicity studies. In the single-dose study, the rats were dosed on day one and sacrificed on day 15 for histologic evaluations. In the 14-day study, the rats were sacrificed 24 hours after the last dose. Histological evaluations of the respiratory system were done in every group and in the remaining organs of the control and high-dose groups only. No significant, treatment-related effects were observed in either study. The NOAEL was 10 and 6.9 mg/kg/day for the single-dose and 14-day repeat-dose exposure.

Table 1. Overview of Mannitol toxicity studies

Study #	Species	Duration	Route	Mannitol (mg/kg/day) ^a	NOAEL
XIS 001/033434	Rat	1 time	IH	0, 2, 8 & 10	10
XIS 002/033951	Rat	14 days	IH	0, 0.9, 2.5, & 6.9	6.9

a.. Estimated Pulmonary deposits.

Special toxicity:

Two studies were conducted to evaluate the eye irritation potential of mannitol. The studies are a Draize eye irritation test in rabbits and a bovine corneal opacity and permeability test in vitro. No significant irritation was observed in either assay. The results demonstrate that mannitol is non-irritating to the eye.

2.6.6.2 Acute Toxicity Study

Single-dose Toxicity Study of by Inhalation Administration to Rats.

Key findings: This is a preliminary acute toxicity study in rats which revealed no abnormal findings at single inhalation mannitol doses up to 10 mg/kg. CD-1 rats (5/sex/dose) were given via nose-only inhalation single pulmonary deposited doses of 0, 2, 8, and 10 mg/kg of mannitol. The rats were sacrificed after an observation period of 14 days.¹ Parameters assessed included clinical observations (clinical signs, food consumption and body weight) and autopsy. No histological examination was conducted. No treatment-related abnormalities were found. This study is not very informative given its design. A detailed review of the study is omitted. The following is the administrative information about the study.

Study number:	XIS 001/033434
Volume #, and page #:	Volume 1.2, Page 2-007
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 1, 2003
Study complete date:	January 19, 2004
Report date:	April 30, 2004
GLP compliance:	Yes, Signed GLP statement

¹ The pulmonary dose was calculated by multiplying the reported achieved doses by 0.1 (deposition fraction). The report states that the achieved doses are 17.6, 80.0 and 98.1 for the low, mid and high dose groups, respectively. The achieved doses were calculated from aerosol mannitol concentrations of 0.48, 2.84 and 3.72 mg/L. At least 71% of the aerosol has MMAD of (b) (4). The duration of exposure was 60 minutes.

QA reports: Yes,
 Drug lot # & purity: 3M05, 98- 102% purity

2.6.6.3 Repeat-Dose Toxicity

Study Title: Toxicity Study by Repeated Daily Inhalation Administration to CD rats for 2 weeks

Key findings: CD-1 rats (10/sex/dose) were given via nose-only inhalation pulmonary deposited doses of 0, 0.9, 2.5 and 6.9 mg/kg of mannitol for 14-days. The rats were sacrificed after 24 hours after the last dosing. No treatment-related abnormalities were found. The NOAEL is 6.9 mg/kg/day.

Study number: XIS 002/033951
 Volume #, and page #: Volume 1.2, Page 2-112
 Conducting laboratory and location: (b) (4)
 Date of study initiation: November 6, 2003
 Study complete date: February 27, 2004
 Report date: June 14, 2004
 GLP compliance: Yes, Signed GLP statement
 QA reports: Yes,
 Drug lot # & purity: 3M05, 98- 102% purity
 Formulation/vehicle:

Methods: Animals were dosed by oral inhalation once per week for 6 weeks.

Dosing:

Species/strain: Rats (CrI:CD[®] (SD) IGS BR),
 #/sex/group: 10
 Age: 6 - 7 weeks
 Weight: M: 26. – 324g; F: 168 - 205g
 Doses in administered units: (See Table 2, next page, for dose estimates)
 Route, form: Nose-only IH, dry powder, 60 minutes/day

Observations and times:

Clinical signs: Daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: None
EKG: Pre-treatment and week 5
Minute volume: For 15 minutes pretreatment using Buxco Electronics LS-20 system

<i>Hematology:</i>	Week 2
<i>Clinical chemistry:</i>	Week 2
<i>Urinalysis:</i>	Week 2
<i>Gross pathology:</i>	Sacrifice
<i>Sacrifice method:</i>	Pentobarbitone (IP)
<i>Organs weighed:</i>	Adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid lobes (with parathyroids) and uterus
<i>Histopathology:</i>	A complete panel for the control and high dose groups; respiratory system for all groups.
<i>Toxicokinetics:</i>	None
<i>Other:</i>	Aerosol concentration and particle size distribution were measured in each exposure period from representative animal exposure positions. Particles were generated by a scraper from a compressed powder and a streamed air flowing over the scraped dust. Particle sizes were determined with an Anderson cascade impactor.

Results

Dose estimates: Table 2 (below) presents the dose estimates of the study. The estimated pulmonary deposition was 0.9, 2.5 and 6.9 mg/kg/day for the low, mid and high dose groups, respectively.

Table 2. Estimated Pulmonary Deposits in the 14 day IH Study in Rats

Treatment	Dose	Aerosol			Exposure (mg/kg/day)	
		MMAD (µm)	GSD	Drug (mg/L)	Achieved Dose ^a	Pulmonary Deposit ^b
Air	0	-	-	-	-	-
Mannitol	LD	2.4	2.37	0.264	9.0	0.9
	MD	3.7	2.46	0.877	25.2	2.5
	HD	4.7	2.80	2.796	69.3	6.9

a. Achieved dose reported by the sponsor.

b. Converted from the achieved dose. Pulmonary deposits (mg/kg/day) = Achieved dose (mg/kg/day) x 0.1 (pulmonary deposition factor). For example, the pulmonary deposition for the HD = 69.3 x 0.1 = 6.9 mg/kg/day.

Mortality: None.

Clinical Signs: No treatment-related effects were observed.

Body Weights: No treatment-related effects were observed. The respective terminal mean body weight was 329, 340, 345, 343 grams in males and 215, 204, 200 and 201 grams in females.

Clinical pathology: No remarkable findings were noticed.

Hematology: No treatment-related effects were observed.

Clinical chemistry: No treatment-related effects were observed.

Urinalysis: No treatment-related effects were observed.

Organ weights: No treatment-related effects were observed.

Gross pathology: No treatment-related effect was observed.

Histopathology:

No significant abnormalities were observed in the respiratory system in the treatment groups (Table 3). Increased incidences of inflammation cells in the heart and kidney were seen in the high dose group, but the significance of these findings is unknown given the lack of systemic toxicity of mannitol from non-inhalation route of administration.

Table 3. Noticeable Pathology findings of the 14 –day inhalation Study of Mannitol

Mannitol (mg/kg/day, Pulmonary)	Male				Female			
	0	0.9	2.5	6.9	0	0.9	2.5	6.9
N	10	10	10	10	10	10	10	10
Adrenal: cortical vacuolation	8	-	-	10	10	-	-	6
Brain: vascular inflammation	0	-	-	1	0	-	-	0
Epididymides: inflammation cells	6	-	-	9				
Heart: Inflammation cells in myocardio.	1	-	-	4	0	-	-	2
Kidney: cortical tubular basophilia	1	-	-	4	1	-	-	2
Interstitial inflammatory cells	2	-	-	5	2	-	-	2
Lungs: Sub-pleural inflammation cells	2	7	4	6	6	5	4	9
Spleen: prominent extramedullary hemapoiesis	4	-	-	7	10	-	-	10

- indicates not examined.

2.6.6.4 Special toxicity studies

Study Title: Eye Irritation to the Rabbits (Report No. 004/034088)

Three white New Zealand rabbits were administered 78 mg (0.1 ml in volume) of mannitol in one eye and observed for ocular irritation for 72 hours post administration. The opposite eye served as a control. Parameters evaluated include corneal opacity, iridial lesions, and conjunctival redness and chemosis. No remarkable findings were observed in any of the rabbits. Mannitol is considered non-irritant to the eye under the conditions tested.

Study Title: Bovine Corneal Opacity and Permeability Assay (Report No. 003/034081)

This assay assessed the ocular irritancy potential of mannitol *in vitro*. It is not a standard test. Some consider it an alternative to the Draize *in vivo* eye irritation test. Isolated bovine corneas (obtained from slaughter houses) were incubated with mannitol powder, 20% imidazole (positive control) or 0.9% saline on the anterior side but the culture medium on the posterior side at 32°C for 4 hours. Opacity was determined by the light transmission through the cornea. Permeability was measured by the rate of sodium fluorescein crossing the cornea

with a spectrophotometer. (Cornea was incubated at 5 mg/ml sodium fluorescein at 32°C for 90 minutes.) A composite score was derived for each cornea based on its opacity and permeability reading. A score value of less than 25 was considered non-irritant. A score of greater than 25 was considered irritant. The composite score of 0.2, 152.4 and not applicable was obtained for the mannitol, imidazole and saline, respectively. The report states that mannitol is classified as “a negative potential eye irritant” according to the criterion.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

The available nonclinical data of this application is insufficient to support the safety of the proposed clinical trial of mannitol. The deficiency is primarily the lack of appropriate inhalation toxicity studies in a non-rodent species and the lack of adequate safety margins between the observed-no-adverse-effect-level in animals and the proposed clinical dose in humans. The lack of sufficient safety margin is indicated by the proposed clinical dose (635 mg/patient, or 12.7 mg/kg/day) being greater than the NOAEL in monkeys (6.9 mg/kg/day from a 14-day inhalation toxicity study). However, significant clinical experience of the proposed use of mannitol exists. The clinical experience appears to support the proposed use of mannitol.

Toxicology:

The toxicology of non-inhalation use of mannitol is well understood. Mannitol is non-carcinogenic and non-mutagenic. F344/N rats and B6C3F1 mice fed with up to 5% D-mannitol in diet for 103 weeks did not reveal any evidence of tumorigenicity. Mannitol used as a nutrient and/or dietary supplement in animal drugs, feeds, and related products is generally recognized as safe [21 CFR 582.5470 (4/1/97)]. Medically, mannitol has been used as a laxative, diuretic and excipient. As an excipient, mannitol is present in many oral, parenteral (e.g., IV, and IP), and topical products.

However, toxicological characterization of inhaled mannitol is limited. There is no information in the literature regarding to the toxicity of inhaled mannitol. The sponsor conducted inhalation toxicity studies of mannitol in rats for the treatment duration of up to 14 days. Respective D-mannitol doses (pulmonary deposition) for the low, mid and high dose groups were 2, 8 and 10 mg/kg/day in the single-dose study and 0.9, 2.5 and 6.9 mg/kg/day in the 14-day study. No significant, treatment-related effects were observed in either study. The NOAEL was 10 and 6.9 mg/kg/day for the single-dose and 14-day repeat-dose exposure.

Clinical Experience

According to the sponsor, approximately 1,400 asthmatics and normal volunteers received via inhalation up to 635 mg mannitol/patient. No significant adverse effects associated with the mannitol treatment were observed.

Conclusion

The proposed trial of mannitol is safe from the nonclinical viewpoint. The application does not contain sufficient nonclinical data to support the safety of all the proposed clinical doses of mannitol. The inadequacy includes the lack of toxicity studies in a second species (a non-rodent species) and the lack of adequate safety margins between the NOAEL in rats and the portion of the proposed clinical doses ($> 35 \text{ mg/kg/day}$), based on a NOAEL of 7 mg/kg/day ($7 \text{ mg/kg/day} \div 10 \text{ safety factor} \times 50 \text{ kg/patient} = 35 \text{ mg/patient}$). However, sufficient clinical experience appears to support the safety of all the proposed doses and compensates the inadequacy in nonclinical data. Also, the expected adverse effect associated with the proposed use of mannitol – bronchoconstriction – is readily monitorable clinically. The proposed trial is reasonably safe.

Recommendation:

The initial IND protocol is considered to be reasonably safe to proceed.

Luqi Pei, Ph.D.
Pharmacologist/toxicologist

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I concur.

Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-22368

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