CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208151Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208151
Supporting document/s:	SDN001 (eCTD 0000) SDN008 (eCTD 0007)
Applicant's letter date:	SDN001: 2-12-2016 SDN008: 8-31-2016
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Product:	Atropine sulfate ophthalmic solution 1%
Indication:	• For use in producing cycloplegia and mydriasis, (b) (4) • Penalization of the healthy eye in the treatment of amblyopia. • (b) (4)
Applicant:	Alcon Research LTD 6201 South Freeway Fort Worth, TX 76134
Review Division:	Division of Transplant and Ophthalmology Products
Reviewer:	Aaron M Ruhland, PhD
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1 Executive Summary

1.1 Introduction

The subject of this New Drug Application is Isopto® Atropine 1% (Atropine Sulfate Ophthalmic Solution, 1%). The proposed indications are for use in producing cycloplegia and mydriasis and treating amblyopia (b) (4). The applicant filed the NDA as a 505(b)(2) application. All nonclinical pharmacology/toxicology data included in the application are derived from published literature sources. The Applicant relies only on published data. No listed drugs were referenced.

1.2 Brief Discussion of Nonclinical Findings

The receptors antagonized by atropine are the peripheral structures that are stimulated or inhibited by muscarine (i.e., exocrine glands and smooth and cardiac muscle). Findings in nonclinical studies reflect this mechanism of action including mydriasis, tachycardia, decreased water intake, water retention and decreased urine volume. Decreased salivation was also observed. Chronic exposure results in decreased weight gain and death at doses much higher than those expected following topical ophthalmic exposure.

Publications submitted by the applicant indicate that atropine sulfate showed no genotoxic potential and was not carcinogenic.

The systemic administration of atropine was associated with decreases in male fertility. Nonclinical data suggest anticholinergic effects on contraction of vas deferens and seminal vesicle during emission resulting in decreased sperm volume and altered composition of the ejaculate. Administration of atropine in female rats resulted in marked vascular congestion, epithelial necrosis and fibrous tissue proliferation of the uterine tissue. Atropine administration was associated with a reduction of uterine parameters like uterine diameter, thickness of myometrium and endometrium and surface epithelial cell height. The results suggest that the anticholinergic effects of atropine may interfere with the rhythmic release of pituitary gonadotropins and result in decreased estrogen.

Teratology studies of atropine were limited. A study in mice reported that exposure to atropine on Day 8 or Day 9 of gestation was associated with an increase in skeletal anomalies, including one occurrence of axial skeletal fusion, and one occurrence of a soft tissue anomaly, exencephaly. The authors of the paper, however, concluded that atropine was not teratogenic. Given the low incidence of each anomaly and inadequate study design, a definitive conclusion regarding teratogenicity cannot be reached. As such, the labeling should state that the potential for fetal harm remains unknown and atropine should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus.

1.3 Recommendations

1.3.1 Approvability: The application is approvable from a Pharmacology/Toxicology perspective.

1.3.2 Applicant's proposed labeling (sections relevant to nonclinical Pharmacology/Toxicology)

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no adequate and well-controlled studies with ISOPTO® Atropine 1% in pregnant women. (b) (4) . ISOPTO® Atropine 1% should be used (b) (4) if the (b) (4) benefit (b) (4) the potential risk (b) (4)

8.2 Lactation

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Atropine was negative in the (b) (4)

13.2 Animal Toxicology and/or Pharmacology

A neurotoxicology study in rats utilizing the Morris water maze demonstrated minimal behavioral effects, altered swim path, during 6 days of intraperitoneal dosing with atropine at 5 mg/kg (108 times the recommended human ophthalmic dose).

1.3.3 FDA's proposed changes to labeling (Redline version of sections relevant to nonclinical Pharmacology/Toxicology). Details regarding proposed changes are included in this review in the relevant sections. Suggested deletions are notated as a strikethrough font and suggested additions are notated as a thick underlined font (blue).

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no adequate and well-controlled studies with ISOPTO® Atropine 1% administration in pregnant women to inform a drug-associated risk. Adequate animal development and reproduction studies have not been conducted with atropine sulfate. (b) (4) In humans, 1% atropine sulfate is systemically bioavailable following topical ocular administration *[see section 12.3 Pharmacokinetics].* ISOPTO® Atropine 1% should only be used (b) (4) during pregnancy if the (b) (4) potential benefit (b) (4) justifies the potential risk (b) (4) to the fetus.

8.32 Lactation

There is no information regarding the presence of atropine in human milk following ocular administration, the effects on the breastfed infants, or the effects on milk production to inform risk of ISOPTO® Atropine 1% to an infant during lactation. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ISOPTO® Atropine 1% and any potential adverse effects on the breastfed child from ISOPTO® Atropine 1% or from the underlying maternal conditions. (b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Atropine sulfate was negative in the (b) (4) Salmonella/microsome mutagenicity test. (b) (4)
Studies to evaluate carcinogenicity and impairment of fertility have not been conducted.

(b) (4)

2 Drug Information

2.1 Drug

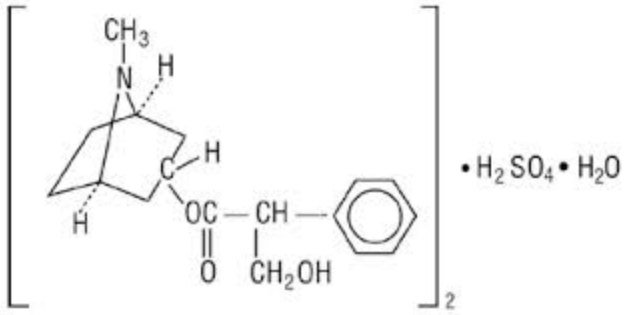
CAS Registry Number: 55-48-1

Generic Name: Atropine Sulfate Ophthalmic Solution USP, 1%

Chemical Name: (b) (4) sulfate (2:1) salt monohydrate; α-(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, *endo*-(±)-, sulfate (2:1) (salt), monohydrate

Molecular Formula/Molecular Weight: $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ / 694.83 g/mol

Structure:



Pharmacologic Class: Anticholinergic; cholinergic muscarinic antagonist

2.3 Drug Formulation

Table 1: Drug product formulation of ISOPTO® Atropine 1% (atropine sulfate monohydrate ophthalmic solution)

Ingredient	Function	Concentration (%)
Atropine sulfate	Active pharmaceutical ingredient	1.0%
Benzalkonium chloride	Preservative	0.01%
Hypromellose	(b) (4)	(b) (4) %
Boric Acid		%
Hydrochloric acid and/or sodium hydroxide	pH adjustment	To pH (b) (4)

2.4 Comments on Novel Excipients

All excipients have been previously qualified for topical ophthalmic use.

2.6 Proposed Clinical Population and Dosing Regimen

Isopto® Atropine 1% is an anti-muscarinic agent indicated for:

- Mydriasis
- Cycloplegia
- Amblyopia
- (b) (4)

(b) (4)

3 Studies Submitted

The following studies were submitted in the original NDA submission (SDN001) or in a subsequent submission (SDN008).

3.1 Studies Reviewed

General Toxicology

- Study T715-TR 112:7320:75/76: Ocular irritation potential of atropine sulfate 2% steri-unit in rabbits after multiple topical ocular instillation
- Boyd, C., and E. Boyd, 1962, "The chronic toxicity of atropine administered intramuscularly to rabbits", *Toxicol Appl Pharmacol*, 4: 457 – 467.
- Boyd, C., 1963, "The acute intramuscular toxicity of atropine in albino rats", *Toxicol Appl Pharmacol*, 5: 772.
- Lulham, G., *et al*., 1990, "A subchronic toxicity study of two inhaled aerosolized atropine sulfate formulations in rats and dogs", *Drug Chem Toxicol*, 13(1): 19 – 42.

Developmental and Reproductive Toxicology

- Ratnasooriya, W., 1989, "Effects of atropine on fertility of male rats", *Vidyodaya J, Sci*, 1: 47 – 55.
- Ban, Y., *et al*., 2002, "Impairment of male fertility induced by muscarinic receptor antagonists in rats", *Reprod Toxicol*, 16: 757 – 765.
- Sato, T., *et al*., 2005, "Atropine-induced inhibition of sperm and semen transport impairs fertility in male rats", *J Toxicol Sci*, 30: 207 – 212.
- Schlough, J., 1969, "Delayed implantation in the rat induced by atropine", *Biol Reprod*, 1: 315 – 319.
- Patil, M., *et al*., 2009, "Atropine sulfate induced changes in uterine, adrenal, liver and thyroid gland in female albino rats", *J Pharmacol Toxicol*, 4: 236 – 245.
- Dziuk, P., and T. Mann, 1963, "Effect of atropine on the composition of semen and secretory function of male accessory organs in the boar", *J Reprod Fertil*, 5: 101 – 108.
- Black, D., and R. Duby, 1965, "Effect of oxytocin, epinephrine, and atropine on the oestrous cycle of the cow", *J Reprod Fertil*, 9: 3 – 8.
- Rauch, T., 1990, "Atropine sulfate impairs selective parameters of performance in the Morris water maze", *J Neural Transm Park Dis Dement Sect*, 2: 159.

Genotoxicity

- McCann, J., *et al*, 1975, "Detection of carcinogens as mutagens in the *Salmonella* / microsome test: Assay of 300 chemicals", *Proc Nat Acad Sci*, 72: 5135 – 5139.

Carcinogenicity

- Schmähl, D. and M. Habs, 1976, "Life-span investigations for carcinogenicity of some immune-stimulating, immunodepressive and neurotropic substances in Sprague-Dawley rats", *Z Krebsforsch*, 86: 77 – 84.

3.2 Studies Not Reviewed

- American Hospital Formulary Service (AHFS), 2012, "AHFS Drug Information 2012", American Society of Health-System Pharmacists, pp. 1 – 14.
- Ali-Melkkila, T., *et al*., 1993, "Pharmacokinetics and related pharmacodynamics of anticholinergic drugs", *Acta Anaesthesiol Scand*, 37: 633.
- Arcuri, P. and R. Gautieri, 1973, "Morphine-induced fetal malformations III: possible mechanisms of action", *J Pharm Sci*, 62: 1626.
- Brown, J., 1990, "Chapter 8: Atropine, Scopolamine, and related anti-muscarinic drugs", *Publication unknown*
- Bueker, E., and W. Platner, 1956, "Effect of cholinergic drugs on development of chick embryo", *Proc Soc Exp Biol Med*, 91: 539.
- Chen, H., *et al*., 2006, "Sensitive and specific liquid chromatographic–tandem mass spectrometric assay for atropine and its eleven metabolites in rat urine", *J Pharm Biomed Anal*, 40: 142.
- Christen, P., 2000, "Tropane alkaloids: Old drugs used in modern medicine", *Studies in Natural Products Chemistry*, 22: 717.
- Drug Bank database: Atropine (DB00572)
- Fujita, M., *et al*., 1979, "Enhancement of gastric carcinogenesis in dogs given *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine following vagotomy", *Cancer Res*, 39: 811.
- Gabourel, J. and R. Gosselin, 1958, "The mechanism of atropine detoxication in mice and rats", *Arch Int Pharmacodyn*, 115: 416.
- Gosselin, R., *et al*., 1955, "The metabolism of ^{14}C-labeled atropine and tropic acid in mice", *J Pharmacol Exp Ther*, 115: 217.
- Kalser, S., *et al*., 1957, "Further studies of the excretion of atropine-*alpha*-C^{14}", *J Pharmacol Exp Ther*, 121: 449.
- Kalser, S., *et al*., 1965, "Drug metabolism in hypothermia. I. Biliary excretion of ^{14}C-atropine metabolites in the intact and nephrectomized rat", *J Pharmacol Exp Ther*, 147: 252.
- Kanto, J., and U. Klotz, 1988, "Pharmacokinetic implications for the clinical use of atropine, scopolamine, and glycopyrrolate", *Acta Anaesthesiol Scand*, 32: 69.
- (b) (4)
- (b) (4)
- Meisner, D., *et al*., 1989, "Liposomal ophthalmic drug delivery. III. Pharmacodynamic and biodisposition studies of atropine", *Int J Pharmaceutics*, 55: 105.
- Prete, M., *et al*., 1987, "Plasma atropine concentrations via intravenous, endotracheal, and intraosseous administration", *Am J Emerg Med*, 5: 101.
- (b) (4)
- Registry of Toxic Effects of Chemical Substances (RTECS): Atropine, 2012

- Schaeffel, F., 2005, “Pupillographic evaluation of the time course of atropine effects in the mouse eye”, *Optom Vis Sci*, 82: 215.
- Stadtbäumer, K., *et al*., 2006, “Effects of mydriatics on intraocular pressure and pupil size in the normal feline eye”, 2006, *Vet Ophthalmol*, 9: 233.
- Tatsuta, M., *et al*., 1989, “Inhibition by neostigmine and isoproterenol and promotion by atropine of experimental carcinogenesis in rat stomach by N-methyi-N' -nitro-N-nitrosoguanidine”, *Int J Cancer*, 44: 188.
- Whelan, N., *et al*., 2011, “Efficacy of tropicamide, homatropine, cyclopentolate, atropine, and hyoscine as mydriatics in Angora goats”, *New Zealand Veterinary Journal*, 59: 328.
- Wurzburger, R., 1977, “Radioimmunoassay of atropine in plasma”, *J Pharmacol Exp Ther*, 203: 435.

4 Pharmacology

4.1 Primary Pharmacology

Atropine is commonly classified as an anticholinergic or antiparasympathetic (parasympatholytic) drug. More precisely, however, it is termed an antimuscarinic agent since it antagonizes the muscarine-like actions of acetylcholine and other choline esters.

Atropine inhibits the muscarinic actions of acetylcholine on structures innervated by postganglionic cholinergic nerves, and on smooth muscles which respond to endogenous acetylcholine but are not so innervated. As with other antimuscarinic agents, the major action of atropine is a competitive or surmountable antagonism which can be overcome by increasing the concentration of acetylcholine at receptor sites of the effector organ (e.g., by using anticholinesterase agents which inhibit the enzymatic destruction of acetylcholine). The receptors antagonized by atropine are the peripheral structures that are stimulated or inhibited by muscarine (i.e., exocrine glands and smooth and cardiac muscle). Responses to postganglionic cholinergic nerve stimulation also may be inhibited by atropine but this occurs less readily than with responses to injected (exogenous) choline esters.

Reviewer’s note: Following topical application to the eye, atropine sulfate blocks the cholinergic responses of the pupillary sphincter muscle of the iris (mydriasis) and the ciliary muscle controlling accommodation (cycloplegia).

4.2 Secondary Pharmacology

No references were provided which describe the secondary pharmacology of atropine sulfate.

4.3 Safety Pharmacology

A study was cited which measured ECG in Beagle dogs following inhalation of atropine (see below). While heart rate was increased in response to atropine sulfate, no other changes in cardiac function were attributed to the test article.

5 Pharmacokinetics/ADME/Toxicokinetics

No nonclinical studies determined ocular distribution or systemic exposure to atropine sulfate following topical ocular administration. However, human data was provided to inform systemic bioavailability.

6 General Toxicology

Study title: Ocular irritation potential of atropine sulfate 2% steri-unit in rabbits after multiple topical ocular instillation

Study no.:	T715-TR 112:7320:75/76
Study report location:	SDN001; Section 4.2.3.2
Conducting laboratory and location:	Alcon Laboratories, Fort Worth, TX
Date of study initiation:	Not specified
GLP compliance:	Not specified
QA statement:	No
Drug, lot #, and % purity:	Drug Lot #VI752

Methods

Doses:	2% Atropine sulfate Steri-Unit (Alcon)
Frequency of dosing:	q20 minutes for 6 hours (18 doses over 6 hours)
Route of administration:	Topical ocular drop
Dose volume:	0.05mL/dose
Formulation/Vehicle:	Drug formulation similar to Isopto Atropine except this test article did not contain benzalkonium chloride or hypromellose
Species/Strain:	Rabbit / New Zealand
Number/Sex/Group:	6 eyes / treatment group
Age:	Not specified
Weight:	~ 2 kg

Observations and Results

Clinical Signs (Ocular examination per Draize scoring every 2 hours; iris, anterior chamber, cornea, lens, and light reflex scored 6 hours after final instillation per Baldwin, McDonald and Beasley, 1973).

- minimal conjunctival congestion
- minimal discharge
- minimal impaired light reflex (expected pharmacologic response; also observed in untreated contralateral eye due to systemic absorption)
- minimal corneal cloudiness

Table 2: Clinical signs following topical ophthalmic instillation of 2% Atropine sulfate Steri-Unit: Two hours post-instillation

Parameter	Vehicle control		Atropine Steri-Unit	
	Incidence	Mean Score	Incidence	Mean Score
Conjunctival Congestion (max score = 6)	6/6	2.0	1/6	0.3
Conjunctival Discharge (max score = 6)	0/6	0.0	0/6	0.0

Table 3: Clinical signs following topical ophthalmic instillation of 2% Atropine sulfate Steri-Unit: Four hours post-instillation

Parameter	Vehicle control		Atropine Steri-Unit	
	Incidence	Mean Score	Incidence	Mean Score
Conjunctival Congestion (max score = 6)	4/6	1.3	4/6	1.3
Conjunctival Discharge (max score = 6)	0/6	0.0	0/6	0.0

Table 4: Clinical signs following topical ophthalmic instillation of 2% Atropine sulfate Steri-Unit: Six hours post-instillation

Parameter	Vehicle control		Atropine Steri-Unit	
	Incidence	Mean Score	Incidence	Mean Score
Conjunctival Congestion (max score = 6)	4/6	1.3	6/6	4.0
Conjunctival Discharge (max score = 6)	0/6	0.0	2/6	1.0
Light Reflex (max score = 2)	0/6	0.0	6/6	2.0
Corneal cloudiness (severity; max score = 4)	0/6	0.0	2/6	0.3
Corneal cloudiness (area; max score = 4)	0/6	0.0	2/6	0.8

Study title: The acute intramuscular toxicity of atropine in albino rats

Study report location:	*Toxicology and Applied Pharmacology*, 5: 772
Conducting laboratory and location:	Carl E Boyd, Department of National Health and Welfare, Ottawa, Canada
Date of publication:	1963
GLP compliance:	Not stated
QA statement:	No
Drug, lot #, and % purity:	Not provided

Methods

Doses:	5 – 1200 mg/kg
Frequency of dosing:	Single injection
Route of administration:	Intramuscular injection
Dose volume:	1.0 mL / kg
Formulation/Vehicle:	Not specified
Species/Strain:	“Albino”
Number/Sex/Group:	10 – 15 / treatment group (males only)
Age:	Not specified
Weight:	207 ± 55 g

Observations and Results

Mortality

Mortality was categorized as “early” occurring at < 3 days and “delayed” occurring > 3 days. An LD_{50} was calculated for early mortality as 1068 ± 77 mg/kg with mortality observed at doses ≥ 600 mg/kg. The immediate cause of death in early mortalities was respiratory failure usually preceded by tremors and convulsive twitching. Delayed deaths occurred at all doses and appeared independent of the dose administered. The overall LD_{50} for mortality was calculated as 995 ± 61 mg/kg.

Table 5: The percentage incidence of mortality for the early and delayed reactions, singly and combined, in relation to the dose of atropine

[a] Calculated from doses of from 600 to 1200 mg/kg.

Clinical Signs

- 6 hours
  - mydriasis
  - hyperkinesia
  - irritability
  - convulsive movements
  - ataxia

- 24 hours
  - irritability
  - pallor
  - diarrhea
  - exophthalmos
  - hyperkinesia
  - penile erection

- 1 – 2 weeks
  - Survivors and delayed decedents

        - pallor
        - diarrhea
        - penile erection
        - chromodacryorrhea

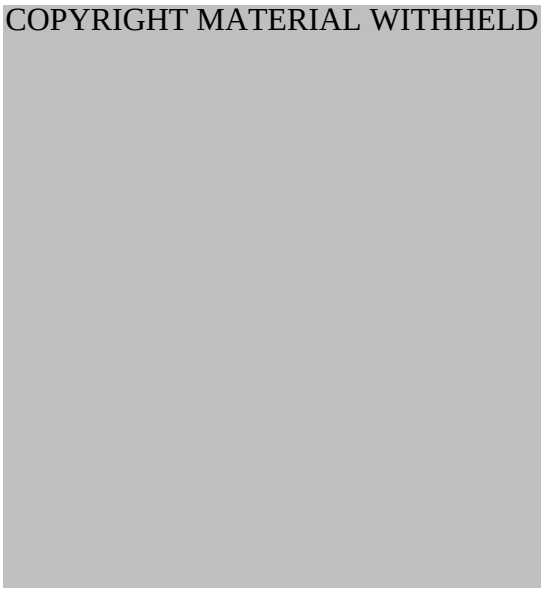
    - Delayed decedents only
        - irritability

The authors also note local lesions in the injected left leg starting in as little as 30 minutes following drug administration. Clinical observations include flaccidity, edema, cyanosis, local anesthesia, and a tendency for the animal to chew at or chew off, its toes or other portions of the limb. Many of these signs appeared as early as 30 minutes following injection. At 1 week, necrosis, a tendency for the left foot to be fixed in a position of mid extension, and hyperesthesia were present, along with lesser degrees of the signs seen at 1 day. By 1 month, most of the lesions were healed or healing, leaving many animals with atrophied or sloughed thighs and self-amputated digits or feet. The local lesions were more marked in animals receiving the higher doses of atropine sulfate.

Body Weights

- At 24 hours following dose administration, body weight was decreased up to 62 ± 17 g/kg/24hours (range +3 to -62 g/kg/24hours) with no obvious dose dependency
- In survivors, a dose dependent decrease in body weight gain was observed compared to controls, with consistent body weight loss in those animals which progressed to delayed death

Figure 1: Mean change in body weight in survivors and delayed deaths non-survivors



Feed and water consumption (24-hours post-injection)

- A dose dependent decrease in food and water intake was apparent at 24 hours

Study title: The chronic toxicity of atropine administered intramuscularly to rabbits

Study report location:	*Toxicology and Applied Pharmacology*, 4: 457 - 467
Conducting laboratory and location:	Carl E Boyd and Eldon M Boyd Department of Pharmacology. Queen's University, Kingston, Ontario, Canada
Date of publication:	1962
GLP compliance:	Not stated
QA statement:	Not provided
Drug, lot #, and % purity:	Not provided

Key Study Findings

- Systemic administration of atropine caused mydriasis, anemia, fever, leukocytosis, hypercholesterolemia, and alkaluria.
- Atropine induced dose dependent loss of body weight partially explained by decreased food intake
- Pathologic changes were noted as loss of weight and edema of most organs examined, hepatitis, pulmonary thrombosis, inhibition of spermatogenesis, thymic atrophy, and histologic changes in the gall bladder, spleen, and pancreas.

Methods

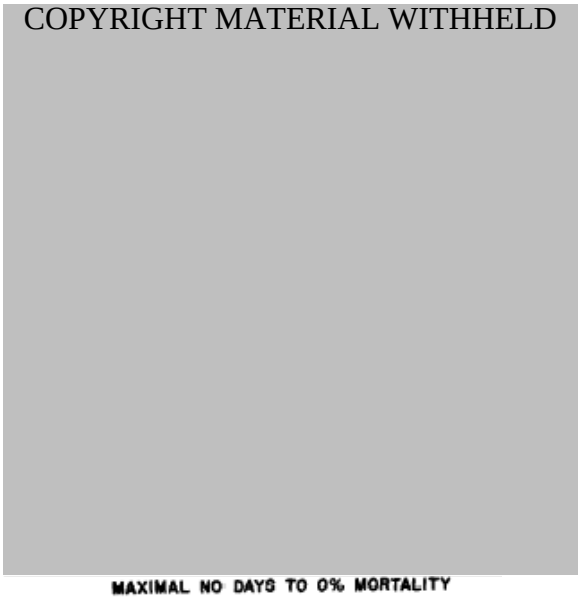
Doses:	44, 59, 74, 88, 118 mg/kg
Frequency of dosing:	Once daily for 100 days
Route of administration:	Intramuscular injection
Dose volume:	1 mL / kg
Formulation/Vehicle:	Water
Species/Strain:	CBL rabbit
Number/Sex/Group:	10 males / group
Age:	“young”
Weight:	1 – 1.5 kg
Satellite groups:	Pair-fed controls and controls fed “ad libitum”

Observations and Results

Mortality

Mortality was observed. Sixty per cent of the deaths were characterized as convulsions and respiratory failure occurring within a few minutes of the time of the injection of atropine. The remaining deaths occurred overnight (i.e. not observed). On Day 100 (day of last dose), 48 % mortality was found across all drug-treated rabbits, 16% in the pair-fed controls, and 0 in the controls fed ad libitum for 100 days. Regression of dose producing 50% mortality on a given observation day was logarithmic. The dose estimated to produce 10% mortality at 100 days was 65 ±10 mg/kg/day compared with the pair-fed controls and 46 ± 5 mg/kg/day compared with the controls fed ad libitum. The highest dose, 118 mg/kg, approximated the dose estimated to kill 50% of rabbits in 10 days. A regression analysis of the daily dose against the maximal number of days to no deaths was presented:

Figure 2: Regression analysis of daily dose against maximal number of days to no deaths



Clinical Signs

- Mydriasis
- Diminished pupillary light reflex
- Increase in colonic temperature
- Impaired flexion of the left hind limb developed after 8-11 weeks of atropine injection into that limb. This persisted until death or sacrifice.

Body Weights

- Inhibition of growth noted in atropine treated rabbits
- The dose that produced 50% incidence was calculated by linear regression and plotted against the time in days at which each had been estimated. At 100 days, food intake was depressed in 50% of animals by a daily dose of 52.6 ± 8.4 mg/kg. The authors note that anorexia and starvation are features of chronic atropine intoxication, but since pair fed controls lost less weight, the reduction in growth was not completely due to reduced food intake.

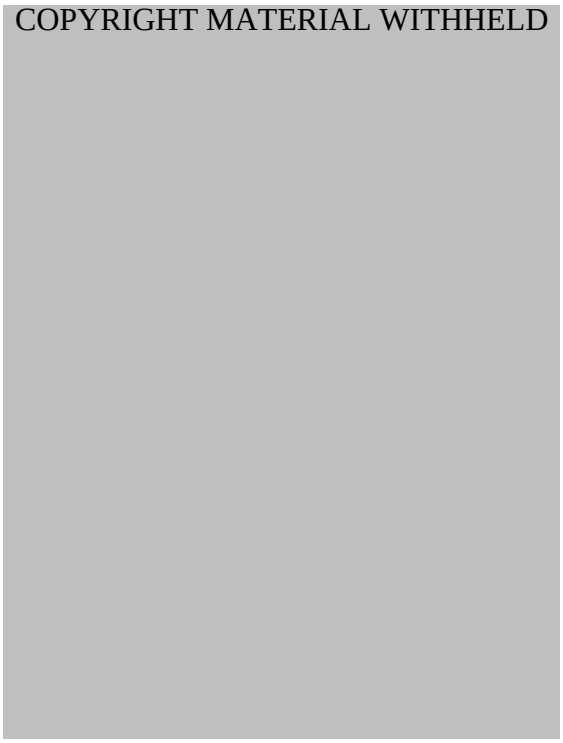
Feed and water consumption (24 hour assessment once weekly)

- Decreased water consumption
- The authors estimated that at 100 days food intake was depressed in 50% of animals by a daily dose of 52.6 ± 8.4 mg/kg

Hematology (hemoglobin, hematocrit, leukocyte count once every 2 weeks)

- The authors note that after 2 – 3 months a decrease in hemoglobin, and to a lesser degree hematocrit, was due "mostly to starvation". The authors plot the dose dependency of the effect. At the high dose, it appears that hemoglobin was decreased by ~10% in pair-fed control (open circles) and ~25% compared to rabbits fed "ad libitum" (solid circles).

Figure 3: Percentage change from pair-fed controls (open circles) and controls fed ad libitum (filled circles) in the concentration of blood hemoglobin after 9 – 12 weeks of daily administration of atropine sulfate



- Leukocytosis: Increase in leukocytes by 38%, no other details were provided

Clinical Chemistry (serum cholesterol)

- "Slightly lower" serum cholesterol at 21 and 84 days in two groups.

Urinalysis (analysis of 24 hour collection every two weeks)

- Decreased urine volume
- The pH of urine was slightly ($p = 0.05$) increased over values in pair-fed controls. There were no significant changes in urinary albumin, glucose, acetone, occult blood, or bilirubin.

Gross Pathology

In atropine treated animals, the authors note that most organs were edematous but the degree of edema was not related to dose.

Organ Weights

Organs were noted as smaller in weight, but edematous. Measurements of weight and water content of the organs of surviving animals were summarized. A dose dependent decrease in the weight compared to those of pair-fed controls was reported for many organs.

Organ weight

Table 6: Shifts in the weight of organs of surviving rabbits

Organ	**Daily dose of atropine sulfate**			
	44.2	**58.8**	**73.7**	**88.3**
Submaxillary salivary glands	+ 17.0	- 4.4	- 12.8	- 24.9 *
Esophagus	- 4.1	- 14.9	- 21.1 *	- 0.1
Stomach	+ 2.9	- 17.8	- 26.3 *	- 7.3
Duodenum	+ 0.5	- 5.9	- 23.0	- 29.3
Jejunum	- 19.6	- 1.4	- 24.4	- 28.2
Ileum	- 13.7	- 19.7 *	- 22.5	- 21.7
Cecum	+ 5.1	- 16.0 *	- 24.5	+ 6.4
Appendix	- 8.6	- 23.7	- 41.0 *	- 42.6
Colon	+ 7.4	- 8.1	- 3.7	0
Liver	- 18.4	- 23.7 *	- 37.2 *	- 36.4 *
Gall bladder	+ 1.1	+ 6.8	- 4.3	+ 15.3
Kidneys	- 12.0	- 14.3	- 26.5 *	- 26.5 *
Heart	- 14.3	- 29.7 *	- 35.2 *	- 31.9 *
Trachea	- 2.1	- 19.7 *	- 38.6*	- 2.8
Lungs	+ 1.1	- 21.4 *	- 36.8 *	- 16.1
Spleen	- 0.3	+ 11.0	- 41.9 *	- 35.5
Adrenal glands	- 21.0	- 7.3	+ 5.7	+ 6.5
Testicles	- 1.8	- 0.8	- 32.5 *	- 11.9
Thymus	- 15.7	- 18.2	- 54.1 *	- 28.3
Brain	-	- 28.0 *	- 5.7	-
Total Body	- 9.8	- 18.7 *	- 26.7 *	- 28.9 *

* indicates that the mean difference was significant at $p \leq 0.05$ by *t*-test.

Water weight (% of control; measured as grams water per 100 gram dry weight of organ)

Table 7: Shifts in the water levels of organs of surviving rabbits

Organ	Daily dose of atropine sulfate			
	44.2	**58.8**	**73.7**	**88.3**
Submaxillary salivary glands	0	+ 7.6	+ 2.8	+ 13.4
Esophagus	- 3.2	+ 9.5	+ 5.7	+ 15.4 *
Cardiac Stomach	- 7.9	- 21.3 *	- 9.5 *	+ 40.6 *
Pyloric Stomach	+ 8.7 *	- 1.2	- 0.7	+ 9.6 *
Duodenum	- 7.2	- 1.7	+ 13.8 *	- 2.0
Jejunum	+ 15.1 *	- 1.5	+ 10.1	+ 3.5
Ileum	- 0.7	+ 17.5 *	+ 15.3	+ 22.2
Cecum	+ 9.1	+ 19.9 *	+ 0.9	- 3.8
Appendix	+ 1.4	+ 5.8	0.0	+ 3.4
Colon	+ 9.0	+ 18.1	+ 1.3	+ 13.3
Liver	- 3.0	+ 5.0	- 2.6	+ 2.4
Gall bladder	+ 23.8 *	+ 21.8 *	- 9.4	+ 30.6 *
Kidneys	+ 6.9	+ 5.5	- 4.3	- 15.1 *
Heart	+ 1.0	+ 2.3	+ 1.2	+ 11.6 *
Trachea	+ 7.3	+ 16.5 *	+ 46.9 *	+ 50.0 *
Lungs	+ 17.4 *	+ 11.0	- 0.8	+ 2.2
Spleen	- 1.1	+ 2.4	+ 0.6	+ 1.9
Adrenal glands	- 11.0	+ 3.8	- 8.5	+ 5.6
Testicles	- 0.6	+ 1.2	- 4.8	- 0.8
Thymus	+ 56.5 *	+ 13.2	- 2.5	+ 138.5 *
Cerebrum	-	+ 1.3	+ 0.9	-
Cerebellum	-	+ 6.5	+ 19.4	-
Muscle	-	+ 14.1 *	+ 11.8	-
Skin	- 0.6	+ 2.8	+ 17.1	+12.1 *

* indicates that the mean difference was significant at $p \leq 0.05$ by *t*-test.

Histopathology

Histological Findings:

Dose related lesions were described though incidence/dose dependence was not reported:

- Liver: mild central lobular degeneration, hepatic fibrosis. The authors note that fibrosis was uncommon.
- Lungs: venous thrombosis and cell infiltrations
- Testes: degeneration of spermatogonia cells and spermatocytes with few or no

free sperm.

- Gall bladder: Necrotic changes in the columnar epithelium.
- Spleen: volume of white pulp reduced,
- Thymus: thymocytes reduced.
- Pancreas: deficiency of zymogenic granules in the acinar glands; small cysts but no fibrosis were seen in the pancreas of 2 rabbits.
- The impaired flexion of the left hind limb was found due to fibrosis of areas of the gluteal muscles, sometimes accompanied by, and probably following, hemorrhage and leukocytic infiltration. The subendothelium of the smaller arteries in this region was markedly hypertrophied, partially obliterating the lumen of the vessel.

Special Evaluation

- **Respiratory tract fluid**
    - The volume and chloride content of respiratory tract fluid were measured on survivors of groups receiving 59 and 74 mg/kg/day. Values did not differ significantly from those in pair-fed controls.

The authors summarize the results as a mean percentage change from control at the time when 50% of rabbits had died from daily dosing with atropine sulfate. For the 44 mg/kg group, the calculations were made from measurements recorded after 98 days of atropine administration, when no rabbits had died.

Table 8: Mean percentage changes from pair-fed and ad libitum fed controls of survivors at the time when 50% of rabbits had died from daily dosing with atropine sulfate

Measurement	Control for comparison	Daily dose of atropine sulfate (mg/kg)				
		44 mg/kg	59 mg/kg	74 mg/kg	88 mg/kg	118 mg/kg
Body weight	Ad-lib fed	- 6	- 29	- 35	- 41	- 33
	Pair fed	- 10	- 19	- 26	- 30	- 14
Food intake	Ad-lib fed	- 9	- 5	+ 8	+6	- 45
Water intake	Ad-lib fed	- 33	- 18	- 56	- 46	- 31
	Pair fed	- 18	- 21	- 55	- 35	- 42
Urine output	Ad-lib fed	- 68	- 24	- 63	- 64	- 47
	Pair fed	- 46	- 47	- 57	- 67	- 31
Colonic temperature	Ad-lib fed	- 0.39	+ 0.097	- 0.9	- 0.096	- 0.097
	Pair fed	+ 0.53	+ 0.73	+ 0.58	+ 0.81	- 0.39

Based on these results, the authors estimates the dose of atropine which would produce the stated effect in 50% of rabbits compared with pair-fed controls after a stated number of days of daily intramuscular injections:

Table 9: Estimates of doses of atropine sulfate producing effect in 50% of rabbits, compared with pair-fed controls, after daily intramuscular injection of atropine sulfate

Days of administration	Effect				
	Inhibition of growth	Inhibition of water intake	Inhibition of urine output	Augmentation of colonic temperature	Death
10	86 ± 4	73 ± 10	91 ± 14	-	127 ± 9
20	83 ± 4	71 ± 10	87 ± 14	-	110 ± 8
30	80 ± 4	69 ± 10	84 ± 14	-	101 ± 7
40	77 ± 4	67 ± 10	80 ± 14	80 ± 20	95 ± 6
50	74 ± 4	65 ± 10	76 ± 14	75 ± 20	90 ± 6
60	72 ± 4	63 ± 10	73 ± 14	69 ± 20	87 ± 6
70	69 ± 4	61 ± 10	69 ± 14	63 ± 20	84 ± 6
80	66 ± 4	59 ± 10	66 ± 14	58 ± 20	82 ± 5
90	63 ± 4	57 ± 10	62 ± 14	52 ± 20	79 ± 5
100	60 ± 4	55 ± 10	57 ± 14	46 ± 20	78 ± 5

Study title: A subchronic toxicity study of two inhaled aerosolized atropine sulfate formulations in rats and dogs

Study report location:	*Drug Chem Toxicol*, 4: 457 - 467
Conducting laboratory and location:	Lulham, G., *et al.,* Bio-research Laboratories Ltd. (Quebec, Canada), US Army Medical Material Development Activity (Fort Detrick, MD), Riker Labs/3M Company (St. Paul, MN)
Date of publication:	1990
GLP compliance:	Not stated
QA statement:	Not provided
Drug, lot #, and % purity:	Not provided

The subchronic (21 days) toxicity of inhaled metered aerosol formulations (solution and suspension) of atropine sulfate was investigated in rats and dogs.

Key Study Findings

- No mortality
- The expected mydriatic effect of atropine sulfate was seen in both species and, similarly, the pupillary light reflex was impaired in rats and dogs receiving atropine sulfate at all dose levels.
- Reduced salivation was noted in both species

- Ophthalmologic examinations in both species were unremarkable.
- In dogs, atropine sulfate caused tachycardia, but there was no evidence of an adverse effect on the electrocardiogram or on systolic blood pressure.
- In both species, atropine sulfate did not alter body weight, food consumption or clinical pathology parameters.
- Necropsy observations and histopathological findings revealed no effect of atropine sulfate in either species although, in the rat, adrenal gland hypertrophy in both sexes followed inhalation was reported for the suspension at both dose levels.

Rats

Methods

Doses:	Solution: 0.78 and 2.5 mg/kg/day Suspension: 1.4 and 3.2 mg/kg/day Authors note that doses 37 – 47-times higher than human dose of 2.0 mg BID (0.067 mg/kg via autoinjector)
Frequency of dosing:	Daily, approximately 2 hours
Route of administration:	Inhalation apparatus
Formulation/Vehicle:	Metered aerosol
Species/Strain:	Rat: Sprague Dawley
Number/Sex/Group:	10/sex/group
Age:	6 – 7 weeks
Weight:	Not available

Observations and Results

Mortality (twice daily)

- No mortalities occurred.

Clinical Signs (including salivation, twice daily)

- Reduced salivation

Ophthalmoscopy (only pupillary diameter and pupillary light reflex assessed prior to exposure and approximately one-half hour following exposure on study days 1, 4, 7, 17, and 21)

- Marked pupillary dilatation (from baseline1 - 2mm to ~5 mm) was observed post-dosing in all rats. In general, diameter returned to baseline by the time of the next day's dose.

- Following inhalation of atropine sulfate, light reflex was slow or absent.

Hematology (red/white cell counts, hemoglobin, hematocrit, prothrombin time, platelet count at necropsy)

- No changes attributed to the test article

Clinical Chemistry (urea, protein, albumin, alkaline phosphatase, ALT, AST, glucose, bilirubin, cholesterol, LDH, sodium potassium, calcium, chloride)

- No changes attributed to the test article

Gross Pathology

- No changes attributed to the test article

Organ Weights

- An increase in adrenal weight (relative to body weight) was noted in both sexes receiving the suspension formulation. No histopathological correlate was noted. Rats receiving the inhaled solution had normal adrenal weights.

Histopathology

- No changes attributed to the test article

Dogs

Doses:	Solution and Suspension: 0.5 and 1.3 mg/kg/day; authors note that doses 7 and 19-fold higher than human dose of 2.0 mg BID (0.067 mg/kg via autoinjector)
Frequency of dosing:	Daily
Route of administration:	Inhaled
Formulation/Vehicle:	Metered aerosol
Species/Strain:	Dog: Beagle
Number/Sex/Group:	4 males/group
Age:	6 – 7 months
Weight:	Not available

Observations and Results

Mortality

One dog developed severe acute bacterial enteritis and was euthanized on Day 22. The authors cannot exclude atropine as potentially contributing to the infection since atropine has been shown to decrease intestinal motility.

Clinical Signs (including assessment of salivation; twice daily)

- Reduced salivation

Pupillary diameter and pupillary light reflex (pre-dose and immediately post-dose on Days 1, 4, 7, 14 and 21)

- Pupillary dilatation (from baseline 6 – 8 mm to ~9.5 - 11 mm) was observed post-dosing in all dogs. Many times the pupils remained dilated up until the time of the next day's dose.
- Dogs receiving the high-dose had slowed or absent pupillary light reflex which remained at the subsequent pre-dose assessment. In low dose animals, the light reflex was also impaired pre-dosing and post-dosing but to a lesser severity.

Body Weights (twice weekly)

- No changes attributed to the test article

Feed Consumption (daily)

- No changes attributed to the test article

Ophthalmoscopy (pre-treatment and final week of study)

- No changes were attributed to the test article

ECG (pre-dose and 30 minutes post-dose)

- No changes or toxicity attributed to the test article

Heart Rate (pre-dose and immediately post-dose on Days 1, 4, 7, 14 and 21)

- Marked post-dose elevations in heart rate were noted at the low dose and higher doses. The low dose (0.5 mg/kg) represents an 8-fold margin over 100% absorption of the proposed clinical dose in adults and a 4-fold safety margin for juveniles.

Table 10: Mean heart rate in male dogs in subchronic toxicity study of inhaled atropine sulfate

Group	Study Day									
	1		4		7		14		21	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-

Group 1: Sham (air) control; Group 2: Placebo aerosol (solution); Group 3: Atropine sulfate solution (0.5 mg/kg); Group 4: Atropine sulfate solution (1.3 mg/kg); Group 5: Placebo Aerosol Suspension; Group 6: Atropine sulfate suspension (0.5 mg/kg); Group 7: Atropine sulfate suspension (1.3 mg/kg)

Hematology (same as for rat described above)

- No changes attributed to the test article

Clinical Chemistry (same as for rat described above)

- No changes attributed to the test article

Urinalysis (pre-treatment, at sacrifice)

- No changes attributed to the test article

Gross Pathology

- No changes attributed to the test article

Organ Weights

- No changes attributed to the test article

Histopathology

- No changes attributed to the test article

7 Genetic Toxicology

Study Title: Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals

Study report location:	*Proc Nat Acad Sci*, 72: 5135 - 5139
Conducting laboratory and location:	Joyce McCann, Edmund Choi, Edith Yamasaki, Bruce Ames, Biochemistry Department, University of California, Berkeley, CA.
Date of publication:	1975
GLP compliance:	Not stated
QA statement:	Not provided
Drug, lot #, and % purity:	Not provided

Strains: TA100, TA1537, TA1535, TA98

Results

This survey review of 300 chemicals reported that atropine sulfate with or without metabolic activation (Aroclor activated S9 fraction of rat liver microsomes) was not mutagenic in the *Salmonella* strains tested. The authors also note that atropine was found not to be carcinogenic but note that limited data are available.

8 Carcinogenicity

Study title: Life-span investigations for carcinogenicity of some immune-stimulating, immunodepressive and neurotropic substances in Sprague-Dawley rats

Study report location:	*Z. Krebsforsch*, 86: 77 – 84.
Conducting laboratory and location:	D. Schmähl and M. Habs Institut für Toxikologie und Chemotherapie Am Deutschen Krebsforschungszentrum, D-6900 Heidelberg, Im Neuenheimer Feld 280 Federal Republic of Germany
Year of publication:	1976
GLP compliance:	Not stated
QA statement:	Not included

Key Study Findings

- The carcinogenic potential of atropine sulfate was assessed along with 11 other substances. No increase in tumor incidence over controls was noted in atropine treated rats.

Methods

Doses:	6 mg/kg
Frequency of dosing:	Once weekly
Route of administration:	Intraperitoneal
Basis of dose selection:	5% of the LD_{50} dose
Species/Strain:	Rat: Sprague-Dawley
Number/Sex/Group:	36/sex/group
Age:	12 days
Paradigm for dietary restriction:	Ad libitum

Observations and Results

Table 11: Tumor incidence in rats treated with weekly intraperitoneal atropine sulfate

Substance		Number of animals	Mean survival time (weeks)	Survival p value	Animals bearing tumors	
					Number	Percentage
Control	Males	36	96 ± 17	-	1	3
	Females	33	94 ± 15	-	3	11
Atropine sulfate	Males	30	88 ± 19	> 0.05	0	0
	Females	31	89 ± 14	> 0.3	3	10

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Effects of atropine on fertility of male rats

Study report publication:	*Vidyodaya J*, 1: 47 – 55
Authors and location:	(b) (4) [redacted]
Year of publication:	1989
GLP compliance:	Not stated
QA statement:	Not included

Key Study Findings

Methods

Doses:	25% and 50% rod content
Frequency of dosing:	Continual
Dose volume:	Rod: 3.5 mm diameter. 10 – 12 mm length
Route of administration:	Single rod inserted adjacent to each epididymis
Formulation/Vehicle:	Silastic rod (50 – 75 % polysiloxane polymer)
Species/Strain:	Rat: Sprague-Dawley
Number/Sex/Group:	Males: 25% rod: n= 6 50% rod: n = 11 Control rod: n = 14

Observations and Results

Atropine release from the rods was confirmed with the average release rate of atropine from 50% atropine rods as 60.3 ± 2.8mg after 7 days. If release kinetics were zero order, this would be equivalent to daily release of 8.4 ± 0.35 mg.

Clinical observations
Nine of the 11 rats with the 50% atropine rods developed inflammation and necrosis of the tunica vaginalis and scrotal sacs around the operation site. This lesion was not noted in control rod implanted rats or 5 of the 6 rats implanted with the 25% atropine sulfate rod. The lesions were treated with Polybactrin R spray.

Libido, ejaculatory competence and fertility
Assessed on Days 3 and 7 and then approximately at weekly intervals by pairing each male overnight with a pro-estrus female which has at least three consecutive 4-day vaginal cycles. The sexual behavior pattern of the paired rats was noted 2 – 3 hours later. The presence of spermatozoa in the vaginal smears of paired females on the following morning was used as evidence of successful mating. In the absence of spermatozoa, daily vaginal smearing was undertaken to determine occurrence of pregnancy or pseudopregnancy. At 9 – 12 days post-coitum, the females were laparotomized and the number of fetuses present was recorded. For every group of treated males, a fertility index was calculated. The fertility index = total number of fetuses / number of successful matings at a given time.

The fertility of rats fitted with drug- free rods was normal (6-10 fetuses). In contrast, every drug treated male became subfertile (1-4 fetuses) on several occasions and completely sterile at least on one occasion. This effect on fertility was significant (during the first two matings with the 25% atropine rods and at all matings with 50% atropine rods).

Mating performance, i.e. percentage of successful matings, was normal with control and 25% atropine rods. However, performance was significantly reduced in male rats implanted with 50% atropine rods. In contrast to the mating performance, precopulatory courtship behavior did not appear to be altered by atropine. Atropine rod implanted rats showed normal sniffing and grooming behaviors of female genital areas, palpation of females' sides with forepaws and actual attempts of mounting and intromission.

Vaginal sperm counts
For select overnight matings (n = 6/treatment), the following morning the vaginas were lavaged with saline and the number of sperm present was counted.

Vaginal sperm count of females paired with males fitted with drug-free rods was 5.06 ±0.73 million. In contrast, the vaginal sperm content of females paired with atropine treated males (25% or 50% rods) was ≤ 0.1 million (n= 6, for each group).

Motility of epididymal sperm (n = 6 / treatment group)
On day 7 following implantation, the motility of the spermatozoa from the left and right epididymides were scored using a subjective scale from 0 (immotile) to 5 (greatest motility ever observed).

In control rats, motilities of epididymal spermatozoa were 4.60 - 4.75 ± 0.25 and those on the treated sides were 1.25 ± 0. 47 with 50 % atropine rods and 2.3 ± 0.32 with 25 % atropine rods. These differences were statistically significant (Student's t-test). Drug treatment did not cause any obvious morphological abnormalities in epididymal spermatozoa as judged by light microscopy.

Histology of testes
On day 7, a subset of rats was sacrificed and the testes examined histologically (n=6).

The testes of rats fitted with 50% atropine rods appeared similar to that of rats with drug-free rods, with no obvious interference in the spermatogenic process. In addition, germinal epithelium showed no signs of desquamation.

Accessory organ weight
Seminal vesicles along with coagulatory glands, epididymides vasa deferentia and testes were isolated and weighed (n = 6).

The average wet weights of testes, epididymides, vasa deferentia and seminal vesicular-coagulatory gland complex of rats fitted with drug-free rods were 943 ± 135 grams,446 ± 67 ,83 ± 7 and 560 ± 66 grams, respectively. In rats implanted with 50% atropine rods, the corresponding values were 1248 ± 46g, 533 ± 65g, 88 ± 09g and 440 ± 51 grams. There was no significant alteration in the wet weights of any of these organs (Student's t-test).

Nerve-induced mechanical response of isolated vasa deferentia
Isolated vasa deferentia were stimulated with platinum ring electrodes. A typical response which is partially adrenergic is characterized by an initial rapid contraction and a slower more sustained secondary response. Atropine sulfate was added to increase concentration every 15 minutes (10, 20 50, and 100 µg/mL) and the response to electrical simulation recorded.

In another experiment, responses to electrical stimulation in the presence of a single 25% (n = 6) or 50% (n= 8) atropine rod placed within the organ bath. Contractile responses in the presence of drug, or drug containing rods, were expressed as percentage reduction of their respective pre-drug controls.

Both components of the contractile response were inhibited by free atropine and by rods containing atropine. Repeated washing (4-5 min) subsequent to the addition of 100 µg atropine restored the initial contraction by 65.7 ± 9 % and the secondary contraction by 78.8 ± 8.4% respectively, indicating that the inhibition of the response is reversible.

Necropsy
The rats with 50% atropine rods were sacrificed and their reproductive systems examined grossly for abnormalities.

In the 9 rats with the scrotal lesions described above, bilateral spermatic granulomas of considerable size were evident in the vasa deferentia close to the vasa/cauda epididymal junction. In the other 2 rats, the cauda epididymides were seen to be distended but no granulomas were evident. In all 11 rats, the rods were seen almost at the site of implantation, encapsulated in dense connective tissue covering.

Study title: Impairment of fertility induced by muscarinic receptor antagonists in rats

Study report publication:	*Reprod Toxicol*, 16: 757 – 765.
Authors:	Y. Ban, T. Sato, T. Nakatsuka, M. Kemi, K/ Samura, H. Matsumoto, M. Cukierski, M. van Zwieten
Year of publication:	2002
GLP compliance:	Not stated
QA statement:	Not included

Methods

Doses:	62.5 and 125 mg/kg
Frequency of dosing:	Daily for 7-days prior to cohabitation with untreated females and continued through cohabitation period until scheduled sacrifice 7 – 9 days after confirmation of mating.
Route of administration:	Oral gavage
Species/Strain:	Rat: Crj:CD(SD)IGS
Number/Sex/Group:	20 /group

Key Study Findings

- Study of muscarinic receptor antagonist referred to as Compound A (not atropine sulfate) though study did include a substudy of atropine sulfate for comparative purposes. Only atropine sulfate data is presented in this review.
- Two (of 20) males died in the high dose atropine sulfate treatment group (125 mg/kg/day) on Day 1 and Day 3.
- Significant decreases in body weight (up to ~40%) were reported at both doses and were considered to be related to decreased food consumption which may have been due to decreased salivation
- Sperm numbers in the cauda epididymis, sperm motility in the vas deferens, testicular weight and histopathology were not affected by treatment
- Significant differences in fecundity and fertility indices were observed following mating males treated with 125 mg/kg/day with untreated females.
- Copulatory plug weights were decreased in untreated females following mating with atropine treated males
- Atropine treatment of males was associated with increased percentage of pre-implantation losses and a decrease in the number of implants and live fetuses
- A NOAEL was not established as significant effects were reported at the low dose, 62.5 mg/kg. This dose represents a 185-fold margin over the maximum adult ophthalmic dose (3.28 mg/day) and 91-fold margin for juveniles at the same dose (assuming 100% absorption of the applied dose).

Table 12: Findings in males following treatment with atropine for 16 or 17 consecutive days

Groups	Atropine (mg/kg/day)		
	0 (control)	62.5	125
Number of males examined	20	20	20

Table 13: Reproductive performance of males following treatment with atropine for 7 days and reproductive findings in untreated females

Groups	Atropine (mg/kg/day)		
	0 (control)	62.5	125
Number of males examined	20	20	18

Study title: Atropine-induced inhibition of sperm and semen transport impairs fertility in male rats

Study report publication:	*J Toxicol Sci*, 30: 207 – 212
Authors and location:	T. Sato, Y. Ban, M. Uchida, E. Gondo, M. Yamamoto, Y. Sekiguchi, A. Sakaue, M. Kemi, and T. Nakatsuka
Year of publication:	2005
GLP compliance:	Not stated
QA statement:	Not included

Key Study Findings

- A low pregnancy rate associated with a decreased number of implants was observed in females that mated with the atropine-treated males.
- The average number of sperm in the vas deferens was increased in the atropine-treated males.
- The average seminal vesicle weight in the atropine- treated males was greater than that of controls.
- The copulatory plug weights were decreased in the atropine-treated males.

Methods

Doses:	125 mg/kg
Frequency of dosing:	Daily for 10 before mating with untreated females; atropine treatment continued until mating was confirmed (maximum of 5 additional days)
Dose volume:	5 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat: Crj:CD(SD)IGS
Number/Sex/Group:	Atropine: 20 males Control: 13 males (8 mated; 5 non-mated)

Observations and Results

Mortality

- No animals were reported to have died prior to scheduled sacrifice though no specific mention of documenting mortality was made.

Copulatory plug weight
The average copulatory plug weight in the atropine group (54± 38 mg) was significantly less than that in the control group (133 ± 43 mg). Copulatory plug weight was decreased by 59%.

Seminal vesicle weight and histopathology
Seminal vesicle weight was less in mated males compared to non-mated males in the control group. The average seminal vesicle weight in the mated atropine group (1.13 ± 0.36 g) was 1.2-fold greater than that of mated controls but did not reach statistical significance. No treatment-related changes were observed in the seminal vesicles from any male rats in the atropine group when examined microscopically.

Sperm count in vas deferens
Following mating, sperm count was decreased compared to non-mated males in the control group. The average number of sperm per gram vas deferens in the atropine group (332.0 ± 141.4 x 10^5) was significantly greater than that of mated controls (~2.3-fold).

Mated females

A significant treatment-related decrease in fecundity index (-45%) was observed in the atropine group when compared to controls. There were treatment-related but non-significant (at $p>0.05$) changes in percentage of preimplantation loss (13 .1 ± 22.0% versus 3.0 ± 4.5% in controls), number of implants per pregnant female (13.3 ± 4.2 versus 15.5 ± 1.3 in controls) and number of live fetuses per pregnant female (12.4 ± 4.0 versus 15.0 ± 1.4 in controls) in the atropine group. There were no treatment-related effects on the mating index, number of corpora lutea per pregnant female, number of

resorptions and dead fetuses per pregnant female and percent of postimplantation loss in the atropine group.

Table 14: Reproductive performance of males following treatment with atropine sulfate and reproductive findings in untreated females

Groups	Atropine (mg/kg/day)	
	0 (control)	125
Number of mated females	COPYRIGHT MATERIAL WITHHELD	
Number of pregnant females		
Fecundity index (%)		
Number of corpora lutea / litter		
Pre-implantation loss / litter (%)		
Number of implants		
Number of implants / litter		
Number of resorptions and dead fetuses / litter		
Post-implantation loss / litter (%)		
Number of live fetuses / litter		

Study title: Effect of atropine on the composition of semen and secretory function of male accessory organs in the boar

Study report publication:	*J Reprod Fertil*, 5: 101 – 108
Authors, laboratory and location:	P. Dziuk and T. Mann A.R.C. Unit of Reproductive Physiology and Biochemistry, School of Veterinary Medicine and Molteno Institute, University of Cambridge
Date of publication:	1963
GLP compliance:	Not stated
QA statement:	Not included

Methods

Doses:	25, 37.5 and 50 mg
Frequency of dosing:	Once every 3 - 4 days, semen collected 30 minutes after injection
Route of administration:	Intravenous injection
Species/Strain:	Boar: “Large White” inbred strain
Number/Sex/Group:	2 males (12 semen collections over 6 weeks)
Special protocol	Semen collected following mating with “dummy” sow

Clinical observations

- An immediate effect of atropine administration was noted during the collection of semen: an almost complete absence of salivation and foaming at the mouth which normally accompanies the act of copulation in the boar

Sperm count, volume and content of semen

- Semen volume, 30 min after atropine injection was reduced to one-fourth of normal, and contained little gel and reduced fluid
- Sperm concentration was increased at least 4 times so the number of spermatozoa present in the total ejaculate remained almost the same
- The authors note that the disappearance of gel indicated that atropine suppresses the activity of the bulbourethral glands
- The chloride content of the ejaculate was lower following atropine injection which may be attributable to the inhibition of the secretory activity of the urethral glands. While the seminal vesicles and epididymides store their secretion, the urethral gland produces it during ejaculation presumably as an immediate response to parasympathetic stimuli and hence the smaller volume and lower chloride content of the semen following atropine injection.

Table 15: Effect of atropine on the volume and sperm density of semen collected from boar 30 minutes after injection of atropine sulfate

Date of semen collection		Atropine treatment (mg)	Semen volume (mL)			Spermatozoa	
			Total	Fluid	Gel	per mL (x 10^6)	per ejaculate (x 10^9)
Boar A	Oct 20	COPYRIGHT MATERIAL WITHHELD					
	Oct 23						
	Oct 26						
	Oct 30						
	Nov 2						
	Nov 6						
	Nov 9						
	Nov 13						
	Nov 16						
	Nov 20						
	Nov 23						
	Nov 27						
Boar B	Oct 20						
	Oct 23						
	Oct 26						
	Oct 30						
	Nov 2						
	Nov 6						
	Nov 9						
	Nov 13						
	Nov 16						
	Nov 20						
	Nov 23						
	Nov 27						

Male fertility conclusions

- Implantation of atropine rods appeared to alter the motility of sperm without altering histopathology of the testes, such findings were not reported following systemic administration of atropine
- Atropine sulfate treatment of male rats decreased vaginal plug weight though the significance of this effect on ejaculate content/volume in humans is unknown
- Mating performance was not significantly affected by treatment of mating males with atropine sulfate
- The average number of sperm in the vas deferens was increased in the atropine-treated male rats.
- The average seminal vesicle weight in the atropine- treated male rats was greater than that of controls.

- Males exposed to atropine were less fertile as evidenced by increased pre-implantation loss and subsequent decreased number of implants and live fetuses.
- Overall, these results suggest that inhibition of sperm and semen transport from the vas deferens and seminal vesicle to the urethra during the process of emission result in reduced male fertility.
- Atropine sulfate may pharmacologically inhibit the contraction of vas deferens and seminal vesicle during emission in rats and the bulbourethral gland in boars

Female fertility

Study title: Delayed implantation in the rat induced by atropine

Study report publication:	*Biol Reprod*, 1: 315 – 319
Authors, laboratory and location:	J Schlough Department of Biology, Wisconsin State University
Date of study publication:	1969
GLP compliance:	Not stated
QA statement:	Not included

Key Study Findings

- Administration of atropine sulfate to mated female rats resulted in delayed implantation
- Administration of human chorionic gonadotropin (HCG) reverses the effect of atropine causing implantation to occur at the usual time

Methods

Doses:	700 mg/kg
Frequency of dosing:	Presence of vaginal sperm was considered Day 1. Single doses were administered at specific time points afterward to separate groups.
Route of administration:	Subcutaneous injection
Species/Strain:	Rat: “Albino”
Number/Sex/Group:	4 – 15 females/group
Special study design:	In some rats, 10 IU HCG was administered 3 hours after atropine administration on Day 3

Observations and Results

- Treatment of mated female rats from approximately 6 pm on the day after confirmation of successful mating (Day 2) through approximately 2:00 pm on Day 3 resulted in impaired implantation as evidenced by a decreased number of rats with confirmed implantation sites and an increase in the number of unimplanted blastocysts.

- Treatment with HCG partially reversed the effect of atropine and allowed implantation to occur in an increased number of dams
- The effect of atropine on implantation may be inhibition of neuronally stimulated pituitary gonadotropin release or its subsequent action which under normal circumstances stimulates estrogen secretion allowing implantation

Table 16: Effect of atropine on time of implantation

Treatment / treatment day	Treatment time	Number of rats	Number with implantation sites	Average number of implantation sites	Number with blastocysts (unimplanted)	Average number of blastocysts
Day 2 (Atropine)	10:00 am	COPYRIGHT MATERIAL WITHHELD				
	2:00 pm					
	6:00 pm					
Day 3 (Atropine)	10:00 am					
	Noon					
	2:00 pm					
	6:00pm					
Day 4	10:00 am					
	2:00 pm					
Day 3 Atropine (noon) + HCG (3:00 pm)						

Study title: Atropine sulphate induced changes in uterine, adrenal, liver and thyroid gland in female albino rats

Study report publication: *J Pharmacol Toxicol*, 4: 236 – 245
Authors: M. Patil, S.J. Patil, S.B. Patil
Date of study publication: 2009
GLP compliance: Not stated
QA statement: Not included

Key Study Findings

- Intraperitoneal atropine sulfate treatment (1mg/kg/day or 2mg /kg/day) resulted in marked vascular congestion, epithelial necrosis and fibrous tissue proliferation of the uterine tissue. The fibrosis was extensive resulting into compression of endometrial glands. Desquamation of glandular epithelium was also observed.
- Atropine administration was associated with a reduction of uterine parameters like diameter, thickness of myometrium and endometrium and surface epithelial cell height.
- Atropine administration may interfere with the rhythmic release of pituitary gonadotropins and result in decreased estrogen ad prolonged diestrus with associated delays in proestrus, estrus and metestrus

Methods

Doses:	1, 2 mg / kg
Frequency of dosing:	Daily for 30 days (~6 estrus cycles); treatment started during estrus phase as Day 1
Dose volume:	0.2 mL
Route of administration:	Intraperitoneal injection
Formulation/Vehicle:	Saline
Species/Strain:	Rat: Wistar (albino)
Number/Sex/Group:	6 females / group

Observations and Results

Body weight

- No changes in body weight were attributed to the test article

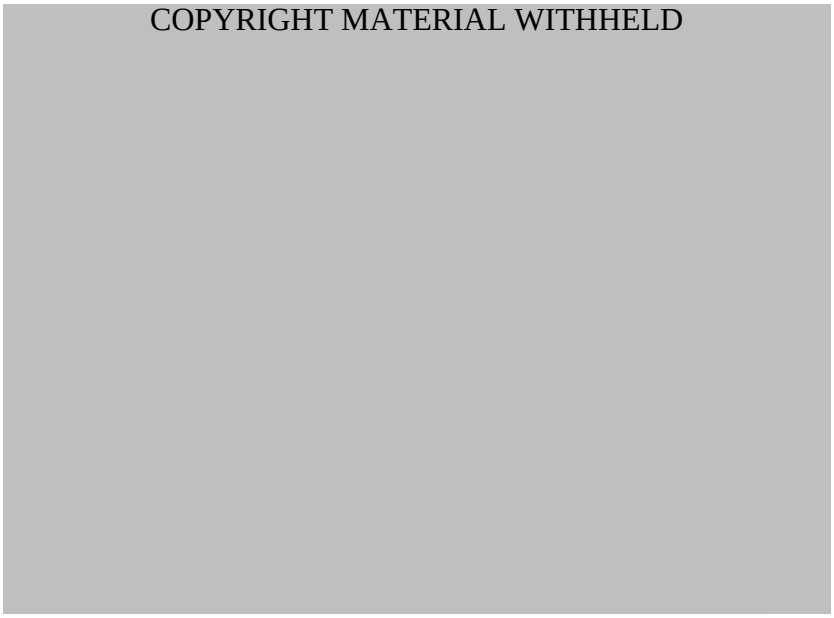
Uterus, adrenal, liver and thyroid weights

- Uterus weight was significantly decreased by 28.96% and 53.90% following treatment with 1 mg/kg and 2 mg/kg atropine sulfate

Estrous cycle

- The duration of proestrus, estrus, and metestrus phases were significantly reduced with atropine treatment whereas the diestrus phase was increased significantly with atropine treatment (1 and 2 mg/kg/day).

Figure 4: Effect of atropine sulfate on the duration of stages of estrus cycle in albino rats

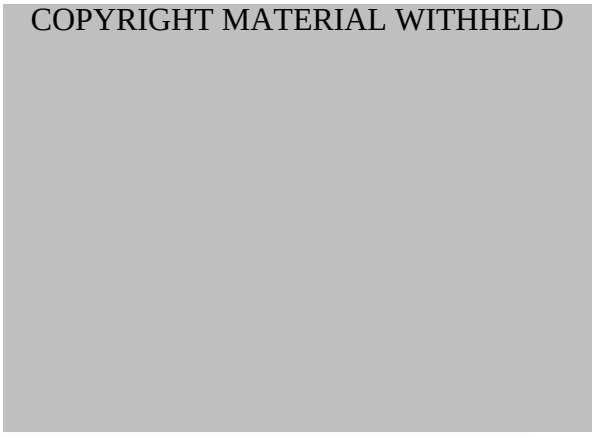


(Note: best image available; doses are portrayed as 0.1 and 0.2 mg / 100 g body weight i.e. 1 and 2 mg/kg body weight).

Uterus weight

Administration of atropine sulfate significantly reduced the weight of the uterus by 28.9 and 53.9 percent for the 0.1 and 0.2 mg treatment groups, respectively.

Figure 5: Effect of atropine sulfate on weight of uterus

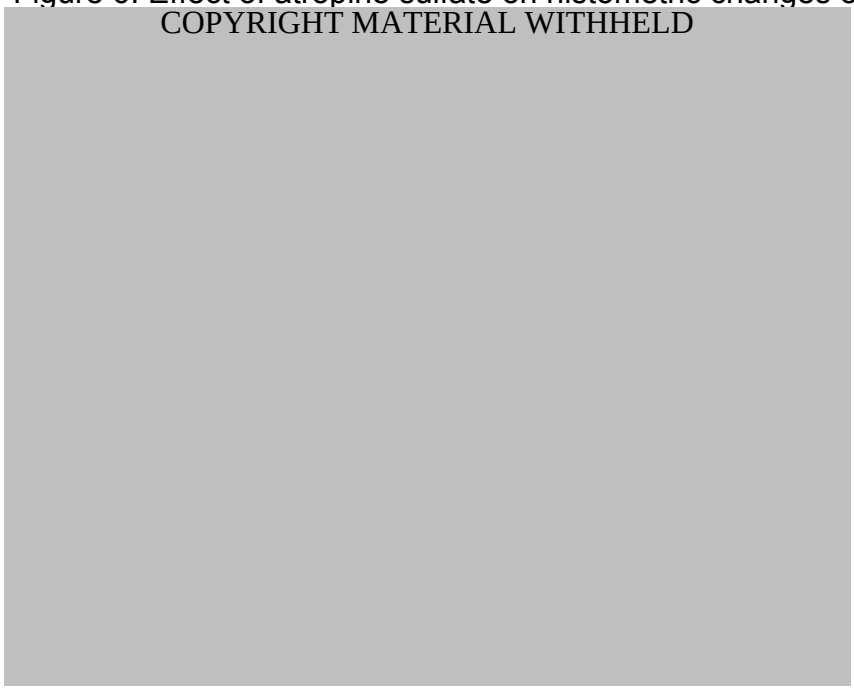


Uterus: histological, cytological, and histometrical (uterus diameter, endometrial thickness, myometrial thickness, endometrial endothelial cell height) analyses

- The uterine tissue of rats treated with atropine sulfate showed hypertrophied endometrium, endometrial glands and luminal epithelium characterized by marked vascular congestion, epithelial necrosis and fibrous tissue proliferation. Fibrosis was extensive and resulted in compression of the endometrial glands. Desquamation of the glandular epithelium was evident.

- Uterus diameter, thickness of the myometrium and endometrium as well as the epithelial cell height were significantly reduced

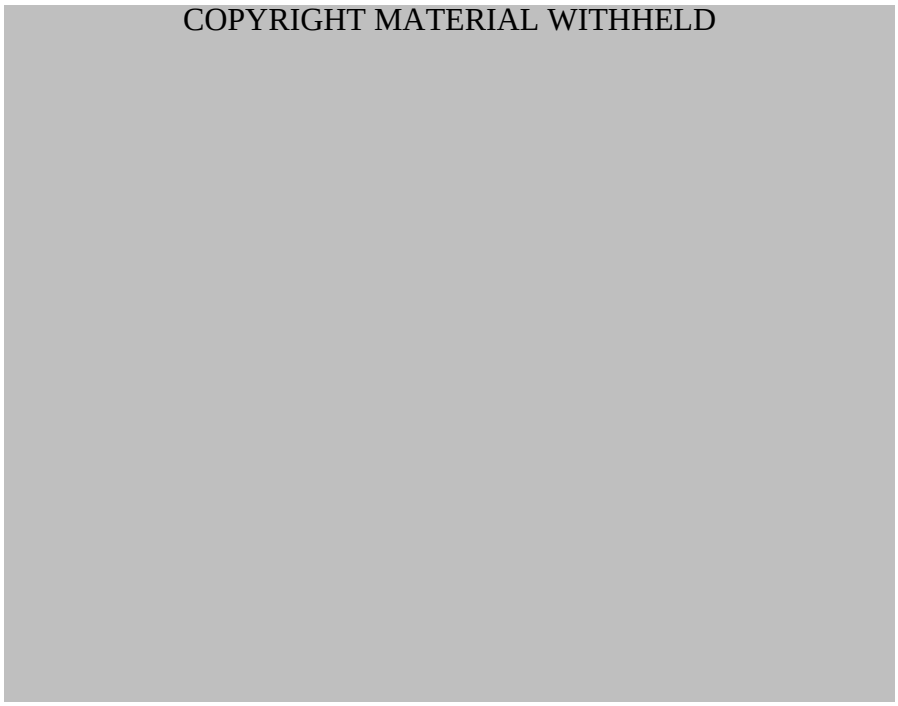
Figure 6: Effect of atropine sulfate on histometric changes of uterus



Other organ weights

- Significant increases in adrenal, thyroid and liver weights were also reported.

Figure 7: Effect of atropine on weight of adrenal gland, liver and thyroid gland



9.2 Embryonic Fetal Development

Study title: Morphine-induced Fetal Malformations III: Possible Mechanisms of Action

Study report publication:	*J Pharm Sci*, 62: 1626 - 1634
Conducting laboratory and location:	P. Arcuri and R. Gautieri
Date of publication:	1973
GLP compliance:	Not stated
QA statement:	Not included

Key Study Findings

- Although multiple treatment combinations were used in this study, they are not relevant to this IND. This reviewer will focus on atropine alone group. Treatment of dams with atropine sulfate (50 mg/kg, subcutaneous; n=6) on Day 8 or Day 9 of gestation was associated with an increase in skeletal anomalies. The authors note increases in delayed ossification in the phalanges, sternebrae, and skull, especially the supraoccipital bone, and rib and vertebral fusions but occurrence of specific findings in the atropine monotherapy treatment group was not reported.

- When atropine was administered on Day 8 of gestation, one instance of exencephaly was reported among 6 litters (61 total fetuses) examined
- When atropine was administered on Day 9 of gestation, one instance of axial skeletal fusion among 6 litters (33 total fetuses) examined was reported.
- A NOAEL was not established. The 50 mg/kg dose in the mouse represents a 115-fold margin over the maximum daily dose in adults proposed for this indication assuming 100% absorption.

Methods

Doses:	50 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	Distilled water
Species/Strain:	Mouse / CF-1 (albino)
Number/Sex/Group:	Not stated, but data presented indicate 5 - 6 litters examined per treatment
Study design:	• Atropine administered on Day 8 or 9 of gestation • Skeletal and soft tissues malformations determined with Staples and Wilson techniques, respectively.

Observations and Results (results only included for atropine sulfate alone treatment group)

Mortality

- Atropine alone (50 mg/kg s.c.) did not cause mortality.

Clinical Signs

- Subcutaneous administration of 50 mg/kg atropine sulfate did not produce readily observable maternal effects, except an apparent increased heart rate and a coldness of the tail

Body Weight

- Maternal body weight was not significantly affected by a single treatment with atropine (see Table A below)

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- A single injection of atropine on Day 8 or Day 9 of gestation was not associated with an increase in resorptions compared to saline controls.

Offspring (Malformations, Variations, etc.)

- The authors present the data in two separate ways: mean number of abnormalities observed (Table A, below) and the occurrence of specific abnormalities observed (Table B, below)
- For mean number of abnormalities (Table A), the authors report that atropine treatment on Day 8 or Day 9 was associated with an increase in skeletal abnormalities compared to saline injected controls (1.5 and 5.5 for Day 8 treatment, and 0.8 and 5.2 for Day 9 treatment, for saline control and atropine treatment, respectively).
- The authors note that the most common skeletal defects observed were delayed ossification in the phalanges, sternebrae, and skull, especially the supraoccipital bone, and rib and vertebral fusions. Actual occurrence for the atropine alone treatment was not specified.
- The authors state that atropine injection alone resulted in the production of no "significant anomalies". The authors report one exencephalic fetus and one fetus exhibiting axial skeletal fusions induced following 50 mg/kg atropine on Days 8 and 9, respectively.

Table 17: Mean values of test groups receiving single injections of atropine on Day 8 or Day 9

* denotes statistical significance from saline control

Table 18: Occurrence of Exencephaly, cryptorchid testes and axial skeletal fusions with atropine treatment on Day 8 or Day 9 of gestation

Treatment	Exencephaly				Cryptorchid testes				Axial Skeletal fusions			
	Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
	N	A	N	A	N	A	N	A	N	A	N	A
Day 8												
Control (untreated)	COPYRIGHT MATERIAL WITHHELD											
Saline control												
Atropine (50 mg/kg)												
Day 9												
Saline control												
Atropine (50 mg/kg)												

N=normal; A=abnormalites

9.3 Prenatal and Postnatal Development

Study title: Atropine sulfate impairs selective parameters of performance in the Morris water maze

Study report location:	*J Neural Transm Park Dis Dement Sect*, 2: 159
Conducting laboratory and location:	Rauch, T; U.S. Army Research Institute of Environmental Medicine, Natick, MA
GLP compliance:	Not specified
QA statement:	No
Drug, lot #, and % purity:	Atropine sulfate (Sigma Chemical Co.)

Key Study Findings

- A significant drug effect on swim distance was observed in animals treated with atropine sulfate. Mean swim distance and escape latencies were increased.
- Swim speed was reduced in rats which were administered 75 or 100 mg/kg atropine. Atropine also significantly increased length of swim path.

Methods

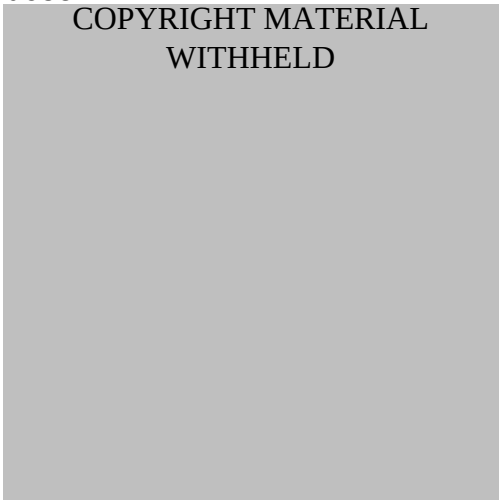
Doses:	5, 25, 50, 75, 100 mg/kg
Frequency of dosing:	30 minutes prior to challenge in water maze; all animals received each treatment with 2 day rest period between doses (Latin square design)
Route of administration:	Intraperitoneal injection
Formulation/Vehicle:	Saline
Species/Strain:	Rat / CD (Charles River)
Number/Sex/Group:	Males only:
Age	Mean age: 140 days (naive adults)
Weight:	300 – 500 g

Observations and Results

Rats were trained to learn the location of a spatially fixed platform hidden in a Morris water maze. Throughout training, (i.e., days 1-6), all animals rapidly learned to swim to the hidden escape platform. Over 60 trials the mean escape latency declined from 90 to 4 seconds. All animals achieved asymptotic performance and swam a straight (linear) path to the escape platform.

There was a significant drug effect on swim distance. Mean swim distance for the 50, 75, and 100 mg/kg atropine sulfate doses was significantly greater than the saline control. Coinciding with increased swim distance, escape latencies were also increased.

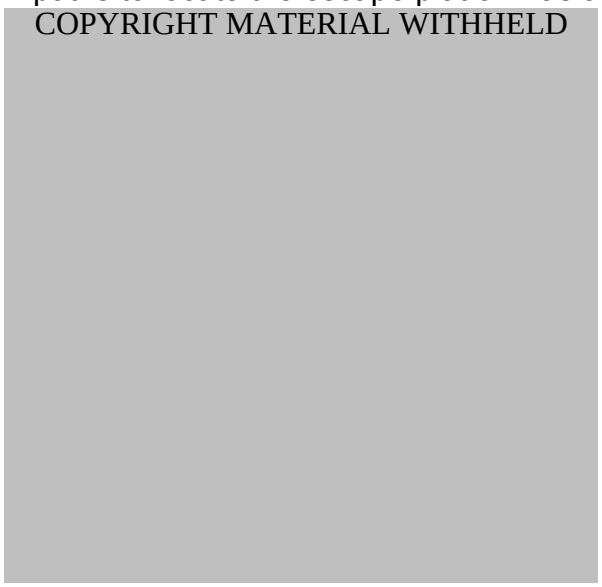
Figure 8: Mean escape latencies and swim distances as a function of atropine sulfate dose



Swim speed was reduced in rats which were administered 75 or 100 mg/kg atropine. Atropine also significantly increased length of swim path observed as the number of loops to the platform. Atropine sulfate treated animals (5, 25, 50, 75, and 100mg/kg dose levels) reached the platform by swimming toward its position but frequently looping 360 ~ at least once until the platform was reached. The looping strategy was usually

within the designated alley leading directly to the platform. The saline treated animals never looped and swam a straight (linear) path to the platform.

Figure 9: Mean swim speed (cm/sec) and the number of animals swimming looping paths to locate the escape platform as a function of atropine sulfate dose



The authors suggest that cholinergic blockade may significantly disrupt the processing of visual cues which rats use in place navigation tasks.

10 Integrated Summary and Safety Evaluation

The applicant has submitted a 505(b)(2) NDA application for topical ophthalmic atropine sulfate 1% for use in producing cycloplegia and mydriasis (b) (4) Additionally, the Applicant is seeking approval for penalization of the healthy eye in the treatment of amblyopia (b) (4) .
Topical ophthalmic atropine sulfate has an extensive clinical history dating back several decades.

The receptors antagonized by atropine are the peripheral structures that are stimulated or inhibited by muscarine (i.e., exocrine glands and smooth and cardiac muscle). Findings in nonclinical studies reflect this mechanism of action including mydriasis, tachycardia, increased body temperature, decreased water intake, water retention and decreased urine volume. Decreased salivation was also observed. Chronic exposure results in decreased weight gain and death at doses higher than those expected following topical ophthalmic exposure.

Publications submitted by the applicant indicate that atropine sulfate showed no genotoxic potential and was not carcinogenic.

The systemic administration of atropine was associated with decreases in male fertility. Nonclinical data suggest anticholinergic effects on contraction of vas deferens and seminal vesicle during emission resulting in decreased sperm volume and altered composition of the ejaculate. Administration of atropine in female rats resulted in marked vascular congestion, epithelial necrosis and fibrous tissue proliferation of the uterine tissue. Atropine administration was associated with a reduction of uterine parameters like uterine diameter, thickness of myometrium and endometrium and surface epithelial cell height. The results suggest that the anticholinergic effects of atropine may interfere with the rhythmic release of pituitary gonadotropins and result in decreased estrogen.

Teratology studies of atropine were very limited. A sub-study in mice in a single publication indicated that exposure to atropine on Day 8 or Day 9 of gestation was associated with an increase in skeletal anomalies which included one occurrence of axial skeletal fusion (17% of litters treated on Day 8) and one occurrence of a soft tissue anomaly, exencephaly (17% of litters treated on Day 9). Neither abnormality was reported in control groups, however the authors of the paper concluded that atropine was not teratogenic. Additionally, systemic administration of atropine was associated with decreased learning performance in rats challenged in a water maze.

Table 199: Safety margins over toxicities reported in the nonclinical studies[1]

Toxicity	Species	NOAEL (mg/kg) M/F	Exposure Margin over LOAEL (based on 100% absorption of topical ophthalmic dose*)
Skeletal malformations / exencephaly [1]	Mouse	-No NOAEL established, effects seen at only dose studied: 50 mg/kg	75-fold
Tachycardia	Dog	No NOAEL established, effects seen at only dose studied: 0.5 mg/kg	Adults: 5-fold Juveniles: 2.5-fold
Female Fertility[1] • Altered Estrus cycling • Reduced thickness of myometrium/ endometrium, reduced uterine weight	Rat	No NOAEL established, effects seen at lowest dose studied: 1 mg/kg	3-fold
Male Fertility[1] • Reduced fecundity	Rat	No NOAEL established, effects seen at only dose studied: 125 mg/kg	370-fold

* maximum clinical dose is 3.28 mg/day if administered QID and bilaterally

[1] *The studies represented in this Table were not GLP, had low numbers (≤6) and were limited in regard to establishing definitive effect on function. Additionally, historical data were not provided to facilitate interpretation of teratology data. As such, the integrity of these data is uncertain.*

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/s/

AARON M RUHLAND
11/07/2016

LORI E KOTCH
11/07/2016

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA Number: 208151 **Applicant: Alcon Research LTD** **Stamp Date: 2-12-2016**

Drug Name: Isopto (atropine sulfate ophthalmic solution) 1 **NDA Type: 505(b)(2); original**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	✓		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		
4	Are all required and requested IND studies in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?			N/A - The 'requirement' for nonclinical data will depend on gaps identified in clinical datasets during review. An IND was not submitted.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).		✓	No – nonclinical formulations in published literature and that used in the one submitted original study are not identical to the clinical formulation. However, concentrations of the API (2%) and Boric acid (1.6%) were greater in the original toxicology study, as compared to the clinical formulation. BAC and hypromellose were not included in the nonclinical formulation. Clinical data are being relied upon to support this application and appropriate clinical data using the clinical formulation may be available.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	✓		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?		✓	Applicant relying on published studies that were not conducted in accordance with GLP regulations. Additionally, one original study report was submitted which does not appear to be GLP-compliant (study conducted in 1976, prior to US requirement for GLP data).
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			Not applicable.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		✓	An attempt was made to label using PLLR format. Exposure multiples appear to be based on a mg/kg dose comparison.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)			No impurity issues have been raised to date. Product specifications appear to be within ICH guidance limits. We defer to CMC regarding the identification of impurity concerns during the review of this application.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?		✓	Applicant is relying on published literature to support this application, and has provided one original study report. Extensive clinical history with the marketed product is provided as further support of safety. No listed drugs were provided on Form 356h.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

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/s/

AARON M RUHLAND
04/11/2016

LORI E KOTCH
04/11/2016