PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: 206289
Supporting document/s: SDN001 (eCTD 0000)
Applicant’s letter date: 10-22-2013
CDER stamp date: 10-23-2013
Product: Atropine sulfate ophthalmic solution 1%
Indication: For use in producing cycloplegia and mydriasis, and for pupil dilation.
Applicant: Akorn Inc
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Lake Forest, IL 60045
Review Division: Division of Transplant and Ophthalmology Products
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1 Executive Summary

1.1 Introduction

The subject of this New Drug Application is Atropine Sulfate Ophthalmic Solution USP, 1%. The proposed indications are for use in producing cycloplegia and mydriasis and for pupillary dilation. 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.

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 sub-study in mice in a single publication reported 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 and one occurrence of a soft tissue anomaly, exencephaly. The authors of the paper, however, concluded that atropine alone 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

Pregnancy Category C: Animal reproduction studies have not been conducted with atropine. It is not known whether atropine can cause fetal harm.

Atropine sulfate ophthalmic solution, 1% should be

8.3 Nursing Mothers

Traces of atropine have been found in human milk administration of atropine solution for injection. Because some systemic absorption occurs from topical administration, caution should be exercised when atropine sulfate ophthalmic solution, 1% is administered to a nursing woman.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Atropine negative in the mutagenicity test. Studies carcinogenicity and impairment of fertility have not been conducted.

13.3 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

Pregnancy Category C:
There are no adequate and well-controlled studies of atropine sulfate in pregnant women. Animal development and reproduction studies have not been conducted with Atropine. Since it is not known whether topically administered atropine sulfate can cause fetal harm, Atropine sulfate ophthalmic solution, (1%) should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus.

8.3 Nursing Mothers
Traces of atropine have been found in human milk following administration of atropine solution for injection. Because some systemic absorption occurs from following topical administration, caution should be exercised when atropine sulfate ophthalmic solution, 1% is administered to a nursing woman.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Atropine sulfate was negative in the Salmonella/microsome mutagenicity test. Studies to evaluate carcinogenicity and impairment of fertility have not been conducted.

2 Drug Information

2.1 Drug
CAS Registry Number: 55-48-1

Generic Name: Atropine Sulfate Ophthalmic Solution USP, 1%

Chemical Name: \(1\alpha H, 5\alpha H\)-Tropan-3-\(\alpha\)-ol (\(\pm\))-tropate (ester), sulfate (2:1) salt monohydrate; \(\alpha\)-(hydroxymethyl)- 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, \(\text{endo-}(\pm)\)-, sulfate (2:1) (salt), monohydrate

Molecular Formula/Molecular Weight: \((\text{C}_{17}\text{H}_{23}\text{NO}_{3})_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O} / 694.83 \text{ g/mol}

Structure:
Pharmacologic Class: Anticholinergic; cholinergic muscarinic antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

- DMF [letter of authorization provided]: Atropine sulfate drug substance
- NDA 021-146: Atropine sulfate injection
  - Approved 7-9-2001
    - Pharmacology/Toxicology review is brief though the reviewer notes: “The only currently approved injectable atropine product is Meridian’s AtroPen Auto-Injector, which is approved for only one of the seven (acute use) indications sought by Abbott (treatment of anti-cholinesterase poisoning from organophosphorus insecticides). Although we would like to see some non-clinical safety data in support of Abbott’s application, specifically genetic toxicity and developmental toxicity data, which appear not have been provided in support of the approved Meridian product, it would certainly be inconsistent on the part of the Agency to deny approval of the pending NDA on the basis of the absence of nonclinical safety data while permitting the continued use of the marketed product in the absence of similar data”.
    - See APPENDIX A for relevant pharmacology/toxicology information contained within the label for Atropine sulfate injection.
2.3 Drug Formulation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Function</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine sulfate (b)(4)</td>
<td>Active pharmaceutical ingredient</td>
<td>(b)(4) %</td>
</tr>
<tr>
<td>Hypromellose 2910 (b)(4)</td>
<td></td>
<td>(b)(4) %</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>Preservative</td>
<td>0.01%</td>
</tr>
<tr>
<td>Dibasic sodium phosphate (b)(4)</td>
<td></td>
<td>(b)(4) %</td>
</tr>
<tr>
<td>Monobasic sodium phosphate (b)(4)</td>
<td></td>
<td>(b)(4) %</td>
</tr>
<tr>
<td>Edetate disodium (b)(4)</td>
<td></td>
<td>(b)(4) %</td>
</tr>
<tr>
<td>Sodium hydroxide (b)(4)</td>
<td>pH adjuster</td>
<td>To pH 3.5 – 6.0</td>
</tr>
<tr>
<td>Hydrochloric acid (b)(4)</td>
<td>pH adjuster</td>
<td>To pH 3.5 – 6.0</td>
</tr>
<tr>
<td>Water for injection (b)(4)</td>
<td></td>
<td>(b)(4) %</td>
</tr>
</tbody>
</table>

2.4 Comments on Novel Excipients

Per the FDA inactive ingredient database, monobasic sodium phosphate (b)(4) is qualified (b)(4) % in ophthalmic solution. The concentration of monobasic sodium phosphate (b)(4) % in the applicant’s proposed formulation (b)(4) % is The applicant has been marketing the proposed formulation for human use since 1995 under “grandfather” status so the formulation and its contents should be considered qualified.

2.5 Comments on Impurities/Degradants of Concern

Impurity specifications

The CMC reviewer requested nonclinical advice regarding drug product specifications (including leachables/extractable).

The total daily dose of the drug product, Atropine Ophthalmic Solution, 1% is 1 drop administered (b)(4). If a drop size is assumed at 0.035 mL, then the total daily dose of drug substance in 3 drops is 1.05 mg/eye/day (2.1 mg TDI if administered bilaterally). Therefore, per ICH Q3RB, (b)(4)

The applicant proposes the following release and stability specifications for the drug product:
Note that the applicant’s proposed specification is confidential.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NMT %</td>
<td>No</td>
<td>NMT %</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>NMT %</td>
<td>Yes</td>
<td>NMT %</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>NMT %</td>
<td>No</td>
<td>NMT %</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Highest unknown impurity</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Total Impurity</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

The applicant provides the following justifications for the proposed specifications:

- USP Monograph for drug substance, Atropine Sulfate
- USP Monograph for drug product, Atropine Sulfate Ophthalmic Solution
- Drug substance manufacturer’s specifications for Atropine Sulfate
- Stability data for the exhibit batches of drug product stored at accelerated and long term stability study conditions
- ICH guidelines Q3B(R2) for Impurities in New Drug Products
- Internal scientific rationale and specifications for similar ophthalmic solutions manufactured by Akorn

The applicant states that all specifications are designed to assure that the marketed drug products comply with USP monograph for drug product as well as with other current regulatory requirements. Stability ranges were set based upon the stability data from stability studies performed for proposed drug product at Accelerated Testing conditions.

No impurity specifications were found for Atropine Sulfate ophthalmic solution when searching the USP website. Impurity acceptance criteria were found for atropine sulfate USP Monograph for Atropine Sulfate and are listed as follows:
Organic Impurities - Current Akorn Specifications for Atropine Sulfate, USP

<table>
<thead>
<tr>
<th>Name</th>
<th>Acceptance criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropic acid</td>
<td>0.2</td>
</tr>
<tr>
<td>7-hydroxyhyoscyamine</td>
<td>0.2</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>0.2</td>
</tr>
<tr>
<td>6-hydroxyhyoscyamine</td>
<td>0.2</td>
</tr>
<tr>
<td>Hyoscyamine related compound A</td>
<td>0.3</td>
</tr>
<tr>
<td>Littorine</td>
<td>0.2</td>
</tr>
<tr>
<td>Apoatropine</td>
<td>0.2</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>0.1</td>
</tr>
<tr>
<td>Total impurities</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The proposed release specification and the proposed stability specifications exceed the ICHQ3B qualification threshold. Additionally, the proposed release and stability specifications of “highest unknown impurity” at NMT % or % appear to exceed the identification threshold set by ICHQ3RB though since this is not a qualification issue, Pharmacology/Toxicology deferred to CMC regarding this aspect.

In the batch analyses provided by the CMC reviewer, exhibit batches of atropine ophthalmic solution 1% contained “degradant” (i.e. ) at release. The former specifications listed “degradant” as NMT % in the finished product and NMT % for stability at 24 months. This applicant signed specification document was dated 2-9-2011. In the justification document presented for the updated specifications, the applicant notes that the “degradant” listed in the former specification document has been identified as %. The applicant proposes to raise the release and stability specifications of the drug product with release and stability specifications of % and %, respectively, then it appears clinical use of the marketed product would qualify these specifications at these concentrations. If the applicant wishes to raise the release specification of %, then they should provide safety data to qualify this level (nonclinical or clinical data) using a drug product containing %.

The applicant has revised the finished product release and stability specifications on October 10, 2013 to include one other impurity, , in addition to . The proposed specifications and the release specification fall below the qualification threshold. The applicant proposes a stability specification which exceeds the qualification threshold of %. It appears this was not identified in batch analyses prior to October 2013. The applicant should either lower the specification to % or provide safety data to support the higher specification. It is unknown whether concentration data from previously manufactured batches is available, as clinical use may justify the proposed specifications.
Extractables/ Leachables
The applicant provided a report on extractables/leachables. Regarding extractables/leachables, the CMC reviewer asked whether the qualification threshold and conclusions on page 9 are reasonable in that they do not need to include extractables/leachables in the drug product specifications. The CMC reviewer forwarded the specification, batch analysis, justification and the report on extractables/leachables.

Qualification thresholds have not been established for extractables or leachables. The applicant proposes that these compounds not exceed and the CMC has agreed. Since no threshold has been set for extractables/leachables, it appears that ICH Q3R2 was used (which may not be appropriate) to set the qualification threshold at . Using a % uncertainty, the applicant set the specification lower to , as advised by CMC. Pharmacology/Toxicology has no basis to reject this specification.

Reviewer’s note: The applicant uses drops per day as the maximum daily dose, however in the prescribing information in the labeling, it appears that the maximum daily dose is 6 drops.

Conclusions:

- The proposed specification for (stability) exceeds ICHQ3 Qualification Threshold. The applicant should submit safety data to support the proposed specification, or reduce specification to ≤ICHQ3 qualification threshold.

- The proposed specification (NMT %) of exceeds ICHQ3 qualification threshold. Since the applicant has been marketing the drug product with release and stability specifications of % and %, respectively, then it appears clinical use of the marketed product would qualify these specifications at these concentrations. If the applicant wishes to the release specification of %, then they should provide safety data to qualify this level.

- The level of “highest unknown impurity” exceeds the identification threshold. It would seem that specification should be reduced to ≤ICHQ3 identification threshold. However, this not a qualification issue, and the Pharmacology/Toxicology discipline defers to CMC discipline.

- Total impurity level is not a qualification issue, and Pharmacology/Toxicology discipline defers to CMC discipline on the adequacy of these levels.
• In the absence of guidance defining a definitive qualification threshold, the Pharmacology/Toxicology discipline has no basis for objection to using as the qualification limit for leachables/extractables.

2.6 Proposed Clinical Population and Dosing Regimen

Atropine sulfate ophthalmic solution USP, 1% is indicated for use in patients requiring mydriasis. The drug product is also indicated for pupillary dilation, for example, for penalization of the healthy eye in amblyopia.

The highest indicated total daily dose of Atropine Ophthalmic Solution USP, 1% is 1 drop administered. If a drop size is assumed at 0.035 mL, then the total daily dose of drug substance in 3 drops is 1.05 mg/eye/day (2.1 mg TDI if administered bilaterally).

3 Studies Submitted

3.1 Studies Reviewed

General Toxicology

Developmental and Reproductive Toxicology

**Genotoxicity**

**Carcinogenicity**

### 3.2 Studies Not Reviewed

  o Review of clinical adverse events

  o Human pharmacokinetic data

• Goodma and Gilman’s *The Pharmacological Basis of Therapeutics*, 12th edition, Section II. Neuropharmacology > Chapter 9. Muscarinic receptor agonists and antagonists > Muscarinic receptor antagonists
  o Book chapter on pharmacology and uses of atropine and other muscarinic receptor antagonists

• Basic and Clinical Pharmacology, 12th edition (McGraw-Hill Education, LLC), Chapter 8. Cholinoceptor-blocking drugs > Basic Pharmacology of the muscarinic receptor-blocking drugs
  o Book chapter on basic pharmacology of atropine and other muscarinic receptor antagonists

  o Book chapter on clinical applications and pharmacology of atropine and other muscarinic receptor antagonists

• Basic and Clinical Pharmacology, 12th edition (McGraw-Hill Education, LLC), Chapter 8. Cholinoceptor-blocking drugs > Summary: Drugs with anticholinergic actions
  o Summary book chapter

• Vaughan & Asbury’s *General Ophthalmology*, 18th edition (McGraw-Hill Education, LLC), Chapter 22. Ophthalmic Therapeutics > Commonly used eye medications > Mydriatics and cycloplegics
  o Book chapter
4 Pharmacology

4.1 Primary Pharmacology

The applicant wishes to reference the relevant sections of the approved labeling for atropine sulfate injection, 0.05% and 0.1% (NDA 21-146), for the pharmacology of atropine. The applicant cites numerous medical textbooks regarding the pharmacology of atropine.

The labeling approved for NDA 21-146 (Atropine sulfate for injection) states the following regarding the primary pharmacology of atropine sulfate:

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
One 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.

6 General Toxicology

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doses</td>
<td>44, 59, 74, 88, 118 mg/kg</td>
</tr>
<tr>
<td>Frequency of dosing</td>
<td>Once daily for 100 days</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Intramuscular injection</td>
</tr>
<tr>
<td>Dose volume</td>
<td>1 mL / kg</td>
</tr>
<tr>
<td>Formulation/vehicle</td>
<td>Water</td>
</tr>
<tr>
<td>Species/strain</td>
<td>CBL rabbit</td>
</tr>
<tr>
<td>Number/sex/group</td>
<td>10 males / group</td>
</tr>
<tr>
<td>Age</td>
<td>“young”</td>
</tr>
<tr>
<td>Weight</td>
<td>1 – 1.5 kg</td>
</tr>
<tr>
<td>Satellite groups</td>
<td>Pair-fed controls and controls fed “ad libitum”</td>
</tr>
</tbody>
</table>

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 terminal injection of atropine. The remaining deaths occurred at night. At the time atropine administration was stopped, 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 time 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:
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).
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>
<thead>
<tr>
<th>Organ</th>
<th>Daily dose of atropine sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>44.2</td>
</tr>
<tr>
<td>Submaxillary salivary glands</td>
<td>+ 17.0</td>
</tr>
<tr>
<td>Esophagus</td>
<td>- 4.1</td>
</tr>
<tr>
<td>Stomach</td>
<td>+ 2.9</td>
</tr>
<tr>
<td>Duodenum</td>
<td>+ 0.5</td>
</tr>
<tr>
<td>Jejunum</td>
<td>- 19.6</td>
</tr>
<tr>
<td>Ileum</td>
<td>- 13.7</td>
</tr>
<tr>
<td>Cecum</td>
<td>+ 5.1</td>
</tr>
<tr>
<td>Appendix</td>
<td>- 8.6</td>
</tr>
<tr>
<td>Colon</td>
<td>+ 7.4</td>
</tr>
<tr>
<td>Liver</td>
<td>- 18.4</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>+ 1.1</td>
</tr>
<tr>
<td>Kidneys</td>
<td>- 12.0</td>
</tr>
<tr>
<td>Heart</td>
<td>- 14.3</td>
</tr>
<tr>
<td>Trachea</td>
<td>- 2.1</td>
</tr>
<tr>
<td>Lungs</td>
<td>+ 1.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>- 0.3</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>- 21.0</td>
</tr>
<tr>
<td>Testicles</td>
<td>- 1.8</td>
</tr>
<tr>
<td>Thymus</td>
<td>- 15.7</td>
</tr>
<tr>
<td>Brain</td>
<td>-</td>
</tr>
<tr>
<td>Total Body</td>
<td>- 9.8</td>
</tr>
</tbody>
</table>

* 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>
<thead>
<tr>
<th>Organ</th>
<th>Daily dose of atropine sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>44.2</td>
</tr>
<tr>
<td>Submaxillary salivary glands</td>
<td>0</td>
</tr>
<tr>
<td>Esophagus</td>
<td>- 3.2</td>
</tr>
<tr>
<td>Cardiac Stomach</td>
<td>- 7.9</td>
</tr>
<tr>
<td>Pyloric Stomach</td>
<td>+ 8.7 *</td>
</tr>
<tr>
<td>Duodenum</td>
<td>- 7.2</td>
</tr>
<tr>
<td>Jejunum</td>
<td>+ 15.1 *</td>
</tr>
<tr>
<td>Ileum</td>
<td>- 0.7</td>
</tr>
<tr>
<td>Cecum</td>
<td>+ 9.1</td>
</tr>
<tr>
<td>Appendix</td>
<td>+ 1.4</td>
</tr>
<tr>
<td>Colon</td>
<td>+ 9.0</td>
</tr>
<tr>
<td>Liver</td>
<td>- 3.0</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>+ 23.8 *</td>
</tr>
<tr>
<td>Kidneys</td>
<td>+ 6.9</td>
</tr>
<tr>
<td>Heart</td>
<td>+ 1.0</td>
</tr>
<tr>
<td>Trachea</td>
<td>+ 7.3</td>
</tr>
<tr>
<td>Lungs</td>
<td>+ 17.4 *</td>
</tr>
<tr>
<td>Spleen</td>
<td>- 1.1</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>- 11.0</td>
</tr>
<tr>
<td>Testicles</td>
<td>- 0.6</td>
</tr>
<tr>
<td>Thymus</td>
<td>+ 56.5 *</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>-</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-</td>
</tr>
<tr>
<td>Muscle</td>
<td>-</td>
</tr>
<tr>
<td>Skin</td>
<td>- 0.6</td>
</tr>
</tbody>
</table>

* indicates that the mean difference was significant at p ≤ 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
  
  o 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>
<thead>
<tr>
<th>Measurement</th>
<th>Control for comparison</th>
<th>Daily dose of atropine sulfate (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>44 mg/kg</td>
</tr>
<tr>
<td>Body weight</td>
<td>Ad-lib fed</td>
<td>- 6</td>
</tr>
<tr>
<td></td>
<td>Pair fed</td>
<td>- 10</td>
</tr>
<tr>
<td>Food intake</td>
<td>Ad-lib fed</td>
<td>- 9</td>
</tr>
<tr>
<td></td>
<td>Pair fed</td>
<td>- 18</td>
</tr>
<tr>
<td>Water intake</td>
<td>Ad-lib fed</td>
<td>- 33</td>
</tr>
<tr>
<td></td>
<td>Pair fed</td>
<td>- 18</td>
</tr>
<tr>
<td>Urine output</td>
<td>Ad-lib fed</td>
<td>- 68</td>
</tr>
<tr>
<td></td>
<td>Pair fed</td>
<td>- 46</td>
</tr>
<tr>
<td>Colonic temperature</td>
<td>Ad-lib fed</td>
<td>- 0.39</td>
</tr>
<tr>
<td></td>
<td>Pair fed</td>
<td>+ 0.53</td>
</tr>
</tbody>
</table>

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:
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

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

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 baseline 1 - 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>
<thead>
<tr>
<th>Group</th>
<th>Pre-1</th>
<th>Post-1</th>
<th>Pre-4</th>
<th>Post-4</th>
<th>Pre-7</th>
<th>Post-7</th>
<th>Pre-14</th>
<th>Post-14</th>
<th>Pre-21</th>
<th>Post-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>138</td>
<td>138</td>
<td>132</td>
<td>130</td>
<td>132</td>
<td>136</td>
<td>127</td>
<td>133</td>
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<tr>
<td>2</td>
<td>131</td>
<td>131</td>
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<td>132</td>
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<td>134</td>
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<tr>
<td>3</td>
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<td>206</td>
<td>134</td>
<td>206</td>
<td>135</td>
<td>255</td>
<td>133</td>
<td>191</td>
<td>131</td>
<td>179</td>
</tr>
<tr>
<td>4</td>
<td>136</td>
<td>270</td>
<td>137</td>
<td>247</td>
<td>132</td>
<td>260</td>
<td>132</td>
<td>234</td>
<td>131</td>
<td>211</td>
</tr>
<tr>
<td>5</td>
<td>140</td>
<td>133</td>
<td>134</td>
<td>138</td>
<td>133</td>
<td>131</td>
<td>134</td>
<td>135</td>
<td>133</td>
<td>134</td>
</tr>
<tr>
<td>6</td>
<td>151</td>
<td>190</td>
<td>136</td>
<td>190</td>
<td>133</td>
<td>258</td>
<td>134</td>
<td>209</td>
<td>134</td>
<td>231</td>
</tr>
<tr>
<td>7</td>
<td>138</td>
<td>231</td>
<td>141</td>
<td>212</td>
<td>133</td>
<td>232</td>
<td>137</td>
<td>220</td>
<td>134</td>
<td>196</td>
</tr>
</tbody>
</table>

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

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 LD50 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>
<thead>
<tr>
<th>Substance</th>
<th>Number of animals</th>
<th>Mean survival time (weeks)</th>
<th>Survival p value</th>
<th>Animals bearing tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Males</td>
<td>36</td>
<td>96 ± 17</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>33</td>
<td>94 ± 15</td>
<td>3</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td>Males</td>
<td>30</td>
<td>88 ± 19</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>31</td>
<td>89 ± 14</td>
<td>3</td>
</tr>
</tbody>
</table>
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: W. Ratnasooriya
Department of Zoology
University of Colombo
Colombo 3, Sri Lanka

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-estrous 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 flushed five times 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 response 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

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 through Day 7 and during cohabitation with untreated females (Day 7 – 16/17)
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 290-fold margin over the maximum adult ophthalmic dose (2.1 mg/day) and 142-fold margin for juveniles at the same dose (assuming 100% absorption of the applied dose).
<table>
<thead>
<tr>
<th>Groups</th>
<th>Atropine (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
</tr>
<tr>
<td>Number of males examined</td>
<td>20</td>
</tr>
<tr>
<td>Number of dead males</td>
<td>0</td>
</tr>
<tr>
<td>Body weight gain (g, days 1 – 7)</td>
<td>8.9 ± 3.7</td>
</tr>
<tr>
<td>Terminal body weight (g)</td>
<td>361.1 ± 12.4</td>
</tr>
<tr>
<td>Absolute testicular weight (g)</td>
<td>3.28 ± 0.29</td>
</tr>
<tr>
<td>Testicular weight relative to body weight</td>
<td>0.90 ± 0.07</td>
</tr>
<tr>
<td>Sperm number (cauda epididymis) (x10^8)</td>
<td>2.15 ± 0.33</td>
</tr>
<tr>
<td>Sperm number per gram cauda epididymis (x10^8)</td>
<td>8.00 ± 0.87</td>
</tr>
<tr>
<td>Percent motile sperm</td>
<td>92.4 ± 2.6</td>
</tr>
<tr>
<td>Histopathology of testes</td>
<td>Not remarkable</td>
</tr>
<tr>
<td>Histopathology of epididymis</td>
<td>Not remarkable</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Atropine (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
</tr>
<tr>
<td>Number of males examined</td>
<td>20</td>
</tr>
<tr>
<td>Number of females cohabited</td>
<td>20</td>
</tr>
<tr>
<td>Number of mated females</td>
<td>20</td>
</tr>
<tr>
<td>Number of pregnant females</td>
<td>19</td>
</tr>
<tr>
<td>Copulation index (%)</td>
<td>100</td>
</tr>
<tr>
<td>Fecundity index (%)</td>
<td>95</td>
</tr>
<tr>
<td>Fertility index (%)</td>
<td>95</td>
</tr>
<tr>
<td>Copulatory plug weight / mated male (mg)</td>
<td>182 ± 88</td>
</tr>
<tr>
<td>Number of corpora lutea / litter (%)</td>
<td>15.3 ± 2.1</td>
</tr>
<tr>
<td>Preimplantation loss / litter (%)</td>
<td>3.7 ± 12.4</td>
</tr>
<tr>
<td>Number of implants / litter</td>
<td>15.4 ± 2.7</td>
</tr>
<tr>
<td>Number of resorptions and dead fetuses / litter</td>
<td>1.6 ± 2.3</td>
</tr>
<tr>
<td>Postimplantation loss / litter (%)</td>
<td>9.6 ± 13.3</td>
</tr>
<tr>
<td>Number of live fetuses / litter</td>
<td>13.8 ± 3.0</td>
</tr>
</tbody>
</table>
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

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 10⁵) 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>
<thead>
<tr>
<th>Groups</th>
<th>Atropine (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
</tr>
<tr>
<td>Number of mated females</td>
<td>8</td>
</tr>
<tr>
<td>Number of pregnant females</td>
<td>8</td>
</tr>
<tr>
<td>Fecundity index (%)</td>
<td>100</td>
</tr>
<tr>
<td>Number of corpora lutea / litter</td>
<td>15.9 ± 1.5</td>
</tr>
<tr>
<td>Pre-implantation loss / litter (%)</td>
<td>3.0 ± 4.5</td>
</tr>
<tr>
<td>Number of implants</td>
<td>124</td>
</tr>
<tr>
<td>Number of implants / litter</td>
<td>15.5 ± 1.3</td>
</tr>
<tr>
<td>Number of resorptions and dead fetuses / litter</td>
<td>0.5 ± 0.8</td>
</tr>
<tr>
<td>Post-implantation loss / litter (%)</td>
<td>3.2 ± 4.9</td>
</tr>
<tr>
<td>Number of live fetuses / litter</td>
<td>15.0 ± 1.4</td>
</tr>
</tbody>
</table>
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

Key Study Findings

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>
<thead>
<tr>
<th>Date of semen collection</th>
<th>Atropine treatment (mg)</th>
<th>Semen volume (mL)</th>
<th>Spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Fluid</td>
</tr>
<tr>
<td>Boar A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct 20</td>
<td>-</td>
<td>316</td>
<td>260</td>
</tr>
<tr>
<td>Oct 23</td>
<td>-</td>
<td>450</td>
<td>330</td>
</tr>
<tr>
<td>Oct 26</td>
<td>25.0</td>
<td>85</td>
<td>80</td>
</tr>
<tr>
<td>Oct 30</td>
<td>-</td>
<td>330</td>
<td>250</td>
</tr>
<tr>
<td>Nov 2</td>
<td>-</td>
<td>297</td>
<td>237</td>
</tr>
<tr>
<td>Nov 6</td>
<td>37.5</td>
<td>74</td>
<td>52</td>
</tr>
<tr>
<td>Nov 9</td>
<td>-</td>
<td>340</td>
<td>270</td>
</tr>
<tr>
<td>Nov 13</td>
<td>-</td>
<td>243</td>
<td>206</td>
</tr>
<tr>
<td>Nov 16</td>
<td>50.0</td>
<td>76</td>
<td>60</td>
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<tr>
<td>Nov 20</td>
<td>-</td>
<td>230</td>
<td>162</td>
</tr>
<tr>
<td>Nov 23</td>
<td>-</td>
<td>390</td>
<td>250</td>
</tr>
<tr>
<td>Nov 27</td>
<td>-</td>
<td>320</td>
<td>270</td>
</tr>
<tr>
<td>Boar B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct 20</td>
<td>-</td>
<td>332</td>
<td>276</td>
</tr>
<tr>
<td>Oct 23</td>
<td>-</td>
<td>322</td>
<td>260</td>
</tr>
<tr>
<td>Oct 26</td>
<td>-</td>
<td>342</td>
<td>268</td>
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<tr>
<td>Oct 30</td>
<td>25.0</td>
<td>57</td>
<td>54</td>
</tr>
<tr>
<td>Nov 2</td>
<td>-</td>
<td>348</td>
<td>294</td>
</tr>
<tr>
<td>Nov 6</td>
<td>-</td>
<td>212</td>
<td>174</td>
</tr>
<tr>
<td>Nov 9</td>
<td>37.5</td>
<td>45</td>
<td>43</td>
</tr>
<tr>
<td>Nov 13</td>
<td>-</td>
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<td>Nov 16</td>
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<td>370</td>
<td>300</td>
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<tr>
<td>Nov 20</td>
<td>50.0</td>
<td>45</td>
<td>42</td>
</tr>
<tr>
<td>Nov 23</td>
<td>-</td>
<td>326</td>
<td>256</td>
</tr>
<tr>
<td>Nov 27</td>
<td>-</td>
<td>154</td>
<td>132</td>
</tr>
</tbody>
</table>

**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 effected 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>
<thead>
<tr>
<th>Treatment / treatment day</th>
<th>Treatment time</th>
<th>Number of rats</th>
<th>Number with implantation sites</th>
<th>Average number of implantation sites</th>
<th>Number with blastocysts (unimplanted)</th>
<th>Average number of blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2 (Atropine)</td>
<td>10:00 am</td>
<td>15</td>
<td>15</td>
<td>9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2:00 pm</td>
<td>5</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6:00 pm</td>
<td>12</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Day 3 (Atropine)</td>
<td>10:00 am</td>
<td>14</td>
<td>2</td>
<td>6</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Noon</td>
<td>10</td>
<td>1</td>
<td>11</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2:00 pm</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6:00 pm</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Day 4</td>
<td>10:00 am</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2:00 pm</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Day 3 Atropine (noon) + HCG (3:00 pm)</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**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
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).
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.
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
Other organ weights

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

9.2 Embryonic Fetal Development

Reviewer's note: The applicant did not submit any nonclinical studies which characterized the effect of atropine sulfate on embryonic or fetal development. A search of the literature revealed a single study which examined teratogenic effects of atropine sulfate.

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

<table>
<thead>
<tr>
<th>Study report publication:</th>
<th>J Pharm Sci, 62: 1626 - 1634</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conducting laboratory and location:</td>
<td>P. Arcuri and R. Gautieri</td>
</tr>
<tr>
<td>Date of publication:</td>
<td>1973</td>
</tr>
<tr>
<td>GLP compliance:</td>
<td>Not stated</td>
</tr>
<tr>
<td>QA statement:</td>
<td>Not included</td>
</tr>
</tbody>
</table>

Reference ID: 3482794
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) 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.
- One instance of exencephaly among 60 fetuses examined and one instance of axial skeletal fusion among 32 fetuses examined were reported following atropine treatment.
- 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, sternaebrae, 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 A: Mean values of test groups receiving single injections of atropine on Day 8 or Day 9

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Maternal Weight Ratio (starting / terminal)</th>
<th>Fetal Ratio, Left Horn / Right Horn</th>
<th>Resorption ratio, Left Horn / Right Horn</th>
<th>Average fetal weight (g)</th>
<th>Sex ratio, M / F</th>
<th>Soft Tissue abnormalities</th>
<th>Skeletal abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 8(^1/)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>25.5 / 48.0</td>
<td>6.2 / 3.7</td>
<td>0.17 / 0.33</td>
<td>1.07</td>
<td>4.0 / 5.8</td>
<td>0.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Saline control</td>
<td>26.8 / 51.0</td>
<td>5.8 / 6.2</td>
<td>0.33 / 0.50</td>
<td>1.13</td>
<td>6.5 / 5.5</td>
<td>0.17</td>
<td>1.3</td>
</tr>
<tr>
<td>Atropine (50 mg/kg)</td>
<td>25.5 / 46.0</td>
<td>5.8 / 4.8</td>
<td>0.07 / 0.83</td>
<td>1.16</td>
<td>6.0 / 4.2</td>
<td>0.33</td>
<td>5.5</td>
</tr>
<tr>
<td>Day 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>25.2 / 49.7</td>
<td>6.2 / 4.0</td>
<td>0.50 / 0.33</td>
<td>1.20</td>
<td>5.7 / 4.5</td>
<td>0.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Atropine (50 mg/kg)</td>
<td>26.8 / 49.0</td>
<td>5.2 / 5.8</td>
<td>0.00 / 0.33</td>
<td>1.20</td>
<td>6.2 / 4.8</td>
<td>0.0</td>
<td>5.2*</td>
</tr>
</tbody>
</table>

* denotes statistical significance from saline control

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\(^1\) Reference ID: 3482794
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 patients requiring mydriasis. The drug product is also indicated for pupillary dilation, for example, in penalization of the healthy eye in amblyopia. Topical ophthalmic atropine sulfate has an extensive clinical history dating back several decades though not been yet been FDA approved.

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 alone was not teratogenic.
Table 1: Safety margins over toxicities reported in the nonclinical studies

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Species</th>
<th>NOAEL (mg/kg) M/F</th>
<th>Exposure Margin over LOAEL (based on 100% absorption of topical ophthalmic dose*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal malformations / exencephaly</td>
<td>Mouse</td>
<td>- No NOAEL established, effects seen at only dose studied: 50 mg/kg</td>
<td>115-fold</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>Dog</td>
<td>No NOAEL established, effects seen at only dose studied: 0.5 mg/kg</td>
<td>Adults: 8-fold Juveniles: 4-fold</td>
</tr>
<tr>
<td>Female Fertility⁴</td>
<td>Rat</td>
<td>No NOAEL established, effects seen at lowest dose studied: 1 mg/kg</td>
<td>4.63-fold</td>
</tr>
<tr>
<td>• Altered Estrus cycling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Reduced thickness of myometrium/ endometrium, reduced uterine weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male Fertility⁴</td>
<td>Rat</td>
<td>No NOAEL established, effects seen at only dose studied: 125 mg/kg</td>
<td>579-fold</td>
</tr>
<tr>
<td>• Reduced fecundity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹The 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.

11 Appendix/Attachments

APPENDIX A: Information relevant to Pharmacology/Toxicology in the current labeling for Atropine sulfate injection (Alcon Laboratories)

PRECAUTIONS
Carcinogenesis, Mutagenesis, Impairment of Fertility
There have been no long-term studies done using Atropine Sulfate in animals to evaluate carcinogenic potential.

**Pregnancy**

**Pregnancy Category C.** Animal reproduction studies have not been conducted with Atropine Sulfate. It is also not known whether these ingredients can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. STERI-UNITS® Solutions containing these ingredients should be given to a pregnant woman only if clearly needed.

**Nursing Mothers**

It is not known whether these drugs are excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Atropine Sulfate or Pilocarpine Hydrochloride is administered to a nursing woman.

**OVERDOSAGE**

Systemic atropine toxicity is manifested by flushing and dryness of the skin (a rash may be present in children), blurred vision, a rapid and irregular pulse, fever, abdominal distention in infants, mental aberration (hallucinosis) and loss of neuromuscular coordination. Atropine poisoning, although distressing, is rarely fatal even with large doses of atropine, and is self-limited if the cause is recognized and the atropine medication discontinued. Treatment includes supportive measures including maintaining a patent airway and assisting respiration if needed. Treat hyperthermia, coma and seizures if they occur.¹ In infants and children, the body surface must be kept moist. Excitement may be controlled by diazepam or a short acting barbiturate. For ingestion, activated charcoal can be used to prevent drug absorption. If necessary, ipecac or another cathartic may be useful for drug removal during initial treatment.¹ ² Physostigmine is used as an antidote to the systemic effects of atropine and may be administered parenterally to provide more prompt relief of intoxication. Parenteral physostigmine may be particularly useful in cases of pronounced hallucinations, agitation in which a patient may be dangerous to himself or others, arrhythmias resulting in uncontrolled hemodynamic instability, and intractable seizures.

**REFERENCES**

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

/s/

AARON M RUHLAND
04/03/2014

LORI E KOTCH
04/03/2014
**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement**

**NDA/BLA or Supplement**

- **NDA Number:** 20-6289  
- **Applicant:** Akorn, Inc.  
- **Stamp Date:** 10-23-2013  
- **Drug Name:** Atropine sulfate ophthalmic solution  
- **NDA/BLA Type:** New NDA; NME; (SD1)

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

<table>
<thead>
<tr>
<th>Content Parameter</th>
<th>Yes</th>
<th>No</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>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?</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Is the pharmacology/toxicology section legible so that substantive review can begin?</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>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)?</td>
<td>✓</td>
<td></td>
<td>Applicant states nonclinical repeat ocular dose studies were not found in literature. Applicant wishes to rely on extensive human experience for ocular safety. Only a single literature reference regarding genotoxicity was provided (Ames assay). No teratogenicity studies were submitted by the applicant. A literature search has revealed that limited teratogenicity data exist. Data (publications) obtained by this reviewer will be used to inform label.</td>
</tr>
<tr>
<td>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).</td>
<td>✓</td>
<td></td>
<td>As stated above, nonclinical ocular studies were not submitted. The excipients used in formulation are qualified for ophthalmic use. The applicant will rely on clinical studies to establish ocular safety.</td>
</tr>
<tr>
<td>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?</td>
<td>✓</td>
<td></td>
<td>See above.</td>
</tr>
</tbody>
</table>

Reference ID: 3412443
# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

<table>
<thead>
<tr>
<th>Content Parameter</th>
<th>Yes</th>
<th>No</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>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?</td>
<td>✔️</td>
<td></td>
<td>None of the non-clinical literature references cited were conducted in accordance with GLP.</td>
</tr>
<tr>
<td>8 Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?</td>
<td>✔️</td>
<td></td>
<td>Literature references addressing female fertility were submitted as requested, however literature on developmental toxicity (teratogenicity) was not provided. A lack of data regarding developmental toxicity is also reflected in the approved labeling for Duodote® (NDA 021983).</td>
</tr>
<tr>
<td>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?</td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)</td>
<td>✔️</td>
<td></td>
<td>To date, no impurity issues have arisen.</td>
</tr>
<tr>
<td>11 Has the applicant addressed any abuse potential issues in the submission?</td>
<td></td>
<td></td>
<td>Not applicable.</td>
</tr>
<tr>
<td>12 If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?</td>
<td></td>
<td></td>
<td>Not applicable.</td>
</tr>
</tbody>
</table>

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

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None.

---

Aaron M Ruhland, PhD 11-25-13  
Reviewing Pharmacologist  

Lori Kotch, PhD 11-25-13  
Team Leader/Supervisor  

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File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908
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/s/

AARON M RUHLAND
11/25/2013

LORI E KOTCH
11/27/2013