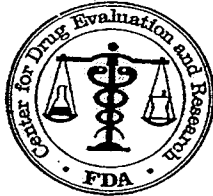


**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

NDA 22-103

PHARMACOLOGY REVIEW(S)



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

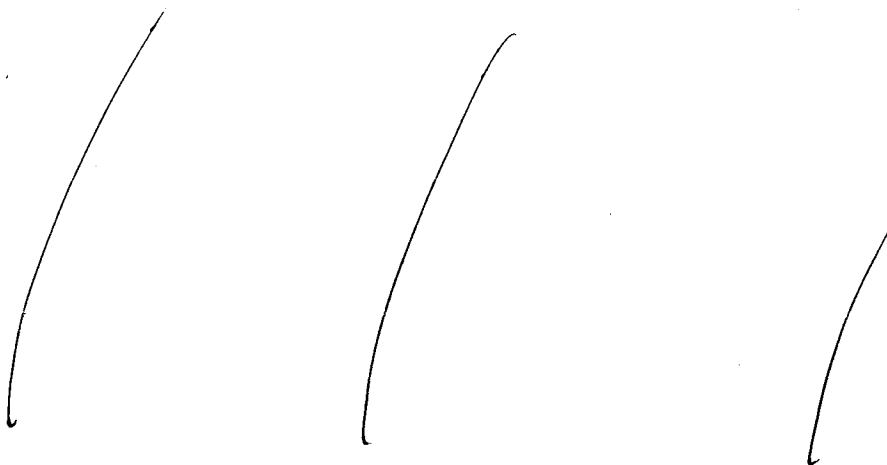
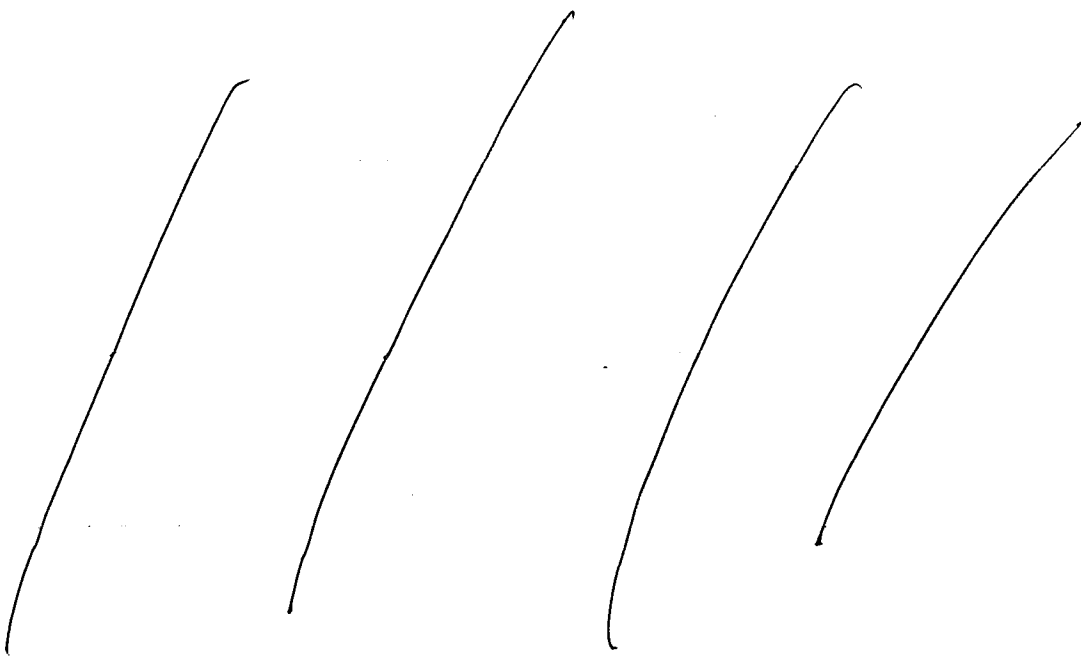
NDA NUMBER: 22-103
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 10/12/06
PRODUCT: Trospium chloride
INTENDED CLINICAL POPULATION: Men and women with overactive bladder
SPONSOR: Indevus Pharmaceuticals, Inc.
DOCUMENTS REVIEWED: Electronic submission
REVIEW DIVISION: Division of Reproductive and Urologic Products
(HFD-580)
PHARM/TOX REVIEWER: Laurie McLeod-Flynn
PHARM/TOX SUPERVISOR: Lynnda Reid
DIVISION DIRECTOR: Scott Monroe
PROJECT MANAGER: Jean Makie

Date of review submission to Division File System (DFS): 23 May 2007

EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: There are no impediments to approval of this application from a pharmacology/toxicology perspective.
- B. Recommendation for nonclinical studies: There are no recommendations for further nonclinical studies.
- C. Recommendations on labeling:



1 Page(s) Withheld

 Trade Secret / Confidential

 Draft Labeling

 Deliberative Process

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

General toxicology:

No histological effects were observed in rats or dogs at any dose tested. In rats, the high doses in chronic studies were greater than 16 multiples (via AUC) of the clinical dose, and in dogs the high dose exposures exceeded 150 multiples (via AUC) of the clinical exposures. The primary significant effects were attributable to the pharmacological action of the drug and include mydriasis, decreased mucus production, decreased intestinal motility, and increased heart rate.

The IC₅₀ for trospium chloride in a hERG study was 22 µM, and a small potential for QT prolongation of lesser magnitude than haloperidol was demonstrated in a rabbit isolated perfused heart study.

Genetic toxicology:

No evidence of genetic toxicity was observed in a battery of assays including Ames tests, a mouse lymphoma assay, and a micronucleus assay in rats.

Carcinogenicity:

No evidence of any neoplastic effect was observed after treatment with trospium chloride up to doses equivalent to the average clinical exposure (via AUC) in male and female rats and about 16 times the average clinical exposure (via AUC) in male and female mice, the maximally tolerated dose in both species.

Reproductive toxicology:

In a rat study, no effect on fertility was observed up to 200 mg/kg/day (16 multiples of the clinical dose via AUC).

In a segment II study in rats, no maternal or fetal effects were observed at 200 mg/kg or 16 multiples of the clinical dose (by AUC).

In a segment III study in rats, a no effect level for maternal and fetal toxicity was 20 mg/kg/day. At 200 mg/kg/day (~ 16 times the expected clinical exposure by AUC), signs of maternal toxicity and death were observed, and survival of fetuses to Day 4 was decreased. No developmental effects were observed at any dose.

In a rabbit segment II study, no maternal or fetal effects were observed up to 20 mg/kg/day (5 mg/ml)(approximately equivalent to the expected clinical exposure via AUC). Maternal toxicity (reduction in food consumption and fecal output, hunched posture, diarrhea, a stained, matted and wet perigenital region, and slight suppression in

body weight) was observed at 200 mg/kg/day (50 mg/ml)(approximately 32 times the expected clinical exposure via AUC). The following malformations were observed, but were not considered to be statistically significant:

Nominal dose	0 mg/kg/day	2 mg/kg/day	20 mg/kg/day	200 mg/kg/day
Concentration	0 mg/ml	0.5 mg/ml	5 mg/ml	50 mg/ml
Total no. of fetuses	56	63	61	71
Multiple malformations, no. of fetuses	0	1 *	1 **	1 ***

* gross deformities of the spinal column, the pelvic girdle, and the back legs plus severely retarded ossification of the cranial bones and edema in the neck region

**umbilical hernia with prolapse of the liver and defective development of the phalanges

*** umbilical hernia with prolapse of the liver

- B. Pharmacologic activity: Trospium chloride antagonizes the effect of acetylcholine on cholinergically innervated organs in a dose-dependent manner. Its parasympatholytic action reduces the tonus of smooth muscles.
- C. Nonclinical safety issues relevant to clinical use: Mydriasis was observed in female dogs at or below the average expected clinical exposures and in male dogs at about 4 multiples of the average expected clinical exposure. An estimate could not be made regarding the potential for QT prolongation in humans based on animal data; however, a clinical QT study was conducted.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22103

Review number: 1

Sequence number/date/type of submission: 000 / 12 October 2006 / original submission

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Indevus Pharmaceuticals, Inc.

Manufacturer for drug substance: Indevus Pharmaceuticals, Inc.

Reviewer name: Laurie McLeod-Flynn

Division name: Division of Reproductive and Urologic Products

HFD #: 580

Review completion date: 16 May 2007

Drug:

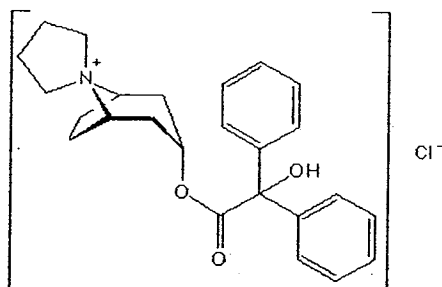
Trade name: Sanctura XR

Generic name: Trospium chloride

Chemical name: 3 α -benziloyloxyspiro [nortropane-8, 1'-pyrrolidinium] chloride

Molecular formula/molecular weight: C₂₅H₃₀ClNO₃ / 427.97

Structure:



Relevant INDs/NDAs/DMFs: NDA 21595, IND 61381

Drug class: anticholinergic

Intended clinical population: men and women with overactive bladder

Clinical formulation: — 60 mg extended release capsules

Route of administration: oral

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

Studies not reviewed within this submission:

All studies were previously reviewed under NDA 21595.

Labeling review:

Proposed label:



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 Draft Labeling

 Deliberative Process

APPENDIX/ATTACHMENTS

Sponsor's Tabulated Summaries

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**APPEARS THIS WAY
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Pharmacology studies:

Species (strain/material) No./Dose Age/Weight Report Number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Primary activity – in vitro binding studies			
Rat – isolated cortex, heart and salivary glands Ref 1 (March 1994) (IND Ref 1) Ref 2 (IND Reference 2)	Tropium chloride MPS4-WG71 10^{-10} – 10^{-6} M water	Objectives: Determine the in vitro receptor binding affinity of tropium chloride for muscarinic M ₁ , M ₂ , and M ₃ receptor subtypes and to nicotinic receptors. Design: Muscarinic receptors M ₁ , M ₂ and M ₃ were isolated from rat cortex, heart, and salivary glands and nicotinic receptors were isolated from rat cortex. The affinity of tropium chloride for muscarinic and nicotinic receptors was determined. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~) non-GLP study	Tropium chloride binds with high affinity to the muscarinic M ₁ , M ₂ , and M ₃ receptors (K _d = 1.0, 2.0, and 2.5 x 10 ⁻⁸ M, respectively). While tropium chloride had little-to-no affinity for the nicotinic receptor at a concentration >10 ⁻⁶ M.
CHO-K1 cell lines ~~XXXXXXXXXXXXXXXXXXXX~~	Tropium chloride Difenhydramine Tolterodine Oxybutynin Propiverine HEPES buffer	Objectives: Determine the binding affinity of various antimuscarinic agents against the human recombinant receptor subtypes M ₁ – M ₃ . Design: Binding affinity was determined from competition experiments using 12 concentrations of antagonist. Non-specific binding was defined using 1 μM atropine. IC ₅₀ values were determined from competition curves. K _d values were derived from IC ₅₀ values using the Cheng-Prusoff IC ₅₀ correction. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~) non-GLP study	Tropium chloride had the greatest affinity for the M ₃ receptor when evaluated against the M ₁ , M ₂ , M ₃ , and M ₅ receptors. The pK _i for tropium at the M ₃ receptor was 9.3 compare with 9.1, 8.6, 8.9, and 6.4 for difenhydramine, tolterodine, oxybutynin, and propiverine, respectively.
Primary activity – in vitro binding studies, continued			
Human myocytes isolated from the detrusor muscle of the bladder ~~XXXXXXXXXXXXXXXXXXXX~~ (IND Ref 3)	Tropium chloride 5.1 nM – 1000 μM Reference cpds Oxybutynin 0.1 nM – 1000 μM	Objectives: Evaluate the anticholinergic effect of tropium chloride and oxybutynin in enzymatically isolated myocytes of the human bladder. Design: Human bladder tissue was surgically removed, enzymatically digested, and myocytes isolated. Using patch-clamp techniques and the Ca ²⁺ -sensitive fluorescence dye ~~XXXXXXXXXXXX~~ , the anticholinergic potency of tropium chloride and oxybutynin were evaluated. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~) non-GLP study	In this system, tropium chloride was approximately 20-fold more potent than oxybutynin with EC ₅₀ 's of 43±12 nM and 890±80 nM, respectively, at blocking the acetylcholine-induced Ca stimulation. Tropium chloride and oxybutynin also relaxed smooth muscles irrespective of the competitive blockage of cholinergic receptors.
Primary activity – in vitro effects on isolated organs			
Porcine urinary bladder – German Landrace pigs Human detrusor smooth muscle ~~XXXXXXXXXXXXXXXXXXXX~~ 1093 (IND Ref 1)	Tropium chloride 10^{-10} to 10^{-4} M (ethanol) Reference cpds Flavoxate (ethanol) Oxybutynin (ethanol) Vipacostin (ethanol) Papaverine (methanol) 10^{-8} to 10^{-4} M	Objectives: To evaluate the inhibitory effect of various spasmodic agents on muscarinic contractions of the isolated porcine and human detrusor smooth muscle. Design: Porcine and human urinary bladder tissues were collected and strips (0.5 x 0.5 x 0.3 cm) were prepared and mounted in an organ bath and connected to an isometric force transducer with a tension of 1 g. The tissues were allowed to equilibrate for 30 min. Isometric responses of the tissues were amplified and recorded for analysis. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~) non-GLP study	The potency, EC ₅₀ , measured by the relaxation of the smooth muscle tissues, was determined. On the porcine detrusor strips, tropium chloride was 4000, 8000, and 16,000 times more potent than oxybutynin, papaverine, and vipacostin, respectively. On the human detrusor tissue, tropium chloride was 3000 and 36,000- to 50,000-fold more potent than oxybutynin, and papaverine and vipacostin, respectively.
Primary activity – in vitro effects on isolated organs, continued			
Human isolated urinary bladder ~~XXXXXXXXXXXXXXXXXXXX~~ 1091 (IND Ref 1)	Tropium chloride 10^{-10} to 10^{-4} mg/mL Reference cpds Oxybutynin Propiverine 10^{-10} to 10^{-4} mg/mL	Objectives: To demonstrate and compare the direct action of tropium chloride, oxybutynin, and propiverine on the isolated human urinary bladder. Design: Muscle strips (15 mm long x 5 mm wide) were excised, mounted in a tissue bath, and connected to an isometric force transducer. The tissues were prestretched with 2 g tension over 1 hour. The anticholinergic effects of tropium chloride, propiverine, and oxybutynin were evaluated over a concentration of 10^{-10} to 10^{-4} mg/mL in the presence of acetylcholine. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~) non-GLP study	Addition of tropium chloride, propiverine, or oxybutynin to the tissue bath resulted in antagonism of the hyperreflexic bladder produced by acetylcholine. This activity was observed for all compounds at similar concentrations.
Primary activity – in vitro effects on isolated organs, continued			
Rat (Wistar) isolated tracheal chain 10 F19099 Ref 7 (IND Ref 3)	Tropium chloride (batch 3045) 5×10^{-9} 7.5×10^{-9} 1×10^{-8} mol/mL Reference cpds Atropine 5×10^{-9} 7.5×10^{-9} 1×10^{-8} mol/mL Buscopan 5×10^{-9} 7.5×10^{-9} 1×10^{-8} mol/mL Tyrode buffer	Objectives: To evaluate the antispasmodic activity of tropium chloride on the rat isolated tracheal chain. Design: Rats were stunned by a neck blow. The thoracic cavity and neck opened and the trachea exposed, removed and placed into Tyrodes buffer. The cartilage rings were individually cut and six rings joined with silk thread to form a chain such that the muscle part appeared alternately on the left and right sides. The chain was suspended in a tissue bath with one end connected to a force transducer. Concentration-activity curves were prepared to methacholine chloride and serotonin. The antispasmodic effects of tropium chloride, atropine, and Buscopan were determined. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~) non-GLP study	Tropium chloride and atropine antagonized the cholinergic spasms produced by methacholine chloride by 33% to 46% at 10^{-8} mol/mL. Buscopan was effective at 10^{-8} mol/mL. Tropium chloride did not inhibit the tracheal contractions produced by serotonin.

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Species (strain)/material No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Primary activity – In vitro effects on isolated organs, continued			
Rat (Wistar) Isolated ileum <i>Ref 8</i> [IND Ref 4]	Tropium chloride (Batch 5045) 1.185 x 10 ⁻⁶ 5.4 x 10 ⁻⁶ 7.5 x 10 ⁻⁶ g/mL <u>Reference cpds</u> Buscopan (Hyosidine butylbromide) 7.5 x 10 ⁻⁶ g/mL Atropine 7.5 x 10 ⁻⁶ 2.55 x 10 ⁻⁶ g/mL Demineralized water	Objective: Determine the antispasmodic activity of tropium chloride, Buscopan, and carbacol on acetylcholine-induced contraction of the isolated rat ileum. Design: Rats were killed by a neck blow, the abdomen opened, and a 10-cm length of ileum removed and rinsed. Two 2-cm sections were mounted in organ baths with a 0.5 g load and allowed to rest 30 min. A concentration-activity curve was prepared by incubating the tissue with increasing concentrations of carbacol (4 x 10 ⁻⁶ to 6.25 x 10 ⁻⁶ g/mL) or acetylcholine (8 x 10 ⁻⁶ to 6.25 x 10 ⁻⁶ g/mL). After a 30-min rest, tropium chloride, Buscopan, or atropine was added to the bath 3 min prior to challenge with acetylcholine or carbacol. The effect on contraction of the ileum was determined. (Laboratory – ~~██████████~~) non-GLP study	Tropium chloride, Buscopan, and atropine all inhibited the contractions produced by acetylcholine and carbacol. Tropium chloride and atropine were equally effective at inhibiting contraction of the ileum, while Buscopan was approximately 103-fold less active.
Primary activity – In vitro effects on isolated organs, continued			
Rat (Wistar) Isolated ileum Female 200 - 300 g <i>Ref 9</i> [IND Ref 5]	Tropium chloride (Batch 5045) 1 x 10 ⁻⁶ 3.16 x 10 ⁻⁶ 1 x 10 ⁻⁷ M <u>Reference cpds</u> Atropine 1 x 10 ⁻⁶ 3.16 x 10 ⁻⁶ 1 x 10 ⁻⁷ M Tyrode buffer	Objective: Assess the anticholinergic efficacy of tropium chloride and atropine on the isolated rat ileum. Design: Rats were stunned by a neck blow, the abdomen opened, ileum isolated and removed and rinsed with Tyrode solution. Segments 1.5 cm long, were excised and suspended in an organ bath attached to a pressure transducer. A force of 1 g was placed on the tissue and the tissue allowed to rest. Concentration-activity curves were prepared to acetylcholine. The anticholinergics tropium chloride and atropine were evaluated. (Laboratory – ~~██████████~~) non-GLP study	The molar potency and receptor affinity of tropium chloride (pA ₂ = 5.565) was similar but significantly greater (p < 0.05) than that of the reference compound atropine (pA ₂ = 6.173).
Primary activity – In vitro effects on isolated organs, continued			
Rat (Wistar) Isolated vas deferens Male 250 g <i>Ref 10</i> [IND Ref 7]	Tropium chloride (Batch 5045) 3.16 x 10 ⁻⁶ 1 x 10 ⁻⁶ mol/mL <u>Reference cpds</u> Atropine 3.16 x 10 ⁻⁶ 3.16 x 10 ⁻⁶ 1 x 10 ⁻⁶ mol/mL Buscopan 5 x 10 ⁻⁷ 7.5 x 10 ⁻⁷ 1 x 10 ⁻⁶ mol/mL Krebs-Henseleit buffer	Objective: Determine the inhibitory effect of tropium chloride, atropine and Buscopan on acetylcholine-induced contractions of the isolated rat vas deferens. Design: Rats were killed by a neck blow, the abdomen opened, and the right and left vas deferentia ligated and removed. The tissues were placed in a tissue bath and attached to a transducer. A concentration-response curve was prepared with acetylcholine (1 x 10 ⁻⁶ to 1 x 10 ⁻⁵ mol/mL). Tropium chloride, atropine, and Buscopan were incubated with the tissue for 5 minutes and again challenged with acetylcholine. The effect on acetylcholine-induced spasms was determined. (Laboratory – ~~██████████~~) non-GLP study	Cholinergic spasms of the vas deferens were inhibited by up to 50% with tropium chloride 1 x 10 ⁻⁶ mol/mL, atropine at 3.16 x 10 ⁻⁶ mol/mL, and Buscopan at 1 x 10 ⁻⁶ mol/mL.
Primary activity – In vitro effects on isolated organs, continued			
Rat (Wistar) Isolated uterus Female 150 - 250 g <i>Ref 11</i> [IND Ref 8]	Tropium chloride (Batch 5045) 10 ⁻⁶ - 10 ⁻⁸ g/mL <u>Reference cpds</u> Atropine 10 ⁻⁶ , 10 ⁻⁸ g/mL Buscopan 8.12 x 10 ⁻⁷ 1 x 10 ⁻⁷ 4 x 10 ⁻⁷ g/mL Demineralized water	Objective: Assess the antispasmodic efficacy of tropium chloride compared with atropine and Buscopan on the isolated rat uterus. Design: Rats were killed by a neck blow, the abdomen opened, uterus removed and 1 cm sections cut from each horn and suspended in an organ bath with one end attached to a pen lever. A load of 0.5 g was placed on the tissue and it was allowed to rest 30 minutes. Concentration-activity curves were prepared to carbacol and acetylcholine. The antispasmodic activity of tropium chloride, Buscopan and atropine were evaluated. (Laboratory – ~~██████████~~) non-GLP study	Tropium chloride and atropine inhibited cholinergic spasms in a comparable manner over the concentration range of 10 ⁻⁶ to 10 ⁻⁸ g/mL. Buscopan was 100- to 500-fold less effective in this test system.
Primary Activity – In vitro effects on isolated organs, continued			
Guinea pig (Pinaright) Male and Female 350 g <i>Ref 12</i>	Tropium chloride 2 x 10 ⁻⁶ g/mL stock Azoniaspirantrorpanol 10 ⁻⁶ g/mL stock <u>Reference cpds</u> Atropine 2 x 10 ⁻⁶ g/mL stock Carbachol 10 ⁻⁶ g/mL stock	Objective: Assess the antispasmodic efficacy of tropium chloride and the primary metabolite azoniaspirantrorpanol on the guinea pig ileum. Design: Guinea pigs were killed by a neck blow, the abdomen opened, 5 cm sections of the ileum removed and cut into sections 2 to 2.5 cm in length. The tissues were suspended in an organ bath with contractions recorded with a paper recorder. Contractions of the ileum were produced by the addition of carbacol. The antispasmodic activity of tropium chloride, azoniaspirantrorpanol, and atropine were evaluated. (Laboratory – ~~██████████~~) non-GLP study	Tropium chloride exhibited a mean spasmolytic effect of 75% on the isolated ileum at a concentration of 5.37 x 10 ⁻⁶ g/mL in the intestinal bath, while azoniaspirantrorpanol exhibited the same effect at a concentration of 2.625 x 10 ⁻⁶ g/mL. The spasmolytic efficacy of azoniaspirantrorpanol was weaker than that of tropium chloride by a factor = 4000.

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Species (strain/material) No./Dose Age/Weight Report number (Reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) QLP status	Results
Primary activity – In vivo effects			
Rat (Wistar) In situ urinary bladder 6F 200-250 g Ref 14 [IND Ref 6]	Tropium chloride (batch 834E) 0.3125, 0.625, 0.938, 0.1 mg/kg as a 0.9% saline iv 5 mL/kg	Objective: To evaluate the antispasmodic activity of tropium chloride on the rat urinary bladder <i>in situ</i> . Design: Rats were anesthetized and the urinary bladder isolated. The bladder was emptied, filled with saline and the free end of the bladder attached to a transducer. After a 30-min stabilization, acetylcholine (5 x 10 ⁻⁶ g/mL) was dropped onto the urinary bladder over 2 min with a 50-min washout to establish a baseline. Rats were administered tropium chloride 1/2 and 2 minutes later the bladder was challenged with acetylcholine. This was repeated 5 times in 10-min cycles for all doses of tropium chloride. (Laboratory – ~~XXXXXXXXXX~~); non-GLP study	Tropium chloride produced a concentration- and time-dependent inhibition of the acetylcholine-induced increase in intravesical pressure. A dose of 0.1 mg/kg produced an antispasmodic effect which lasted >52 minutes.
Primary activity – In vivo effects, continued			
Dog (Mongrel) 5M, 7F 3-5 kg 1999 [IND Ref 13]	Tropium chloride 0.03, 0.1, 0.3 mg/kg or 0.1, 0.3, and 1.0 mg/kg Saline 0.9% iv	Objective: Effect of tropium chloride on urodynamic parameters in vivo in decerebrate dogs. Design: Animals were anesthetized, immobilized face-up and instrumented for delivery of tropium chloride and measurement of urodynamic parameters and blood pressure. Animals were righted, immobilized feet down, and decerebrated. Tropium chloride was administered and bladder volume, threshold pressure, maximum bladder pressure, voided volume and residual urine measured. (Laboratory – ~~XXXXXXXXXX~~); non-GLP study	Threshold pressure (0.1 mg/kg) and maximum bladder pressure (0.03, 0.1, and 0.3 mg/kg) declined significantly while residual urine (0.3 mg/kg) increased significantly after administration of tropium chloride suggesting tropium chloride reduces bladder contractility. Tropium chloride has no significant effect on heart rate, blood pressure, or respiration.
General safety pharmacology – CNS effects			
Mouse (NMRI) 3 F/group 18-25 g Ref 19 [IND Ref 13]	Tropium chloride 1 mg/kg; 10 mL/kg Reference compounds Atropine sulphate Buscopan 1 mg/kg; 10 mL/kg Saline 0.9% SC	Objective: Evaluate the sedative activity on the central nervous system. Design: Mice were placed on a rotating rod (13 cm in diameter and 15.5 cm long; 25 rotations per minute) and given two preliminary runs at 30-minute intervals to determine the amount of time they could remain on the rod. The animals were given test or reference compound by SC injection, and 30 and 120 minutes later, the amount of time the mice could remain on the rod determined. (Laboratory – ~~XXXXXXXXXX~~); non-GLP study	Tropium chloride, like the reference compounds atropine sulphate and Buscopan, had no discernible sedative activity on the central nervous system.
Mouse (NMRI) 10 F/group 25-28 g Ref 20 [IND Ref 20]	Tropium chloride 1 mg/kg; 10 mL/kg Reference compounds Atropine sulphate Buscopan 1 mg/kg; 10 mL/kg Saline 0.9% SC	Objective: Effect on pentetrazol-induced convulsions. Design: Mice were treated for 30 minutes with the test or reference compounds (1 mg/kg, SC). 30 minutes later, pentetrazol (50 mg/kg, IP) was administered and the number of mice displaying convulsions determined. (Laboratory – ~~XXXXXXXXXX~~); non-GLP study	Tropium chloride and the reference compounds atropine sulphate and Buscopan did not alter the seizure rate produced by administration of pentetrazol.
General safety pharmacology – CNS effects, continued			
Mouse (NMRI) 10 F/group 20-25 g Ref 21 [IND Ref 21]	Tropium chloride 1.0, 3.16 mg/kg Reference compounds Atropine sulphate Buscopan 1 mg/kg; 10 mL/kg Saline 0.9% SC	Objective: Investigate the potential influence of the test compound on hexobarbital-induced sleeping time. Design: Mice were injected SC with the test or reference compound and 30 minutes later injected with 100 mg/kg hexobarbital sodium. The time to recovery of the righting reflex was individually measured. (Laboratory – ~~XXXXXXXXXX~~); non-GLP study	Tropium chloride at 1 and 3.16 mg/kg SC produced no significant effect on hexobarbital-induced sleep time. Similarly, the reference compounds atropine sulphate and Buscopan (1 mg/kg, SC) were without effect.
Rat 10 F/group 250-300 g Ref 22 [IND Ref 22]	Tropium chloride 0.1, 0.5 mg/kg Reference compounds Atropine sulphate 1 mg/kg Bicyclohexylamine 2, 10 mg/kg Buscopan 2, 10 mg/kg iv	Objective: Determine if tropium chloride crosses the blood-brain barrier in centrally active concentrations, and if so, the relevance of those doses to man. Design: Rats were anesthetized, immobilized, ventilated and individual filaments isolated from an anterior root of the lower lumbar or upper sacral spine. Electrodes were attached to record the discharge of alpha and gamma impulses. Atropine, Buscopan, and tropium chloride were injected 10 minutes prior to physostigmine injection. (Laboratory – ~~XXXXXXXXXX~~); non-GLP study	Physostigmine (0.3 mg/kg, iv) raised the reflex discharge of impulses from the alpha-motoneurons and reduced the activity of the gamma-motoneurons. Atropine inhibited the physostigmine-induced discharge of alpha motoneurons and increased the number of gamma discharges. Buscopan was tested as it, like tropium chloride, contains a quaternary ammonium group. Buscopan at 10 mg/kg, but not 2 mg/kg, resulted in a weak inhibition of the physostigmine-induced effects on the alpha and gamma motoneurons. Tropium chloride at 0.5 mg/kg (500 µg/mL based on a 0.25 kg rat) did not affect the alpha activity produced by physostigmine and had a weak effect on gamma activity. From these results, it is unlikely that tropium chloride in pharmacologically active doses would exhibit central anticholinergic activity.
General safety pharmacology – Cardiovascular and respiratory effects			
HEK293 cells (transfected with ~~XXXXXXXXXX~~) Ref 23 [Report 021206.JOE]	Tropium chloride (batch 86310) 0, 1, 10, 30, 100 µM Reference compounds Terfenadine 50 µM Sterile water	Objective: In vitro effect of tropium chloride on the HERG channel current in HEK293 cells. Design: HEK293 cells were transfected with ~~XXXXXXXXXX~~ , cultured, and incubated with test compound or the positive control for 10 min at 35 °C. Cells stably expressing HERG were held at -60 mV for at least 30 sec before addition of tropium chloride or the positive control, terfenadine. The % inhibition and IC50 were determined. (Laboratory – ~~XXXXXXXXXX~~); GLP	Tropium chloride inhibited the HERG current in a dose-dependent manner with 0.2%, 12.1%, 30.5%, 58.4% and 81.4% inhibition observed at 0, 1, 10, 30, and 100 µM. The IC50 was determined to be 23.1 µM, which is approximately 5500 to 6335 times greater than the expected clinical plasma concentrations of 1 to 15 ng/mL. The positive control, terfenadine produced a 71.7% block at a concentration of 50 µM.

Species (strain)/material No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
General safety pharmacology – Cardiovascular and respiratory effects, continued			
Rabbit (New Zealand White) Female 1500 – 1800 g Ref 24	Tropium chloride (batch 5045) 10, 100, 200, 1000, 2000 nM Reference compounds Haloperidol 10, 100, 200, 1000, 2000 nM	Objective: To evaluate the risk for induction of torsade de pointes in isolated rabbit hearts. Design: Rabbits were administered 1000 U/kg heparin, IV, 5 min prior to receiving a sharp blow to the neck with subsequent exsanguination. The heart was excised, prepared, and perfused according to the Langendorff technique at a constant pressure of 73 cm H₂O, with Tyrode solution at 37°C. APCI electrodes were attached to the heart and recordings collected for 120 min. Hypokalemia and hypomagnesemia were simulated by reducing extracellular K⁺ and Mg²⁺ to 0.5 mM. A slight bradycardia and sinusoidal tone were induced by the addition of 10⁻⁶ M norepinephrine and 10⁻⁷ M carbachol. Cumulative concentrations of either haloperidol or tropium chloride at 10, 100, 200, 1000, and 2000 nM were tested. (Laboratory - ██████████) non-GLP	Torsade de pointes arrhythmias developed in 3/5 hearts treated with haloperidol at concentrations >200 nM. In hearts treated with tropium chloride, no torsade de pointes arrhythmias occurred at concentrations up to 2000 nM. Tropium chloride produced weak effects on cardiac electrophysiology and on functional parameters of the heart, however, tropium chloride did not significantly prolong the activation recovery interval (ARI), enhance dispersion, lead to a right-left difference in ARI or promote bradycardia. The most notable effect was an increase in heart rate probably resulting from the anticholinergic effect against carbachol-induced bradycardia. None of the risk factors of torsade de pointes arrhythmias were promoted by tropium chloride in this study at concentrations up to 2000 nM. This concentration is approximately 500 to 63 times greater than the expected plasma concentration range of ~1-15 ng/mL in humans.
General safety pharmacology – Cardiovascular and respiratory effects, continued			
Guinea pig (Frodoight white) 26 M/gross 250g Ref 25 IND Ref 181	Tropium chloride (batch 5045) 1, 10, 100 µg/mL/kg Reference compounds Atropine 10, 31.5, 100 µg/mL/kg Buscopan 100, 315.2, 1000 µg/mL/kg Saline 0.9% IV	Objective: To evaluate the bronchodilator, blood pressure and heart rate effects of tropium chloride in the guinea pig. Design: Animals were anesthetized and instrumented for drug administration, measurement of blood pressure, and measurement of tracheal pressure. Methacholine chloride (10 µg/mL/kg, IV, 0.5 mL/min) was infused at 30-minute intervals to establish baseline. Thirty minutes later, the test or reference compounds were given and the effects on heart rate, blood pressure and bronchial pressure determined. (Laboratory - ██████████) non-GLP study	Tropium chloride inhibited the bronchospasm induced by methacholine chloride in a dose and time dependent manner. At 10 minutes postdose, 100% inhibition was observed at 10 and 100 µg/mL, with 100% inhibition still apparent 40 minutes post 100 µg/mL administration. Atropine produced 100% inhibition at 100 µg/mL 10 min post administration, and 56% inhibition at 40 minutes. Buscopan at 100 µg/mL was less effective in this model producing approximately 80% inhibition at 10 minutes with no inhibition present 40 minutes postdose. All compounds produced a dose-related inhibition of the reduction in mean arterial blood pressure occurring in response to methacholine. This effect was still present 40 minutes after administration, except at the lowest dose tested in each group. No effect of the test compounds on the decrease in heart rate produced by methacholine was noted.
General safety pharmacology – Cardiovascular and respiratory effects, continued			
Cat (Cross-bred European domestic) 3/sex/group 2.5 kg Ref 26 IND Ref 171	Tropium chloride (batch 5045) 0.1, 0.315, 1.0, 3.15 mg/kg Reference compounds Atropine 0.1, 0.315, 1.0, 3.15 mg/kg Saline 0.9% IV	Objective: Determination of hemodynamic effects and modification of acetylcholine effects compared with atropine and Buscopan. Design: Animals were anesthetized and instrumented for measurement of heart rate and hemodynamic parameters. After establishment of baseline, animals were challenged with acetylcholine (2 mg/kg/mL) and measurements taken at 2, 3, 4, 5, 10, and 15 minutes postdose. The animals were then dosed with tropium chloride, atropine, or Buscopan and measurements recorded immediately, 5, and 10 minutes postdose. (Laboratory - ██████████) non-GLP study	Atropine, Buscopan, and tropium chloride all induced dose-related decreases in heart rate along with reductions in myocardial contractility, left-ventricular pressure, and peripheral arterial pressure. The highest dose of all 3 substances prevented the acute effects of the acetylcholine injection (increase in heart rate, reductions in peripheral and left ventricular pressures, and a reduction in contractility).
General safety pharmacology – Gastrointestinal effects			
Mouse (NMRI) 3-16 P/gross 20-25 g Ref 27 IND Ref 141	Tropium chloride (batch 5045) 5, 15.8, 50, 158 mg/kg, PO; 0.205, 0.625, 2.0, 6.25 mg/kg, IV Reference compounds Atropine sulphate 0.25, 0.5, 1.5, 5.0 mg/kg, PO; 0.25, 0.5, 1.5 mg/kg, IV Buscopan 15.8, 50.0, 158 mg/kg, PO; 0.5, 1.5, 5.0 mg/kg, IV 20 mL/kg, PO/IV PO deionized water IV Saline 0.9%	Objective: Effects of anticholinergic agents on intestinal motility in the mouse after oral and IV administration. Design: Female mice were dosed orally or intravenously 30 or 10 minutes, respectively, before challenge with tetracanth carmine red and carbachol. After 25 minutes the animals were killed and the intestines removed. The length of the stained portion of the intestine was measured and calculated in relation to the total length of the gut. (Laboratory - ██████████) non-GLP	Tropium chloride, atropine, and Buscopan all inhibited intestinal motility in a dose dependent manner. After oral administration, atropine was the most effective; however, after IV administration, tropium chloride had the greatest effect on intestinal motility. Buscopan was less effective than either tropium chloride or atropine in this system by a factor of approximately 200.
General safety pharmacology – Gastrointestinal effects, continued			
Rat (Wistar) 5-14/group 150-200 g Ref 28 IND Ref 151	Tropium chloride (batch 5045) 5 mg/kg WQ 71 (batch 840720) 5 mg/kg 5 mL/kg Saline 0.9% IP/PO	Objective: To determine the effects of tropium chloride and WQ 71 on intestinal motility after PO and IP administration. Design: Wistar rats were cannulated intraduodenally for drug administration. Tropium chloride or WQ 71 were administered orally and intraduodenally at 5 mg/kg, 15 and 30 minutes before administration of the tetracanth carmine red suspension in one group of animals, carbachol was administered SC to stimulate intestinal motility. The stomach and total intestines were removed at different timepoints and the distance traversed by the carmine red dye determined. (Laboratory - ██████████) non-GLP study	No difference was observed in intestinal transit time between normal animals and animals that received an intraduodenal catheter. In animals where intestinal motility was not stimulated by carbachol, the inhibitory activity of tropium chloride was 16% and 13% after intraduodenal and oral administration, respectively. Animals given carbachol to stimulate intestinal motility had a reduced intestinal transit time of 32.5%, respectively, after intraduodenal administration. Tropium chloride reduced intestinal motility regardless of the route of administration. An increase in the inhibition of motility was observed when animals were given carbachol to stimulate intestinal motility.

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Species (Strain)/Material No./Dose Age/Weight Report number (Reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
General Safety pharmacology – Gastrointestinal effects, continued			
Dog (Monogre) 6 animals 12-18 kg Ref 29 IND Ref 145	Tropium chloride (batch 5717) 0.5, 0.1, 0.5 mg/kg, IV 1, 5, 10 mg/kg, PO and ID Buscopan 0.5 mg/kg, IV 1, 5, 10 mg/kg, PO and ID Atropine 0.1, 0.2 mg/kg, IV	Objective: To evaluate in the unanesthetized dog the effects of tropium chloride on motility of the small intestine and colon under fasted and fed conditions and with stimulation by carbachol or morphine. Design: Dogs were anesthetized and fitted with indwelling intraparietal electrodes, strain gauges, and in addition, four animals were fitted with an intraduodenal catheter. Electrodes were implanted in the duodenum, jejunum, ileum and colon at various positions to measure intestinal and colonic motility. After an 8 to 10 day recovery period, animals were fasted for 18 hours or fed and challenged 3 hours postprandially with tropium chloride, Buscopan, carbachol, atropine, and morphine. Colonic motility was determined 2 hours prior to and 4 hours after injection. Intestinal motility was recorded. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~ non-GLP	The effects of tropium chloride were dependent upon the route of administration and digestive state of the animal. The inhibitory effects of tropium chloride were more evident after intravenous administration in the postprandial animals, while oral and intraduodenal administrations were most effective in fasted animals. The efficacy of tropium chloride was superior (≈10-fold, IV) with a longer duration of action compared with that of Buscopan. The anticholinergic properties of tropium chloride were evident by the complete inhibition of carbachol stimulation of colonic motility. Morphine-induced stimulation of colonic motility was inhibited by tropium chloride with comparable activity to atropine.
General Safety pharmacology – Genitourinary effects			
Rat (Wistar, BPF) 10 M/group 250-300 g Ref 30 IND Ref 233	Tropium chloride (batch 5717) 1 mg/5 mL/kg Reference compounds Atropine Buscopan 1 mg/5 mL/kg Saline 0.9% IV	Objective: Effects of tropium chloride on renal function in anesthetized rats. Design: Male Wistar rats were anesthetized and the ureters and jugular vein cannulated. Inulin and PAH were injected into the jugular vein and 20 min later an infusion of physiological saline started. Tropium chloride or the reference compounds were given IV 30 min after the start of the saline infusion. Urine and blood samples were collected at 20 and 50 minutes postdose. The following parameters were determined: urinary flow rate, glomerular filtration rate calculated from inulin clearance, absolute and fractional reabsorption of fluid, absolute and fractional reabsorption/secretion and clearances of Na ⁺ , K ⁺ , Cl ⁻ , glucose, urea, and PAH. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~ non-GLP	No discernible effects of tropium chloride, atropine, or Buscopan were observed on urinary flow rate, GFR, or on the reabsorption/secretion amounts and clearances of Na ⁺ , K ⁺ , Cl ⁻ , glucose, urea, or PAH, suggesting little effect on renal function.
General Safety pharmacology – Genitourinary effects, continued			
Rat (Wistar, BPF) 10 M/group 225-300 g Ref 31 IND Ref 243	Tropium chloride (batch 5717) 1 mg/5 mL/kg Reference compounds Atropine Buscopan 1 mg/5 mL/kg Saline 0.9% IV	Objective: Effect of tropium chloride on renal function in conscious rats. Design: Rats were tested before treatment with tropium chloride, atropine, or Buscopan followed by water loading (20 mL/kg). The animals were placed in diuresis cages and urine collected in 30-minute fractions for 6 hours. Urinary flow, osmolality, and Na ⁺ , creatinine and glucose concentrations determined. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~ non-GLP	No effects of tropium chloride, atropine, or Buscopan on any of the excretory functions measured in conscious rats were observed.
General Safety pharmacology – Other effects			
In vivo/in vitro Rat (Wistar W/L) 10 F 160-230 g Ref 32 IND Refs 25 & 26)	Tropium chloride (batch 5717) 10 ⁻⁶ , 10 ⁻⁷ , 10 ⁻⁸ g/mL Krugers solution	Objective: Effect of tropium chloride on the release of histamine from isolated mast cells of sensitized rats. Design: Rats were injected intraperitoneally with 1 mL 4% ovalbumin. The animals were exsanguinated under anesthesia and 10 mL Kruger solution injected into the abdomen and the abdomen massaged for 1.5 min. The abdominal fluid was collected and the cells pelleted, resuspended, and aliquots incubated with tropium chloride or buffer. Histamine release was measured using the isolated guinea pig leucine bioassay. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~ non-GLP	Incubation of tropium chloride at concentrations of 10 ⁻⁶ , 10 ⁻⁷ , and 10 ⁻⁸ g/mL, resulted in no release of histamine from sensitized rat mast cells when compared with control.
General Safety pharmacology – Other effects, continued			
Guinea pig (BFAZ/4) Hansen 5 F/group ≈350 g Ref 32 IND Ref 25 & 26)	Tropium chloride (batch 5717) 10 mg/kg Saline 0.9% IP	Objective: To determine if tropium chloride had an effect on the onset, course or duration of anaphylactic shock in sensitized guinea pigs. Design: Guinea pigs were sensitized with a SC injection of 0.6 mg ovalbumin at two sites on the back. Two days later the same dose was divided and given IM at each hind limb. After 22 days, five animals received an IP injection of 10 mg/kg tropium chloride (0.5% solution) in physiological saline. The remaining 5 animals served as control and were injected with physiological saline. Fifteen minutes later the animals were challenged with an IP injection of ovalbumin (1 mg/mL) and 2 days later surviving animals received an intrazodiac injection of 0.1 mg ovalbumin. (Laboratory – ~~XXXXXXXXXXXXXXXXXXXX~~ non-GLP	Tropium chloride had no effect on the profile or severity of the anaphylactic reaction produced by injection of ovalbumin in guinea pigs.

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Species (strain/material) No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
General safety pharmacology - Other effects, continued			
Rabbit (New Zealand White) 4 F/group 3.0-3.6 kg Ref 32 IND Refs 25 & 26	Tropium chloride (batch 5717) 1.0 mg/kg Control Physiological saline IV	Objective: To determine if tropium chloride has an effect on circulating antibodies in sensitized rabbits. Design: Female rabbits were sensitized by IM injection of 0.5 mg ovalbumin in 1 mL saline given as a divided dose into each hind leg. Four days later the same ovalbumin dose was given SC at 2 sites on the back followed 2 days later by IM injections as before. Four weeks later, the same dose of ovalbumin was given IM as a booster. At the end of the sensitization period, animals were challenged with tropium chloride (1.0 mg/kg) or saline. Twenty minutes later blood was collected, serum separated, and plated on petri dishes (5 holes/dish) containing agar gel. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Tropium chloride had no effect on circulating antibodies in sensitized rabbits.
General safety pharmacology - Other effects, continued			
Rat (Wistar) 10/group 150-175 g Ref 33 IND Ref 27	Tropium chloride 1 mg/5 mL/kg Reference cpds Atropine Buscopan 1 mg/5 mL/kg DC	Objective: Evaluation of the anti-inflammatory effects of tropium chloride on viscain carrageenin or ovalbumin induced rat paw edema. Design: Rats were anesthetized, right hind leg shaved, cleaned, and the paw volume measured. Animals were pretreated with tropium chloride, atropine or Buscopan 20 minutes before injection of the irritant, viscain carrageenin (0.6% solution) or ovalbumin (2% suspension). The irritants were administered by intraplantar injection between the second and third toe at a dose of 0.1 mL/dorsal. Edema was evaluated 2 or 4 hours after ovalbumin challenge or 2, 5, 7, and 24 hours after viscain carrageenin induced edema. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Pretreatment of rats with tropium chloride, atropine, or Buscopan (1 mg/kg, SC) produced no significant inhibition of ovalbumin- or viscain carrageenin-induced rat paw edema.
General safety pharmacology - Other effects, continued			
Rat (Wistar) 15-15 F/group 150-175 g Ref 34 IND Ref 28	Tropium chloride 1 mg/5 mL/kg Reference cpds Atropine sulphate Buscopan 1 mg/5 mL/kg Hydrocortisone 5 mg/5 mL/kg Saline 0.5% DC	Objective: Evaluation of the anti-inflammatory effects of tropium chloride in the cotton pellet test of delayed inflammation. Design: Rats were implanted in the lower rib arch with a pellet of cotton which had been saturated with viscain-carrageenin to produce a delayed inflammatory reaction. Rats were treated with tropium chloride, atropine, Buscopan or hydrocortisone concurrently with implantation of the cotton pellet, and then daily at 24-hr intervals from day 2 through 10. At the end of the 11 th day, the animals were sacrificed, pellet removed and inflammatory response determined. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Tropium chloride decreased the inflammatory response by 21.5% compared with atropine (12.1%) and Buscopan which had no activity. The positive control, hydrocortisone, inhibited inflammation by 48.7%.
General safety pharmacology - Other effects, continued			
Rat (Wistar) 10 M/group 300 g Ref 35 IND Ref 29	Tropium chloride 1 mg/kg Reference cpds Atropine sulphate Buscopan 1 mg/kg Saline 0.9% IV	Objective: Evaluation of the effect of tropium chloride on permeability of the plasma-lymph barrier and impairment of the lymphagogic effect of bradykinin in anesthetized rats. Design: Male Wistar rats were fasted for 15 hours, anesthetized, injected IV with 500 mg/kg polyvinylpyrrolidone, and the trachea, right carotid artery and left external jugular vein cannulated. The thoracic duct was cannulated for determination of lymph flow. Lymph was collected in 60-minute intervals over 4 hours. Sixty minutes after the lymph collection began, animals were administered tropium chloride or atropine (1 mg/kg, IV). One group received tropium chloride at different times prior to the injection of bradykinin. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	No effects on intrinsic lymphagogic or lymphostatic activities were observed after injection of tropium chloride, atropine or Buscopan. In animals treated with bradykinin, tropium chloride had no effect on the lymphagogic activity of bradykinin.
Interactions with other drugs			
In vitro Ref 37 IND Reference 30	Tropium chloride (batch 35225) 20 mg tropium chloride 200.8 µCi ³ H-tropium chloride Maalox 70 20 mL Demineratized water	Objective: In vitro evaluation of the interaction of tropium chloride and the antacid Maalox 70. Design: ³ H-labeled tropium chloride was incubated with Maalox 70 for 8 hours at room temperature. Samples were taken at 0, 0.25, 0.5, 1, 3, 5, and 8 hours during the incubation period. The samples were analyzed by liquid scintillation counting and thin layer chromatography. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Evaluation of samples by liquid scintillation counting and thin layer chromatography indicated no adsorption, absorption or degradation of tropium chloride over the 8-hour incubation period.
In vitro Ref 38 IND Reference 31	³ H-tropium chloride 1.5 x 10 ⁴ dpm/mL CaCl ₂ , MgCl ₂ , AlCl ₃ , FeCl ₃ , Water	Objective: In vitro evaluation of the interaction of tropium chloride with various two and three-valent cations. Design: ³ H-labeled tropium chloride was placed into a dialysis chamber and allowed to equilibrate for 24 hours at room temperature. The solutions in both dialysis chambers were analyzed by scintillation counting. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Incubation of tropium chloride with the two and three-valent cations did not result in a change in lability or sedimentation of the solution. Incubation of labeled tropium chloride with the two and three-valent cations for 24 hours resulted in no accumulation of tropium chloride in the chamber containing the salt and suggested no interaction with the salts that were tested.

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Pharmacokinetics studies:

Species (strain)/material No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Pharmacokinetics - single dose			
Rat (Wistar) Sex/group 250 - 320 g Ref 39 [IND Ref 38 & 53]	[¹⁴ C]-tropicam chloride (batch 7343) 0.2, 2, 20, 200 mg/kg, PO 1, 2, 5, 5 mCi/kg 10 mL/kg Double-distilled water	Objective: Pharmacokinetics in the rat after single dose oral administration. Design: Following administration, blood samples were collected at 8 timepoints over the 1 st hour and at 1.5, 2, 3, 5, 8, 12, and 24 hours. Absorption and pharmacokinetics were evaluated. Activity was determined by liquid scintillation counting. (Laboratory - ~~XXXXXXXXXXXX~~) non-GLP	The blood levels of tropicam chloride were largely dose-related over the entire dose range. T _{1/2} was determined to be between 1.0 and 1.5 hours after dosing with similar values obtained for males and females. The maximum concentration was 20.67 x 10 ³ cpm/mL for the males and 21.03 x 10 ³ cpm/mL for the females. Sex-related differences were marginal with a faster reduction in the blood levels of the females than males between hours 3 and 8, however, after 10 hours the blood levels were once more concurrent. The absorption rate of tropicam chloride was approximately 5% from the intestinal tract.
Rat (Wistar) Sex/group 250 - 220 g Ref 40 [IND Ref 37 & 52]	[¹⁴ C]-tropicam chloride (batch 7343) 5, 50, 500 µg/kg, IV 50 µCi/kg, 5 mCi/kg 2.5 mL/kg Phosphate buffered saline	Objective: Pharmacokinetics in the rat after single dose intravenous administration. Design: Following administration blood samples were collected at 8 timepoints over the 1 st hour and at 1.5, 2, 3, 4, 6, 8, 10, and 24 hours postdose. Absorption and pharmacokinetics were evaluated. Activity was determined by liquid scintillation counting. (Laboratory - ~~XXXXXXXXXXXX~~) non-GLP	The blood levels decreased in three phases after IV administration and suggested a very rapid distribution of tropicam chloride. Mean AUCs and total clearance were similar after 5 and 50 µg/kg. The AUC was smaller and the clearance greater after 5 µg/kg. Tropicam chloride was rapidly reduced to 1% of the peak levels between 4 and 8 hours postdose in both males and females.
Pharmacokinetics - single dose, continued			
Dog (Beagle) 3-F group 3.5 - 8.5 months 5.5 - 11.8 kg Ref 41 and Ref 42 [IND Ref 38] [IND Ref 43]	[¹⁴ C]-tropicam chloride (batch 7343) 1 mCi/kg, PO 0.5 - 0.7 mL capsule/dose 50 µCi/kg, IV and IM 0.5 mL/kg, IV 1.0 mL/dog, IM Phosphate buffered saline	Objective: Pharmacokinetics in the dog after oral, intravenous, and intramuscular administration. Design: Dogs were administered tropicam chloride orally, intravenously, or intramuscularly. Blood levels were determined at various timepoints over 48 hours postdose. Absorption and pharmacokinetics were evaluated. Activity was determined by liquid scintillation counting. (Laboratory - ~~XXXXXXXXXXXX~~) non-GLP	Oral administration: Peak blood levels after oral administration were reached at 2.15 hours postdose with a maximum concentration of 5.73 µg/mL. Clearance was determined to be 18.53 mL/min. Intravenous administration: Plasma levels declined rapidly with a terminal half-life of 20 hours. Clearance was determined to be 21.93 mL/min. Intramuscular administration: Peak blood levels were reached at 0.125 hours postdose with a C _{max} of 38.25 µg/mL. The clearance was determined to be 12.75 mL/min. The oral absorption was estimated to be 5.5%.
Pharmacokinetics - repeat dose			
Rat (Wistar) 10 M 212 - 237 g Ref 43 Ref 44 [IND Ref 42] [IND Ref 42 and 54]	[¹⁴ C]-tropicam chloride (batch 7343) 0.2 mg/kg 1 mCi/kg 2 mL/kg PO Double distilled water	Objective: Pharmacokinetics in the rat after repeat dose oral administration. Design: Rats were dosed orally with tropicam chloride at 10 timepoints over 4 days (hour 1, hour 7, hour 14, on days 1-3 and at hour 1 on day 4). Blood was collected at various timepoints over the 4-day period. Total and non-voidable activity profiles were determined. (Laboratory - ~~XXXXXXXXXXXX~~) non-GLP	Peak blood levels were reached approximately 48 hours after administration of the first dose. The t _{1/2} was similar after administration of the first dose (3.5 hours) or the last dose (3.8 hours), and also similar to the values received after single dose administration (2.9 to 3.9 hours). Clearance after the 1 st dose was 40.58 mL/min and after the 10 th dose, 41.10 mL/min. These data suggest no accumulation of tropicam chloride after repeated dose administration of 0.2 mg/kg.
Protein binding			
In vitro Human serum Ref 45 Ref 46 [IND Ref 50] [IND Ref 51]	[¹⁴ C]-tropicam chloride Equilibrium dialysis: 10.3, 109.42, 1094.5 µg/L Ultracentrifugation 0.5, 5.0, 50.0 µg/L Gel filtration: [¹⁴ C]-labeled tropicam chloride in 20 mL human serum Saline buffered to pH 7.3	Objective: To determine the serum protein binding in human serum in vitro employing equilibrium dialysis, ultracentrifugation and gel filtration. Design: Using the equilibrium dialysis method labeled tropicam chloride was dialyzed against human serum for 24 h at room temperature. The concentration in serum and buffer were determined. With the ultracentrifugation method, [¹⁴ C]-tropicam chloride was added to human serum and mixed thoroughly for 30 min, then centrifuged for 6 h at 40,000 rpm and binding determined. The gel filtration method mixed [¹⁴ C]-tropicam chloride in 20 mL human serum and applied to a Sepharose column. Fractions were collected and counted. Activity was determined by liquid scintillation counting. (Laboratory - ~~XXXXXXXXXXXX~~) non-GLP	Equilibrium dialysis: Using this method, 53% to 49% binding was observed over the concentration range 10 to 1000 µg/L. Ultracentrifugation: the binding was determined to be about 72% over the concentration range of 0.5 to 50 µg/L. Gel filtration: two binding values were obtained - 53% and 76%. While there was some variability regarding the binding rates of tropicam chloride to serum proteins, none of the rates were sufficiently high to suggest an interference with other drugs.
Protein binding, continued			
Human serum Ref 46 [IND Ref 51]	[¹⁴ C]-tropicam chloride 0.01, 0.039, 0.1, 0.43, 1.0, 7.5, 10 µg/mL in vitro	Objective: Determination of the in vitro binding of tropicam chloride to human serum proteins. Design: [¹⁴ C]-tropicam chloride was added in different amounts to human serum. 1 mL samples were removed, centrifuged at 4000 rpm for 30 min at room temperature and the activity in the ultratrate and serum determined. (Laboratory - ~~XXXXXXXXXXXX~~) non-GLP	The binding of [¹⁴ C]-tropicam chloride is inversely proportional to the concentration with 50% bound at 0.01 µg/mL and 40% bound at 1 µg/mL, the "physiological" concentration range of tropicam chloride.
Dog (Beagle) 3-F group 3.5 - 8.5 months 5.5 - 11.8 kg Ref 41 [IND Ref 38]	[¹⁴ C]-tropicam chloride (batch 7343) 2 mg/kg 1 mCi/kg, PO Capsule 50 µg/kg 50 µCi/kg, IV Phosphate buffered saline	Objective: Determination of the in vivo binding of tropicam chloride and its metabolites with plasma proteins and distribution into erythrocytes. Design: [¹⁴ C]-tropicam chloride was administered orally or intravenously to dogs and 0.25, 2, and 6 hours later blood collected. Samples were dialyzed against sodium phosphate buffer for 24 hrs at room temperature. Determination of plasma protein binding by gel filtration was performed. (Laboratory - ~~XXXXXXXXXXXX~~) non-GLP	The in vivo plasma protein binding data in the dog after oral and intravenous dosing suggest 30% to 50% binding to plasma proteins regardless of the route of administration. These results correlate well with the in vitro findings in human serum, where the plasma protein binding was estimated to be 40% to 70%.

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Species (Strain)/Material No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/Vehicle	Study design (Laboratory) (GLP status)	Results
Tissue distribution/Accumulation			
Rat (Wistar) 3 M Ref 45 [IND Ref 53]	[³ H]-tropicam chloride (batch 7345) 0.16 mg/kg 10 mCi/kg PO	Objective: Determination of the in vivo binding of tropicam chloride between plasma and erythrocytes. Design: [³ H]-tropicam chloride was administered orally to 3 rats and blood samples taken at 0.25, 2, and 8 hours post dose. Binding was determined using centrifugation and scintillation counting. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	The binding of [³ H]-tropicam chloride to erythrocytes was determined to be 5.85%, 13.10% and 13.01% at 0.25, 2, and 8 hours postdose, respectively. This limited accumulation in or on erythrocytes is compatible with the strong hydrophilic nature of tropicam chloride.
Rat (Long Evans) 7M 209 - 246 g Ref 47	[¹⁴ C]-tropicam chloride (batch CPQ73184) 20 mg/kg (19.99 µCi/kg) PO 5 mL/kg Distilled water	Objective: Determine the tissue distribution of [¹⁴ C]-tropicam chloride following a single oral administration. Design: Male pigmented Long Evans rats were dosed with 20 mg/kg (19.99 µCi/kg) [¹⁴ C]-tropicam chloride. A single rat was sacrificed at 0.5, 1, 2, 24, 72, 120, and 325 hours after dosing. Tissue distribution was determined by quantitative whole-body autoradiography. (Laboratory - ~~XXXXXXXXXX~~) GLP	Following oral administration of [¹⁴ C]-tropicam chloride, the distribution of radioactivity was limited. Concentrations of drug-derived radioactivity were evaluated in 56 tissues and fluids. The table below represents a summary of the pharmacokinetic parameters for total [¹⁴ C]-tropicam chloride in the tissues and organs in which it was detected. Systemic exposure to [¹⁴ C]-tropicam chloride-derived radioactivity was low, in line with the low oral bioavailability of the compound. The feces was the primary route of elimination, while renal excretion played a minimal role. Absorbed [¹⁴ C]-radioactivity did not cross the blood-brain barrier, and no radioactivity was associated with the testes, epididymis or seminal vesicles. There was no evidence that [¹⁴ C]-tropicam chloride was associated with melanin bearing tissues such as eyes or pigmented skin. Elimination of [¹⁴ C]-activity was essentially complete by 72-hours postdose.
Tissue distribution/Accumulation, continued			
Rat (Wistar) 6Sex/group 250 - 320 g Ref 39 [IND Ref 38 and 53]	[³ H]-tropicam chloride (batch 7345) 0.2, 2, 20 and 200 mg/kg, PO 5 mCi/kg 10 mL/kg Double-distilled water	Objective: To determine the distribution of tropicam chloride in the rat following single dose oral administration. Design: [³ H]-tropicam chloride was administered to rats and tissue distribution determined at 0.25, 2, 5, and 24 hours postdose and at 24 hours postdose at different concentrations. Tissues were homogenized and extracted with methanol and activity determined by liquid scintillation counting. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	The highest levels of activity were in the excretory organs including the liver and kidneys followed by the lungs and heart. Only trace activity was detected in the brain suggesting neither tropicam chloride or its metabolites cross the blood-brain barrier. Tissue levels fell quickly and between 2 and 8 hours and by 24 hours postdose ranged from 1.5% to 2.9% of the administered activity (excluding the gastrointestinal tract). No clear sex-related differences were observed.
Rat (Wistar) 10 M 212 - 237 g Ref 43 [IND Reference 42]	[³ H]-tropicam chloride (batch 7345) 0.2 mg/kg 1 mCi/kg 2 mL/kg PO Double distilled water	Objective: Distribution in the rat after repeated dose oral administration. Design: Rats were dosed orally with tropicam chloride at 10 timepoints over 4 days (hour 1, hour 7, hour 14, on days 1-3 and at hour 1 on day 4). Tissues were collected at 8 and 24 hours after the final dose. Total and non-volatile activity profiles were determined. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Organ concentrations were approximately 10-fold higher after repeated dose administration compared with single dose administration. Organs with the highest concentrations were the heart, liver, adrenals, and kidneys. The concentration in the brain remained low after repeated administration.
Tissue distribution/Accumulation, continued			
Rat (Wistar) 6Sex/group 250 - 320 g Ref 40 [IND Ref 37 and 52]	[³ H]-tropicam chloride (batch 7345) 5, 50, 500 µg/kg, IV 50 µCi/kg, 2.5 mL/kg Phosphate buffered saline	Objective: To determine the distribution of tropicam chloride in the rat after single dose intravenous administration. Design: [³ H]-tropicam chloride was administered IV to rats and tissue distribution determined at 0.25, 2, 8, and 24 hours postdose and at 24 hours postdose at different concentrations. Selected organs were removed, homogenized, and extracted with methanol. Activity was determined by liquid scintillation counting. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Evaluating first the effect of concentration on the distribution profile, it was found that regardless of dose, the kidneys had the highest total activity of all organs evaluated. The next highest activities in decreasing order were heart, liver, muscle, and lungs. No activity was found in the brain of any of the doses tested. The effect of time on tissue distribution was also evaluated. The highest total activity was found in the kidneys and liver at 0.25 hr postdose. At 2 hours postdose, the total activity had declined, but remained highest in the kidneys. The liver, heart, and lungs had the next highest levels of total activity. By 24 hours postdose, the highest activity levels remained in the kidneys. Whole body activity 24 hours postdose was 1.8% in males and 3.5% in females. No sex-related differences were apparent.
Rat (Hanan Cdx) P(V) 15 M 7 - 8 weeks 160 - 200 g Ref 48 [IND Ref 47]	[³ H]-tropicam chloride (batch TRQ6757) 1 mg/kg (batch 44404) 50 µg/kg 18.5 MBq/kg IV Phosphate buffered saline	Objective: Evaluation of the tissue distribution of tropicam chloride in the rat following IV administration. Design: A single IV dose of 50 µg/kg (18.5 MBq/kg) was administered to each rat and at 1, 3, 10, 24, and 56 hours postdose, 3 rats were sacrificed and selected organs and tissues removed. At 56 hours postdose urine, feces and expired ³ H ₂ O were collected. (Laboratory - ~~XXXXXXXXXX~~) non-GLP	Following intravenous administration, the highest concentrations of total radioactivity occurred in the small intestine, kidneys, liver and large intestine 1-hour postdose. By 3 hours postdose the levels were highest in the large intestine, small intestine, kidneys and liver. At the 56-hour timepoint, although the tissue concentrations had decreased significantly, there was still measurable activity in the large intestine, kidneys, liver, eyes, thyroid, and adrenals. The mean total recovery at the 56-hour time point was 98.75%. Radioactivity in pigmented and non-pigmented skin was similar suggesting no association of tropicam chloride with melanin.

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Species (strain/material) No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Tissue distribution/Accumulation, continued			
Gestating Rats (Wistar) 15 F 220 - 320 g Ref 49 [IND Reference 49]	[³ H]-trospium chloride (batch 7045) 50 µg/kg 50 µCi/kg 5 mL/kg IV Phosphate buffered saline	Objective: Evaluation of the distribution and placental permeability of trospium chloride in gestating rats after intravenous administration. Design: Female gestating rats were given trospium chloride intravenously on the 18 th , 19 th , or 20 th day of gestation. The dams were killed 0.5, 2, or 8 hours after injection and concentrations of total activity and non-volatile activity determined in selected tissues, placenta, and fetuses. (Laboratory - [redacted]) non-GLP	The distribution pattern in the gestating rat was similar to that observed in non-pregnant animals, in that the highest levels detected were in the liver and kidneys. The barrier function of the placenta increases from gestation day 18 to 20 with placental concentrations 45-fold higher than the fetus (32.83 vs 0.53 dpm x 10 ³ g, respectively). When fetal tissue was evaluated the levels were highest in the liver (1.22 dpm x 10 ³ g wet wt) and kidneys (1.64 dpm x 10 ³ g wet wt), consistent with the observations in the adult animals.
Lactating Rats (Sprague-Dawley) 5 F/group 250 - 400 g Ref 50 [IND Reference 45]	[³ H]-trospium chloride (batch 7045) 2 mg/kg 1 mCi/kg PO 50 µg/kg 50 µCi/kg 0.86 mg/rat 1 mCi/rat IV Phosphate buffered saline	Objective: Evaluation of the distribution of trospium chloride into the milk of lactating rats. Design: [³ H]-trospium chloride was administered PO or IV to lactating rats on days 7, 8, and 9 after parturition. Blood (IV administration only) and milk samples were collected at 5 and 30 min and 1, 2, 4, 6, 8, 10, and 24 hours postdose. Activity was determined by liquid scintillation counting. (Laboratory - [redacted]) non-GLP	Regardless of the route of administration, less than 1 part per thousand of the administered dose was recovered from the milk over the time frame evaluated. After oral administration of [³ H]-trospium chloride peak concentrations were reached in the milk approximately 8 hours postdose. Over a 24-hour period 2.22 x 10 ⁻³ % was excreted into the milk. After IV administration of [³ H]-trospium chloride peak concentrations in the milk occurred approximately 8 hours post dose. The percentage of non-volatile activity recovered over a 24-hour period was 4.36 x 10 ⁻³ %. Based on these results, it is unlikely that trospium chloride in the milk would produce adverse effects on the development of young rats.
Enzyme induction/inhibition			
Human liver microsomes (P3, P20) Ref 51 [IND Reference 32 and 55]	Trospium chloride 0, 37, 111.1, 333.3, 1000, 3000 µM 0.1 M KH ₂ PO ₄ buffer in vitro	Objective: Evaluation of the effect of trospium chloride on the P430 enzyme CYP 1A2. Design: Human liver microsomes (P3 and P20) were incubated for 15 minutes at 37°C in a medium of 0.1 M KH ₂ PO ₄ buffer and 3% DMSO with caffeine (500 µM) as substrate and trospium chloride. The formation of the caffeine metabolite paraxanthine was measured by HPLC. (Laboratory - [redacted]) non-GLP	Trospium chloride, at the highest concentration of 3000 µM, resulted in a 25% inhibition of caffeine turnover. No effects were observed at concentrations ≤333.3 µM. The IC ₅₀ for P3 was 8347±3154 µM and for P20 was 8432±1518 µM. Therapeutic concentrations of trospium chloride are <50 nM, therefore it is unlikely that an effect on CYP 1A2 will be observed.
Human liver microsomes (P11, P13) Ref 52 [IND Reference 33]	Trospium chloride 0, 37, 333, 3000 µM 0.1 M KH ₂ PO ₄ buffer, 3% DMSO in vitro	Objective: Evaluation of the effect of trospium chloride on the P430 enzyme CYP 2C19. Design: Human liver microsomes (P11 and P13) were incubated for 60 minutes at 37°C in a medium of 0.1 M KH ₂ PO ₄ buffer and 3% DMSO with S-(+)-mephenytoin (20 µM) as substrate and trospium chloride. The formation of the 4-hydroxymephenytoin metabolite was measured by HPLC. (Laboratory - [redacted]) non-GLP	Trospium chloride, at a concentration of 333 µM, resulted in a decrease in the formation of 4-hydroxymephenytoin by 25% and 24% for P11 and P13, respectively. At 37 µM, the lowest concentration tested, a 15% decrease in the formation of 4-hydroxymephenytoin was observed in the P11 liver sample but not the P13 sample. The IC ₅₀ values determined were 1756.3±884.1 µM and 1958.6±486.6 µM for the P11 and P13 liver microsomes, respectively. As the therapeutic concentration of trospium chloride is <50 nM, it is unlikely that an effect on CYP 2C19 will occur.
Enzyme induction/inhibition, continued			
Human liver microsomes (P10, P20) Ref 53 [IND Reference 34]	Trospium chloride 0, 37, 111.1, 333.3, 1000, 3000 µM 0.1M KH ₂ PO ₄ buffer, 3% DMSO in vitro	Objective: Evaluation of the effect of trospium chloride on the P450 enzyme CYP 2D6. Design: Human liver microsomes (P10 and P20) were incubated for 20 minutes at 37°C in a medium of 0.1 M KH ₂ PO ₄ buffer and 3% DMSO with dextromethorphan (0, 0.4, 2, 10, 50, 400, 2000 µM) as substrate and trospium chloride (37 to 3000 µM). The formation of dextrophan was evaluated by HPLC with fluorometric detection. (Laboratory - [redacted]) non-GLP	Trospium chloride exhibits a pronounced inhibitory effect on the high affinity, but not the low affinity binding sites of CYP 2D6. The inhibitory concentrations for the high affinity site were 20.6 µM for P20 and 51.3 µM for P10. The inhibitory concentration at the low affinity binding sites was 762.5 µM for P20 and 165.6 µM for P10. As the therapeutic concentration of trospium chloride is <50 nM it is unlikely an effect on CYP 2D6 will occur.
Human liver microsomes (P3, P21) Ref 54 [IND Reference 35]	Trospium chloride 0, 37, 111.1, 333.3, 1000, 3000 µM 0.1M KH ₂ PO ₄ buffer, 3% DMSO in vitro	Objective: Evaluation of the effect of trospium chloride on the P450 enzyme CYP 2E1. Design: Human liver microsomes (P3 and P21) were incubated for 20 minutes at 37°C in a medium of 0.1 M KH ₂ PO ₄ buffer and 3% DMSO with chlorzoxazone (50 µM) as substrate and trospium chloride (37 to 3000 µM). The formation of the 6-hydroxychlorzoxazone metabolite was measured by HPLC. (Laboratory - [redacted]) non-GLP	The rate of metabolism of chlorzoxazone in the absence of trospium chloride was 216 µM. The addition of trospium chloride (111 to 3000 µM) to P21 microsomes resulted in a fluctuation of the metabolism rate between 156 and 208 pmol/min/mg protein. Trospium chloride (333 to 3000 µM) decreased metabolism 15 to 18%, while 111 µM had no effect on metabolism in the P3 microsomes. As the therapeutic concentration of trospium chloride is <50 nM it is unlikely that an effect on CYP 2E1 will occur.
Human liver microsomes (P3, P20) Ref 55 [IND Reference 36]	Trospium chloride 0, 37, 111.1, 333.3, 1000, 3000 µM 0.1M KH ₂ PO ₄ buffer, 3% DMSO in vitro	Objective: Evaluation of the effect of trospium chloride on the P450 enzyme CYP 3A4. Design: Human liver microsomes (P3 and P20) were incubated for 20 minutes at 37°C in a medium of 0.1 M KH ₂ PO ₄ buffer and 3% DMSO with deslorfenipine (50 µM) as substrate and trospium chloride (37 to 3000 µM). The formation of the deslorfenipine metabolite was measured by HPLC. (Laboratory - [redacted]) non-GLP	Human liver microsomes incubated with deslorfenipine formed the metabolite pyridine deslorfenipine through the action of the CYP 3A4 enzyme. Addition of trospium chloride at 3000 µM resulted in 30% inhibition of the microsomes of P3 and P20, however at a concentration of 1100 µM; no effect on deslorfenipine metabolism was detected. The IC ₅₀ values were determined to be 23677±10315 µM for the P3 and 14529±5901 µM for the P20 microsomes. Based on this it is not anticipated that trospium chloride would inhibit CYP 3A4 at therapeutic doses (<50 nM).

Species (Strain/Material No./Dose Age/Weight Report number (reference))	Test compound (Batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Enzyme induction/inhibition, continued			
Human liver microsomes <i>Ref 56</i>	Trosopium chloride 0, 37, 111, 1, 333, 1, 1033, 3000 µM 0.1M KH ₂ PO ₄ buffer, 1.5% DMSO Coumarin 2 µM in vitro	Objective: Evaluation of the effect of trosopium chloride on the P450 enzyme CYP 2A6. Design: The incubation medium consisted of 0.1 M KH ₂ PO ₄ buffer, 1.5% DMSO, and a protein content of 0.25 mg/mL in a volume of 500 µL. After preincubation for 2 minutes at 37 °C, the reaction was started by addition of substrate and stopped 20-minute later by addition of 500 µL of a 25% trichloroacetic acid solution (3 min). A 500 µL sample of the supernatant was diluted with 500 µL 1.55 M glycine-sodium hydroxide buffer, pH=10.3, and transferred into a disposable polystyrene vial for fluorescence analysis at room temperature. (Laboratory - ██████████) non-GLP	The metabolism rate of coumarin to 7-OH-coumarin in the absence of trosopium chloride was 500 to 820 pmol/min/mg protein. When trosopium chloride was added to the incubation mixture the rate of formation of the coumarin metabolite was decreased and an IC ₅₀ of 2.3 to 2.5 mM determined for trosopium chloride. Therefore, at therapeutic concentrations of trosopium chloride (450 µM) no effect on CYP 2A6 metabolism is expected.
Human liver microsomes <i>Ref 56</i>	Trosopium chloride 0, 37, 111, 1, 333, 1, 1033, 3000 µM 0.1M KH ₂ PO ₄ buffer, 3% DMSO Warfarin 5 µM in vitro	Objective: Evaluation of the effect of trosopium chloride on the P450 enzyme CYP 2C9. Design: The incubation medium consisted of 0.1 M KH ₂ PO ₄ buffer with 3.0% DMSO, pH 7.4, and a protein content of 0.8 mg/mL. After preincubation for 2 minutes at 37 °C, the reaction was started by addition of the substrate and stopped after a further 20-minute incubation by addition of 10 µL of a 70% perchloric acid solution (50 min). A 500 µL sample of the supernatant was transferred into a disposable polystyrene vial for detection by HPLC. (Laboratory - ██████████) non-GLP	The metabolism rate of warfarin to 7-OH-warfarin in the absence of trosopium chloride was 0.37 to 0.50 pmol/min/mg protein. When trosopium chloride was added to the incubation mixture the rate of formation of the warfarin metabolite was decreased and an IC ₅₀ of 2.4 to 2.5 mM was determined. Therefore, at therapeutic concentrations of trosopium chloride (450 µM) no effect on CYP 2C9 metabolism is expected.
Enzyme induction/inhibition, continued			
Rat, dog, and human liver microsomes <i>Ref 57</i>	¹⁴ C-trosopium chloride (Batch CFQ3184) 10 µM Ret compound: Tolbutamide 100 µM	Objective: Evaluation of the effect of GYP 2C9 on the metabolism of ¹⁴ C-trosopium chloride in rat, dog, and human liver microsomes. Design: Microsomes were incubated with ¹⁴ C-trosopium chloride for 60 minutes at 37°C with and without the GYP 2C9 inhibitor sulfaphenazole. A positive control, tolbutamide, was used as the GYP 2C9 substrate in place of ¹⁴ C-trosopium chloride to confirm the assay. Samples were analyzed by radio-chromatography and electrospray mass spectrometry. (Laboratory - ██████████) non-GLP	Incubation of liver microsomes from rat, dog, and human livers with ¹⁴ C-trosopium chloride for 60 minutes resulted in approximately 8%, 55%, and 94% of the trosopium chloride remaining, respectively. Incubation of liver microsomes from rat, dog, and human in the presence of the GYP 2C9 inhibitor sulfaphenazole had no effect on the extent of metabolism of ¹⁴ C-trosopium chloride within each species, but in some cases the peak abundance was slightly altered. The extent of metabolism in both dog and human was very small with greater than 95% of the radioactivity still present as trosopium chloride at the end of the incubation period in the presence or absence of the GYP 2C9 inhibitor sulfaphenazole.
Metabolism			
In vitro <i>Ref 46</i> [IND Reference 51]	³ H-trosopium chloride 5 µL (10 µCi) 20 µL pig liver esterase 0.75 mL fresh rat plasma Sodium borate buffer (pH 8.7)	Objective: Determination of the enzymatic stability of the trosopium chloride ester bond. Design: ³ H-trosopium chloride was incubated for 24 hours at 25°C with pig liver esterase, pig liver esterase in fresh rat plasma, or fresh rat plasma to determine the stability of the ester bond. Samples were analyzed by thin-layer chromatography. (Laboratory - ██████████) non-GLP	In vitro incubation for 24 hours with rat plasma, with or without pig liver esterases, resulted in no cleavage of the ester bond.
Metabolism, continued			
In vitro rat, dog, and human liver S9 microsomal preparations <i>Ref 57</i>	¹⁴ C-trosopium chloride (Batch CFQ3184) 10 µM S9 microsomal preparations (5 mg/mL protein) 0.1M potassium phosphate buffer, pH 7.4	Objective: Determination of the in vitro metabolism of ¹⁴ C-trosopium chloride in liver S9 microsomal preparations. Design: ¹⁴ C-trosopium chloride was incubated with liver S9 microsomal preparations (5 mg/mL protein) for 0 or 60 minutes at 37°C. At the end of 60 minutes, samples were analyzed by radio-chromatography with electrospray mass spectroscopy. (Laboratory - ██████████) non-GLP	Following incubation of S9 liver preparations from rat, dog, and human livers with ¹⁴ C-trosopium chloride, the amount of parent compound remaining was 8, 56, and 55%, respectively. Eight metabolite peaks were identified; 7 metabolite peaks in rat, 2 metabolite peaks in dog and human). The three largest peaks appeared in the rat samples and constituted approximately 15, 25, and 36% of the radioactivity in the chromatogram. These results suggest limited metabolism of trosopium chloride by liver microsomes from the dog and human and extensive metabolism by rat liver microsomes.
Rat (Wistar) strain/group 250 - 320 g <i>Ref 59</i> [IND Reference 38 and 53]	¹⁴ C-trosopium chloride (Batch 7045) 0.2, 2, 20 and 200 mg/kg 5 mL/kg 10 mL/kg Double-distilled water	Objective: Metabolism in the rat after single dose oral administration. Design: Following administration, blood samples were collected at 5 timepoints over the 1 st hour and at 1.5, 2, 3, 6, 10, and 24 hours. Urine, feces, bile and specified tissues were collected at various times postdose and metabolism determined. (Laboratory - ██████████) non-GLP	The activity in the plasma was too low to evaluate. The primary metabolite in the urine after doses of 2 and 20 mg/kg was azoniaspiro representing 78.9% and 52.4%, while trosopium chloride accounted for 3.1% and 0.8% of the activity, respectively. At 200 mg/kg azoniaspiro accounted for 21.7% and trosopium chloride 27.1% of the activity. One unidentified peak made up 22.4% of the total. In bile the azoniaspiro made up 37.8% and 25.2% of the activity while trosopium chloride comprised 12.0% and 13.9% at 2 and 20 mg/kg, respectively. Increasing the dose to 200 mg/kg increased the trosopium chloride level to 29.4% and decreased the azoniaspiro activity to 3.8%. Two unidentified metabolites made up 36.5% and 10.5% of the remaining activity.

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Species (strain)/material No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Metabolism, continued			
Rat (Wistar) 3-Sig group 250 - 320 g Ref 40 [IND Reference 37 and 52]	[¹⁴ C]-trospium chloride (batch 7345) 75.8 µg/kg 5 mL Citra IV 5.50 µg/kg 50 µg/kg IV Phosphate buffered saline	Objective: Metabolism in the rat after single dose intravenous administration. Design: Rats were administered trospium chloride IV (75.8 µg/kg) and 0, 1, 1, and 6 hours later the metabolic profile in plasma determined. The metabolic profile in the heart, lungs, liver, kidneys, adrenal glands and testes were determined 6 hours postdose. Urine was collected over 24 hours and bile collected at 1, 2, 3, 4, 5, 6, and a composite over 24 hours after doses of 5 and 50 µg/kg. Metabolic profiles were determined by thin-layer chromatography. (Laboratory - ~~XXXXXXXXXX~~ non-GLP)	Plasma profile: At 10 minutes trospium chloride activity ranged from 36.4% - 69.3% and azoniaspino 13.2% - 29.8%. One hour postdose trospium chloride represented 12.4% - 15.3% and azoniaspino 39.5% - 45.8%. By 6 hr trospium chloride activity was 19%-69.8% and azoniaspino 12.2% to 22.3%. Organ profile: Trospium chloride accounted for 32% to 35% of the activity in the heart and lungs, respectively, with no azoniaspino identified. In the kidneys trospium chloride made up 53% of the activity with only trace amounts of azoniaspino. In the liver trospium chloride comprised 20% and azoniaspino 35% of the activity. In the adrenals and testes trospium chloride activity was 10.2% and 21.7% while the metabolite azoniaspino comprised <2.6% in the adrenals and was not detected in the testes. Urine and bile profile: Over 24 hours trospium accounted for 33.5% and 31.9% of the activity in urine and bile, respectively. The metabolite azoniaspino accounted for 4% and 10% of the activity in urine and bile. Five to six additional metabolites were present which were not identified.
Lactating Rats (Sprague Dawley) 250 - 400 g Ref 50 [IND Ref 43]	[¹⁴ C]-trospium chloride (batch 7345) 0.86 mg/0.5mL rat 1 mL Citra, 5mL rat IV 2 mg/kg 1 mL/kg PO	Objective: Determine the metabolism of trospium chloride in lactating rats. Design: Rats were doses PO and IV on day 7 through 9 post-parturition. Blood and milk samples were collected at 6 and 3 hours postdose. Samples were analyzed by thin-layer chromatography. (Laboratory - ~~XXXXXXXXXX~~ non-GLP)	<1% of the administered activity was excreted into the milk of lactating rats after oral or intravenous administration of [¹⁴ C]-trospium chloride. Of the activity present in the milk, trospium chloride accounted for 72% and 55% 6 and 3 hours postdose, respectively.
Metabolism, continued			
Dog (Beagle) 3 - 5 F 3.5 - 8.5 months 6.5 - 11.8 kg Ref 41 and Ref 42 [IND References 39 and 40]	[¹⁴ C]-trospium chloride (batch 7345) 1 mL Citra 2 mg/kg PO 0.5 - 0.7 mL capsule/eq 50 µCi/50 µg/kg IV and IM 0.5 mL/kg IV 1.0 mL/kg IM Phosphate buffered saline	Objective: Metabolic profile in the plasma and feces of the dog after oral, intravenous, and intramuscular administration. Design: Dogs were administered trospium chloride orally, intravenously or intramuscularly. Blood was collected at 15 min and 6 hrs postdose while feces was collected over 0-24 hr and 24-48 hr periods. The metabolic profile was determined by thin-layer chromatography. (Laboratory - ~~XXXXXXXXXX~~ non-GLP)	Plasma: Oral administration - 40% of the activity was identified as trospium chloride IV administration - N/A IM administration - Trospium chloride was the primary peak with some activity present as azoniaspino. Feces: Oral administration - trospium chloride accounted for 57.7% and 57.2% of the activity from 0-24 and 24-48 hr, respectively. Azoniaspino accounted for <5% over both time periods. IV administration - Over the 0-24 hr timepoint trospium chloride accounted for 18.1% and azoniaspino 8.8% of the activity. An unidentified metabolite represented 40.3% of the total activity. From 24 to 48 hours postdose trospium chloride decreased to 5.2% and azoniaspino remained constant at 10.5%. An unidentified metabolite represented 32.8% of the activity. IM administration - Trospium chloride was present at 41.6% and 32.3% 0-24 and 24-48 hrs postdose. Azoniaspino represented 15.9% and 19.2% over the same timeframes. One additional peak represented 28.3% and 26.3% of the activity from 0-24 and 24-48 hours, respectively.
Excretion pattern			
Rat (Wistar) 12-16 M group 250 - 320 g Ref 39 [IND References 38 and 53]	[¹⁴ C]-trospium chloride (batch 7345) 0.2, 2, 23, 200 mg/kg 10 mL/kg PO Double-distilled water	Objective: Excretion in the rat after single dose oral administration. Design: Following administration, urine and feces were collected over 24-hours while bile was collected at various times postdose. (Laboratory - ~~XXXXXXXXXX~~ non-GLP)	Following oral administration of 0.2, 2, 20, and 200 mg/kg trospium chloride the excretion of total activity (urine + urine + feces) within 24-hours was 2.63%, 3.35%, 2.79%, and 3.15% of the administered dose, respectively, indicating little effect of dose on urinary excretion. The excretion of activity in the bile over a 24-hour period was considered dose-related based on the following: 1.26%, 1.85%, 3.91%, and 14.55% excreted after 0.2, 2, 20, and 200 mg/kg, respectively. Evaluation of the activity of trospium chloride in the feces suggested excretion of 44.71%, 32.43%, 46.45%, and 14.11%, respectively, primarily due to the low absorption from the GI tract. The decrease in fecal excretion at 200 mg/kg may be related to the pharmacological activity of the compound causing a slower intestinal passage and higher absorption rate. Total recovery of activity ranged from 68% to 83% over the concentration range tested.
Rat (Wistar) 5-12 sex/group 250 - 320 g Ref 40 [IND References 37 and 52]	[¹⁴ C]-trospium chloride (batch 7345) 5.50, 500 µg/kg 50 µg/kg or 5 mL Citra IV 2.5 mL/kg IV Phosphate buffered saline	Objective: Excretion in the rat after single dose intravenous administration. Design: Following administration, urine and feces were collected over 24 hours while bile was collected at various times postdose. (Laboratory - ~~XXXXXXXXXX~~ non-GLP)	After IV administration of 5, 50, or 500 µg/kg [¹⁴ C]-trospium chloride, the recovery in the urine was similar across doses with activity ranging from 36.5% to 44.6%. Biliary excretion was measured at various timepoints over a 24-hour period after administration of 5 or 50 µg/kg. Excretion at these doses in male rats was 25.6% and 25.6%, respectively. In female rats, excretion at 5 µg/kg was slightly lower than the males at 19.2%. Total excretion after 24 hours was determined from urine, bile, feces, intestinal tract, and carcass of animals receiving 5 and 50 µg/kg. At 5 and 50 µg/kg the total excretion was 87.3% and 82.6%, respectively.

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Species (Strain/Material) No./Dose Age/Weight Report number (Reference)	Test compound (Batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Excretion pattern, continued			
Rats (Hartn O/Sac FV6) 16 M 7-8 weeks 160-200 g Ref 48 (IND Ref 47)	¹⁴ C-trospium chloride (batch TRQ6767) 50 µg/kg (19.5 MBq/kg) IV Phosphate buffered saline	Objective: Evaluation of the excretion of trospium chloride in the rat after IV administration. Design: Fifteen male rats were administered 50 µg/kg (19.5 MBq/kg) trospium chloride and 3 rats sacrificed at 1, 3, 10, 24, and 96 hours postdose. A selection of organs and tissues were removed at each timepoint. In addition, at the 96-hour timepoint urine, feces, and expired ¹⁴ H ₂ O were collected. (Laboratory - ██████████) GLP	Excretion took place primarily in the first 24 hours postdose. The majority (53.65%) of radioactivity was recovered in the feces, while the excretion into the urine accounted for a mean of 36.55%. An average of 0.12% of the dose was associated with the ¹⁴ H ₂ O, while 0.83% was found in the carcass. The total recovery of 96 h postdose was 98.76%
Dog (Beagle) 3 - 5 F 5.3 - 11.8 kg Ref 42 (IND Reference 35)	¹⁴ C-trospium chloride (batch 7043) 1 mCi/2 mg/kg, PO 0.3 - 0.7 mL capsule/dog 50 µCi/50 µg/kg, IV and IM 0.5 mL/kg IV 1.0 mL/500 IM Phosphate buffered saline	Objective: Excretion in the dog after oral, intravenous, and intramuscular administration. Design: Dogs were administered trospium chloride orally, intravenously or intramuscularly, and excretion into the urine and feces determined over a 48-hour period. (Laboratory - ██████████) non-GLP	Elimination was predominantly fecal with 85.25%, 47.73%, and 64.38% eliminated after oral, IV, and IM administration. The high levels following oral administration primarily represent unabsorbed drug. The high levels in the feces following IV and IM administration probably represent biliary excretion. Urinary excretion represented 1.45%, 35.07%, and 25.77%, after oral, IV and IM administration, respectively. Total recovery of activity ranged from 90.5% to 101.82%.
Toxicokinetics			
Mouse (CD-1) 42/sex/group 4 weeks 18 - 21 g Ref 55 (IND Reference 92)	Trospium chloride (batch 43554) 2, 20, 200 mg/kg/day Oral (diet)	Objective: Determination of plasma levels of trospium chloride after 28-days oral administration in the diet. Design: Mice were dosed daily with trospium chloride by oral administration (diet). Blood samples were taken from 2 mice/sex/timepoint on days 3 (10Z, 14:15, 28:28) at timepoints of 0, 5, 6, 7, 12, 16 and 20 hours postdose. The plasma was frozen at -20°C until analysis by HPLC with fluorometric detection. (Laboratory - ██████████)	Plasma levels of total trospium chloride were below the quantitation limit for samples in the low (2 mg/kg/day) and intermediate (20 mg/kg/day) dose groups. At 200 mg/kg/day the data obtained 7 hours postdose on days 1, 14, and 28, had plasma values of 28.34, 62.23, and 34.24 ng/mL, respectively, suggesting exposure similar to that observed in rats at the same timepoints.
Toxicokinetics, continued			
Rat (Sprague Dawley) 14/sex/group 240 - 300 g Ref 59 (IND Reference 44)	Trospium chloride (batch 39338) 2, 20, 200 mg/kg/day Oral (diet)	Objective: Determination of plasma levels of trospium chloride after 28-days oral administration in the diet. Design: Rats were dosed daily with trospium chloride by oral administration (diet). Blood samples were taken from 2 rats/sex/timepoint from the orbital sinus on days 9 (10Z, 14:15, 28:28). Plasma concentrations of trospium chloride were determined at 20:00 (8:00 pm), 00:00 (12:00 am), 3:00 am, 5:00 am, 7:00 am, 12:00 pm, and 4:00 pm on days 3 (10Z, 14:15, and 28:28). The plasma was frozen at -20°C until analysis by HPLC with fluorometric detection. (Laboratory - ██████████) GLP study	Plasma levels of total trospium chloride were below the quantitation limit for the majority of samples in the low dose group (2 mg/kg/day) at all time points. There was no indication of dose proportionality and dose linearity could not be calculated. No indication of enzyme induction was observed. Plasma concentrations after dosing with 200 mg/kg/day for 28 days suggest some accumulation of trospium chloride, which may be related to increased tissue binding. No differences between males and females were observed.
Toxicokinetics, continued			
Rabbit 6 F/group Ref 60 (IND Reference 45)	Trospium chloride (batch 39338) (43594) 20, 20, 200 mg/kg/day Oral (gavage)	Objective: Determination of plasma levels of trospium chloride after oral administration to pregnant rabbits on day 6 through 18 of gestation. Design: Rabbits were dosed daily with trospium chloride by oral (gavage) administration on gestation day 6 through 18. Blood samples were taken from 6 rabbits on day 6, 12, and 18 at 1, 2, 3, and 4 hours postdose. The plasma was frozen at -20°C until analysis using a validated HPLC method with fluorometric detection. (Laboratory - ██████████) GLP study	At 200 mg/kg/day the C _{max} was reached between 2 and 3 hours postdose. The concentrations in the pregnant rabbit after oral gavage administration were generally 10 times higher than in the rat and mouse following continuous administration in the diet. No indication of drug accumulation or dose proportionality was observed.
Dog (Beagle) 2 M/2 F Ref 61 (IND reference 46 and 62)	Trospium chloride (batch 39338, 43594) 0.5, 5, 50 mg/kg/day Oral (gelatin capsule)	Objective: Toxicokinetics from a 28-day oral toxicity study in the dog. Design: Two male and 2 female beagle dogs were dosed orally for 28 days and blood samples collected at 0.5, 1, 2, 3, 4, and 5 hours postdose on days 1, 14 and 28. A validated fluorometric HPLC method was used for sample analysis. (Laboratory - ██████████) GLP study	Plasma concentrations (C _{max} and AUC) were determined and found to increase over the dosing interval in animals receiving 0.5, 5, and 50 mg/kg/day. The AUC (area under the plasma concentration data curve) and C _{max} increased over the time period from 1 to 28 days. The increases in these values over time were thought to result from the pharmacological activity of the compound. The T _{max} after administration of 0.5 or 5 mg/kg/day was approximately 1 hour postdose, and after 50 mg/kg/day the T _{max} was 0.5 to 3 hours with higher peak concentration values. No differences between males and females were observed.

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Toxicology studies:

Species (Strain)/Material No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulations Vehicle	Study design (Laboratory) GLP status	Results
Single-dose toxicity			
Mouse (CF1) 10 M/group 17 - 24 g Ref 62 [IND Reference 51]	Trospium chloride 378, 398, 500, 630 mg/kg 2.5% in distilled water Oral (gavage)	Objectives: Acute oral toxicity in male mice. Design: Single dose administration with observations up to 14 days. Mortality, clinical signs, and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The oral LD ₅₀ after 24 hours was 430 mg/kg and after Day 14 was 425 mg/kg in males. Clinical signs included hyperactivity, tremor, spasms, tonic-clonic convulsions and impaired coordination, postural abnormalities, lowering of grip strength and tone of extremities, piloerection, cyanosis, and reduction in body temperature. 1-hr postdose a decrease in activity was observed.
Mouse (CF1) 10 F/group 17 - 33 g Ref 63 [IND Reference 52]	Trospium chloride 250, 314, 398, 500, 630 mg/kg 2.5% in distilled water Oral (gavage)	Objectives: Acute oral toxicity in female mice. Design: Single dose administration with observations up to 14 days. Mortality, clinical signs, and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The oral LD ₅₀ 24 hours and 14 days postdose was 365 mg/kg in no late mortalities were observed. Clinical signs included hyperactivity, tremor, spasms, tonic-clonic convulsions and impaired coordination, postural abnormalities, lowering of grip strength and tone of extremities, piloerection, cyanosis, and reduction in body temperature. 1-hr postdose a decrease in activity was observed.
Mouse (CF1) 10 M/group 22 - 26 g Ref 64 [IND Reference 53]	Trospium chloride 5.00, 6.30, 7.54, or 10.00 mg/kg 3.12% in distilled water IV	Objectives: Acute intravenous toxicity in male mice. Design: Single dose administration with observations up to 14 days. Mortality, clinical signs, and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The intravenous LD ₅₀ was determined to be 7.6 mg/kg for both the 24 hour and 14 day timepoints. Clinical signs included hyperactivity, restlessness, tremor, spasms, tonic-clonic convulsions and impaired coordination, postural abnormalities, lowering of grip strength and tone of the extremities, reduced elicitation of reflexes, exophthalmos, cyanosis, piloerection, and reductions in body temperature. By 20 minutes postdose a decrease in activity had occurred.
Single-dose toxicity, continued			
Mouse (CF1) 10 F/group 20 - 28 g Ref 65 [IND Reference 54]	Trospium chloride 5.00, 5.30, 7.54, 10.00 mg/kg 0.05% in distilled water, pH 6.0 IV	Objectives: Acute intravenous toxicity in female mice. Design: Single dose administration with observations up to 14 days. Mortality, clinical signs, and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The intravenous LD ₅₀ in female mice was determined to be 5.4 mg/kg at 24 hours and 14 days postdose. Clinical signs included hyperactivity, restlessness, tremor, spasms, tonic-clonic convulsions and impaired coordination, postural abnormalities, lowering of grip strength and tone of the extremities, reduced elicitation of reflexes, exophthalmos, cyanosis, piloerection, and reductions in body temperature. By 20 minutes postdose a decrease in activity was observed.
Rat (Wistar) 10 M/group 150 - 160 g Ref 66 [IND Reference 57]	Trospium chloride 0.62, 0.754, 1.0, 1.26 g/kg 10% in distilled water, pH 6.0 Oral (gavage)	Objectives: Acute oral toxicity in male rats. Design: Single dose oral administration with observations up to 14 days. Mortality, clinical signs and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The oral LD ₅₀ in male rats was determined to be 0.64 g/kg at 24 hours and 14-days postdose. Clinical signs regardless of dose, occurred approximately 10 minutes postdose and included hyperactivity, tremor, spasms, and tonic-clonic convulsions. Approximately 1-hour postdose a decrease in activity was observed. Impaired coordination, postural abnormalities, changes in color of skin and mucosa, piloerection, and reductions in body temperature were observed. Some symptoms remained for 24 hours.
Rat (Wistar) 10 F/group 140 - 160 g Ref 67 [IND Reference 58]	Trospium chloride 0.6, 0.63, 0.754, 1.0 g/kg 10% in distilled water, pH 6.0 Oral (gavage)	Objectives: Acute oral toxicity in female rats. Design: Single dose oral administration with observations up to 14 days. Mortality, clinical signs and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The oral LD ₅₀ in female rats was determined to be 0.8 g/kg at 24 hours and 14-days postdose. Clinical signs regardless of dose, occurred approximately 10 minutes postdose and included hyperactivity, tremor, spasms, and tonic-clonic convulsions. Within 1 hour a decrease in activity was observed. Postural abnormalities, reduced reflex excitability, lowering of grip strength and tone of the extremities, changes in the color of the skin and mucosa, and reductions in body temperature were observed.
Single-dose toxicity, continued			
Rat (Wistar) 10 M/group 145 - 160 g Ref 68 [IND Reference 59]	Trospium chloride 7.54, 10.0, 12.6, 15.9 mg/kg 0.5% and 1% in distilled water, pH 6.0 IV	Objectives: Acute intravenous toxicity in male rats. Design: Single dose intravenous administration with observations up to 14 days. Mortality, clinical signs, and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The intravenous LD ₅₀ in male rats was determined to be 10.7 mg/kg at both 24 hours and 14 days postdose. Clinical signs occurring at all doses included hyperactivity, restlessness, impaired coordination, postural abnormalities, tremor, tonic-clonic convulsions, and spasms. Reductions in elicitation of reflexes, exophthalmos, lowering of grip strength and tone of the extremities, cyanosis, piloerection, bradycardia, and reductions in body temperature were also observed. A decrease in activity was observed 10 minutes postdose. Some signs continued for 3 hours.
Rat (Wistar) 10 F/group 154 - 160 g Ref 69 [IND Reference 59]	Trospium chloride 10.0, 12.6, 15.9, 20.0 mg/kg 1% solution in distilled water, pH 6.0 IV	Objectives: Acute intravenous toxicity in female rats. Design: Single dose intravenous administration with observations up to 14 days. Mortality, clinical signs, and body weight were recorded. (Laboratory - [redacted]) pre-GLP study	The intravenous LD ₅₀ in female rats was determined to be 12.3 mg/kg at both 24 hours and 14 days postdose. Clinical signs occurring at all doses included hyperactivity, impaired coordination, postural abnormalities, tremor, spasms, exophthalmos, and tonic-clonic convulsions. Reductions in grip strength and tone of the extremities, reduced elicitation of reflexes, cyanosis, piloerection, bradycardia, and reductions in body temperature. By 10 minutes postdose a decrease in activity was observed. Some signs continued for 3 hours.
Repeated dose toxicity			
Mouse (CO-1) 4Esex/group 4 weeks old 18 - 21 g Ref 70 [IND Reference 62]	Trospium chloride (batch 43684, 39338) 2, 20, 200 mg/kg/day Oral (diet)	Objectives: To determine the toxicity and toxicokinetics following 28-days of oral administration of trospium chloride. Design: Male and female mice were given trospium chloride in the diet for 28 days. Animals were evaluated for mortality, clinical signs, body weight, food consumption, water consumption, achieved dosages, and toxicokinetics. (Laboratory - [redacted]) GLP study	No notable signs of toxicity as determined by mortality, body weight, food and water consumption were observed. Levels of trospium chloride were below the limit of quantification at 2 and 20 mg/kg/day and for most of the samples at 200 mg/kg/day. Available data did suggest plasma levels at 200 mg/kg/day were similar to those observed in the rat under similar treatment conditions.
Mouse (CO-1) 50Esex/group 4 weeks 18 - 21 g Ref 71 [IND Reference 61]	Trospium chloride (batch 2772) 0, 2, 20, 200 mg/kg/day Oral (diet)	Objectives: To assess the carcinogenic potential of trospium chloride administered to CD-1 mice for 78-weeks in the diet. Design: Mice were administered trospium chloride orally in the diet for 78-weeks. Examined parameters included mortality, clinical signs, body weight, food consumption, water consumption, analysis of formulated diets, gross pathology, and histopathology. (Laboratory - [redacted]) GLP study	No notable effects at 2 mg/kg/day. Mortality in the 20 and 200 mg/kg/day males was statistically different from controls at 38% and 66%. Associated clinical signs in males included a higher incidence of swollen and cyanosed abdomens and enlarged distended intestines. No significant effect on mortality in females. No evidence of a carcinogenic effect in males or females when compared with the combined controls or historical controls was observed.

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Species (Strain/Material No./Dose Age/Weight Report number (Reference))	Test compound (Batch number) Dose/Route Formulation Vehicle	Study design (Laboratory) GLP status	Results
Repeated dose toxicity, continued			
Rat (Sprague Dawley) 18/sex/group 8 weeks 240 - 100 g Ref 73 [IND Reference 55]	Trospium chloride (Batch 43654) 2, 20, 200 mg/kg/day Oral (diet)	Objectives: To assess the toxicity and toxicokinetics of trospium chloride after oral (diet) administration for 4 weeks. Design: Rats were administered trospium chloride daily in the diet. Parameters evaluated included mortality, body weight, food consumption, water consumption, and analysis of dosage levels. The effects of dose, sex, and repeated administration on plasma pharmacokinetic parameters of trospium chloride were also evaluated. (Laboratory - ██████████) GLP study	There were no treatment-related deaths in this study. 200 mg/kg/day: Bodyweight gain was reduced in both males and females with a mean increase in weight of 128 g in control males and 100 g while control females gained 47 g and females in the 200 mg/kg/day group gained 36 g. These decreases in weight gain were correlated with a general reduction in food consumption in animals in this dose group. Plasma levels of trospium chloride were detected at 420 mg/kg/day and increased with dose day and were determined to be 168.1, 560.1 and 752.9 ng/ml on days 1, 14, and 28, respectively.
Rat (Wistar) 23/sex/group 200 - 270 g Ref 74 [IND Reference 55]	Trospium chloride (Batch 782719) 0, 2, 20, 200 mg/kg/day Oral (diet)	Objectives: To assess the oral toxicity of trospium chloride administered in the diet of rats for 35 weeks. Design: Rats were administered trospium chloride in the diet for 35 weeks. Examined parameters included mortality, clinical signs, body weight, clinical chemistry, hematology, urinalysis, gross pathology, organ weights, and histopathology. (Laboratory - ██████████) Pre-GLP study	The only notable difference from control animals was a decrease in body weight gain in males and females given 200 mg/kg/day, with the effect in females reaching significance.
Repeated dose toxicity, continued			
Rat (Sprague Dawley) 50/sex/group 4 weeks 60 - 85 g Ref 75 [IND Reference 83]	Trospium chloride (Batch 1772) 0, 0, 2, 20, 200 mg/kg/day Oral (diet)	Objectives: To assess the carcinogenic potential of trospium chloride administered to Sprague Dawley rats in the diet for 104 weeks. Design: Rats were administered trospium chloride in the diet for 104 weeks. Examined parameters included mortality, clinical signs, body weight, food consumption, water consumption, analysis of formulated diets, gross pathology, and histopathology. (Laboratory - ██████████) GLP study	Trospium chloride did not adversely affect survival of rats over the 104-week treatment period. No clinical signs were associated with oral administration of 2, 20, or 200 mg/kg/day. Body weight was significantly decreased 3% in males and 23% in females at 20 mg/kg/day. At 200 mg/kg/day body weight was decreased by 23% and 37% in males and females, respectively. An increased incidence of hypertrophy of the uterine cervix in the intermediate and high dose groups was the one non-neoplastic significant finding. No evidence of a carcinogenic potential was observed. The NOEL was determined to be 2 mg/kg/day.
Dog (Beagle) 3/sex/group 9.5 - 18.1 kg Ref 76 [IND Reference 69]	Trospium chloride (Batch 6447) 50, 100, 200 mg/kg/day Oral (gelatin capsule)	Objectives: Determine the maximum tolerated dose for repeated dose administration. Design: Male and female dogs (3/sex/group) were orally administered trospium chloride daily for 5 days in gelatin capsules. Animals were observed for 5 hours postdose for clinical signs. (Laboratory - ██████████) non-GLP	There were no deaths. 50 mg/kg/day - was well tolerated with no clinical signs observed. 100 mg/kg/day - vomiting (mild) was observed in 2/6 animals on Day 1. Mild tremor of the hindlegs was noted in 1/6 animals on Day 2. 200 mg/kg/day - Vomiting and sedation described as severe and moderate tonic convulsion and unconsciousness were observed in all animals on Day 1. The limit of tolerance was determined to be between 50 and 100 mg/kg/day.
Repeated dose toxicity, continued			
Dog (Beagle) 2/sex/group Ref 77 [IND Reference 58]	Trospium chloride (Batch 43654, 39338) 0.5, 5, or 50 mg/kg/day Oral (capsule)	Objectives: Determine the toxicity and toxicokinetics of oral repeated dose administration. Design: Male and female Beagle dogs were dosed with trospium chloride daily for 28 days. Mortality, clinical signs, body weight, and food consumption were determined. Plasma samples were taken on Days 1, 14, and 28 for determination of toxicokinetics. (Laboratory - ██████████) GLP study	There were no deaths. Clinical signs included increased heart rate, dilated pupils which did not respond to a light stimulus, and a dry nose and mouth. The frequency, duration, and severity of these signs increased with increasing dose. Decreased bodyweight and food consumption was apparent over the first week of dosing. The addition of water to the dry diet reversed these changes. C _{max} and AUC values were found to increase with increasing dose and dose day over the 28-day period, with the increase in AUC disproportionate compared to the dose.
Repeated dose toxicity, continued			
Dog (Beagle) 4/sex/group 5.1 - 9.0 kg Ref 78 [IND Reference 87]	Trospium chloride (Batch 7545) 0, 0.5, 5, 50 mg/kg/day Oral (gelatin capsule)	Objectives: Determine the toxicity of trospium chloride administered daily for 25 weeks to Beagle dogs. Design: Male and female dogs (4/sex/group) were orally administered trospium chloride daily for 25 weeks. Animals were observed for mortality, clinical signs, body weights, food consumption, water consumption, ophthalmoscopy, electrocardiograms, blood pressure, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, and histopathology. (Laboratory - ██████████) pre-GLP study	There was one death during week 10 in a male receiving 50 mg/kg/day. Acute focal bronchopneumonia was identified as a major factor contributing to the animal's death, however, bacterial mucus production may have made the animal more susceptible to the infection. No clinical signs were observed at 0.5 mg/kg/day. At 5 mg/kg/day mydriasis in 50% of animals beginning 3 weeks postdose. At 50 mg/kg/day mydriasis, loss of pupil accommodation, and ventricular and corneal opacity. Abnormal mucus production from week 10. Body weight gain lower than controls and correlated with a small reduction in food intake. Blood pressure 25 weeks postdose was marginally lower than control (116 mmHg vs 135 mmHg, respectively). Triglycerides increased relative to controls at 5 weeks postdose with a dose-related increase observed 25 weeks postdose. At 25 weeks postdose, total protein, albumin, and calcium were reduced, however group mean values were within normal limits. The NOAEL was determined to be 5 mg/kg/day.

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Species (strain/material) No./Dose Age/Weight Report number (reference)	Test compound (batch number) Dose/Route Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Reproduction toxicity			
Rat (Wistar) 30Sex/group 300 g Ref 79 [IND Reference 73]	Trospium chloride (Batch 7374) 0, 2, 20, 200 mg/kg/day 2 mL/kg Distilled water Oral gavage	Objectives: Effect on fertility and early embryonic development in rats administered trospium chloride. Design: Male and female rats were treated with trospium chloride beginning 61 days (males) and 14 days (females) before mating. Females continued to be treated from gestation to the end of lactation. Animals in the F ₁ generation were allowed to mate and their offspring (F ₂) followed through lactation. Behavior, litter size, body weight, postnatal mortality, and development were recorded. (Laboratory - ~~XXXXXXXXXXXXXXXXXXXX~~ pre-GLP study	There were no deaths attributed to the administration of trospium chloride. Weight gain of males and females dosed with 20 and 200 mg/kg/day were marginally below control, but not statistically different. The number of live offspring of the F ₁ generation was slightly (but not statistically) lower in the 200 mg/kg/day group (mean 6.3) compared with the control group (mean 10.8). One F ₁ offspring of the 20 mg/kg/day group was observed to have low-grade hydrocephalus. No other specific abnormalities or malformations were noted.
Rat (Wistar) 20 F/group 150 - 200 g Ref 80 [IND Reference 73]	Trospium chloride (Batch 7374) 0, 2, 20, 200 mg/kg/day 10 mL/kg Distilled water Oral gavage	Objectives: Effect of trospium chloride on embryotoxicity, fetotoxicity, and teratogenicity after oral dosing in the rat. Design: Female rats received single doses of trospium chloride or vehicle on days 6 to 16 of gestation. The dams were weighed and examined daily for appearance and behavior. On day 20 of pregnancy, the females were sacrificed, uterus removed, and live and dead fetuses examined. All changes in development were recorded. (Laboratory - ~~XXXXXXXXXXXXXXXXXXXX~~ pre-GLP study	No mortality was observed in the dams. Prenatal losses were lowest in the high dose group and highest in the low dose group. The number of resorptions and postimplantation losses was low in all treated rats and less than the control values. No differences were noted on number of live fetuses, fetal weights, mean uterine weight, and placental weights, and no abnormalities or malformations of the skeletal or internal organs were observed.
Reproduction toxicity, continued			
Rabbit (Russian) 11-14 F/group 2.3 g Ref 81 [IND Reference 72]	Trospium chloride (Batch 5758) 0, 2, 20, 200 mg/kg/day 4 mL/kg Distilled water Oral gavage	Objectives: Effect of trospium chloride on embryotoxicity, fetotoxicity, and teratogenicity after oral dosing in the rabbit. Design: Female rabbits received single doses of trospium chloride or vehicle on days 6 to 18 of gestation. All rabbits were weighed on Day 1, and then daily from Day 6 to 26. On gestation day 29, the dams were sacrificed and examined macroscopically. The uterus and fetuses were removed, fetuses weighed, and any abnormalities and/or malformations noted. (Laboratory - ~~XXXXXXXXXXXXXXXXXXXX~~ pre-GLP study	One female in the high dose group died as a result of a dosing error. Postimplantation losses were higher in the 200 mg/kg/day group compared with the control group. In the intermediate dose group one fetus had slightly retarded ossification of the cranial bones. Severe malformations occurred in one fetus at 2 mg/kg/day with gross deformities of the spinal column, the pelvic girdle, and the back legs. This fetus had severely retarded ossification of the cranial bones and edema in the neck region. One fetus in the intermediate dose group had umbilical hernias with prolapse of the liver and defective development of the phalanges. In the high dose group one female had umbilical hernias with prolapse of the liver.
Rabbit (Russian) 6 F/group 4-5 months 1.83 - 2.5 Kg Ref 82 [IND Reference 73]	Trospium chloride (Batch 42654) 0, 2, 20, 200 mg/kg/day 4 mL/kg Deionized water Oral gavage	Objectives: Assessment of the toxicokinetics of trospium chloride following daily oral administration to pregnant rabbits. Design: Female rabbits were dosed daily on gestation days 6 to 18 with 0, 2, 20, or 200 mg/kg trospium chloride by oral gavage. Blood samples were collected on Days 6, 12, and 18 for determination of plasma kinetics. After completion of the Day 18 sampling, surviving animals were killed and checked for pregnancy. (Laboratory - ~~XXXXXXXXXXXXXXXXXXXX~~ pre-GLP study	There were 3 premature deaths, however only one was thought related to the administration of trospium chloride. One animal dosed at 200 mg/kg/day was killed as a result of reduced fecal output, hunched posture, diarrhea, thinness, and a stained, matted and wet perigenital region. No clinical signs were apparent in the low and intermediate dose groups. At 200 mg/kg/day a slight suppression in body weight gain and a marked reduction in food consumption was observed along with stained, matted and wet perigenital region. Caes was reached in the high dose group approximately 2-3 hours post dose. No indication of drug accumulation or dose proportionality was observed.
Reproduction toxicity, continued			
Rats (Sprague Dawley) 20 F/group 8 - 9 weeks 201 - 245 g Ref 83 [IND Reference 74]	Trospium chloride (Batch 73275) 0, 2, 20, 200 mg/kg/day 10 mL/kg Distilled water Oral gavage	Objectives: Assessment of the effects of trospium chloride on perinatal and post-natal development in rats. Design: Female Sprague Dawley rats were dosed with trospium chloride at 0, 2, 20 or 200 mg/kg/day from Day 15 post coitum until Day 21 post partum. Maternal observations included mortality, general condition, and bodyweight. Postnatal observations included duration of gestation, and duration of parturition. Observations in the offspring included mortality, behavior, litter size, bodyweight, sex ratio, physical development, and auditory and visual functions. All animals were killed on Day 25 post partum and examined for macroscopic abnormalities. (Laboratory - ~~XXXXXXXXXXXXXXXXXXXX~~ GLP study	Two females died 1 hour postdose on Days 19 and 21 postpartum in the 200 mg/kg/day group. In both animals there was evidence of fecal compaction in the colon and the kidneys were dark in color with no other macroscopic findings. At 200 mg/kg/day there was a marginal reduction in maternal bodyweight gain during late gestation, however, by the end of the lactation period, no differences were evident. Clinical signs observed at all dose groups included: disturbances of respiration, pupil dilation, and excitability. These observations were dose related in incidence and severity. There was no evidence of embryotoxicity or teratogenic effects in this study.
Genotoxicity			
Salmonella typhimurium Ref 84 [IND Reference 75]	Trospium chloride (Batch 42405) Salmonella typhimurium TA 1538, TA 1537, TA 98, TA 100, TA 102 0, 10, 100, 500, 1000, 2500, 5000 or 5000.0 µg/plate (+X-) D-9 mix in vitro	Objectives: To assess the mutagenic potential of trospium chloride. Design: Mutagenic potential of trospium chloride was tested with five strains of Salmonella typhimurium in the presence and absence of S-9 liver fraction. (Laboratory - ~~XXXXXXXXXXXXXXXXXXXX~~ GLP study	Trospium chloride did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used and was therefore considered to be non-mutagenic under the conditions of this test.
Saccharomyces cerevisiae Ref 85 [IND Reference 77]	Trospium chloride (Batch 73276) 0, 10, 100, 500, 1000, 2500, 5000 and 10,000 µg/plate DMSO in vitro	Objectives: To evaluate the mutagenic effect of trospium chloride in an in vitro system employing Saccharomyces cerevisiae. Design: After colonization of the in vitro toxicity of trospium chloride in the system, the cubique strain C4 was inoculated with trospium chloride in the presence and absence of S-9 mix. After incubation for 4 days the number of revertant colonies was determined. (Laboratory - ~~XXXXXXXXXXXXXXXXXXXX~~ non-GLP study	Trospium chloride did not exhibit genetic activity in any of the assays conducted in this evaluation and was considered not mutagenic under the conditions of this study.

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Species (strain)/material/ No./Dose/ Age/Weight/ Report number (reference)	Test compound (batch number) Dose/Route/ Formulation/ Vehicle	Study design (Laboratory) GLP status	Results
Genotoxicity, continued			
Mouse lymphoma cells Ref 56 [NO Reference 76]	Trosopium chloride (Batch 2046) 0, 15.8, 31.6, 158, 5, 15.8, 31.6, 158, 500, 1580, 5000 µg/mL (*) (*) 0-9 mix 2000, 3000, 4000, 5000 µg/mL (*) (*) 0-9 mix in vitro	Objectives: Trosopium chloride was evaluated for mutagenicity to 6-thioguanine resistance in mouse lymphoma L5178Y cells. Design: Mouse lymphoma L5178Y cells were incubated with various concentrations of trosopium chloride in the presence and absence of 0-9 mix. The mutation of mouse lymphoma cells to 6-thioguanine resistance was determined. (Laboratory - [redacted]) GLP study	Treatment of L5178Y mouse lymphoma cells with up to 5000 µg/mL trosopium chloride in the presence and absence of 0-9 mix failed to induce any increase in mutation at the HGPRT locus in two independent experiments.
Chinese hamster ovary cells Ref 57 [NO Reference 78]	Trosopium chloride (Batch 2046) 0, 15.8, 31.6, 158, 500, 1580, 5000 µg/mL Distilled water	Objectives: To determine the clastogenic potential of trosopium chloride in CHO cells. Design: CHO cells were incubated with trosopium chloride in the presence or absence of 0-9 mix for 2 hours followed by incubation for 22 hours. CHO cells were evaluated for the number of chromosomal aberrations produced. (Laboratory - [redacted]) GLP study	Incubation of CHO cells with trosopium chloride in the presence or absence of 0-9 mix did not induce chromosomal aberrations when tested up to a concentration of 5000 µg/mL.
Genotoxicity, continued			
Rat (Wistar) Sex/group 7 weeks M: 205 g F: 192 g Ref 58 [NO Reference 79]	Trosopium chloride (Batch 25346) 0, 400 mg/kg 4 mL/kg physiological saline Oral (gavage)	Objectives: To evaluate the potential for trosopium chloride to produce micronuclei when administered orally (oral route). Design: Rats were given a single oral dose of 400 mg/kg trosopium chloride. At 24, 48, and 72 hours postdose, animals were sacrificed and bone marrow smears prepared. The smears were evaluated for the number of micronuclei and polychromatic and nonochromatic erythrocytes. Cyclophosphamide (500 mg/kg) was used as the positive control. (Laboratory - [redacted]) GLP study	Trosopium chloride did not produce a biologically significant increase in the number of bone marrow micronuclei in polychromatic erythrocytes and was therefore considered not to induce chromosome aberrations under the conditions of this test.

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/s/

Laurie McLeod
5/23/2007 01:17:53 PM
PHARMACOLOGIST

Lynnda Reid
5/23/2007 02:02:55 PM
PHARMACOLOGIST
I concur with Dr. McLeod-Flynn's recommendations.

NDA: 22103

**45 Day Filing Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY**

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA.	X		
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the-art protocols, etc.)?	X		
6) If the formulation to be marketed is not identical to the formulation used in toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie. adequate repeat studies using the marketed product and /or adequate justification for why such repetition would not be necessary)?	X		

ITEM	YES	NO	COMMENT
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.57? Is information available to express human dose multiples in either mg/m ² or comparative serum/plasma AUC levels?	X		
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item #10 below why it is not.	X		
10) Reasons for refusal to file:			

 Reviewing Pharmacologist / Date

 Supervisory Pharmacologist/Date

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/s/

Laurie McLeod
11/30/2006 03:28:40 PM
PHARMACOLOGIST

Lynnda Reid
12/4/2006 11:14:34 AM
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