

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**21-595**

**PHARMACOLOGY REVIEW**



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

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-595  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 04/26/03  
DRUG NAME: TROSPIUM CHLORIDE  
INDICATION: OVERACTIVE BLADDER  
SPONSOR: INTERNEURON PHARMACEUTICALS, INC.  
DOCUMENTS REVIEWED: VOLUMES 7-12  
REVIEW DIVISION: DIVISION OF REPRODUCTIVE AND  
UROLOGIC DRUG PRODUCTS (HFD-580)  
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Date of review submission to Division File System (DFS): 02/12/04

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***EXECUTIVE SUMMARY***

**1. Recommendations**

1.1 Recommendation on approvability: There are no impediments to approval of this application from a preclinical pharmacology/toxicology perspective.

1.2 Recommendation for nonclinical studies: No new preclinical studies are recommended.

1.3 Recommendations on labeling:

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**2. Summary of nonclinical findings**

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 10 multiples (AUC) of the clinical dose, and in dogs the high dose exposures exceeded 100 multiples (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 IC<sub>50</sub> 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. The potential magnitude of a QT effect in humans could not be accurately estimated from these studies. However, a QT study is being conducted clinically.

#### 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 about 10 multiples of the average clinical exposure (via AUC) in male and female rats and about 20 times the average clinical exposure (via AUC) in male and female mice.

#### Reproductive toxicology:

In a rat study, no effect on fertility was observed up to 200 mg/kg/day (10 multiples of the clinical dose by AUC).

In a segment II study in rats, no maternal or fetal effects were observed at 200 mg/kg or 10 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 (~ 10 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 50 mg/kg/day or ~5-6 times the expected clinical exposure (AUC).

In summary, in rats, maternal toxicity, and a decrease in fetal survival were observed at 200 mg/kg/day, depending on the timing and duration of exposure. In rabbits, maternal toxicity was also observed at 200 mg/kg/day. No treatment-related malformations were observed in rats or rabbits, and no developmental delays were observed in rats.

2.1 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.

2.2 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 at clinical exposures.

## PHARMACOLOGY/TOXICOLOGY REVIEW

### 3.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 21595

**Review number:** 1

**Sequence number/date/type of submission:**

**Information to sponsor:** Yes ( ) No (x)

**Sponsor and/or agent:** Interneuron Pharmaceuticals, Inc.

**Manufacturer for drug substance:** Madeus AG, Germany

**Reviewer name:** Laurie McLeod-Flynn

**Division name:** Division of Reproductive and Urologic Drug Products

**HFD #:** HFD-580

**Review completion date:** 10 February 2004

**Drug:**

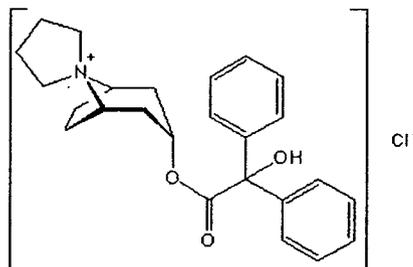
**Trade name:** \_\_\_\_\_

**Generic name:** Trospium Chloride

**Chemical name:** 3 $\alpha$ -benziloyloxyspiro [nortropane-8, 1'-pyrrolidinium] chloride

**Molecular formula/molecular weight:** C<sub>25</sub>H<sub>30</sub>ClNO<sub>3</sub> / 427.97

**Structure:**



**Relevant INDs/NDAs/DMFs:** IND 61381

**Drug class:** anticholinergic

**Indication:** treatment of patients with overactive bladder with symptoms of urinary frequency, urgency, or urge incontinence

**Clinical formulation:** 20 mg sugar-coated tablets

**Route of administration:** oral

**Proposed clinical protocol:** 20 mg bid

**Previous clinical experience:** Trospium chloride was introduced as a spasmolytic agent in Germany in 1967, and is on the market in Germany, Austria, Luxemburg, Spain, and Switzerland. A database of 29 clinical studies includes over 1300 patients treated with trospium chloride.

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

**Studies reviewed within this submission:**

Trospium chloride: Screening for changes induced in the behaviour of mice in the rotating-rod test.

Trospium chloride: Screening for effects on pentetrazol convulsions in mice.

Trospium chloride: Screening for effects on hexobarbitone sleeping time in the mouse.

Report on a study to screen trospium chloride for penetration of the brain and for central anticholinergic activities in the rat.

MP 194, atropine, and hyoscine butylbromide: Investigations for haemodynamic activities in anaesthetized cats.

Effects of Trospium Chloride on Cloned hERG Channels Expressed in Mammalian Cells.

Effects of haloperidol and trospium chloride on QTc and torsade de pointes arrhythmia.

Study title: MP 194, Batch 9045: Screening for bronchodilator activity and for effects on blood pressure and heart rate.

Trospium chloride: Screening for effects on renal function.

Trospium chloride: Excretory functions of the kidneys.

Effects on the motility of the small intestine of female mice in vivo.

Screening of MP 194 and WG 71 for effects on transit time through the small intestines of rats in vivo.

Effect of trospium chloride on intestinocolonic motility in the dog.

Disposition and mass balance of <sup>14</sup>C-Trospium chloride in male Long Evans rats following a single oral dose.

Chronic Toxicity of Orally Administered MP 194 in Male and Female Rats (Administration in the Ration)(35 weeks)

MP 194-S-1: 26 week oral toxicity study in dogs

Salmonella Typhimurium Reverse Mutation Assay with MP94WO

Study to Determine the Ability of MP 194 to Induce Mutations to 6-Thioguanine

Resistance in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay

Mutagenicity Evaluation of MP 194-S-1 Batch 7827/8 in the Mitotic Gene Conversion Assay Using Strain —

Study to Evaluate the Chromosome Damaging Potential of MP 194 by its Effects on Cultured Chinese Hamster Ovary (CHO) Cells Using an *In Vitro* Cytogenetics Assay  
Micronucleus Test in Rats

104 Week Dietary Carcinogenicity Study in Rats

78 Week Dietary Carcinogenicity Study in Mice

Fertility and Generation Study on Rats Given Intragastric Doses of MP 194

Prenatal Toxicity of MP 194 in Rats after Intragastric Administration

Prenatal Toxicity of MP194 in Rabbits after Intragastric Administration

Toxicokinetic Studies in Pregnant Rabbits

MP194-S-1: Effects of Oral Administration Upon Peri- and Post-Natal Development of the Rat

Screening of AS17 (Trospium chloride) for Activity on Isolated Mast Cells of Sensitized Rats

AS17 (Trospium chloride) and Anaphylactic Shock in Guinea Pigs.

AS17 (Trospium chloride) and Effects on Antibodies in Rabbits.

Trospium chloride – Screening for Antiinflammatory Activity in Rat Paw Edema.

Trospium chloride – Screening for Antiinflammatory Activity in the Cotton Pellet Test.

Trospium chloride: Investigations to Screen Trospium chloride for Effects on the Microcirculations, i.e. on the Permeability of the Plasma-lymph Barrier in Rats.

**Studies not reviewed within this submission: NA**

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### 3.2 PHARMACOLOGY

#### 3.2.1 Brief summary

**Pharmacology:** Trospium chloride antagonizes the effect of acetylcholine on cholinergically innervated organs in a dose-dependent manner. Its parasympholytic action reduces the tonus of smooth muscles (e.g. in the bladder, vas deferens, uterus, trachea, and ileum). Receptor assays showed that trospium chloride binds specifically to muscarinic receptors and negligibly to nicotinic receptors. It inhibits the secretion of bronchial mucous, saliva, sweat and, at high doses, gastric acid. Trospium chloride also paralyses accommodation. Due to its quaternary ammonium structure and hydrophilic properties, the substance does not cross the blood-brain barrier. Therefore, trospium chloride is expected to have little effect on the central nervous system.

Receptor	IC <sub>50</sub>
Muscarinic M <sub>1</sub>	1.0 x10 <sup>-9</sup> - 9.3 x10 <sup>-10</sup>
Muscarinic M <sub>2</sub>	2.0 x10 <sup>-9</sup> - 6.7 x10 <sup>-8</sup>
Muscarinic M <sub>3</sub>	2.5 x10 <sup>-9</sup>
Nicotinic	> 10 <sup>-4</sup>

**Effects of Trospium chloride (NC-2200) on the muscarinic receptor binding affinity in guinea pig tissues: (sponsor's tables)**

Table 1 Equilibrium binding parameters for 3H-QNB in guinea-pig urinary bladder, salivary gland, cerebral cortex and atrium

Tissue	No.	Kd(nmol/L)	Bmax(fmol/mg tissue)	Hill coefficient
Urinary bladder	No.1	0.4	8.72	1.1
	No.2	0.5	9.11	1.04
	No.3	0.5	8.97	1.02
	Mean	0.47	8.93	1.05
	S.E.	0.03	0.11	0.02
Salivary gland	No.1	1.29	1.4	0.97
	No.2	1.34	1.4	1
	No.3	1.38	1.53	0.89
	Mean	1.34	1.44	0.95
	S.E.	0.03	0.04	0.03
Cerebral cortex	No.1	0.33	24.85	0.9
	No.2	0.39	24.79	0.88
	No.3	0.31	24.12	0.81
	Mean	0.34	24.59	0.86
	S.E.	0.02	0.23	0.03
Atrium	No.1	0.46	10.09	0.81
	No.2	0.22	9.65	0.75
	No.3	0.42	9.83	0.8
	Mean	0.37	9.86	0.79
	S.E.	0.07	0.13	0.02

Table 2

IC<sub>50</sub> values of various compounds on <sup>3</sup>H-QNB binding to membrane preparations from guinea-pig tissues

Tissue	Compound	IC <sub>50</sub> (mol/L)			Mean(mol/L)	S.E.(mol/L)
		No.1	No.2	No.3		
Urinary bladder	NC-2200	3.44×10 <sup>-9</sup>	3.32×10 <sup>-9</sup>	2.44×10 <sup>-9</sup>	3.07×10 <sup>-9</sup>	3.15×10 <sup>-10</sup>
	Propiverine hydrochloride	2.30×10 <sup>-6</sup>	2.56×10 <sup>-6</sup>	2.85×10 <sup>-6</sup>	2.57×10 <sup>-6</sup>	1.59×10 <sup>-7</sup>
	Oxybutynin hydrochloride	2.83×10 <sup>-8</sup>	2.56×10 <sup>-8</sup>	2.41×10 <sup>-8</sup>	2.60×10 <sup>-8</sup>	1.23×10 <sup>-9</sup>
	Tolterodine tartrate	1.85×10 <sup>-8</sup>	2.27×10 <sup>-8</sup>	1.94×10 <sup>-8</sup>	2.02×10 <sup>-8</sup>	1.28×10 <sup>-9</sup>
	Atropine sulfate	1.68×10 <sup>-9</sup>	2.77×10 <sup>-9</sup>	2.20×10 <sup>-9</sup>	2.22×10 <sup>-9</sup>	3.15×10 <sup>-10</sup>
Salivary gland	NC-2200	5.83×10 <sup>-9</sup>	6.42×10 <sup>-9</sup>	7.62×10 <sup>-9</sup>	6.62×10 <sup>-9</sup>	5.27×10 <sup>-10</sup>
	Propiverine hydrochloride	1.04×10 <sup>-6</sup>	1.14×10 <sup>-6</sup>	8.30×10 <sup>-7</sup>	1.00×10 <sup>-6</sup>	9.13×10 <sup>-8</sup>
	Oxybutynin hydrochloride	1.21×10 <sup>-8</sup>	1.24×10 <sup>-8</sup>	1.17×10 <sup>-8</sup>	1.21×10 <sup>-8</sup>	2.03×10 <sup>-10</sup>
	Tolterodine tartrate	8.06×10 <sup>-9</sup>	8.35×10 <sup>-9</sup>	8.51×10 <sup>-9</sup>	8.31×10 <sup>-9</sup>	1.32×10 <sup>-10</sup>
	Atropine sulfate	2.42×10 <sup>-9</sup>	1.53×10 <sup>-9</sup>	2.10×10 <sup>-9</sup>	2.02×10 <sup>-9</sup>	2.60×10 <sup>-10</sup>
Cerebral cortex	NC-2200	4.62×10 <sup>-9</sup>	5.09×10 <sup>-9</sup>	4.71×10 <sup>-9</sup>	4.81×10 <sup>-9</sup>	1.44×10 <sup>-10</sup>
	Propiverine hydrochloride	7.72×10 <sup>-7</sup>	9.97×10 <sup>-7</sup>	9.99×10 <sup>-7</sup>	9.23×10 <sup>-7</sup>	7.53×10 <sup>-8</sup>
	Oxybutynin hydrochloride	3.87×10 <sup>-9</sup>	4.02×10 <sup>-9</sup>	4.34×10 <sup>-9</sup>	4.08×10 <sup>-9</sup>	1.39×10 <sup>-10</sup>
	Tolterodine tartrate	7.29×10 <sup>-9</sup>	9.23×10 <sup>-9</sup>	9.23×10 <sup>-9</sup>	8.58×10 <sup>-9</sup>	6.47×10 <sup>-10</sup>
	Atropine sulfate	1.16×10 <sup>-9</sup>	1.36×10 <sup>-9</sup>	1.34×10 <sup>-9</sup>	1.29×10 <sup>-9</sup>	6.36×10 <sup>-11</sup>
Atrium	NC-2200	6.51×10 <sup>-9</sup>	6.74×10 <sup>-9</sup>	5.81×10 <sup>-9</sup>	6.35×10 <sup>-9</sup>	2.80×10 <sup>-10</sup>
	Propiverine hydrochloride	3.47×10 <sup>-6</sup>	2.80×10 <sup>-6</sup>	3.33×10 <sup>-6</sup>	3.20×10 <sup>-6</sup>	2.04×10 <sup>-7</sup>
	Oxybutynin hydrochloride	4.03×10 <sup>-8</sup>	3.80×10 <sup>-8</sup>	3.81×10 <sup>-8</sup>	3.88×10 <sup>-8</sup>	7.51×10 <sup>-10</sup>
	Tolterodine tartrate	2.13×10 <sup>-8</sup>	1.87×10 <sup>-8</sup>	2.21×10 <sup>-8</sup>	2.07×10 <sup>-8</sup>	1.03×10 <sup>-9</sup>
	Atropine sulfate	3.10×10 <sup>-9</sup>	3.61×10 <sup>-9</sup>	2.89×10 <sup>-9</sup>	3.00×10 <sup>-9</sup>	6.08×10 <sup>-11</sup>

Table 3

The dissociation constants (K<sub>i</sub>) of various compounds for muscarinic receptors in guinea-pig tissues

Compound	No.	K <sub>i</sub> (mol/L.)			
		Urinary bladder	Salivary gland	Cerebral cortex	Atrium
NC-2200	No.1	1.65×10 <sup>-9</sup>	3.27×10 <sup>-9</sup>	1.85×10 <sup>-9</sup>	2.74×10 <sup>-9</sup>
	No.2	1.59×10 <sup>-9</sup>	3.61×10 <sup>-9</sup>	2.04×10 <sup>-9</sup>	2.83×10 <sup>-9</sup>
	No.3	1.18×10 <sup>-9</sup>	4.29×10 <sup>-9</sup>	1.88×10 <sup>-9</sup>	2.44×10 <sup>-9</sup>
	Mean	1.47×10 <sup>-9</sup>	(1.00)3.72×10 <sup>-9</sup>	(2.53)1.92×10 <sup>-9</sup>	(1.31)2.67×10 <sup>-9</sup> (1.82)
	S.E.	1.47×10 <sup>-10</sup>	3.00×10 <sup>-10</sup>	5.76×10 <sup>-11</sup>	1.18×10 <sup>-10</sup>
Propiverine hydrochloride	No.1	1.10×10 <sup>-6</sup>	5.83×10 <sup>-7</sup>	3.09×10 <sup>-7</sup>	1.46×10 <sup>-6</sup>
	No.2	1.23×10 <sup>-6</sup>	6.42×10 <sup>-7</sup>	3.99×10 <sup>-7</sup>	1.18×10 <sup>-6</sup>
	No.3	1.38×10 <sup>-6</sup>	4.67×10 <sup>-7</sup>	4.00×10 <sup>-7</sup>	1.40×10 <sup>-6</sup>
	Mean	1.24×10 <sup>-6</sup>	(1.00)5.64×10 <sup>-7</sup>	(0.45)3.69×10 <sup>-7</sup>	(0.30)1.35×10 <sup>-6</sup> (1.09)
	S.E.	8.04×10 <sup>-8</sup>	5.13×10 <sup>-8</sup>	3.03×10 <sup>-8</sup>	8.58×10 <sup>-8</sup>
Oxybutynin hydrochloride	No.1	1.36×10 <sup>-8</sup>	6.78×10 <sup>-9</sup>	1.55×10 <sup>-9</sup>	1.69×10 <sup>-8</sup>
	No.2	1.23×10 <sup>-8</sup>	6.98×10 <sup>-9</sup>	1.61×10 <sup>-9</sup>	1.60×10 <sup>-8</sup>
	No.3	1.17×10 <sup>-8</sup>	6.59×10 <sup>-9</sup>	1.74×10 <sup>-9</sup>	1.60×10 <sup>-8</sup>
	Mean	1.25×10 <sup>-8</sup>	(1.00)6.78×10 <sup>-9</sup>	(0.54)1.63×10 <sup>-9</sup>	(0.13)1.63×10 <sup>-8</sup> (1.30)
	S.E.	5.59×10 <sup>-10</sup>	1.14×10 <sup>-10</sup>	5.54×10 <sup>-11</sup>	3.16×10 <sup>-10</sup>
Tolterodine tartrate	No.1	8.87×10 <sup>-9</sup>	4.52×10 <sup>-9</sup>	2.92×10 <sup>-9</sup>	8.96×10 <sup>-9</sup>
	No.2	1.09×10 <sup>-8</sup>	4.70×10 <sup>-9</sup>	3.69×10 <sup>-9</sup>	7.86×10 <sup>-9</sup>
	No.3	9.40×10 <sup>-9</sup>	4.79×10 <sup>-9</sup>	3.69×10 <sup>-9</sup>	9.29×10 <sup>-9</sup>
	Mean	9.72×10 <sup>-9</sup>	(1.00)4.67×10 <sup>-9</sup>	(0.48)3.43×10 <sup>-9</sup>	(0.35)8.70×10 <sup>-9</sup> (0.90)
	S.E.	6.03×10 <sup>-10</sup>	8.01×10 <sup>-11</sup>	2.59×10 <sup>-10</sup>	4.32×10 <sup>-10</sup>
Atropine sulfate	No.1	8.06×10 <sup>-10</sup>	1.36×10 <sup>-9</sup>	4.64×10 <sup>-10</sup>	1.30×10 <sup>-9</sup>
	No.2	1.33×10 <sup>-9</sup>	8.61×10 <sup>-10</sup>	5.44×10 <sup>-10</sup>	1.27×10 <sup>-9</sup>
	No.3	1.07×10 <sup>-9</sup>	1.18×10 <sup>-9</sup>	5.36×10 <sup>-10</sup>	1.22×10 <sup>-9</sup>
	Mean	1.07×10 <sup>-9</sup>	(1.00)1.13×10 <sup>-9</sup>	(1.06)5.15×10 <sup>-10</sup>	(0.48)1.26×10 <sup>-9</sup> (1.18)
	S.E.	1.51×10 <sup>-10</sup>	1.45×10 <sup>-10</sup>	2.54×10 <sup>-11</sup>	2.56×10 <sup>-11</sup>

K<sub>i</sub> values were calculated using the formula  $K_i = IC_{50} / (1 + I / K_d)$ , where I is the radioligand concentration and K<sub>d</sub> is the radioligand equilibrium dissociation constant. Values in parentheses shows selective ratio.

Effects of trospium chloride (NC-2200) on acetylcholine-induced contractions in isolated guinea pig urinary bladder. (sponsor's tables)

Test or control article	Applied dose (mol/l)		
NC-2200	$10^{-8}$	$3 \times 10^{-8}$	$10^{-7}$
Oxybutynin	$3 \times 10^{-8}$	$10^{-7}$	$3 \times 10^{-7}$
Propiverine	$3 \times 10^{-6}$	$10^{-5}$	$3 \times 10^{-5}$
Atropine	$2 \times 10^{-8}$	$6 \times 10^{-8}$	$2 \times 10^{-7}$
Tolterodine	$3 \times 10^{-8}$	$10^{-7}$	$3 \times 10^{-7}$

Table 1 pA<sub>2</sub> values and Schild slope of NC-2200 and various drugs for antagonism of ACh-induced contraction in isolated guinea-pig urinary bladder smooth muscles

Drugs	pA <sub>2</sub>	Schild slope
NC-2200	9.06	1.04
Oxybutynin	7.83	1.02
Propiverine	5.76	1.57
Tolterodine	8.53	0.65
Atropine	6.81	0.92

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### 3.2.2 Safety pharmacology

#### Neurological effects:

**Study title: Trospium chloride: Screening for changes induced in the behaviour of mice in the rotating-rod test.** Trospium chloride (1 mg/kg), hyoscine butylbromide (1 mg/kg) or atropine sulphate (1 mg/kg) was administered subcutaneously to female NMRI mice (3/group). No effect on duration of rotating rod activity was observed.

**Study title: Trospium chloride: Screening for effects on pentetrazol convulsions in mice.** Trospium chloride (1 mg/kg), hyoscine butylbromide (1 mg/kg) or atropine sulphate (1 mg/kg) was administered subcutaneously to female NMRI mice (10/group) 30 minutes prior to administration of pentetrazol. No effect on seizure rate was observed.

**Study title: Trospium chloride: Screening for effects on hexobarbitone sleeping time in the mouse.** Trospium chloride (1 mg/kg or 3.12 mg/kg), hyoscine butylbromide or

atropine sulphate was administered subcutaneously to female NMRI mice (10/group) 30 minutes prior to administration of hexobarbitone. No effect on sleep time was observed.

**Study title: Report on a study to screen trospium chloride for penetration of the brain and for central anticholinergic activities in the rat.** In rats pretreated with 0.5 mg/kg trospium chloride (about 175 times the maximum human dose) the centrally mediated effects of physostigmine (0.3 mg/kg) were slightly inhibited in anesthetized rats. At 0.1 mg/kg trospium chloride showed no centrally mediated inhibition of physostigmine.

#### Cardiovascular effects:

**Study title: MP 194, atropine, and hyoscine butylbromide: Investigations for haemodynamic activities in anaesthetized cats.** Atropine, Buscopan and MP 194 in doses of equal weights (0.1, 0.316, 1, and 3.16 mg/kg i.v.) induced slight dose-related changes in heart rate and reductions in myocardial contractility, left ventricular pressure and peripheral arterial pressure in cats (6/group).

**Effects of Trospium Chloride on Cloned hERG Channels Expressed in Mammalian Cells.** ( — TestStudy No. 021206, 2/28/03). The effects of trospium chloride on ionic currents in \_\_\_\_\_ HEK293) cells that stably express the human ether-a-go-go-related gene were determined. Trospium chloride produced a concentration dependent inhibition of hERG current (mean + SEM)(n=4):

Dose (µM)	3 µM	10 µM	30 µM	100 µM
Exposure equivalent (ng/ml)	1290	4299	12896	42986
% inhibition	12.1±0.6 %	30.6±3.1%	58.4±4.9 %	81.4±2.4 %

The estimated IC<sub>50</sub> for the inhibitory effect of trospium chloride on the hERG current was 22.1 µM (equivalent to 9500 ng/ml), or approximately 9500-633 times the average expected plasma concentration rang of 1-15 ng/ml.

**Effects of haloperidol and trospium chloride on QTc and torsade de pointes arrhythmia.** In isolated, \_\_\_\_\_ rabbit (female New Zealand White) hearts, QT, QTc, dispersion of QT, and direct induction of torsades de pointes were measured in response to trospium chloride and haloperidol administration.

Measurements of left ventricular pressure (LVP), coronary flow (CF), PQ-interval (delay between the end of the pacing stimulus and the first normal ventricular activation, as a measure for the atrioventricular conduction time), and total ventricular activation time (TAT)(as a measure of the delay between activation of the first and activation of the last electrode): (Dose D1=10 nM, D2=100 nM, D3=200 nM, D4=1000 nM, D5=2000 nM, control "con"= Tyrode's solution, hypokalemia "hyp"= 2.5 mM K<sup>+</sup>/0.5 mM Mg<sup>++</sup>, NA=10<sup>-8</sup> M norepinephrine, Car = 10<sup>-7</sup> M carbachol) (sponsor's tables and figures)

LVP (mm Hg)

	con	hyp	NA	NA/Car	D1	D2	D3	D4	D5
Halo.	103±7	111±8	108±7	106±9	99±10	99±7	94±9	83±15	
TSP.	101±6	93±8	96±11	88±11	79±8	75±7	72±5	64±5	62±5

CF (ml/min)

	con	hyp	NA	NA/Car	D1	D2	D3	D4	D5
Halo.	44±3	43±3	37±3	36±2	35±3	33±2	32±3	34±3	
TSP.	46±3	42±4	41±4	37±3	30±3	33±4	29±3	28±2	27±2

PQ (ms)

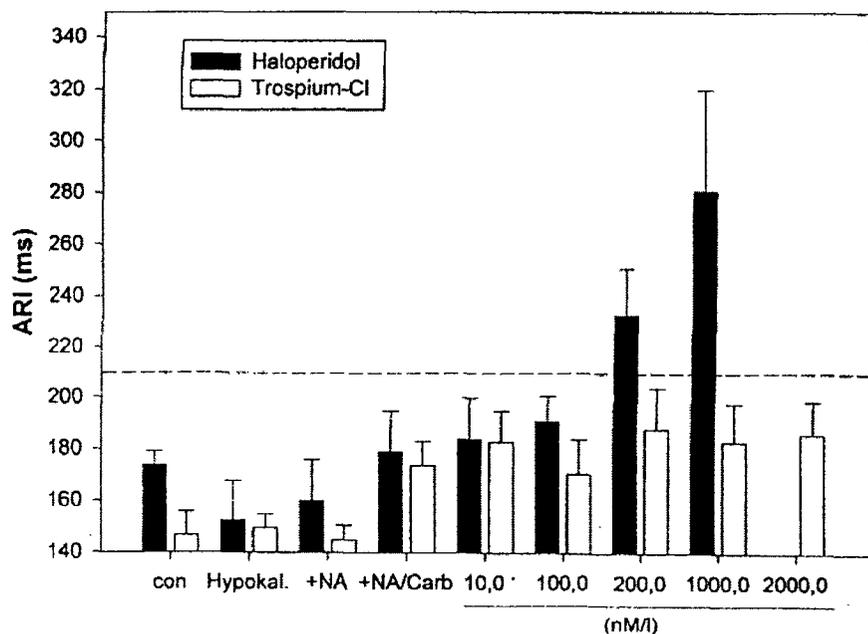
	con	hyp	NA	NA/Car	D1	D2	D3	D4	D5
Halo.	58±1	64±1	60±2	72±1	72±1	75±2	75±2*	75±2*	
TSP.	64±3	66±3	68±2	66±2	64±5	63±2	64±3	61±3	63±3

TAT (ms)

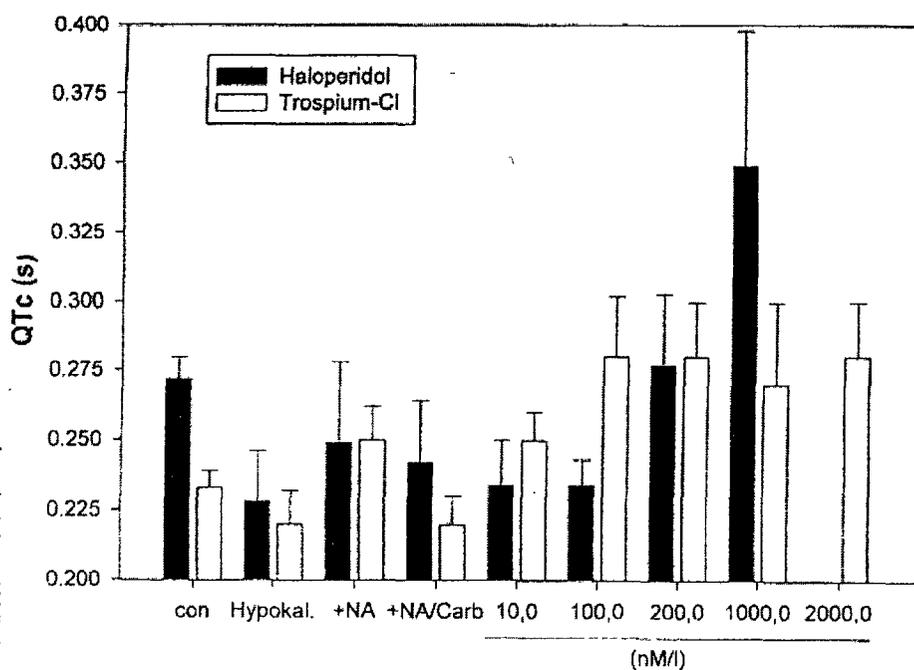
	con	hyp	NA	NA/Car	D1	D2	D3	D4	D5
Halo.	6±0.4	7±0.5	7±1	8±1	7±0.5	7±0.5	7±1	7±2	
TSP.	8±0.7	8±0.8	8±0.8	7±0.7	9±0.9	8±0.9	7±0.6	8±1.1	9±0.5

APPEARS THIS WAY  
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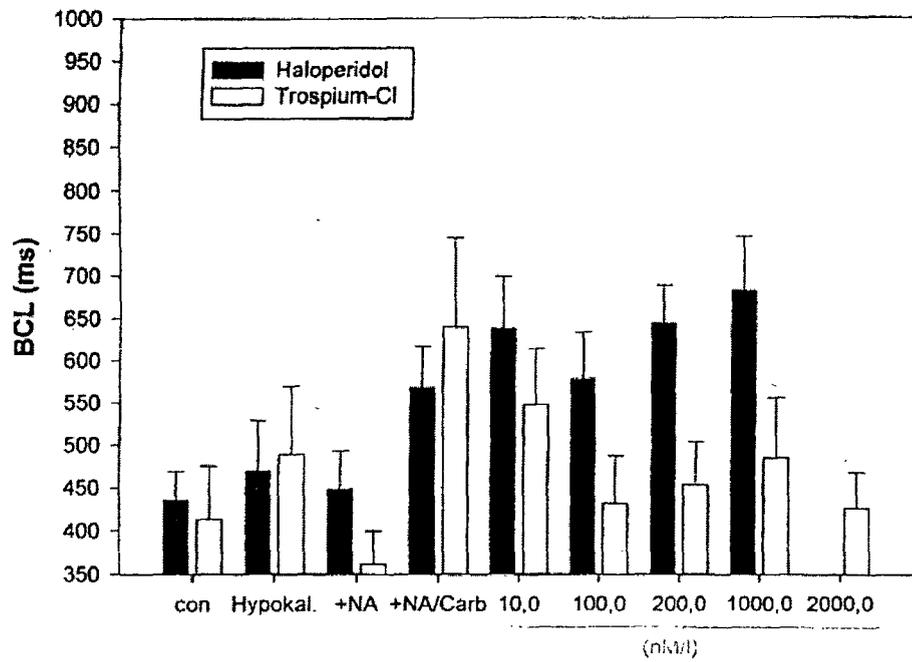
Activation recovery interval (epicardial potential duration):



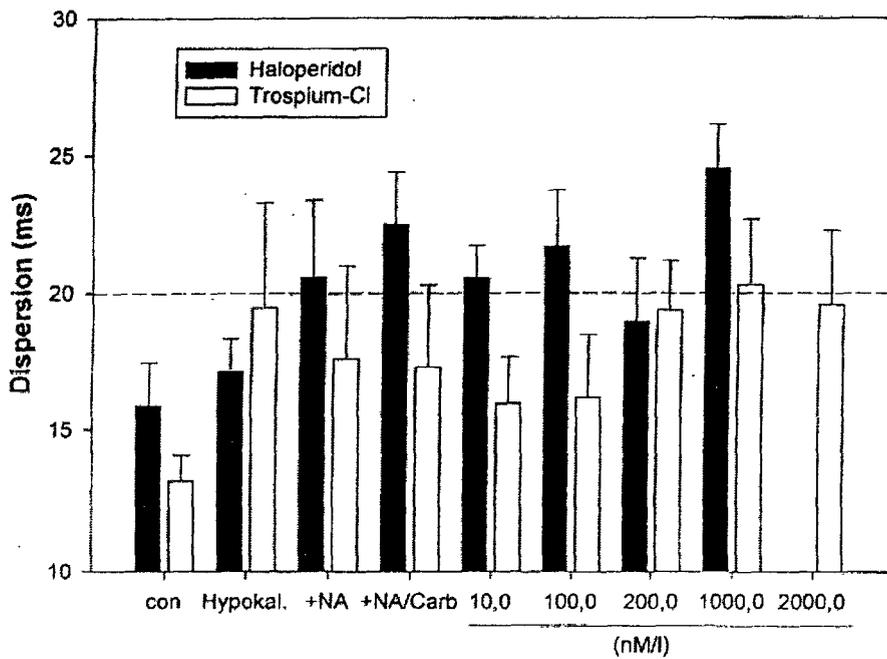
QTc (Bazett's):

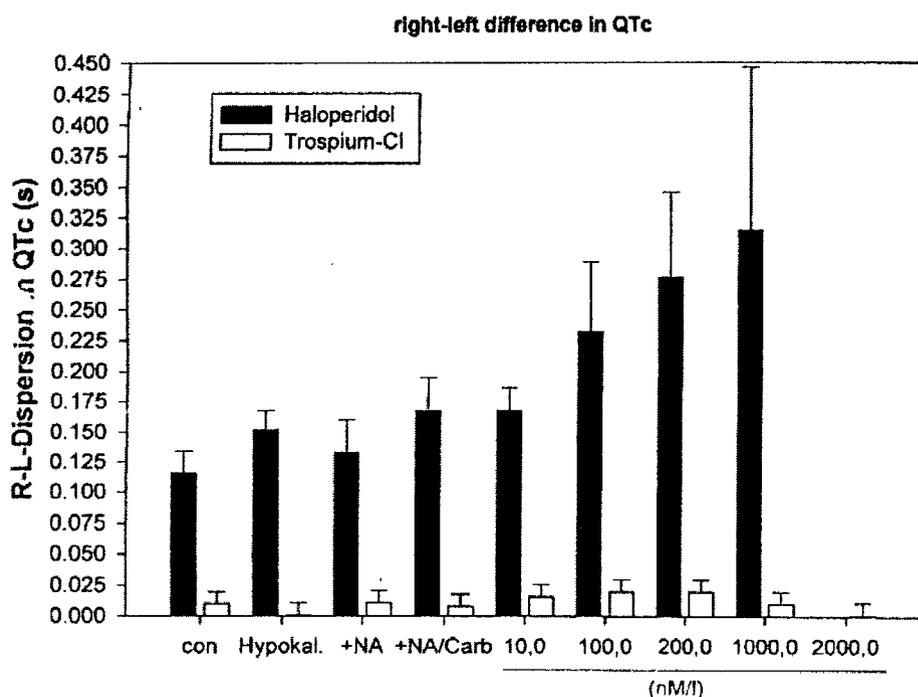


Basic cycle length:



ARI dispersion:





Administration of trospium chloride led to minor but significant prolongations of epicardial potential duration and QTc Bazett's. No Torsades de pointes were initiated at any dose tested (up to 2000 nM). Overall dispersion was not significantly affected by trospium chloride.

The positive control haloperidol induced torsades de pointes in 3/6 rabbit hearts, preceded by early interposing ventricular extrasystoles, increased cycle length, prolonged QT interval and increased dispersion, as expected.

#### Pulmonary effects:

**Study title: MP 194, Batch 9045: Screening for bronchodilator activity and for effects on blood pressure and heart rate.** Prior treatment of anesthetized guinea pigs with 1, 10, or 100 µg/kg MP 194 or with 10, 31.6, or 100 µg/kg atropine or with 0.100, 0.316, or 1 mg/kg Buscopan by i.v. administration resulted in dose related inhibition of cholinergic bronchospasm or approximately similar severity provoked 10 and 40 minutes later by i.v. administration of 10 µg/kg methacholine chloride.

#### Renal effects:

**Study title: Trospium chloride: Screening for effects on renal function.** Trospium chloride (1 mg/kg), Buscopan (1 mg/kg) or atropine (1 mg/kg) was administered intravenously to anesthetized male Wistar rats. No effect on urinary flow rate or

glomerular filtration rate (clearance of inulin, fluid, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, glucose, urea, and PAH) was observed.

**Study title: Trospium chloride: Excretory functions of the kidneys.** Trospium chloride (1 mg/kg), Buscopan (1 mg/kg) or atropine (1 mg/kg) was administered intravenously to conscious male Wistar rats. No effect on renal excretory parameters was observed.

### **Gastrointestinal effects:**

**Study title: Effects on the motility of the small intestine of female mice in vivo.** Test agents were administered i.v. or orally to NMRI female mice, followed after 30 minutes with tragacanth carmine red and carbachol (200 µg/kg). After 25 minutes the animals were sacrificed, and the length of the stained portion of the intestine calculated as a percentage of the total length of the gut.

Effect on intestinal motility (%)						
Dose (mg/kg)	Trospium chloride (batch 9045)		Atropine sulphate		Hyoscine butylbromide	
	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
0.005	-9.19					
0.05	-22.18		-27.72	-15.72		
0.5	-38.53		-35.39	-22.51	-10.58	
5	-47.55	-13.37	-47.95	-40.27	-19.32	
15.8	-45.05	-27.29			-34.26	-14.59
50		-38.42		-42.38	lethal	-12.51
158		-37.71			lethal	-34.37
500						Lethal

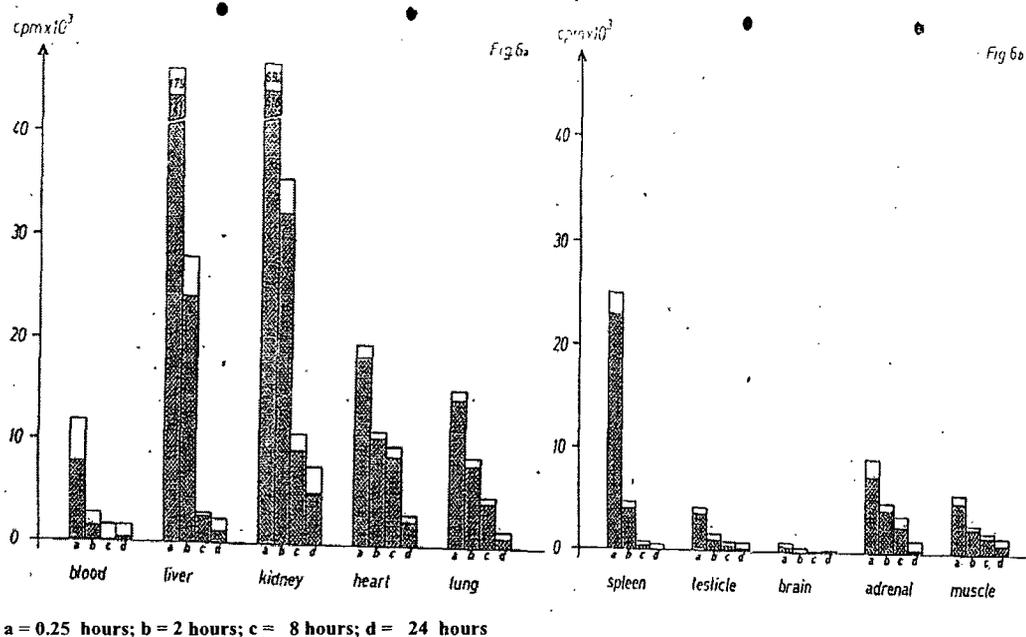
**Study title: Screening of MP 194 and WG 71 for effects on transit time through the small intestines of rats in vivo.** Test agents were administered intraduodenally or orally to Wistar rats. After 15 (i.d.) or 30 (oral) minutes, tragacanth carmine red ± carbachol was administered. Without stimulation of intestinal motility by carbachol, the inhibitory activity of MP 194 (5 mg/kg, batch 9045) was 18.1% intraduodenally and 13% orally. After stimulation of intestinal motility by carbachol, MP 194 or another formulation WG 71 (batch #840720) at 5 mg/kg, intestinal transit time was reduced by 32.5% and 33.3%, respectively.

**Study title: Effect of trospium chloride on intestinocolonic motility in the dog.** Trospium chloride (oral, 1, 5, and 10 mg/kg or intraduodenally, 5 and 10 mg/kg) inhibited intestinal motility in mongrel dogs both in fed and fasted states following stimulation by carbachol or morphine, as measured by electromyography and resistive extensometry.

### 3.3 PHARMACOKINETICS/TOXICOKINETICS

**3.3.1 Absorption:** In rats, absorption is estimated from biliary and renal elimination to be between 3.9 and 6.7%. An estimation of the extent of absorption via comparison of the areas under the blood level curves gives values from 4-5%. In dogs, a comparison of the amounts excreted via the kidneys after oral and intravenous administration gave values of 4-5%. A comparison of AUCs gave an estimate of 6.5%. In humans, about 9.6% of the ingested drug is bioavailable, which parallels levels seen with other tertiary amines.

**3.3.2 Distribution:** Organ distributions were determined after a single dose in albino and pigmented rats (5 µg/kg, 50 µCi/kg). The highest concentrations were observed in the liver and kidneys, followed by heart and adrenal glands. Only traces of nonvolatile activity were observed in the brain. No significant differences were observed in the pattern of distribution between sexes, between i.v. and oral routes of administration, or between pigmented and non-pigmented skin. Only traces were still observed in liver and kidney at 24 hours following administration. (sponsor's figures)



Organ concentrations (nonvolatile radioactivity) were determined 8 and 24 hours after the last of 10 oral doses of 0.2 mg/kg [<sup>3</sup>H]-trospium chloride. In all organs and tissues the levels were higher after repeated doses than after a single dose. Highest levels were measured in the liver, kidney, heart, and adrenal gland. In all organs, concentrations markedly decreased from the 8<sup>th</sup> to the 24<sup>th</sup> hour.

Gestating rats were given 50 µg/kg [<sup>3</sup>H]-trospium chloride by intravenous injection on the 10<sup>th</sup>, 16<sup>th</sup>, and 20<sup>th</sup> days of gestation. At 0.5, 2, or 8 hours, radioactivity was measured in the dams, placentas and fetuses. The concentrations of trospium chloride in the placenta were similar to those in the blood, but lower than in the liver kidneys, and heart. The barrier function of the placenta increased with the duration of pregnancy, resulting in progressively greater differences between the concentrations of radioactivity in the placenta and that in the fetuses or fetal organs. The highest concentrations of radioactivity in the fetal organs were in the liver. The blood cerebrospinal fluid barrier in the brain appeared to develop between the 16<sup>th</sup> and 20<sup>th</sup> days of gestation.

The transfer of [<sup>3</sup>H]-trospium chloride and its metabolites into the milk of lactating rats after i.v. (50 µg/kg) and oral (2 mg/kg) administration was determined between days 7 and 9 postpartum. The total activity of trospium chloride excreted into the milk in a 24 hour period was about 0.5 parts per thousand based on the i.v. dose. Concentration in the milk reached a peak at 6 hours after an i.v. dose and after about 8 hours following an oral dose. Thin layer chromatography showed the presence of trospium chloride (predominant species) and its metabolite azoniaspiro.

Plasma protein binding over a range of concentrations was determined using equilibrium dialysis (10 to 1000 µg/l)(48-53%), ultrafiltration (10 to 1000 µg/l)(41-59 %), ultracentrifugation (0.5 to 50 µg/l)(70-71%), and gel filtration (50-78%). Protein binding rates obtained ex-vivo in dogs ranged from 31-51%.

**Study title: Disposition and mass balance of <sup>14</sup>C-Trospium chloride in male Long Evans rats following a single oral dose.** Male Long Evans rats were administered 20 mg/kg Trospium chloride (oral, 4 mg/ml, 100 µCi/kg, 5 ml/kg). Group I (n=7) was used for profiling blood and plasma at 0.5, 1, 8, 24, 72, 120, and 336 hours and for whole body autoradiography of the carcass. Group II (n=12, 3 per time point) was used for profiling blood and plasma at 1, 8, 24, and 120 hours. Group III (n= 4) was used for collection of urine, feces, and expired air at 0, 4, 8, 24, 48, 72, 96, and 120 hours and for the carcass to calculate mass balance.

Concentration of total <sup>14</sup>C-Trospium chloride-derived radioactivity and pharmacokinetic parameters in tissues and organs of male pigmented rats given a single oral dose of approximately 20 mg/kg body weight (µg equiv/g):

Tissue	0.5 (hr)	1 (hr)	8 (hr)	24 (hr)	72 (hr)	120 (hr)	336 (hr)	C <sub>max</sub> (µg equiv/g)	T <sub>1/2 λz</sub> (hr.)	λz int. (hr.)	AUC <sub>∞</sub> (µg equiv*hr/g)
Kidney	BLQ	BLQ	---	BLQ	BLQ	BLQ	BLQ	---	-	-	-
Liver	---			BLQ	BLQ	BLQ	BLQ	---	17.35	8-24	14.24
Urine	---			BLQ	BLQ	BLQ	BLQ	---	-	-	-
Urinary bladder	BLQ	---		BLQ	BLQ	BLQ	BLQ	---	-	-	-
Cecum	BLQ	BLQ	---	NR	BLQ	---	BLQ	---	16.72	8-120	2131
Cecum contents	BLQ	BLQ	---	NR	BLQ	---	BLQ	---	--	--	--
Esoph. Contents	---		BLQ	BLQ	BLQ	BLQ	BLQ	---	--	--	--
Esophagus	---		BLQ	BLQ	BLQ	BLQ	BLQ	---	-	-	-
Gastric mucosa	---		BLQ	BLQ	BLQ	BLQ	BLQ	---	0.085	0.5-1	90.32

Lg.int.contents	BLQ	BLQ	---	---	BLQ	BLQ	BLQ	---	--	--	--
Large intestine	BLQ	BLQ	---	---	BLQ	BLQ	BLQ	---	3.35	8-24	167.3
Sm.int.contents	---	---	---	---	BLQ	---	BLQ	---	--	--	--
Small intestine	---	---	---	---	BLQ	BLQ	BLQ	---	1.40	1-8	82.30
Stom. Contents	---	---	---	---	BLQ	BLQ	---	---	--	--	--
Stomach	---	---	BLQ	BLQ	BLQ	BLQ	BLQ	---	0.41	0.5-1	39.93
Plasma									0.35	0.5-1	0.021

Radioactivity in all other tissues, including those of the central nervous system, endocrine system, gonads, and eyes, were below the lower limit of quantification.

Mean cumulative percent of radioactivity in excreta at specified times postdose for male pigmented rats following a single oral dose of 14C-Trospium chloride:

Time (hr.)	Urine	Cage rinse	Expired air	Feces	Carcass	Total
4	0.01	0.00	0.00	NA		0.01
8	0.22	0.00	0.00	NA		0.22
24	0.39	0.036	0.00	50.14		50.57
48	0.46	0.036	0.00	91.15		91.65
72	0.47	0.036	0.00	91.79		92.30
96	0.47	0.036	0.00	91.82		92.33
120	0.47	0.036	0.00	91.82	0.00	92.33

**3.3.3 Metabolism:** Biodegradation of trospium chloride was determined by thin layer chromatography and measurement of the radioactive peaks was performed by a thin layer scanner. The main metabolite in rats and dogs was characterized as the ester hydrolysis product, azoniaspironortropanol. The appearance of volatile radioactivity was interpreted as evidence of metabolism of the pyrrolidine ring.

In rats, following i.v. injection, trospium chloride was metabolized quickly to the main metabolite azoniaspironortropanol which was measured in plasma. The metabolic profile of different organs of the same rat at 6 hours post dose showed differences. Hydrolysis of the ester appeared to take place predominantly in the liver, with further metabolic steps occurring in other organs as well. In urine and bile, 7 to 8 radioactive peaks could be identified, the largest being trospium chloride with about one third of the excreted radioactivity. After oral injection, the amount of trospium chloride in urine and bile was smaller than after i.v. injection, but increased with dose.

In dogs, after oral administration, trospium chloride and azoniaspironortropanol were the primary peaks in chromatograms of plasma samples. In feces, following oral administration, a high proportion of trospium chloride was measured. Following i.v. injection, trospium chloride and azoniaspironortropanol were minor peaks. Characterization of smaller metabolites has not been done; however, 80-90 % of the radioactivity in plasma has been shown to be trospium chloride and azoniaspironortropanol. One minor metabolite was identified in clinical studies as being a hydroxy derivative of the benzylic acid moiety.

**3.3.4 Excretion:** After i.v. injection in bile cannulated rats, excretion took place primarily in the first 2-3 hours. After 24 hours, about 40% of the dose was excreted with the urine and 25% with the bile. After i.v. Injection of non-cannulated rats, 31% of the dose was measured in the urine and 54% in the feces. After oral administration, renal excretion was between 2.6 and 3.4 % of dose, while biliary excretion increased in a dose-related manner from 1.35 after a 0.2 mg/kg dose to 14.5% after 200 mg/kg. Total recovery was between 80 and 89%.

After i.v. injection in dogs, 48% was excreted with the feces and 35% with the urine. After oral administration, 1.5% of the dose was in the urine and 85% in the feces. Total recovery was between 91 and 93%.

**3.3.5 Tables and figures to include comparative TK summary**

PK parameters: (human: Cmax ~ 3 ng/ml, AUC ~ 38 ng/ml)

Species	route	Dose mg/kg	T <sub>1/2α</sub> min	T <sub>1/2β</sub> min	T <sub>1/2λ</sub> (hr)	AUC kdpmhr/ml	T <sub>max</sub> hr	C <sub>max</sub> kdpm/ml	Cl <sub>tot</sub> ml/min/kg	Renal ex. % of dose
Rat	i.v. <sup>c</sup>	0.005 • •	0.54	7.7	1.6	34.0			54.0	44.6
		0.05 • •	0.71	16.5	1.7	49.9			36.8	42.2
		0.2 • •	0.65	21.3	1.5	52.6			35.2	36.9
	p.o. <sup>d</sup>	0.2 • •	--	25.7	2.9	--	1.41		--	2.63
		2.0 • •	--	31.4	3.8	406.6	1.46		460f <sup>a</sup>	3.35
		2.0 • •	--	20.1	2.4	282.4	1.09		658f <sup>a</sup>	--
		20 • •	--	36.9	3.1	223.8	1.88		830f <sup>a</sup>	2.78
		200 • •	--	25.4	3.2	235.7	1.38		792f <sup>a</sup>	3.15
Dog	p.o. <sup>c</sup>	2 • •			21.1	100.1	2.2		18.5f <sup>b</sup>	1.45
	i.v. <sup>f</sup>	0.05 • •			20.2	84.4	0.0		21.9f <sup>b</sup>	35.1
	i.m. <sup>f</sup>	0.05 • •			35.7	144.6	0.13		12.8f <sup>b</sup>	26.8

<sup>a</sup>f = fraction absorbed; <sup>b</sup>ml/min; <sup>c</sup>radioactivity dose in all experiments: 1.1x10<sup>5</sup> kdpm/kg  
<sup>d</sup>radioactivity dose in all experiments: 1.1x10<sup>7</sup> kdpm/kg; <sup>e</sup>radioactivity dose: 2.2x10<sup>6</sup> kdpm/kg  
<sup>f</sup>radioactivity dose: 1.1x10<sup>5</sup> kdpm/kg

**Rat: (dietary)**

N=4	Males + females (mg/kg/day)		
	2	20	200
AUC			
day 1-2	--	151.5	368.1
day 14-15		111.5	560.1
day 28-29		65.0	752.9
Cmax			
day 1-2	_____		
day 14-15	_____		
day 28-29	_____		

Dog: (oral gavage)

N=2	Males (mg/kg/day)			Females (mg/kg/day)		
	0.6	6	60	0.6	6	60
AUD (nghr/ml)						
Day 1	14.1-17.5	149.0-268.8	1916.8-3222.0	23.9-27.5	279.7-365.0	3543.2-4526.5
Day 14	15.3-28.0	213.9-284.4	4762.3-10466.3	27.6-36.9	199.7-770.9	4132.6-5271.6
Day 28	43.4-61.9	584.7-990.8	12022.5-12428.2	31.1-42.8	398.4-419.7	6912.1-29339.5
Cmax (ng/ml)						
Day 1	_____					
Day 14	_____					
Day 28	_____					
Tmax (hr)						
Day 1	3	3	1-5	3-4	3-4	0.5-2
Day 14	2-3	2-4	0.5-4	2-4	2-3	1-3
Day 28	3-4	2-3	0.5-2	3-4	3	0.5

### 3.4 TOXICOLOGY

#### 3.4.1 Overall toxicology summary

##### 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 10 multiples (AUC) of the clinical dose, and in dogs the high dose exposures exceeded 100 multiples (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.

While the preclinical toxicology studies were performed prior to the current standards for these studies, there is also an abundance of clinical experience indicating that the adverse effects in clinical studies are probably limited to lowered secretion of perspiratory glands, pupillary dilation, impaired accommodation, lowered salivation, constipation, increased heart rate, micturition disorders.

An unknown potential for QT prolongation is being investigated in a clinical study.

##### Genetic toxicology:

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

##### Carcinogenicity:

No evidence of any neoplastic effect was observed after treatment with trospium chloride up to about 10 multiples of the average clinical exposure (AUC) in male and female rats and about 20 times the average clinical exposure (AUC) in male and female mice.

**Reproductive toxicology:**

In a rat fertility study, treatment of the F0 generation began approximately 61 days (male) and 14 days (females) before mating. The treatment of females continued throughout gestation and to the end of the lactation period. In the 200 mg/kg/day F0 group (10 multiples of the clinical dose by AUC), weight gains were marginally lower as was the number of live fetuses in the F1 generation. No other treatment related effects were observed. No effect on mating behavior or fertility of the F0 or F1 generations was observed at any dose.

In a segment II study in rats, with treatment administered on days 6 – 16 of gestation, no maternal or fetal effects were observed at 200 mg/kg or 10 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 (~ 10 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 50 mg/kg/day or ~5-6 times the expected clinical exposure (AUC).

In rats, maternal toxicity, and a decrease in fetal survival are observed at 200 mg/kg/day, depending on the timing and duration of exposure. In rabbits, maternal toxicity is also observed at 200 mg/kg/day. No treatment-related malformations were observed in rats or rabbits, and no developmental delays were observed in rats.

While the preclinical reproductive studies were performed prior to the current standards for these studies, the design and conduct of the studies is generally consistent with the requirements of a modern GLP study and/or more current data has been added to supplement high dose and pharmacokinetic data. There is also experience with the drug in a reproductively active clinical population and in clinical trials.

**3.4.2 Single-dose toxicity**

Species	Sex	Route	LD <sub>50</sub> (mg/kg)
Rat	M	p.o.	>940
	F	p.o.	>800
	M	i.v.	>10.7
	F	i.v.	>12.3
Mouse	M	p.o.	425
	F	p.o.	365
	M	i.v.	7.5
	F	i.v.	8.4

### 3.4.3 Repeat-dose toxicity

#### Study title: Chronic Toxicity of Orally Administered MP 194 in Male and Female Rats (Administration in the Ration)(35 weeks)

Volume #7, and page #67

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 5 December 1980

GLP compliance:

QA report: yes ( ) no ( )

Drug: batch # 7827/8

Dosing:

Species/strain: Wistar rats

#/sex/group or time point (main study): 23

Weight: 270 g (males) and 200 g (females)

Doses in administered units: 0, 2, 20, and 200 mg/kg/day for 35 weeks

Route, form, volume, and infusion rate: oral, dietary

Results: (summary data submitted) The rats were inspected daily throughout the study and weighed once a week. The hematological and biochemical investigations and urinalysis were carried out before the first dose and at the end of 6, 14, and 35 weeks. At the end of the study the rats were dissected and the organs were weighed. This was followed by a histopathological assessment of the internal organs.

The general condition of the rats was good and their behavior normal throughout the study.

The weight gains of the males and females of the 200 mg/kg/day group was lower than in the control group. The difference in females was statistically significant. No treatment related effects were observed in hematology, clinical chemistry, urinalysis, pathology, or histopathology.

#### Study title: MP 194-S-1: 26 week oral toxicity study in dogs

Study no.: — Project No. 415035

Volume #008, and page #84

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 19 February 1980

GLP compliance: yes

QA report: yes (x) no ( )

Drug: batch # 7049, % pure

Formulation/vehicle: gelatin capsule

Dosing:

Species/strain: Beagle dogs

#/sex/group or time point (main study): 4/sex/group

Weight: 5.1-7.8 kg (males) and 6.1-8.0 kg (females)

Doses administered in units: 0, 0.6, 6, and 60 mg/kg/day

Route, form, volume, and infusion rate: gelatin capsule

Results:

Mortality: One high dose male was found dead day 6 of week 10, after exhibiting signs of abnormal mucus production, bronchial pneumonia, and bacterial infection.

Clinical signs:

N=4	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.6	6	60	0	0.6	6	60
Bronchopneumonia				2				
Temporary reduced food intake and body weight				1				
Mydriasis/impaired pupil accommodation			2	4		2	2	4
Abnormal mucous production				4				4
Vomiting		2		2			2	2

Body weights:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.6	6	60	0	0.6	6	60
Week 26 body weight (kg)	12.6	11.8	11.6	10.2	10.7	11.7	11.3	10.4

Food consumption: A minor reduction in food reduction was observed in high dose males.

Ophthalmoscopy:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.6	6	60	0	0.6	6	60
Corneal opacity				1				
Lenticular opacity								1

Electrocardiography: week 26

N=4	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.6	6	60	0	0.6	6	60
Heart rate (bpm)	96	109	108	115	106	113	124	132
Blood pressure (mmHg)	141	125	141	125	128	133	125	109

Hematology: No treatment related effects were observed.

Clinical chemistry: week 26

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.6	6	60	0	0.6	6	60
Alkaline phosphatase (IU/L)	170.8	247.3	112.5	175.0	174.5	164.3	144.8	291.5
GOT (IU/L)	19	20.2	19.8	16.3	19.5	18.3	22.5	52.5
Cholesterol (mg/dL)	124	114.5	123.3	131.7	122	160.5	138.8	150.3
Triglycerides (mg/dL)	32	37.3	39	94.3	41.5	67.8	70.0	61.0

Organ weights:

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.6	6	60	0	0.6	6	60
Heart (g)	99.4	91.7	94.4	80.7	90.4	91.7	85.7	74.5
Heart (% of BW)	7.9	7.8	8.1	7.9	8.4	7.8	7.6	7.2
Liver (g)	462.8	450.4	387.8	422.8	372.6	431.9	415.5	427.7
Liver (% of BW)	3.69	3.83	3.40	4.24	3.47	3.75	3.68	4.19

Gross pathology: No treatment related effects were observed.  
 Histopathology: No treatment related effects were observed.

Toxicokinetics:

N=2/group	Males (mg/kg/day)			Females (mg/kg/day)		
	0.6	6	60	0.6	6	60
AUD (nghr/ml)						
Day 1	14.1-17.5	149.0-268.8	1916.8-3222.0	23.9-27.5	279.7-365.0	3543.2-4526.5
Day 14	15.3-28.0	213.9-284.4	4762.3-10466.3	27.6-36.9	199.7-770.9	4132.6-5271.6
Day 28	43.4-61.9	584.7-990.8	12022.5-12428.2	31.1-42.8	398.4-419.7	6912.1-29339.5
Cmax (ng/ml)						
Day 1	_____					
Day 14	_____					
Day 28	_____					
Tmax (hr)						
Day 1	3	3	1-5	3-4	3-4	0.5-2
Day 14	2-3	2-4	0.5-4	2-4	2-3	1-3
Day 28	3-4	2-3	0.5-2	3-4	3	0.5

**Toxicology summary:** No histological effects were observed in rats or dogs at any dose tested. In rats the high doses were greater than 10 multiples (AUC) of the clinical dose, and in dogs the high dose exposures exceeded 100 multiples (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.

While the preclinical toxicology studies were performed prior to the current standards for these studies, there is also an abundance of clinical experience indicating that the adverse effects in clinical studies are probably limited to lowered secretion of perspiratory glands, pupillary dilation, impaired accomodation, lowered salivation, constipation, increased heart rate, micturition disorders.

**APPEARS THIS WAY  
ON ORIGINAL**

**Histopathology inventory for IND #61381:**

Study	431827	431811	415035
Species	rat	mouse	dog
Adrenals	X	X	X
Aorta			X
Bone	X	X	X
Brain	X	X	X
Cecum			X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X	X	X
Esophagus	X	X	X
Eye			X
Gall bladder		X	X
Gross lesions	X	X	
Heart	X	X	X
Ileum	X	X	X
Jejunum	X	X	X
Kidneys	X	X	X
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X	X	X
Ovaries	X	X	X
Pancreas	X	X	X
Parathyroid	X	X	X
Pituitary	X	X	X
Prostate	X	X	X
Salivary gland	X	X	X
Sciatic nerve			X
Skeletal muscle			X
Skin	X	X	X
Spinal cord			
Spleen	X	X	X
Sternum	X	X	
Stomach	X	X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid	X	X	X
Tongue			X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X	X	X

**3.4.4. Genetic toxicology****Study title: Salmonella Typhimurium Reverse Mutation Assay with MP94WO**

Study no: \_\_\_\_\_

Volume #9, and page # 148

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 19 January 1994

GLP compliance: yes

QA reports: yes (x) no ( )

Drug: batch # 43406, — pure

Formulation/vehicle: aqua bidest

Methods:

Strains/species/cell line: TA 1535, TA 100, TA 1537, TA 98, TA 102

Dose selection criteria: Doses up to 5000 µg/plate showed normal background growth in TA 98 and TA 100

Metabolic activation system: S9 from Aroclor 1254 induced male Wistar rat liver

Controls:

Vehicle: aqua bidest

Negative controls: solvent controls and non-treated controls

Positive controls: sodium azide (10 µg/plate, TA 1535, TA 100), 4-nitro-o-phenylene-diamine 910 µg/plate, TA 1537, TA 98), or methyl methane sulfonate (5.0 µl/plate, TA 102) in the absence of metabolic activation and 2-aminoanthracene (2.5 µg/plate, all strains) in the presence of metabolic activation

Exposure conditions:

Doses used in definitive study: 0, 33.3, 100, 333.3, 1000, 2500 and 5000 µg/plate

Study design: Both plate incorporation and pre-incubation assays were performed in two independent experiments, in triplicate.

Summary of individual study findings:

Study validity: Positive and negative controls responded as expected.

Study outcome: No increase in the number of revertants was observed at any concentration of test article either in the presence or in the absence of S9. The test article was concluded to be nongenotoxic under the conditions of this assay.

**Study title: Study to Determine the Ability of MP 194 to Induce Mutations to 6-Thioguanine Resistance in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay**

Study no: \_\_\_\_\_

Volume #8, and page #185

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: October, 1985

GLP compliance: yes

QA reports: yes (x) no ( )  
Drug: batch # 2046, \_\_\_\_\_ pure  
Formulation/vehicle: distilled water

Methods:

Strains/species/cell line: Mouse Lymphoma L5178Y cells

Dose selection criteria:

Range finding studies: In a range finding study, the four highest doses, 158, 500, 1580, and 5000 µg/ml were found to be non-toxic in the absence and presence of S-9 and were selected for use in the definitive assay.

Test agent stability: (test conducted within one hour of mixture preparation)

Metabolic activation system: Aroclor 1254 induced rat liver S-9 fraction

Controls:

Vehicle: distilled water

Negative controls: distilled water

Positive controls: 4-nitroquinoline-1-oxide (0.1 and 0.2 µg/ml) in the absence of S-9 and benzo(a) pyrene (2.0 and 3.0 µg/ml) in the presence of S-9

Exposure conditions:

Incubation and sampling times: 2 hour incubation followed by sampling at 7-8 days

Doses used in definitive study: 0, 158, 500, 1580, and 5000 µg/ml in assay 1 and 0, 2000, 3000, 4000, and 5000 µg/ml in assay 2

No. of replicates: 4 plates per dose

Summary of individual study findings:

Study validity: Positive and negative controls responded as expected.

Study outcome: No increase in 6-thioguanine resistance was observed after treatment with any concentration of MP 194, which was therefore judged to be not mutagenic under the conditions of this assay.

**Study title: Mutagenicity Evaluation of MP 194-S-1 Batch 7827/8 in the Mitotic Gene Conversion Assay Using Strain \_\_\_\_\_**

Study no: 20998

Volume #9, and page #211

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 4 November 1981

GLP compliance:

QA reports: yes (x) no ( )

Drug: lot #7827/8

Formulation/vehicle: distilled water

Methods:

Cell line: \_\_\_\_\_

Dose selection criteria: 10,000 µg/ml was not toxic (no change in colony count compared to control) and was used as the high dose.

Metabolic activation system: Aroclor 1254 induced rat liver S-9 fraction

Controls:

Vehicle: distilled water

Negative controls: distilled water

Positive controls: N-methyl-N-nitro, N-nitrosoguanidine, 10 µg in DMSO in the absence of S-9, and 2-anthramine, 2.5 µg in DMSO, in the presence of S-9 (the S-9 fraction was also tested for biological activity in a Salmonella assay).

Exposure conditions:

Doses used in definitive study: 0, 1, 100, 500, 1000, 2500, 5000, and 10,000 µg

Study design: Cells and test article or controls were suspended in a semi solid agar overlay and co-incubated for 4 days.

Analysis:

No. of replicates: 3 plates

Counting method: not stated

Criteria for positive results: The negative control value for convertants must be within the normal range. The test article must produce a positive dose response over three concentrations with the highest increase equal to twice the negative control value. A positive test article response must be reproducible in a separate assay.

Summary of individual study findings:

Study validity: Positive and negative controls responded as expected. Toxicity (change in colony count compared to control) was not observed at the high dose of 10,000 µg/ml.

Study outcome: No test article related increase in the number of convertants was observed. It was concluded that trospium chloride was not mutagenic under the conditions of this study.

**Study title: Study to Evaluate the Chromosome Damaging Potential of MP 194 by its Effects on Cultured Chinese Hamster Ovary (CHO) Cells Using an *In Vitro* Cytogenetics Assay**

Key findings: Trospium chloride did not induce increases in chromosomal aberrations in cultured CHO cells, in the absence or presence of S-9, under the conditions of this study.

Study no: \_\_\_\_\_

Volume #9, and page #225

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: November, 1985

GLP compliance: yes

QA reports: yes (x) no ( )

Drug: batch # 2046, \_\_\_\_\_ pure

Formulation/vehicle: 0.2 ml distilled water

Methods:

Cell line: Chinese Hamster Ovary

Dose selection criteria: 5000 µg/ml was not toxic and was used as the high dose.

Test agent stability: (test conducted within three hours of mixture preparation)

Metabolic activation system: Aroclor 1254 induced rat liver S-9 fraction

Controls:

Vehicle: distilled water

Negative controls: distilled water

Positive controls: MMS, 100 µg/ml in DMSO, in the absence of S-9 and CPA,  
50 µg/ml in DMSO, in the presence of S-9

Exposure conditions:

Incubation and sampling times: two-hour incubation with assay at 24 hours

Doses used in definitive study: 5000, 1580, 500, 158, 50, 15.8, and 5 µg/ml

Study design: CHO cells at approximately 100% confluence were incubated with 0.2 ml test substance, negative control, or positive control in the absence or presence of S-9.

100 metaphases

from each culture were scored for aberrations.

Analysis:

No. of replicates: 2 plates at each dose and for each control

Counting method: not stated

Summary of individual study findings:

Study validity: The substance was tested under OECD guidelines. Positive and negative controls responded as expected. Toxicity (change in mitotic index) was not observed at the high dose of 5000 µg/ml.

Study outcome: Trosipium chloride did not induce increases in chromosomal aberrations in cultured CHO cells, in the absence or presence of S-9, under the conditions of this study.

**Study title: Micronucleus Test in Rats**

Key findings: No trosipium chloride related increase in PCEs was observed under the conditions of this study. It was concluded that trosipium chloride did not induce chromosomal aberrations at doses of 400 mg/kg.

Study no: TX120

Submission #1, volume #9, and page #245

Conducting laboratory and location: Department of Toxicology and Experimental Pathology, MADAUS AG, Ostmerheimer Str. 198, D-5000, Köln 91, FRG

Date of study initiation: 5 August 1991

GLP compliance: yes

QA reports: yes (x) no ( )

Drug: lot # 35348, pure

Formulation/vehicle: 100 mg/ml in distilled water

Methods:

Strains/species: Wistar rat, age 7 weeks, 205 g (male) and 182 g (female)

Dose selection criteria: 400 mg/kg is 0.8 X LD<sub>50</sub> in the test species

Test agent stability: confirmed stable under the conditions and duration of the study

Controls:

Vehicle: distilled water

Negative controls: distilled water

Positive controls: Cyclophosphamide, 100 mg/kg i.p. in saline

Exposure conditions:

Incubation and sampling times: 24, 48, and 72 hours

Doses used in definitive study: 400 mg/kg

Study design: The test substance was given by oral gavage to 15 male and 15 female rats. Twenty four, forty eight, and seventy two hours after treatment, 5 males and 5 females of each group were sacrificed, and bone marrow smears were prepared.

No. of replicates: 5 animals (1000 PCEs were scored for micronuclei per animal)

Summary of individual study findings:

Study validity: The substance was tested under OECD guidelines. Positive and negative controls responded as expected. PCE/NCE ratios did not demonstrate any toxic effect on bone marrow cells.

Study outcome: No trospium chloride related increase in PCEs was observed under the conditions of this study. It was concluded that trospium chloride did not induce chromosomal aberrations under the conditions of this study.

**Summary of Genetic Toxicity Findings:** 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.

### 3.4.5. Carcinogenicity

**Study title: 104 Week Dietary Carcinogenicity Study in Rats**

Study number: 431827

Submission #1, volume #9, and page #274

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 25 September 1986

GLP compliance: yes

QA report: yes ( x ) no ( )

Drug: lot #2772, — pure

Study Type: 2 year bioassay

Species/strain: Sprague-Dawley rats

Number/sex/group; age at start of study: 50/sex/group, 85 $\pm$ 2 g (male) or 60 $\pm$ 2 g (female),  
age 4 weeks

Animal housing: 5/cage

Formulation/vehicle: dietary, in feed

Drug stability/homogeneity: confirmed for duration of study

Doses: 0, 2, 20, and 200 mg/kg/day

Route of administration: oral, dietary

Frequency of drug administration: diet prepared weekly for first 13 weeks and  
then fortnightly thereafter

Dual controls employed: two identical control groups

## Results:

### Mortality:

Males (mg/kg/day)					Females (mg/kg/day)				
0	0	2	20	200	0	0	2	20	200
22	18	21	14	15	28	21	29	18	13

### Clinical signs:

Males (mg/kg/day)					Females (mg/kg/day)				
0	0	2	20	200	0	0	2	20	200

### Body weights:

Males (mg/kg/day)					Females (mg/kg/day)					
0	0	2	20	200	0	0	2	20	200	
$\Delta$ BW (%)	--	--	0.0	-9.0	-23.0	--	--	-8.4	-28.4	-37.1
$\Delta$ BW gain (%)	--	--	0.0	-17.0	-42.5	--	--	-14.0	-45.9	-61.7

### Food consumption:

Males (mg/kg/day)					Females (mg/kg/day)					
0	0	2	20	200	0	0	2	20	200	
$\Delta$ FC (%)	--	--	0	0	+3	--	--	-3	+6	+11

### Gross pathology:

Males (mg/kg/day)					Females (mg/kg/day)					
0	0	2	20	200	0	0	2	20	200	
Lungs, focal discoloration	3	4	6	9	17	3	7	6	10	13
Ovaries, cysts						4	5	6	9	12
Cervix, generalized enlargement						1	3	2	5	6
Intestines, distension	0	0	0	1	2	0	0	2	0	3

Histopathology:  
Non-neoplastic:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Cervix, stromal hypertrophy										
__grade +/-						0		0	0	1
__grade +						0		1	3	2
__grade ++						0		1	2	3
total						0		2	5*	6*
Uterus, squamous metaplasia										
__grade +/-						4		7	6	9
__grade +						3		1	1	3
__grade ++						0		0	0	1
total						7		8	7	13
Uterus, dilated/cystic glands										
__grade +/-						4		6	5	5
__grade +						1		2	1	2
__grade ++						0		2	0	1
__grade +++						0		0	1	0
total						5		10	7	8
Lungs										
__inflammation	26		19	31	32	22		20	27	24
__perivascular lymphocyte cuffing	4		9	3	7	3		11*	4	6
__increased alveolar macrophages	16		14	15	22	16		19	19	21
Pituitary,										
__focal anterior lobe hyperplasia	7		6	10	12	4		7	9	9
Skin/subcutis										
__localized epidermal hyperplasia	0		1	3	1	0		0	0	0
__epidermal/follicular cysts	1		1	2	2	0		1	2	1
Mammary glands, alveolar development										
__grade +/-	4		5	5	10	1		1	1	5
__grade +	3		1	4	3	2		4	1	1
__grade ++	0		0	0	0	2		0	0	2
total	7		6	9	13	5		5	2	8
Thyroid, C-cell hyperplasia										
__grade +/-	17		15	22	26	23		25	24	33
__grade +	18		16	15	13	15		10	17	9
__grade ++	1		9*	4	1	4		6	6	2
total	36		40	41	40	42		41	47	44
Thyroid, ultimobranchial cyst	7		6	6	8	1		4	8*	9*
Parathyroids, vacuolation (very mild)	1		0	2	1	1		1	0	6
Stomach, non-glandular										
__patchy epithelial hyperplasia	0		1	1	6*	1		2	2	1
__focal epithelial dysplasia	0		1	1	3	0		0	1	0
Jejunum, distension	0		0	0	3	1		1	0	3
Ileum, distension	0		1	2	3	2		2	2	6
Colon, distension	0		0	3	2	0		2	1	5

Neoplastic:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Skin/subcutis undifferentiated sarcoma (M)	0		2	1	4	1		1	0	0
Thyroid, C-cell adenoma (B)	10		7	6	1**	3		7	4	7
Uterine polyp (B)						2		1	6	3
Rhabdomyosarcoma (M)										1

Toxicokinetics:

plasma levels in ng/ml (human: Cmax~3 ng/ml)						
	Males (mg/kg/day)			Females (mg/kg/day)		
	2	20	200	2	20	200
Day 1 or 2	BLQ			BLQ		
hour 20				BLQ		
hour 0				BLQ		
hour 3				BLO		
hour 5						
hour 7						
hour 12						
hour 16						
Day 14 or 15	BLQ					
hour 20				BLQ		
hour 0				BLQ		
hour 3				BLQ		
hour 5				BLQ		
hour 7				BLO		
hour 12						
hour 16						
Day 28 or 29	BLQ			BLQ		
hour 20	BLQ				BLQ	
hour 0	BLQ				BLQ	
hour 3	BLQ				BLQ	
hour 5	BLQ				BLQ	
hour 7	BLO				BLQ	
hour 12					BLQ	
hour 16					BLQ	
AUC (human AUC~38 nghr/ml)						
	Males + Females (mg/kg/day)					
	2	20	200	2	20	200
Day 1 or 2	not calculable	151.5	368.1 (9.7 X)			
Day 14 or 15	not calculable	111.5	560.1 (14.7 X)			
Day 28 or 29	not calculable	65.0	752.9 (19.8 X)			

Summary of individual study findings:

This study was reviewed by CAC (see attachment at end of review), who felt that the doses were adequate. Statistical review of the results revealed no statistically significant increases in neoplasms.

**Study title: 78 Week Dietary Carcinogenicity Study in Mice**

Study number: — Project No. 431811 and 451576 (toxicokinetics)

Submission #1, volume # 12, and page #1

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 22 October 1986

GLP compliance: yes

QA report: yes (x) no ( )

Drug: lot #2772, — pure

Study Type: bioassay, 78 weeks

Species/strain: CD-1 mice

Number/sex/group; age at start of study: 50/sex/group, ~4 weeks of age, ~21 g (males) or 18 g (females)

Animal housing: individual

Formulation/vehicle: dietary, in feed

Drug stability/homogeneity: confirmed for duration of study

Methods:

Doses: 0, 2, 20, and 200 mg/kg/day

Basis of dose selection:

Route of administration: oral, dietary

Frequency of drug administration: diet prepared weekly for first 13 weeks and then fortnightly thereafter (every 4 weeks?)

Dual controls employed: two identical

Results:

**Mortality:**

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Premature deaths	10	6	9	19	43	11	12	8	12	15
Premature deaths (%)	20	12	18	38	86	22	24	16	24	30

Clinical signs: No treatment related effects were observed except swollen and cyanosed abdomens in high and intermediate dose male premature decedents.

**Body weights: at 78 weeks**

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Body weights (% of control)	--	--	97	98	90	--	--	92**	96	93**
Body weight gain (% of control)	--	--	91	91	72	--	--	79	88	84

**Food consumption:**

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Total (% of control)	--	--	97	99	96	--	--	98	99	95

## Gross pathology:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Lungs, masses/nodules	5	14	9	9	2	0	6	13	6	3
Abdomen										
__cyanosed	5	2	0	1	8	1	4	2	0	0
enlarged/swollen	9	7	3	8	16	11	5	2	4	6
Rectum, enlarged/distended	0	0	0	2	15	0	0	0	0	3
Colon, enlarged/distended	0	0	0	2	17	0	0	0	0	1
Cecum, enlarged/distended	0	0	0	1	7	0	0	0	0	0
Intestines, enlarged/distended	0	0	0	1	11	1	0	0	0	1
Skin/subcutis, focal/irregular discoloration	0	0	0	3	4	2	1	0	0	2
Gall bladder, distension	0	0	0	0	2	0	0	0	0	0

## Histopathology:

Non-neoplastic: (43 of 50 high dose males died prematurely)

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Colon distension	0		0	4	24	0		1	0	4
Heart										
__dilated chambers	0		0	0	4	0		0	0	0
myocardial degeneration	0		0	0	2	0		0	0	0
Ileum distension	0		1	2	5	0		0	0	1
Liver										
__inflammation	4		8	7	8	5		9	10	5
__coagulative necrosis	0		0	0	10	0		1	0	2
single cell necrosis	0		0	2	13	0		0	0	0
Lungs terminal congestion	0	0	2	0	7	3	0	0	1	10
Mesenteric lymph node										
__lymphocyte necrosis	0		0	1	3	0		0	0	0
__fibrous proliferation	0		0	0	0	0		0	0	3
sinus erythrophagia	7		5	8	7	0		0	2	4
Skin										
__adnexal atrophy	1		1	3	5	0		1	0	0
ulceration	0		3	4	2	1		0	0	2
Spleen lymphocyte necrosis	0		0	0	3	0		0	0	0
Thymus										
__lymphocyte depletion	1		0	1	14	0		1	0	0
__lymphocyte necrosis	0		0	2	1	1		0	0	0
fat replacement	0		0	3	0	0		1	0	0
Urinary bladder proteinaceous plug	1		2	6	3					
Rectum distension	0		0	4	22	0		0	0	4
Cecum distension	0		0	0	9	0		0	0	3

Neoplastic: (43 of 50 high dose males died prematurely)

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Hepatic adenoma	8		4	6	0	1		1	1	0
Hepatocyte carcinoma	0		0	3	1	0		0	0	0
Pulmonary adenoma	4	10	7	11	4	1	4	10	4	3
Pulmonary carcinoma	1	6	2	3	0	1	3	0	2	1
Mammary carcinoma, type C	0		0	0	0	0		0	0	1
Hemangiosarcoma, skin	0		2	0	0	0		0	0	1
Hemangiosarcoma, spleen	0		2	0	0	0		1	0	0
Total hemangiosarcoma	0		4	0	0	0		1	0	1
Multilobar osteoma	0		0	0	0	0		0	0	1

Toxicokinetics: (blood levels similar to rat; ~20 times human AUC)

	Males (mg/kg/day)			Females (mg/kg/day)		
	2	20	200	2	20	200
Day 1 or 2	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
hour 20			BLQ			BLQ
hour 0			BLQ			BLQ
hour 3			BLQ			BLQ
hour 5			BLO			BLQ
hour 7						BLQ
hour 12						BLQ
hour 16			BLQ			BLQ
Day 14 or 15	BLQ	BLQ	BLQ	BLQ	BLQ	BLO
hour 20			BLQ			
hour 0			BLO			BLQ
hour 3						BLQ
hour 5			BLO			BLQ
hour 7						BLQ
hour 12			BLQ			BLQ
hour 16			BLQ			BLQ
Day 28 or 29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
hour 20			BLQ			BLQ
hour 0						BLQ
hour 3			BLQ			BLQ
hour 5			BLQ			BLQ
hour 7						BLQ
hour 12						BLQ
hour 16						BLO

Summary of individual study findings: This study was reviewed by CAC (see attachment at end of review), who felt that the doses were adequate. Statistical review revealed no statistically significant increase in neoplasms.

In addition, the requested re-reading of the lung neoplasm slides was received from the sponsor and was acceptable, as were the historical data received for that tissue. Those data were forwarded to the statistician along with CAC's comments. Analysis incorporated survival data, due to the low rate of survival in some groups and the presence of tumors in those groups. There were no positive trends for neoplasms after incorporating historical data.

**3.4.6. Reproductive and developmental toxicology**

**Fertility and early embryonic development**

**Study title: Fertility and Generation Study on Rats Given Intragastric Doses of MP 194**

Study no.: 8.8.1985

Volume #8, and page #297

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: November 1981

GLP compliance:

QA reports: yes ( ) no ( x)

Drug: lot # 7374, % pure

Formulation/vehicle: distilled water

Methods:

Species/strain: Wistar rats (SPF), ~200 g

Doses employed: 0, 2, 20, and 200 mg/kg/day

Route of administration: intragastric

Study design: Treatment of the F0 generation began approximately 61 days (male) and 14 days (females) before mating. The treatment of females continued throughout gestation and to the end of the lactation period. Half the dams (group A) were sacrificed on Day 20 of gestation and the rest (group B) were allowed to rear their offspring (F1). Two animals from each F1 group were mated at approximately 10 weeks of age. The F2 generation was followed until weaning.

Number/sex/group: 30 F0

Fetal parameters and endpoints evaluated: Fetal survival and body weights, development (coat growth, raising of ears, eye opening, tooth eruption) malformations, reproduction

**Results:**

Mortality: Deaths of 4 high dose males and 2 high dose females were attributed to dosing errors.

Clinical signs: No treatment related effects were observed.

Body weight:

	Males (mg/kg/day)				Females (mg/kg/day)			
Weight following final administration	330.1	324.1	324.9	298.1	201.9	202.4	213.0	207.3
Weight gain (g)	130.5	126.3	125.6	103.9	5.8	4.7	7.1	3.1
Weight gain (%)	65.4	63.9	63.0	53.5	3.0	2.4	3.4	1.5
Group A, wt gain during pregnancy (g)	--	--	--	--	96.5	93.1	94.1	86.7
Group A, wt gain during pregnancy (%)	--	--	--	--	46.8	44.2	43.3	42.4
Group B, wt gain during pregnancy (g)	--	--	--	--	104.4	96.1	96.2	88.9
Group B, wt gain during pregnancy (%)	--	--	--	--	50.1	46.2	43.6	40.4
Group B, wt gain during lactation (g)	--	--	--	--	-5.1	-4.7	-5.1	+1.2
Group B, wt gain during lactation (%)	--	--	--	--	-2.2	-2.1	-2.1	+0.5

**Terminal and necroscopic evaluations:**

Group A	Females (mg/kg/day)			
	0	2	20	200
Total number of fetuses	137	135	111	116
live	136	134	111	116
dead	1	1	0	0
Corpora lutea	154	147	127	136
Implantations	150	141	122	130
Resorptions	13	6	11	14
Uterine weight (g)	3.76	3.60	3.65	3.47
Placental weight (g)	6.31	6.01	5.73	5.81

Group B	Females (mg/kg/day)			
	0	2	20	200
Total number of fetuses	162	155	155	136
live	162	155	154	132
dead	0	0	1	4

**Summary of individual study findings:** In the 200 mg/kg/day F0 group, weight gains were marginally lower as was the number of live fetuses in the F1 generation. No other treatment related effects were observed including no effects on the F2 generation. No effect on mating behavior or fertility of the F0 or F1 generations was observed at any dose.

**Embryofetal development**

**Study title: Prenatal Toxicity of MP 194 in Rats after Intra-gastric Administration**

Study no. 18.1.1982

Volume #9 and page #357

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: August, 1981

GLP compliance:

QA reports: yes ( ) no ( )

Drug: lot #7374, % pure

Formulation/vehicle: distilled water

Methods:

Species/strain: Wistar (SPF) rat, 150-200 g

Doses employed: 0, 2.0, 20.0, or 200.0 mg/kg

Route of administration: intra-gastric

Study design: drug or vehicle administered days 6 – 16 of gestation, sacrifice day 20

Number/sex/group: 20/group

Parameters and endpoints evaluated: Abnormalities and malformations of the skull, face, spinal column, including the tail, the extremities, the thoracic and abdominal regions, as well as the anogenital region

Results:

Mortality/clinical signs: No treatment related effects were observed.

Body weight: No treatment related effects were observed.

Terminal and necroscopic evaluations:

Dams: No treatment related effects were observed on the numbers of corpora lutea, implantations or resorptions. No effects on uterine or placental weight were observed. No effects on the number of live fetuses, preimplantation or postimplantation losses were observed.

Offspring: No mortalities were observed. No effects on fetal weight or litter weight were observed. No fetal malformations were observed.

**Study title: Prenatal Toxicity of MP194 in Rabbits after Intra-gastric Administration**

Study no.: 24.3.1980

Volume #8, page # 383

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 7.2.1978

GLP compliance:

QA reports: yes ( ) no (x)

Drug: lot #5798, % pure

Formulation/vehicle: distilled water

Methods:

Species/strain: Russian rabbits, ~2300 g

Doses employed: 0, 0.5, 5.0, or 50.0 mg/ml

Route of administration: intra-gastric

Study design: drug administered days 6 – 18 of gestation, sacrifice day 28

Number/sex/group: 11-14/group

Parameters and endpoints evaluated: abnormalities and malformations of the skull, face, spinal column, including tail, extremities, thoracic and abdominal regions and anogenital region.

Results:

Mortality: One death in the high dose group was reported to be the result of an error in intubation.

Clinical signs: No treatment related effects were observed.

Body weight: No treatment related effects were observed.

Toxicokinetics: See Toxicokinetic Study in Pregnant Rabbits (TX178)

Terminal and necroscopic evaluations:

Dams:

	0 mg/kg/day	0.5 mg/kg/day	5.0 mg/kg/day	50.0 mg/kg/day
Resorptions	17	17	4	23 (11 from 1 dam)
No. of live fetuses	56	63	61	67
No. of dead fetuses	0	0	0	4 (2 from one dam)
Wt. of uterus (g)	325.6	331.1	313.5	383.7
Wt. of placenta (g)	289.0	308.1	318.5	336.4
Preimplantation losses (%)	28.4	10.1	23.5	13.8
Postimplantation losses (%)	23.3	21.3	6.2	28.7

## Offspring:

	0 mg/kg/day	0.5 mg/kg/day	5.0 mg/kg/day	50.0 mg/kg/day
Litter weight (g)	1716.1	1885.1	1907.2	1968.9
Average fetal weight (g)	373.4	333.1	346.1	383.7
Malformations	0	1 fetus*	1 fetus**	1 fetus***

\* gross deformities of the spinal column, the pelvic girdle, and the back legs plus severely retarded ossification of the cranial bones and an oedema in the neck region (reported as one fetus in the "2.0 mg/kg group" [low dose in rats was 2.0 mg/kg/day], but assumed to be the 0.5 mg/kg/day low dose group in rabbits).

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

\*\*\* umbilical hernia with prolapse of the liver

Summary of individual study findings: No maternal or fetal effects were observed up to 50 mg/kg/day or ~ 5-6 times the expected clinical exposure (AUC, see toxicokinetic study below).

**Study title: Toxicokinetic Studies in Pregnant Rabbits**

Study no.: —Project No. 451597, Madeus Code TX178

Volume #9 and page #001

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 18 January, 1994

GLP compliance: yes

QA reports: yes (x) no ( )

Drug: lot #43694, — pure

Formulation/vehicle: distilled water

**Methods:**

Species/strain: female White Russian rabbits, 4-5 months of age, 1.89-2.50 kg

Doses employed: 2, 20, or 200 mg/kg/day

Route of administration: oral gavage

Study design: drug administered days 6-18 of gestation

Number/sex/group: 6/group

Parameters and endpoints evaluated: Toxicokinetics, clinical signs

**Results:**

Mortality: One high dose animal was killed in extremis due to treatment related clinical signs. One animal was kill in extremis due to a large lump on the ventral neck (low dose group). One animal was found dead after 2 days of dosing (mid dose group, reported to be due to misdosing).

Clinical signs: Reduced fecal output, hunched posture, and diarrhea were observed in the high dose group. Thinness, and a stained, matted and wet perigenital region were also observed in the high dose animal killed in extremis.

Body weight: There was a slight suppression in body weight gain in the high dose group.

Food consumption: There was a marked reduction in body weight gain in the high dose group.

**Toxicokinetics:**

Dose	Day of gestation					
	Day 06		Day 12		Day 18	
	AUC (nghr/ml)	Cmax (ng/ml)	AUC (nghr/ml)	Cmax (ng/ml)	AUC (nghr/ml)	Cmax (ng/ml)
2	43.6	—	74.8	—	42.3	—
20	27.6	—	58.8	—	84.6	—
200	1469.9	—	1312.0	—	1070.8	—

**Prenatal and postnatal development**

**Study title: MP194-S-1: Effects of Oral Administration Upon Peri- and Post-Natal Development of the Rat**

Study no.: 81/MDS001/071

Volume #9, and page #74

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 27 October 1980

GLP compliance: \_\_\_\_\_

QA reports: yes (x) no ( )

Drug: lot #7827/8, — pure

Formulation/vehicle: distilled water

**Methods:**

Species/strain: Sprague-Dawley rats, CD strain, 8-9 weeks of age, 201-249 g

Doses employed: 0, 2, 20, or 200 mg/kg/day

Route of administration: oral gavage

Study design: drug administration day 15 post coitum until day 21 post partum, sacrifice day 21 post partum

Number/sex/group: 20 females/group

Parameters and endpoints evaluated: gestation length, gestation index, parturition, litter size, live births, viability indices, bodyweight of offspring at Day 1 and at weaning, physical development of offspring, responses to auditory and visual stimuli, times of pinna unfolding, hair growth, tooth eruption, eye opening

**Results:**

Mortality: 2 deaths occurred in the high dose group

Clinical signs: Rapid and irregular breathing, pupillary dilatation, and increased excitability were observed in the high dose group.

Body weight: Body weight gains were significantly depressed in the high dose group from post coitum days 15 to 22, with a 5.1% decrease on day 22. Body weights were increased over control in the high dose group during lactation.

**In-life observations:****Offspring:**

	0 mg/kg	2 mg/kg	20 mg/kg	200 mg/kg
Live birth index (%)	99	99	98	100
Viability index (%)				
Day 4 post partum	97	100	99	92
Day 7 post partum	97	100	99	90
Day 11 post partum	97	99	99	90
Day 14 post partum	97	99	99	90
Day 18 post partum	97	99	99	90
Day 21 post partum	97	99	99	90

Summary of individual study findings: A no effect level for maternal and fetal toxicity was 20 mg/kg/day. At 200 mg/kg/day, 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.

**Reproductive toxicology summary:**

In a rat fertility study, treatment of the F0 generation began approximately 61 days (male) and 14 days (females) before mating. The treatment of females continued throughout gestation and to the end of the lactation period. In the 200 mg/kg/day F0 group (10 multiples of the clinical dose by AUC), weight gains were marginally lower as was the number of live fetuses in the F1 generation. No other treatment related effects were observed. No effect on mating behavior or fertility of the F0 or F1 generations was observed at any dose.

In a segment II study in rats, with treatment administered on days 6 – 16 of gestation, no maternal or fetal effects were observed at 200 mg/kg or 10 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 (~ 10 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 50 mg/kg/day or ~5-6 times the expected clinical exposure (AUC).

In rats, maternal toxicity, and a decrease in fetal survival are observed at 200 mg/kg/day, depending on the timing and duration of exposure. In rabbits, maternal toxicity is also observed at 200 mg/kg/day. No treatment-related malformations were observed in rats or rabbits, and no developmental delays were observed in rats.

While the preclinical reproductive studies were performed prior to the current standards for these studies, the design and conduct of the studies is generally consistent with the requirements of a modern GLP study and/or more current data has been added to

supplement high dose and pharmacokinetic data. There is also experience with the drug in a reproductively active clinical population and in clinical trials.

### 3.5 SPECIAL TOXICOLOGY STUDIES

**Study title: Screening of AS17 (Trospium chloride) for Activity on Isolated Mast Cells of Sensitized Rats.** Trospium chloride had no effect on sensitized mast cells (Wistar rat) measured by release of histamine (F, 10/group,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$  g/ml).

**Study title: AS17 (Trospium chloride) and Anaphylactic Shock in Guinea Pigs.** Trospium chloride had no effect on anaphylactic shock in female guinea pigs (5/group, i.p., 10 mg/kg).

**Study title: AS17 (Trospium chloride) and Effects on Antibodies in Rabbits.** Trospium chloride had no effect on antibody titers in New Zealand White rabbits (F, 8/group, 1.0 mg/kg).

**Study title: Trospium chloride – Screening for Antiinflammatory Activity in Rat Paw Edema.** Trospium chloride had no effect on Viscarin-carrageenin or ovalbumin induced paw edema in male or female rats (10/group, s.c., 1 mg/kg).

**Study title: Trospium chloride – Screening for Antiinflammatory Activity in the Cotton Pellet Test.** Trospium chloride showed marginal activity in the formation of granuloma tissue in female Wistar rats (13-15/group, s.c.) at 1 mg/kg.

**Study title: Trospium chloride: Investigations to Screen Trospium chloride for Effects on the Microcirculations, i.e. on the Permeability of the Plasma-lymph Barrier in Rats.** Trospium chloride had no effect on the plasma-lymph barrier in male Wistar rats (10/group, 1 mg/kg).

### 3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions and Recommendation: Approval is recommended.

Suggested labeling: See Executive Summary, p. 6.

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

### 3.7 APPENDIX/ATTACHMENTS

**Executive CAC**

**Date of Meeting:** 1 May, 2001

**Committee:** Joseph DeGeorge, Ph.D., HFD-024, Chair  
 Joseph Contrera, Ph.D., HFD-900, Member  
 Jeri El Hage, Ph.D., HFD-510, Alternate Member  
 Alex Jordan, Ph.D., HFD-580, Team Leader  
 Laurie McLeod, Ph.D., HFD-580, Presenting Reviewer

Author of Draft: Laurie McLeod

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

**IND # 61381**

**Drug Name:** Trospium Chloride

**Sponsor:** Interneuron Pharmaceuticals, Inc.

**DRUG CODE#:** MP 194

**DIVISION:** Division of Reproductive and Urologic Drug Products

**LABORATORY:**

**CARCINOGENICITY STUDY REPORT DATE:** 25 Jun 1991

**THERAPEUTIC CATEGORY:** urinary incontinence

**PHARMACOLOGICAL/CHEMICAL CLASSIFICATION:** anticholinergic

**MUTAGENIC/GENOTOXIC:** negative

**Mouse Carcinogenicity Study**

**MOUSE STUDY DURATION:** 78 weeks

**STUDY STARTING DATE:** 22 October 1986

**STUDY ENDING DATE:** 27 April 1988

**ROUTE:** oral, dietary

**DOSING:** CD-1 mice (50/sex/dose) were administered 0, 0, 2, 20, or 200 mg/kg/day trospium chloride. As shown by effects on survival, 20 mg/kg/day was a maximally tolerated dose in males. Decreases in body weight over that of control mice were seen in all groups of drug treated female mice. No effects on food consumption were observed.

**MOUSE CARCINOGENICITY:** negative, pending statistical survival, background and tumor trend analysis

**MOUSE TUMOR FINDINGS (details): (43 of 50 high dose males died prematurely)**

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Hepatic adenoma	8		4	6	0	1		1	1	0
Hepatocyte carcinoma	0		0	3	1	0		0	0	0
Pulmonary adenoma	4	10	7	11	4	1	4	10	4	3
Pulmonary carcinoma	1	6	2	3	0	1	3	0	2	1
Mammary carcinoma, type C	0		0	0	0	0		0	0	1
Hemangiosarcoma, skin	0		2	0	0	0		0	0	1
Hemangiosarcoma, spleen	0		2	0	0	0		1	0	0
Total hemangiosarcoma	0		4	0	0	0		1	0	1

Multilobar osteoma	0	0	0	0	0	0	0	0	1
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**Rat Carcinogenicity Study / Rat Dose Selection**

RAT STUDY DURATION: 104 weeks

STUDY STARTING DATE: 25 September 1986

STUDY ENDING DATE: 12 October 1988

ROUTE: oral, dietary

DOSING: Sprague-Dawley rats (50/sex/dose) were administered 0, 0, 2, 20, or 200 mg/kg/day trospium chloride. As shown by a decrease in body weights in both sexes, 200 mg/kg/day was a maximally tolerated dose. No effects on food consumption were observed.

RAT CARCINOGENICITY: negative, pending statistical background and tumor trend analysis

**RAT TUMOR FINDINGS:**

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	2	20	200	0	0	2	20	200
Skin/subcutis										
undifferentiated sarcoma (M)	0		2	1	4	1		1	0	0
Thyroid, C-cell adenoma (B)	10		7	6	1**	3		7	4	7
Uterine polyp (B)						2		1	6	3
Rhabdomyosarcoma (M)										1

**Executive CAC Recommendations and Conclusions:**

**Mouse:**

\* The Committee concluded that the mid dose in males was high enough to conclude that the study was valid based on an effect on survival in that group. This was an issue because of the premature deaths in the HD males and the large spread in doses between the MD and HD animals. The dose selection in females was considered adequate based on decreases in body weight and the observation that gavage dosing in other species also causes decreased body weight.

\* The committee was concerned about the examination of the 2<sup>nd</sup> control group only upon observation of an apparent increase in treatment-related tumors compared to the 1<sup>st</sup> control (e.g. as selectively performed to show the absence of trend in lung neoplasms). The sponsor should be asked to provide information on their approach, and if blinded rereading of all slides from the selected tissue was not conducted, such should be undertaken.

\* The committee recommended that the division analyze the tumors with and without the high dose group, considering the sponsor's background incidences from 5 or 6 studies from the same time period (1986) after a similar dosing period (78 weeks). (Note: tumor background incidences have been requested for both mice and rats.)

**Rat:**

\* The Committee felt that a maximally tolerated dose was achieved based on body weight changes, as it has been demonstrated that the drug via gavage dosing in other species also causes decreases in body weight.

Joseph DeGeorge, Ph.D.  
Chair, Executive CAC

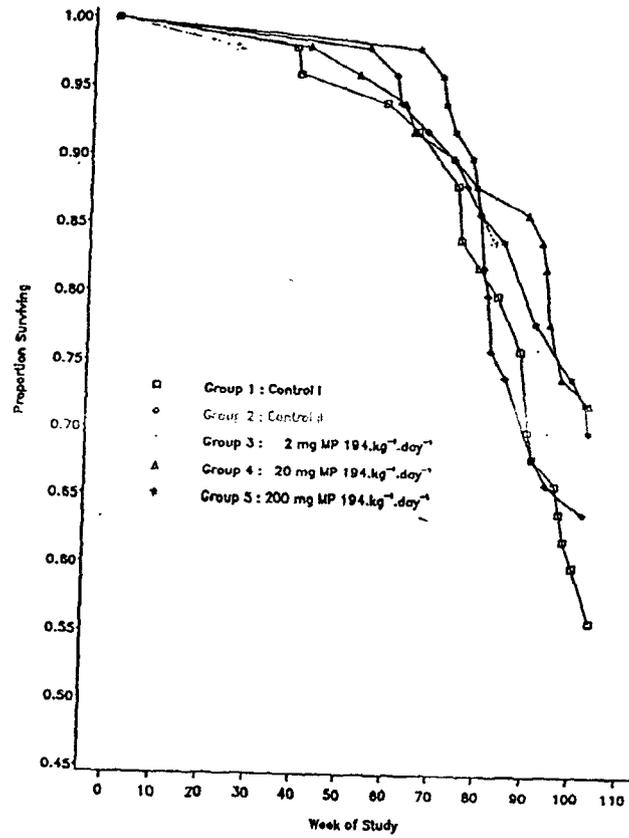
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DRUDP/Division File, HFD-580  
Alex Jordan/Team leader, HFD-580  
Laurie McLeod/Reviewer, HFD-580  
ASeifried, HFD-024

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ON ORIGINAL**

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FIGURE 1  
MP 194  
104 Week Dietary Carcinogenicity Study in Rats  
Kaplan Meier Survival Curve: Males



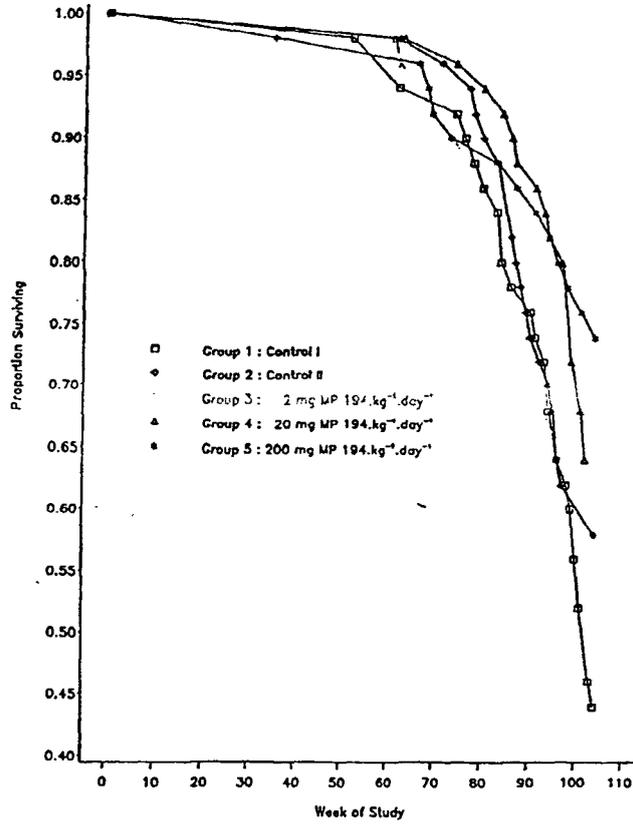
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FIGURE 2  
MP 194  
104 Week Dietary Carcinogenicity Study in Rats  
Kaplan Meier Survival Curve : Females



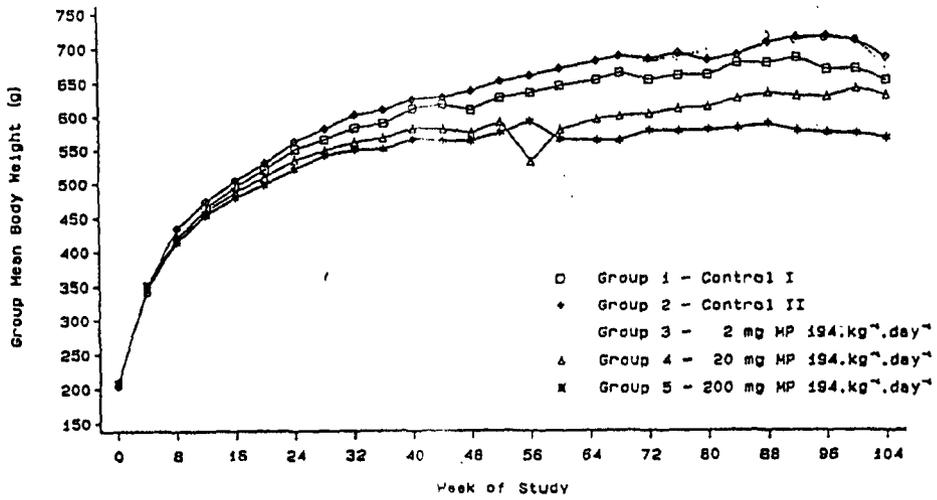
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FIGURE 3  
MP 194  
104 Week Dietary Carcinogenicity Study in Rats  
Group Mean Body Weights (g) : Males



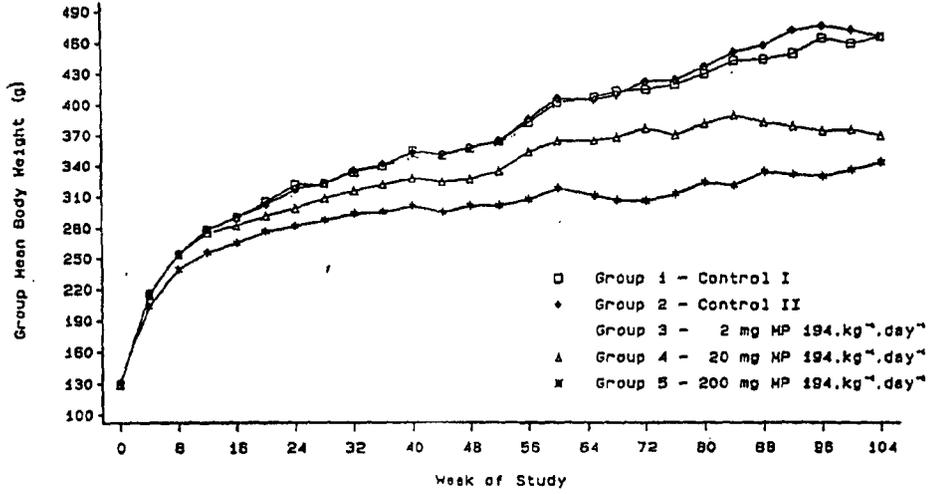
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FIGURE 4  
HP 194  
104 Week Dietary Carcinogenicity Study in Rats  
Group Mean Body Weights (g) : Females

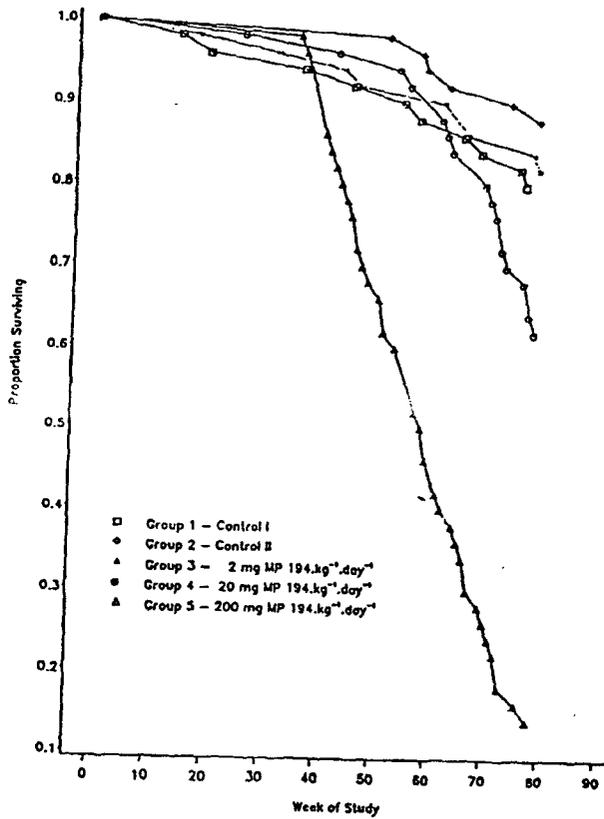


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FIGURE 1  
MP 194  
78 Week Dietary Carcinogenicity Study in Mice  
Kaplan-Meier Survival Curve: Males



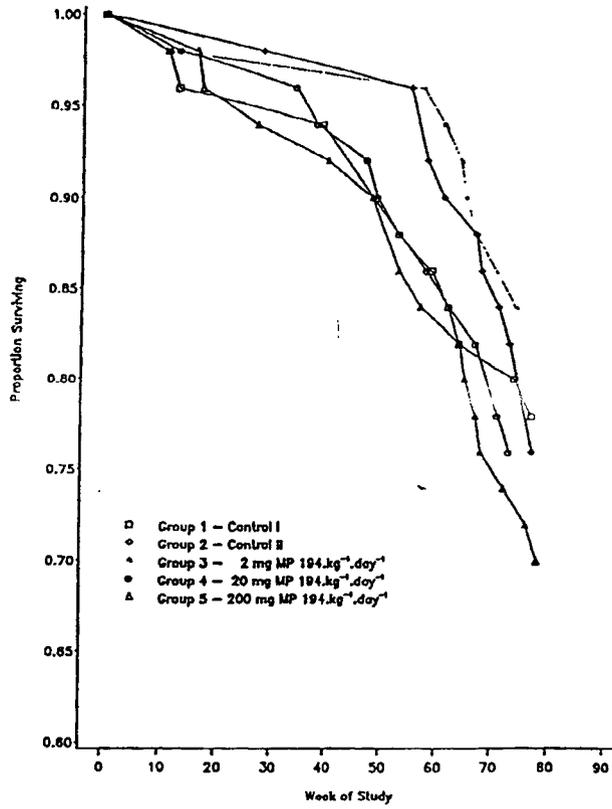
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FIGURE 2  
MP 194  
78 Week Dietary Carcinogenicity Study in Mice  
Kaplan-Meier Survival Curve: Females



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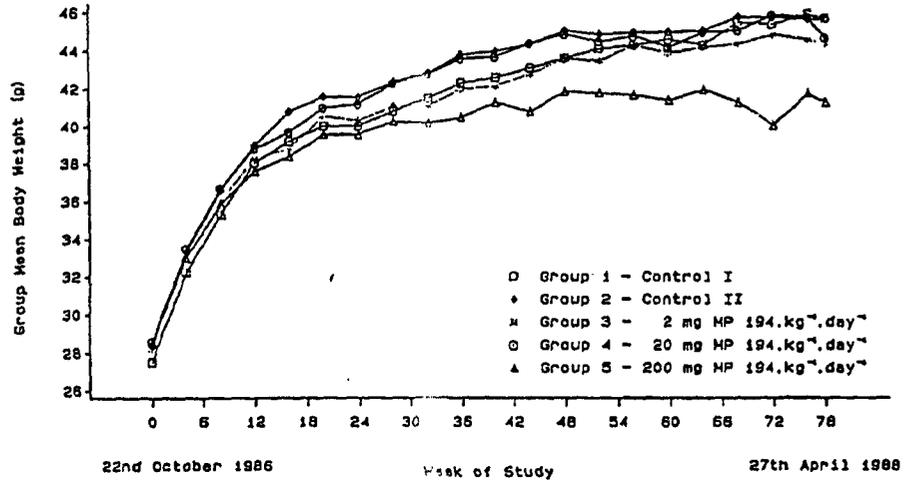
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FIGURE 3

HP 194  
78 Week Dietary Carcinogenicity Study in Mice  
Group Mean Body Weights (g) : Males



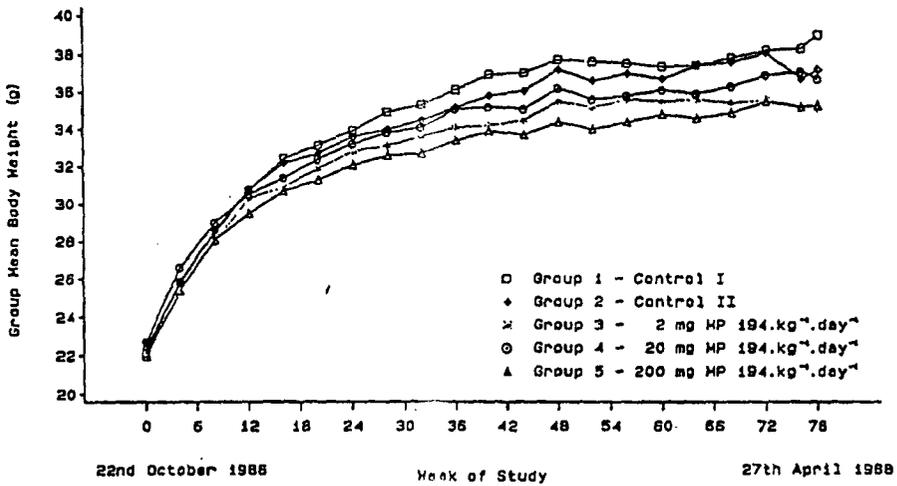
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FIGURE 4  
HP 194  
78 Week Dietary Carcinogenicity Study in Mice  
Group Mean Body Weights (g) : Females



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012 114

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/s/

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Laurie McLeod  
2/12/04 12:02:32 PM  
PHARMACOLOGIST

Lynnda Reid  
2/12/04 12:20:13 PM  
PHARMACOLOGIST