

Table 6.2. Design of the 24-Month Inhalation Carcinogenicity Study in Rats

Groups	I	II	III	IV	V	VI	VII ^a
Treatment	Air	Veh. I	Veh. II	Tiotropium			No
Tiotropium (µg/kg/day) ^b	0	0	0	0.6	1.5	5.3	0
Tiotropium (mg/ml) ^c	0	0	0	0.05 ^c	0.10 ^c	0.40	0
Rats/sex	50	50	50	50	50	50	20

- A group for environmental control.
- The estimated deposited dose. The report describes the dose as 10, 25 and 75 µh/kg/day based on total inhaled doses.
- Tiotropium concentrations in the solution used to generate aerosols. The concentration was 0.10 and 0.20 mg/ml for the low and mid dose groups, respectively, during the initial five weeks.

Methods:

Doses: 0.6, 1.5, 5.3 µg/kg/day. The administered doses were estimated using the mean mass aerodynamic diameter of the aerosol (MMAD), deposition fraction and aerosol tiotropium concentration¹. Aerosol drug concentrations were analyzed (with HPLC) twice a week for first 13 weeks and every other week afterward. Aerosol particle size distributions were determined weekly with the , a laser detection system. The  cascade impaction  was used to measure particle sizes on week 102. The purpose of the  impactor was to generate reference data.

Basis of dose selection: The MTDs in a 13-week inhalation study and a 12-month inhalation study in the same strain.

Restriction paradigm for dietary restriction studies: None.

Route of administration: Nose-only inhalation.

Frequency of drug administration: once daily, 1 hr/exposure for 104 weeks.

Dual controls employed: yes. One air control, two vehicle controls.

Interim sacrifices: No.

Satellite PK or special study group(s): None.

Deviations from original study protocol: Aerosol tiotropium concentrations were decreased after five weeks of exposure. The decrease was from 0.10 and 20 mg/ml to 0.05 and 0.10 mg/ml for the low and mid dose groups, respectively.

¹ The following criteria and parameters were used to derive the administered doses:

$$\text{Dose } (\mu\text{g}/\text{mg}/\text{day}) = (\text{RV} \times \text{CC}) \times \text{DF} / \text{BW},$$

Where: RV = (Respiratory volume, 12 L) = Minute volume (0.2 L/min) x 60 min/day; CC = Mean chamber concentration (µg/L); DF = deposition fraction; BW = Mean mid-week body weight (kg)

Statistical methods:

Data:	Statistical Method
Body weight, food consumption, hematology:	ANOVA
Mortality:	Kaplan-Meier survival curve and Wilcoxon ranking test, $\alpha = 0.05$
Histology data:	Fisher's Exact test
Tumor data:	Fisher's Exact test

Observations and times:

<i>Clinical signs:</i>	Daily.
<i>Body weights:</i>	Weekly for the first 13 weeks, and once every 4 weeks thereafter
<i>Food consumption:</i>	Weekly for the first 13 weeks, and once every 4 weeks thereafter
<i>Ophthalmology:</i>	Weeks 53/53, 78/79, and 103/104
<i>Hematology:</i>	Pre-dosing, weeks 13, 52 and 104
<i>Clinical chemistry:</i>	Not done.
<i>Organ weights:</i>	Not recorded
<i>Gross pathology:</i>	Terminal sacrifice or time of death for prematurely terminated/dead animals
<i>Histopathology:</i>	A complete panel, see histology inventory table for details.
<i>Toxicokinetics:</i>	Months 12 and 23 (5 rats/sex/dose), ca 15 min after exposure

Results:

Doses: Table 6.3 shows the estimated tiotropium doses in the treatment groups. Note that there are significant differences in the pulmonary doses between the study report and the review. The differences are attributed to the deposition fraction employed to derive the estimated pulmonary doses. The report considers 100% of the drug in aerosol particles with aerodynamic diameter of μm as the pulmonary doses while the review considers 7% of the aerosol depositing in the pulmonary region.

Table 6.3. Dosing Estimates of the 24-month Inhalation Carcinogenicity Study in Rats:

Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle	Vehicle	Tiotropium		
Aerosol Tiotropium ($\mu\text{g/L}$) ^a	0	0	0	0.24 ± 0.09	0.56 ± 0.16	1.95 ± 0.44
Mean fraction of aerosol particle with MMAD	-	88.1%	95.8%	87.8%	88.1%	86.9%
Total inhaled dose ($\mu\text{g/kg/day}$)	0	0	0	9.3 ± 5.4	21.7 ± 7.6	74.7 ± 15.8
Pulmonary dose ($\mu\text{g/kg/day}$)						
Reported Dose ^b	0	0	0	9.1 ± 5.1	21.7 ± 8.0	73.2 ± 16.7
Review's Estimated Dose ^c	0	0	0	0.6	1.5	5.3

- a. Mean aerosol tiotropium concentrations based on HPLC analysis of samples taken twice weekly for the first 13 weeks and once every two weeks for the remainder of the study.
- b. These estimates are based on a deposition factor of 100% for particles with aerodynamic diameter of μm

c. These estimates are based on a deposition fraction of 7% for aerosol particles with aerosol diameters of μm . The review uses them as the actual exposure in rats.

Table 6.4 summarizes the characterization of the particle sizes. The MMADs measured with the μm were between μm . Approximately 87% or more of aerosol particles had aerodynamic diameter of μm . Note that the MMAD from the μm impactor is slightly smaller than that from the μm . The reason for the difference is unknown.

Table 6.4 Particle Size Characterization

Groups	MMAD \pm GSD (μm)					
	I	II	III	IV	V	VI
Treatment	Air	Vehicle 1	Vehicle 2	Br 679 BR		
Impactor ^c						

- a. The aerosol chamber concentration of 0.07 $\mu\text{h/L}$ for the week 75 was excluded from the calculation.
- b. The overall mean mass aerodynamic diameter. It excluded those with mean particle size of μm as stated in the study report: "... [T]he sample was considered to be unrepresentative of the test aerosol produced by the μm ... occurred as a result of some artifact of the sampling procedure, [p 29, ... Report 653315 (reissued)]." The report was unclear at the number of excluded points.
- c. The μm cascade impaction was done on week 102.

Mortality

No treatment-related effects were observed. The survival rates between the treated and control groups were similar. See Figure 6.1 for survival-time curve and Table 6.5 for the mortality rate of the study.

Table 6.5 Mortality Rate of 24-month Carcinogenicity study in Rats

Groups	I	II	III	IV	V	VI
Treatment	Controls			Tiotropium		
Tiotropium Dose (mg/kg/day)	0	0	0	0.6	1.5	5.3
Males, Incidence of deaths	6	9	10	9	4	7
Mortality rate (%)	12	18	20	18	8	14
Females, Incidence of deaths	6	12	11	9	9	2
Mortality rate (%)	12	24	22	18	18	4

² Particle sizes measured by the μm cascade impactor was slightly smaller μm , than that of the μm .

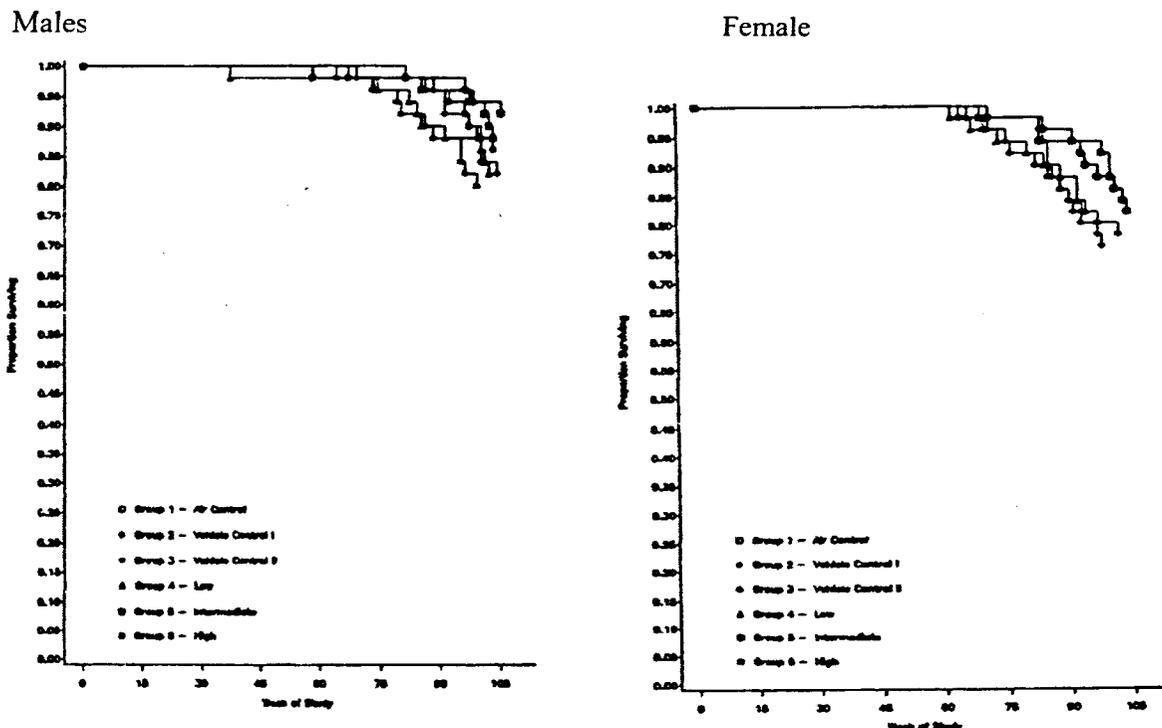


Figure 6.1 Survival curve of the 24-month inhalation study in rats.

Clinical signs: No treatment-related effects were observed. There was no significant difference in the incidence on palpable mass between the control and treated groups (Table 6.6).

Table 6.6 Palpable Masses in 24-month Inhalation Study in Rats

Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicles		Tiotropium (mg/kg/day)		
Aerosol Tiotropium	0	0	0	0.6	1.5	5.3
# Males with masses	14	16	15	9	10	10
# Females with masses	11	13	6	9	5	6

Body weights:

The tiotropium-treated rats showed dose- and treatment duration-dependent decreases in body weights. (Table 6.7). By the end of the treatment period, the high dose males and females showed respective decreases of 19% and 14% in the means of the absolute body weight when compared to the control groups. The vehicle had no effect on body weights (compared to the air control). Figure 6.2 presents a body weight-time course of the study.

Table 6.7 Body Weights in 2-Year Inhalation Carcinogenicity Study in Rats

Groups	I	II	III	IV	V	VI
Treatment	Controls			Tiotropium (mg/kg/day)		
	Air	Vehicle	Vehicle	0.6	1.5	5.3
Male: Week 53 (g)	497	490	494	465 (↓6%) ¹	444 (↓10%)	425 (14%)
Week 104 (g)	532	536	540	488 (↓9%)	458 (↓15%)	435 (↓19%)
Female: Week 53 (g)	262	265	271	251 (↓5%)	247 (↓7%)	242 (↓9%)
Week 104 (g)	292	287	293	263 (↓9%)	254 (↓12%)	253 (↓13%)

1. Numbers in the parenthesis represent changes compared to the average of means of the control groups

Food consumption: No treatment-related effects were observed.

Ophthalmology: No treatment-related effects were observed.

Hematology: No remarkable effects were observed.

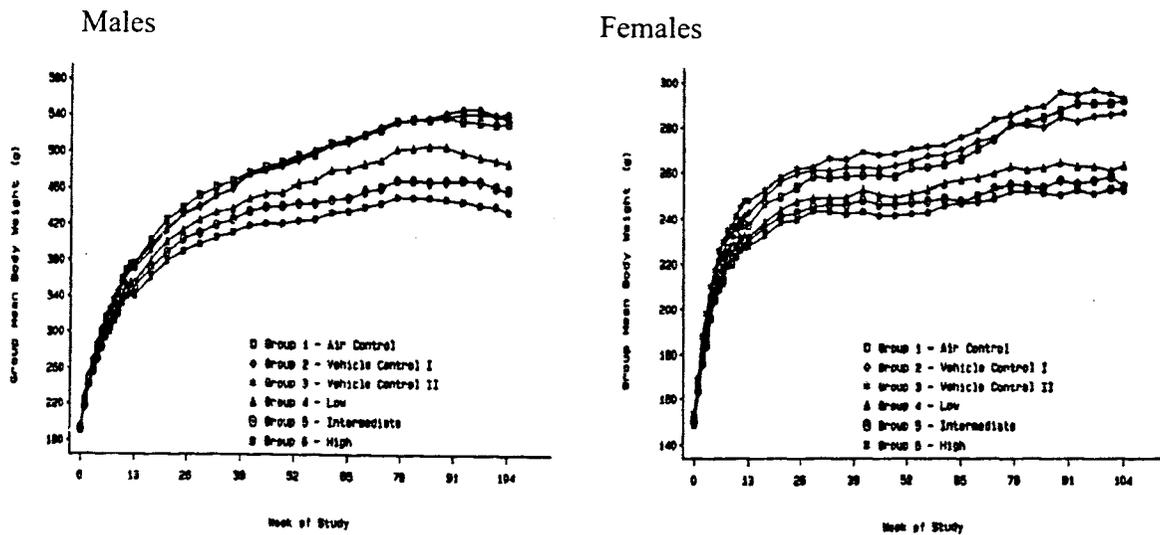


Figure 6.2 Body weights as a function of time of the 2-year carcinogenicity study in rats

Gross pathology: The tiotropium treated rats showed increased incidences of dark foci in the Harderian glands (Table 6.8). The dose-proportionality was, however, not clear.

Table 6.8 Incidences of Dark Focus (one or both sides) in Harderian Glands

Groups	Incidences of Dark Focus (one or both sides)					
	I	II	III	IV	V	VI
Treatment	Controls			Tiotropium (mg/kg/day)		
	0	0	0	0.6	1.5	5.3
Male:	9	6	10	18	13	17
Female:	4	0	1	12	14	16

Histopathology:

Non-neoplastic: Non-neoplastic changes associated with tiotropium treatment were seen in the following organs: the upper respiratory tract, prostate, adrenal glands, gastrointestinal tract, urinary bladder and Harderian glands. Table 6.9 summarizes the non-neoplastic changes.

In the upper respiratory tract, lesions were seen in both the nasal cavity and larynx. The lesions included inflammation, necrosis and hyperplasia and squamous metaplasia of the epithelial cells. These lesions are indicative of an irritant effect of tiotropium. Lesions in the prostate included prostatitis and secretory alterations. Lesions in the adrenal glands were focal cortical hypertrophy and hyperplasia. Dilated or cystic glands were seen in the stomach and increased incidences of parasite infection was noticed in the rectum and caecum. Inflammation and transitional epithelial hyperplasia were observed in the urinary bladder. Also observed were the pigment deposits and inflammation in the Harderian gland. The severity of lesions was generally dose-dependent although most lesions were also present in the low dose group.

Benzalkonium chloride (BAC) appears to be the major contributor to lesions such as the cranial epithelial hyperplasia in the larynx region. The incidence in male rats was: 2/48-air control, 1/49-vehicle minus BAC, 17/50-vehicle with BAC, and 17/49-LD, 12/49-MD, and 13/48-HD. All the tiotropium treatment groups have BAC in the vehicle.

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Table 6.9 Summary of Non-Neoplastic Findings

Findings	Treatment	Incidence of Lesions											
		Males						Females					
		V. I	Air	V. II	LD	MD	HD	V. I	Air	V. II	LD	MD	HD
# animals examined		50	50	50	50	50	50	50	50	50	50	50	50
Adrenal gland:													
Focal cortical hyperplasia/ bilateral/ Very mild		0	3	0	1	2	4	0	0	0	1	0	0
Mild		3	1	0	2	0	2	1	0	1	0	1	0
Moderate		0	0	0	0	2	0	0	0	0	0	0	0
Total		3	4	0	3	4	6*	1	0	1	1	1	0
Focal cortical hypertrophy													
Very mild		5	1	4	4	7	4	5	0	1	1	3	3
Mild		1	3	2	7	8*	2	2	1	2	1	2	0
Moderate		0	0	0	1	0	0	0	0	0	0	0	1
Total		6	4	6	12	15*	6	7	1	3	2	5	4
Caecum: parasites		2	8	4	10*	8	9*	3	3	9	5	6	11*
Kidney: cortical pigment deposit		14	12	26*	24	10	6	0	0	0	0	0	0
Harderian gland:													
Pigment deposit(s)													
Very mild		29	25	29	15	20	7	22	14	10	18	18	6
Mild		11	5	5	24*	24*	31*	3	4	1	28*	25*	34*
Moderate		0	0	0	6*	5	11*	0	0	0	3	4	10*
Severe		0	0	0	0	0	1	0	0	0	0	0	0
Total		40	30	34	49*	49*	50*	25	18	11	49*	47*	50*
Focal inflammation: Very mild		1	1	4	2	6	2	1	0	1	3	0	4
Mild		0	2	1	1	6*	8*	1	1	0	1	0	7
Moderate		0	0	0	0	0	1	0	0	0	0	0	0
Total		1	3	5	3	12*	11*	2	1	1	4	0	11*
Mononuclear cell infiltrate													
Very mild		1	0	0	0	2	6	0	0	0	1	1	11*
Mild		0	0	1	4	0	3	0	0	0	0	0	7*
Total		1	0	1	4	2	9*	0	0	0	1	1	18*
Larynx:		(48)	(49)	(50)	(49)	(49)	(48)	(47)	(43)	(47)	(49)	(49)	(44)
Cranial squamous hyperplasia													
Very mild		1	2	17*	17*	12*	13*	4	2	18*	11*	4	6
Mild		1	1	3	8	25*	17*	0	1	19*	31*	38*	31*
Moderate		0	0	0	1	3	4	0	0	1	1	4	3
Total		2	3	20*	26*	40*	34*	4	3	38*	43*	46*	41*
Cranial epithelial necrosis													
Very mild		0	0	3	3	2	0	0	0	4	1	0	1
Mild		0	0	0	1	2	0	0	0	3	3	7	3
Moderate		0	0	1	0	0	1	0	0	0	2	1	1
Total		4	0	4	4	4	1	0	0	7*	6*	8*	5*
Focal cranial inflammation													
Very mild		0	0	1	0	1	0	0	0	6	13	6	5
Mild		0	0	1	1	3	2	0	0	3	10	15*	19*
Moderate		0	0	0	1	0	0	0	0	0	0	1	0
Total		0	0	2	2	4	2	0	0	9*	23*	22*	24*
Focal inflammation													
Very mild		0	0	1	3	1	6*	0	0	2*	7*	6*	7*
Mild		0	0	1	0	1	4	0	0	0	4	12*	19*
Moderate		0	0	0	0	0	0	0	0	0	1	0	0

Severe	0	0	0	0	0	0	0	0	0	0	1	0
Total	0	0	2	3	2	10*	0	0	2	12*	19*	26*
Squamous metaplasia												
Very mild	0	0	0	0	4	10*	0	0	1	2	7*	6*
Mild	0	0	1	1	2	8*	0	0	1	8*	6*	13*
Moderate	0	0	0	1	1	1	0	0	0	0	4	9*
Severe	0	0	0	0	0	2	0	0	0	0	0	0
Total	1	0	1	2	7	21*	0	0	2	11*	17*	28*
Nasal cavity: (Level 1)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Acute focal inflammation												
Very mild	3	4	2	8	4	3	4	2	1	6	8	10
Mild	1	1	1	18*	23*	25*	0	0	0	8*	4	9*
Moderate	0	0	0	1	0	0	0	0	0	0	0	6*
Severe	0	0	0	1	0	0	0	0	0	0	0	0
Total	4	5	3	35*	46*	48*	4	2	1	14*	12*	25*
Respiratory epithelial squamous metaplasia												
Very mild	3	6	2	4	3	3	0	1	1	4	7*	4
Mild	1	2	1	13*	14*	23*	4	1	0	9	6	12
Moderate	1	0	1	16*	25*	22*	0	0	0	2	3	5
Severe	0	0	0	2	4	1	0	0	0	0	0	0
Total	5	8	4	35*	46*	49*	4	2	1	15*	16*	21*
Goblet-cell hyperplasia												
Very mild	8	6	3	6	9	5	2	0	1	10	10	8
Mild	3	2	1	15*	21*	13	0	0	0	3	0	8*
Moderate	0	0	0	4	13*	12*	0	0	0	0	1	0
Total	11	8	4	25*	43*	30*	2	0	1	13*	11*	16*
Dilated/systic gland, Level 2	11	16	11	19	32*	31*	4	2	2	1	3	8
Pulp impaction, Level 4	2	2	2	3	6	3	4	9	5	14*	14*	12
Prostate: prostatitis	3	4	3	18*	24*	26*						
Secretory alterations	0	0	1	2	10*	17*						
Rectum: parasites	7	5	7	11	12	13	3	12*	6	18*	12	11
Seminal vesicle: inflammation	0	0	0	4	4	5						
Stomach: dilated/systic gland	33	39	29	43*	49*	47*	25	27	23	44*	47*	49*
Among: mild	16	25	9	29*	27*	12	8	7	2	22*	28*	28*
Moderate	3	0	1	9*	17*	32*	0	1	0	3	12*	11*
Submandibular lymph node: Plasmacytosis	6	6	4	6	19*	23*	4	10	9	8	8	15
Thymus: epithelial hyperplasia	4	2	2	2	2	7*	15	15	25	21	21	20
Urinary bladder:												
Subacute inflammation	0	1	0	12*	14*	14*	0	0	0	0	0	1
Transitional epithelial hyperplasia	3	1	4	23*	28*	17*	0	2	1	3	5	4

* Statistically significantly different from the Vehicle II Control group.

1. The level of cut in the nasal cavity.

Neoplastic:

No tiotropium treatment-related tumors were observed. Table 6.10 summarizes the overall tumor incidences among the treatment groups. Table 6.11 lists tumor incidences in each system and treatment group.

Table 6.10. Summary of Tumor Findings in 2-Year Inhalation Carcinogenicity study in Wistar Rats

Findings	Treatment	Males						Females					
		V. II	Air	V. I	LD	MD	HD	V. II	Air	V. I	LD	MD	HD
Number of animals		50	50	50	50	50	50	50	50	50	50	50	50
# animals with tumors		31	34	31	25	24	20	35	39	30	22	29	21
# animals with single tumors		17	21	21	20	19	14	23	20	24	12	22	18
# animals with multiple tumors		14	13	10	5	5	6	12	19	6	10	7	3
# animals with benign tumors		29	27	27	20	19	18	31	33	25	20	20	21
# animals with malignant tumors		5	10	8	6	6	5	7	13	7	3	10	1
# animals with metastasis tumors		2	2	1	0	2	2	1	1	1	2	4	0
Total number of tumors		50	51	43	30	30	28	51	62	38	32	37	27
Total # of benign tumors		45	40	34	24	24	22	42	48	31	29	25	26
Total # of malignant tumors		5	11	9	6	6	6	9	14	7	3	12	1
Total # of metastasising tumors		2	2	1	0	2	3	1	1	1	2	4	0
% animal with tumors		62	68	62	50	48	40	70	78	60	44	58	42
% animal with single tumors		34	42	42	40	38	28	46	40	48	24	44	36
% animal with multiple tumors		28	26	20	10	10	12	24	38	12	20	14	6
% animal with benign tumors		58	54	54	40	38	36	62	66	50	40	40	42
% animal with malignant tumors		10	20	16	12	12	10	14	26	14	6	20	2
% animal with metastasising tumors		4	4	2	0	4	6	2	2	2	4	8	0

* Statistically significantly different from the Vehicle II Control group.

There were no remarkable differences among the groups in terms of the number of animals with tumors, the number of animals with multiple tumors, the number of animals with malignant tumors and metastasising tumors. Nor were there significant increases in tumor incidences in the both the vehicle and tiotropium-treated groups.

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Table 6.11 Tumor Incidences in the 2-Year Inhalation Carcinogenicity Study in Rats

Findings	Treatment	Incidence of Tumors												
		Males						Females						
		V. II	Air	V. I	LD	MD	HD	V. II	Air	V. I	LD	MD	HD	
# animals examined		50	50	50	50	50	50	50	50	50	50	50	50	50
Adrenal: cortical carcinoma (M)							1							
Cortical adenoma (M)			1											
Pheochromocytoma (M)		1	2	1	1				1					
Pheochromocytoma (B)		5	3	4	2			2	1				1	
Brain: meningioma (B)											1			
Asracytoma (B)							1							
Cervix: Stromal sarcoma (M)		-	-	-	-	-	-		1	1			1	
Colon: adenoma (B)						1								
Eye: melnoma (M)			1											
Heart: endocardial schwanoma metastasising ES				1					1			1		
Jejunum: Leiomyosarcoma (M)														i
Kidney: adenocarcinoma/tubular														
Bilateral metastasising (M)			1											
Unilateral metastasising (M)			1			1							1	
Bilateral tub. adenocinoma										1			1	
Nephroblastoma (M)									1					
Liver: Hepatocellular carcinoma				1	1	2								
Hepatocellular adenoma		2	1		1				1					
Lung;alveolar/bronchiolar adenoma														1
Lymphonode: haemangioma/ messenteric		2	2	2	2	2	2						1	1
Mammary gland: adenocarcinoma									2	1		1	1	1
Adenoma									1	3	2	1		1
Fibroadenoma									4	5	6	6	3	4
Other sites:														
Metastasising adenocarcinoma													1	
Metastasising adenocarcinoma											1			
Ovary: Yolk sac carcinoma/ matastasising/ bilateral									1					
Tubulostromal adenoma									3		4	1		4
Pancreas: islet adenoma		2			1									
Thyroid adenoma/ unilateral										1				
Pituitary gland: adenocarcinoma/ Anterior lobe									2	2			1	
Adenoma/anterior lobe		5	10	6	1	2	2	13	17	24*	7	10	9	
Skeletal muscle: rhobdomyosarcoma										1				
Skin and subcutis: various tumors		5	5	10	4	6	4	1	1		1	4		
Spleen: fibrosarcoma		1												
Haemagiosarcoma/metastas.				1										
Haemagiosarcoma				2										
Haemagioma			1			1								
Stamoch: squam. cell papilloma								1		1				
Testis: sertoli cell tumors														
Interstitial cell tumors/bilater.		2	1	1	1	1								
Interstitial cell tumors/unilate.		8	10	11	8	7	9							
Thymus: lymphocytic thymoma		1	4	3	1	1	1		2	2	2	1		
lymphocytic thymoma/mestas.				1										
Thyroid: C-cell crcinoma/						1	1				1			

metastasising/unilateral <i>Invasive/unilateral</i>									1			
C-Cell adenoma/unilateral	5	6	6	6	3	3	3	6	9	4	6	3
C-cell adenoma/bilateral										1		
Trachea: sarcoma							1					
Uterus: Angiosarcoma							1					
Metastasising leiomyosarcoma											1	
Stromal sarcoma								1	1		2	
Stromal polyp							1	3	2	4		1
Schwannoma							1					
Fibroma								1				
Vagina: Stromal polyp												1

* Statistically significantly different from the Vehicle II Control group.

Toxicokinetics:

Table 6.12 summarizes the results of plasma tiotropium concentration. A dose-plasma relationship was apparent.

Table 6.12 Tiotropium Plasma Concentration in Rats (pg/ml, geometric mean)¹

Sex	Month 12			Month 23		
Dose (mg/kg/day)	0.9	2.2	7.5	0.6	1.5	5.3
Males	112.1	237.0	798.2	153.6	261.1	1121.3*
Females	138.7	225.0	852.5	151.1	263.5	1061.2*
Mean	125.4	231	825.4	152.4	262.3	1091.3

1. Plasma drug levels were determined at 12 and 23 months of dosing. One milliliter of blood sample was drawn from five rats/sex/dose via a tail vein 15 minutes post exposure. Biochemie of Beohringer Ingelheim AG analyzed the blood samples for tiotropium levels using HPLC-MSMS. The limit of quantitation was _____

* Significantly higher than levels at 12 months (P <0.05).

Note: Two (of 60) control animals showed detectable amount of plasma tiotropium. The plasma levels of tiotropium were 313.9 (animal no. 3) and 191 pg/ml (animal no. 109). The distribution of the animals was one male air control rat (No. 3) at the 12-month time point and one vehicle II control male rat at the 23-month time point. The report did not identify the potential causes.

Study Summary:

Fifty Wistar rats/sex/dose were given by nose-only inhalation 0.0.6, 1.5 and 5.3 µg/kg/day (pulmonary deposited doses) of tiotropium bromide for 104 weeks. The control was divided into three subgroups (50 rats/sex each subgroup): air, vehicle without benzalkonium chloride (BAC) and vehicle with BAC. Mean plasma tiotropium concentrations were 0.15, 0.26 and 1.09 ng/ml at month 23 for the low, mid and high dose groups, respectively. There were dose-related decreases in body weights in the tiotropium treatment groups. The body weight decrease was

19% and 13% for the high dose males the females at the end of the study. There were no significant differences in the survival rates among the groups. Inflammation of the urinary bladder and prostaticitis were observed in all tiotropium treatment groups. Mild irritation of the upper respiratory tract was seen in groups treated with either BAC alone or BAC in combination with tiotropium bromide. There was no difference in the incidences of tumors between the control and tiotropium treatment groups.

VI.1.B Study Title: Ba 679 BR (Tiotropium) 83 -Week Inhalation Carcinogenicity Study in Female Mice.

Note: The original study title was "...inhalation carcinogenicity study in mice". The review modifies it to more accurately reflect the study. The reason was that tumorogenicity evaluation was formed in the females only. The females were sacrificed at week 83. All males were aborted after 52 weeks of exposure due to high mortality and no tumor evaluation was conducted.

Key findings: No evidence of tiotropium carcinogenicity was found.

Study no: U98-2726
Study type (if not reflected in title): Nose-only inhalation
Volume #, and page #: Volume 61 - 64
Conducting laboratory and location:
Date of study initiation: August 17, 1992
GLP compliance: In compliance with OECD GLP
QA reports: yes (x), no ()
Drug, lot #, radiolabel, and % purity: Batch III; 99.9% peak area in HPLC assay
CAC concurrence: None

Study Type (2 year bioassay, alternative model etc.): 19-month bioassay
Species/strain: CD-1 mice
Number/group; age at start of study: 60; 4 weeks of age
Animal weight at start of exposure: Female: *ca* 22 g.
Animal housing: 20 ± 2°C; 12 hr light cycle; 1/cage
Feed and water: Rat and Mouse (modified) No. 1 diet SQC expanded; feed and water *ad libitum*

Formulation/vehicle (Table 6.13):

Ingredient	Formulation ¹		Vehicle
	I	II	
Tiotropium (mg)	10.0	100.0	-
Hydrochloric acid (0.1 N, ml)	0.8	0.8	0.8
Water for injection (ml)	100	100	100

1. pH of the final formulation was 3.0.

Table 6.14 Design of the 83-Week Inhalation Carcinogenicity Study in Female Mice.

Groups	I	II	III	IV	V	VI	VII ¹
Treatment	Air	Vehicle ¹		Tiotropium (µg/kg/day)			No
Tiotropium (µg/kg/day)	0	0	0	2.7	4.8	9.1	0
#Mice (main study)	60	50	50	60	60	60	20
#Mice (Satellite for PK)	10	10	10	10	10	10	-

1. The two vehicle control groups received identical aerosol treatment.

Methods:

Doses: 2.7, 4.8 and 9.1 µg/kg/day.

Basis of dose selection: The MTDs in a 13-week inhalation study.

Restriction paradigm for dietary restriction studies: None.

Route of administration: Nose-only inhalation.

Frequency of drug administration: Once daily, 1 hr/exposure/day for 83 weeks.

Dual controls employed: yes. One air control, two identical vehicle control groups.

Interim sacrifices: No.

Satellite PK or special study group(s): 10 mice/group for PK.

Deviations from original study protocol:

1. The exposure in the females was terminated at week 83/84 instead of the planned 104 weeks. The study in males was aborted at week 51 of the treatment.
2. Tiotropium concentrations in the solution used to generate the aerosols for the mid and high dose groups were raised from 0.35 and 0.60 mg/ml to 0.55 and 1.0 mg/ml from week 13 and onward, respectively.
3. Particle size distribution was not done for exposure weeks one to five.

Statistical methods:Data:

Body weight, food consumption,
hematology:

Mortality:

Histology data:

Tumor data:

Statistical Method:

ANOVA, Student *t*-test

Kaplan-Meier survival curve and Wilcoxon
ranking test, $\alpha = 0.05$

Pairwisid comparison

Peto's time adjusted method

Observations and times:

Chamber drug conc. Twice weekly for the first 13 week and biweekly afterward

Clinical signs: Daily.

Body weights: Weekly for the first 13 weeks, and once every 4 weeks
thereafter

Food consumption: Weekly for the first 13 weeks, and once every 4 weeks
thereafter

<i>Ophthalmology:</i>	Weeks 13, 52 and 82
<i>Hematology:</i>	Weeks 52 and 83
<i>Clinical chemistry:</i>	Not done.
<i>Organ weights:</i>	Not recorded
<i>Gross pathology:</i>	Terminal sacrifice or time of death for premature deaths
<i>Histopathology:</i>	A complete panel, see histology inventory table for details.
<i>Toxicokinetics:</i>	Weeks 58 and 83, ca 15 min after exposure. 5 mice/group

Results:

Doses: The mice received 2.7, 4.8 and 9.1 µg/kg/day of tiotropium chloride. Table 6.15 summarizes the dose estimates of the study.

Table 6.15 Dose Estimates

Treatment	Air	Vehicle		Tiotropium		
Groups	I	II	III	IV	V	VI
MMAD ¹	-					
Mean fraction of aerosol ¹ particle	-	86.2%	88.8%	95.8%	98.1%	100%
Aerosol tiotropium (µg/L) ²	0	0	0	1.35 ± 0.32	2.61 ± 0.97	5.23 ± 1.87
Achieved total dose (µg/kg) ³	0	0	0	54.2 ± 12.4	96.1 ± 34.2	180.6 ± 44.8
Pulmonary dose (µg/kg) Reported ⁴	0	0	0	52.1 ± 13.7	106 ± 31.9	211.8 ± 61.4
Review Estimates ⁵	0	0	0	2.7	4.8	9.1

1. Particle sizes were not evaluated during weeks 1 – 5 because of equipment malfunction and sampling errors.
2. Mean aerosol tiotropium concentrations based on HPLC analysis of samples taken twice weekly for the first 13 weeks and once every two weeks for the remainder of the study.
3. Based on a minute volume of 1.2 liter, the exposure duration of 1 hour, and the measured drug concentrations.
4. Note that the pulmonary doses were higher than the total achieved doses. The report explained that “due to equipment malfunction or sampling errors, ... for some weeks no estimated values for the pulmonary fraction have been calculated. Consequently, the mean pulmonary fraction appears higher the estimated total achieved dose due to the mean values presented being derived from different numbers of the sample values”. Such a practice is questionable.
5. Derived as the 5% of the total achieved doses. Note that the sponsor use a deposition factor of 100%.

Mortality:

The high dose group showed a statistically significant increase in mortality starting from week 60 (See Statistical review by Dr. Feng Zhou dated October 1, 2001 in IND 46,687). There was no difference in mortality between the controls, low and mid dose groups for the remaining period. Table 6.16 summarizes mortality rate during the study. Figure 6.3 presents the Kaplan-Meier survival curve of the study.

Table 6.16 Mortality (%) of 83-week Carcinogenicity study in Female Mice¹

Time	Groups	I	II	III	IV	V	VI
	Treatment	Controls			Tiotropium (µg/kg/day)		
		Air	Vehicle	Vehicle	2.7	4.8	9.1
Weeks: 0 - 52		1.7	6.0	2.0	5.0	6.7	13.1
53 - 78		16.7	28.0	18.0	25.0	30.0	47.5
79 - 82		18.3	30.0	32.0	30.0	40.0	59.0
(P value) ²		0.075		0.85	0.82	0.27	0.035

1. Source: Dr. Feng Zhou's review dated October 1, 2001 in IND 46,687.
2. Compared to Group 2 (Vehicle Group 1).

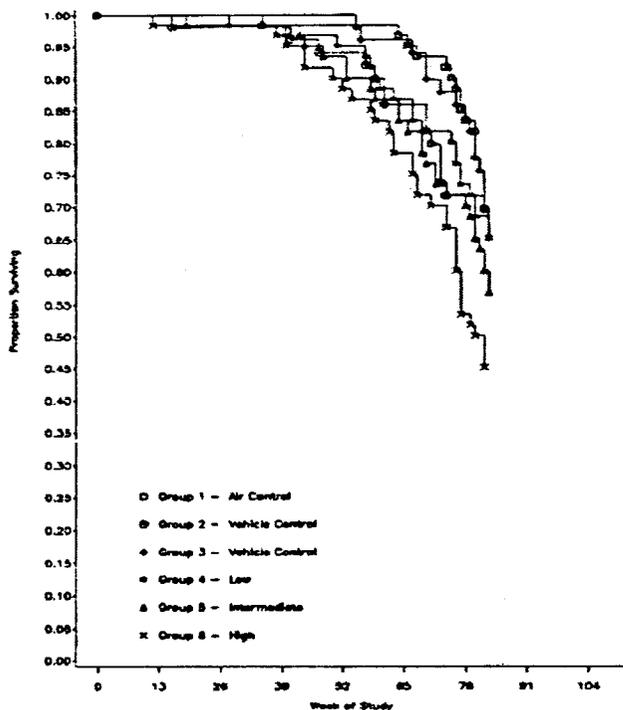


Figure 6.3 Kaplan-Meier survival curve of the 83-week inhalation carcinogenicity study of tiotropium in female mice.

Clinical signs: No treatment-related effects were observed.

Palpable masses: No treatment-related effects were observed.

Body weight:

There was no treatment-related change in body weight in the female mice. Figure 6.4 presents the animal body weight as a function of treatment time. There was no apparent difference in body weight between treatment and control groups.

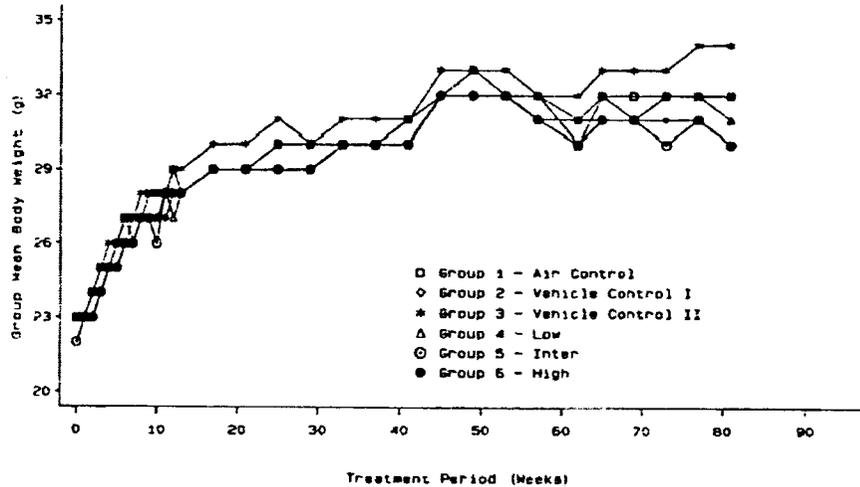


Figure 6.4 Body weight as a function of time in the 83-week carcinogenicity study in female mice.

Food consumption: No treatment-related effects were observed.

Water consumption: No treatment-related effects were observed.

Ophthalmology: No treatment-related effects were observed.

Hematology: No remarkable changes were observed.

Gross pathology: Tiotropium-treated groups showed distended lumen of intestine and urinary bladder (Table 6.17).

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Table 6.17 Gross Pathology Findings (incidence)
in the 83-Week Inhalation Toxicity Study in Female Mice

Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle		Tiotropium ($\mu\text{g}/\text{kg}/\text{day}$)		
				2.7	4.8	9.1
Swollen Abdomen	22	16	21	33	27	37
Distended intestine	1	0	0	3	10	14
Liver enlargement/masses	3	1	0	1	2	2
Ovary cysts and masses	18	13	8	24	20	20
Uterus distension	17	8	16	24	10	17
Urinary bladder distension	1	0	0	2	5	3

Histopathology:

Non-neoplastic Changes:

Non-neoplastic changes associated with tiotropium treatment in female rats were distended lumen of the intestine, amyloidosis of various organs, liver cell necrosis, and some other changes. Table 6.18 summarizes most remarkable non-neoplastic changes in the study.

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Table 6.18 Non-Neoplastic Findings (incidence) of the 83-Week Inhalation Toxicity Study in Female Mice

Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle		Tiotropium (µg/kg/day)		
				2.7	4.8	9.1
Number of animals examined	60	50	50	60	60	60
Heart: Amyloidosis	6	5	10	2	12	9
Nasal cavity:						
Amyloidosis (level 1)	28	10	17	13	41	14
Hyaline inclusions (level 1)	19	15	17	22	32	28
Level 3	4	3	8	8	7	19
Inflammation (level 1)	11	2	2	4	13	4
Lung: congestion	7	3	4	11	15	6
Stomach: hyperkeratosis	0	0	0	0	1	2
Intestine:						
Distended lumen						
Duodenum	0	0	0	0	1	3
Jejunum	0	0	0	0	2	8
Ileum	0	0	0	0	2	8
Cecum	0	0	0	0	14	22
Colon	4	0	0	3	17	22
Rectum	3	1	0	4	19	22
Inflammation/ rectum	0	0	0	1	2	6
Mucosal hyperplasia/ rectum	0	1	0	1	11	11
Liver: multicellular necrosis	3	4	2	0	8	12
Congestion	1	1	3	1	3	5
Gallbladder: goblet cell metaplasia	0	0	0	2	2	4
Pancreas: islet cell hyperplasia	1	2	3	1	0	4
Spleen: diminished size/atrophy	0	3	1	2	8	10
Thymus: medullary cysts	0	0	0	3	0	3
Amyloidosis	0	0	0	3	3	2
Mesenteric lymph node:						
lymphoid hyperplasia	5	2	7	15	11	4
Mammary gland: amyloidosis	1	3	3	0	6	5

Neoplastic Findings:

No tiotropium-associated tumors were observed in the 83-week inhalation carcinogenicity study in female mice. Table 6.19 summarizes the overall tumor incidences and Table 6.20 lists all tumors observed in the study. There are no remarkable increases in tumor incidences in the tiotropium treatment groups when compared to the control groups.

Table 6.19 Neoplastic Findings (incidence) of the 83-Week Inhalation Toxicity Study in Female Mice

Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle		Tiotropium (µg/kg/day)		
				2.7	4.8	9.1
Number of animals	60	50	50	60	60	60
# animals with neoplasms	40	31	26	28	26	24
# animals with more than one neoplasms	12	4	6	6	1	5
# animals with benign tumors	15	7	15	8	5	5
# animals with malignant tumors	34	26	15	25	22	21
# animals with metastasis tumors	0	0	0	0	1	1
Total % animals with tumors	66.7	62.0	52.0	46.7	43.3	39.3
% animals with more than one neoplasms	20	8	12	10	1.7	8.2
% animals with metastasis tumors	0	0	0	0	1.7	1.6

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Table 6.20 Neoplastic Findings (incidence) of the 83-Week Inhalation Toxicity Study in Female Mice						
Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle		Tiotropium (µg/kg/day)		
				2.7	4.8	9.1
Number of animals examined	60	50	50	60	60	60
Liver: Hemangiosarcoma		1				
Hepatocellular carcinoma			1			
Sarcoma					1	
Lung: alveolar/bronchiolar adenoma	5	2	5	1	1	2
Alveolar/bronchiolar carcinoma	6	5	2		2	1
Pancreas: islet cell adenoma		1				
Ovary: Leiomyoma	1					
Cystadenoma	1					
Granulosa-theca cell tumor (benign)					1	
Theca cell tumor (malignant)	1					
Hemangiosarcoma				1	1	
Luteoma	2	1				
Spleen: hemangiosarcoma	1					1
Uterus: Adenoma				1		
Adenocarcinoma	1			2	1	1
Hemangioma	1					
Hemangiosarcoma	3	2		1	1	1
Endometrial/stromal polyp(s)	2		7	2		1
Leiomyoma	3	1	1	1	2	
Granular cell tumor	1	1		1		1
Stromal sarcoma	3		1	2		3
Malignant fibrous histiocytoma						1
Squamous cell carcinoma				1		
Pituitary gland: adenoma/pars distalis	1			1		
Adrenal medullas: medullary tumors (benign)					1	
Medullary tumor (malignant)				1		
Hemolymphoretical system: thymus lymphoma	20	19	8	19	14	14
Non-thymus lymphoma	1					
Non-specified lymphoma	1		1			
Histiocytic sarcoma	2		1	1	1	1
Myeloid leukemia				1		
Mesenteric lymph node: hemangioma			1			1
Hardarian gland: adenoma		1	2			2
Adenocarcinoma			1			
Adipose tissue: lipoma					1	
Mammary gland: carcinoma			1			
Skin/subcutis: fibrosarcoma			1			
Hemangiosarcoma					1	
Sarcoma			1		1	1

Toxicokinetics:

Table 6.21 summarizes the results of plasma tiotropium concentrations. There was no clear dose-proportionality between the mid and high dose groups although there appeared to be a dose-related increase in plasma tiotropium concentration between the low and mid dose groups.

Table 6.21 Tiotropium Plasma Concentration in Female Mice (ng/ml, geometric mean)^{1,2}

Dose (µg/kg/day)	2.7	4.8	9.1
Week 57	8.3 (6.9 – 10.0)	31.3 (11.1 – 88.8)	38.4 (23.4 – 63.0)
N	5	5	5
Week 83	16.4 (13.6 – 19.7)	32.1 (24.3 – 42.4)	32.2 (13.5 – 67.6)
N	4	2	2

1. Tiotropium was detected in four of 27 control samples. The levels of tiotropium in the control samples, however, was generally low (_____, ng/ml).
2. Plasma drug levels were determined at weeks 57 and 83 of dosing. Blood sample was drawn from five mice/dose 15 minutes post exposure. The limit of quantitation was _____
Beohringer Ingelheim AG analyzed tiotropium levels in the blood samples using HPLC-MSMS.

Study Summary: Sixty female CD-1 mice/dose were given by nose-only inhalation 0, 2.7, 4.8 and 9.1 µg/kg/day (pulmonary deposited doses) of tiotropium bromide for 83 weeks. The control was divided into two subgroups groups: air (60) and vehicle (100). The vehicle control was further divided into two identical groups of 50 mice each. The vehicle lacked BAC that was present in the previous rat study. Mean plasma tiotropium concentrations (determined from satellite groups) were 16.4, 32.1 and 32.2 ng/ml for the low, mid and high dose groups, respectively. There were no differences in body weights between the control and the tiotropium treatment groups. However, the high dose group showed statistically higher mortality, starting at week 60 and onward. The mortality rate at the termination of the study (week 83) was 18%, 30%, 32%, 30%, 40% and 59% for the air, vehicles 1 and 2, low, mid and high dose groups, respectively. Histological evaluation at terminal sacrifice showed that following in the mid and high dose groups: distended intestine lumen, rectum mucosal hyperplasia, diminished size or atrophy of the spleen, and multicellular necrosis in the liver. No treatment-related tumors were identified.

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VI.1.C Study Title: Ba 679 BR (Tiotropium) 101-Week Inhalation Carcinogenicity Study in Male Mice.

Key findings: No evidence of carcinogenicity was found.

Study no: U99-1464
Study type (if not reflected in title): Nose-only inhalation
Volume #, and page #: Volume 68 - 75
Conducting laboratory and location:
Date of study initiation: June 23, 1994
Report Date: August 27, 1999
GLP compliance: In compliance with OECD GLP
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: Batch II; 99.8% peak area in HPLC assay

CAC concurrence: None

Study Type (2 year bioassay, alternative model etc.): 23-month bioassay
Species/strain: CD-1 mice
Number/group; age at start of study: 50 - 60; 4 weeks of age
Animal weight at start of exposure: Male: *ca* 21 – 24 g.
Animal housing: 20 ± 2°C; 12 hr light cycle; 1/cage
Feed and water: Rat and Mouse (modified) No. 1 diet SQC expanded; feed and water *ad libitum*
Formulation/vehicle: Same as Study No. 98-2726

Design: See Table 6.22.

Table 6.22 Design of the 101-Week Inhalation Carcinogenicity Study in Female Mice.

Groups	I	II	III	IV	V	VI	VII ¹
Treatment	Air	Vehicle ¹		Tiotropium			No
Tiotropium (µg/kg/day) ¹	0	0	0	0.02	0.07	0.31	0
#Mice (main study)	60	50	50	60	60	60	20
#Mice (Satellite for Salivation)	30	30	30	30	30	30	-

1. The two vehicle control groups received identical aerosol treatment.

METHODS:

Doses: 0.02, 0.07 and 0.31 µg/kg/day.

Basis of dose selection: The MTDs in a 13-week inhalation study in the same species, and the aborted carcinogenicity study (Study No. U98-2726).

Restriction paradigm for dietary restriction studies: None.

Route of administration: Nose-only inhalation.**Frequency of drug administration:**

- 1) Once daily.
- 2) Table 6.23 presents the duration of exposure for each group. The mid and high dose groups were exposed for 60 minutes/exposure/day during the first 63 weeks. The duration was reduced to 30 minutes/exposure/day for the remaining treatment period. For the remaining 30 minutes of each treatment episode, the animals were exposed to air.
- 3) The low dose was exposed to aerosol for 15 minutes followed by either 15 or 45 minutes of air.

Table 6.23 Duration of Aerosol Exposure

Treatment	Air	Vehicle		Tiotropium		
Groups	I	II	III	IV	V	VI
Weeks 1- 62						
Air	60	-	-	45	-	-
Aerosol	-	60	60	15	60	60
Weeks 63 - 101						
Air	60	-	-	45	30	30
Aerosol	-	60	60	15	30	30

Dual controls employed: yes. One air control, two identical vehicle control groups.

Interim sacrifices: No.

Satellite PK or special study group(s): 30 male mice/group for inhibition of salivation, a pharmacological effect of the drug.

Deviations from original study protocol:

1. Tiotropium doses were reduced at week 63 and onward. The mid and high dose groups were exposed for 60 minutes/exposure/day for first 63 weeks. High mortality occurred and prompted a dose reduction. The reduction was achieved by shortening the duration of from 60 minutes to 30 minutes/exposure/day (Table 6.24) and a slight reduction in aerosol tiotropium concentration in the high dose group. Table 6.25 shows that mean aerosol tiotropium concentration for the respective periods.

**Table 6.24 Mean Aerosol Tiotropium Concentrations ($\mu\text{g/L}$)^a
in the 24-month Inhalation Carcinogenicity Study in Male Mice**

Time (weeks)	Low Dose	Mid Dose	High Dose
Week 1 - 62	0.04 (\pm 0.02)	0.06 (\pm 0.02)	0.27 (\pm 0.07)
Week 63 - 101	0.05 (\pm 0.01)	0.05 (\pm 0.01)	0.19 (\pm 0.07)

a. Based on HPLC chemical analysis of aerosol tiotropium concentration in chamber samples.

2. Tiotropium concentrations in solutions used to generating aerosols in the low and high dose groups were adjusted twice as shown in the Table 6.25.

Table 6.25 Tiotropium Concentrations (mg/L)¹ in solutions for generating Aerosols in the 24-month Inhalation Carcinogenicity Study in Male Mice

Time (weeks,)	Low Dose	Mid Dose	High Dose
1 - 2	5	10	35
3 - 8	10	10	50
9 - 101	10	10	40

3. The high dose group was terminated at week 94 of the treatment due to high mortality (26/60 survival).

Statistical methods:

Data:	Statistical Method
Body weight, food consumption, hematology:	ANOVA, Student <i>t</i> -test
Mortality:	Kaplan-Meier survival curve and Wilcoxon ranking test, $\alpha = 0.05$
Histology data:	Pairwise comparison of tumor incidence
Tumor data:	Peto's time adjusted method

Observations and times:

<i>Chamber drug conc.</i>	Twice weekly for the first 13 week and biweekly afterward
<i>Clinical signs:</i>	Daily.
<i>Body weights:</i>	Weekly for the first 13 weeks, and once every 4 weeks thereafter
<i>Food consumption:</i>	Weekly for the first 13 weeks, and once every 4 weeks thereafter
<i>Ophthalmology:</i>	Weeks 14, 52 and 101
<i>Hematology:</i>	Weeks 55, 79 and 101 for groups 1 – 3 and 6.
<i>Pilocarpine-induced salivation:</i>	2 hr post exposure on Weeks 13, 52 and 97
<i>Clinical chemistry:</i>	Not done.
<i>Organ weights:</i>	Not recorded
<i>Gross pathology:</i>	Terminal sacrifice or time of death for premature deaths
<i>Histopathology:</i>	A complete panel, see histology inventory table for details. High dose groups at week 97 and the remaining animals week 101
<i>Toxicokinetics:</i>	Not done

Results:

Doses: The estimated pulmonary exposure was 0.02, 0.07, and 0.31 $\mu\text{g}/\text{kg}/\text{day}$ for the low, mid and high dose groups, respectively. Table 6.26 summarizes the dose estimates of the study.

Table 6.26 Dose Estimates

Treatment	Air	Vehicle		Tiotropium		
Groups	I	II	III	IV	V	VI
MMAD	-	1.23	1.24	1.13	1.20	1.15
Mean fraction of aerosol ¹ · particle ≤	-	85.4%	81.2%	84.8%	85.1%	85.2%
Aerosol tiotropium (µg/L)						
Weeks 1 - 62	0	0	0	0.04	0.06	0.27
Weeks 63 - 101	0	0	0	0.05	0.05	0.19
Duration of treatment (min)						
Weeks 1 - 62	60	60	60	15	60	60
Weeks 63 - 101	60	60	30	15	30	30
Achieved total dose (µg/kg) ²						
Weeks 1 - 62	0	0	0	0.4	1.9	8.3
Weeks 63 - 101	0	0	0	0.3	0.7	2.9
Mean (Weeks 1 - 101) ³	0	0	0	0.36	1.4	6.21
Pulmonary dose (µg/kg/day) ⁴	0	0	0	0.02	0.07	0.31

1. Mean aerosol tiotropium concentrations based on HPLC analysis of samples taken twice weekly for the first 13 weeks and once every two weeks for the remainder of the study.
2. Based on a minute volume of 1.2 liter, the exposure duration of 60 or 30 min., and the measured drug concentrations, and mid-term body weight.
3. Time-weighted averages for the entire study: $C_{\text{mean}} (\text{mg/kg/day}) = [C_{\text{week 1-62}} \times 62 + C_{\text{week 63-101}} \times 39]/101$
4. Derived as the 5% of the means of the achieved total doses.

Mortality:

Tiotropium caused a dose-dependent increase in mortality in male mice. Table 6.27 summarizes the survival rates in several time-points of the study. Figure 6.5 shows its Kaplan-Meier Survival curves. According to the statistical analysis of Dr. Feng Zhou (review dated 1-OCT-2001 in IND 46,687), the mid and high dose groups started to have significant high percentage of deaths starting at week 55.

Table 6.27 Survival Rate (%) of 101-week Carcinogenicity study in Male Mice¹

Time	Groups	I	II	III	IV	V	VI
	Treatment	Controls			Tiotropium (mg/kg/day)		
		Air	Vehicle	Vehicle	0.02	0.07	0.31
Weeks: 80		87	86	82	80	68	57
94		72	68	74	72	53	43
101		70	62	64	63	45	-

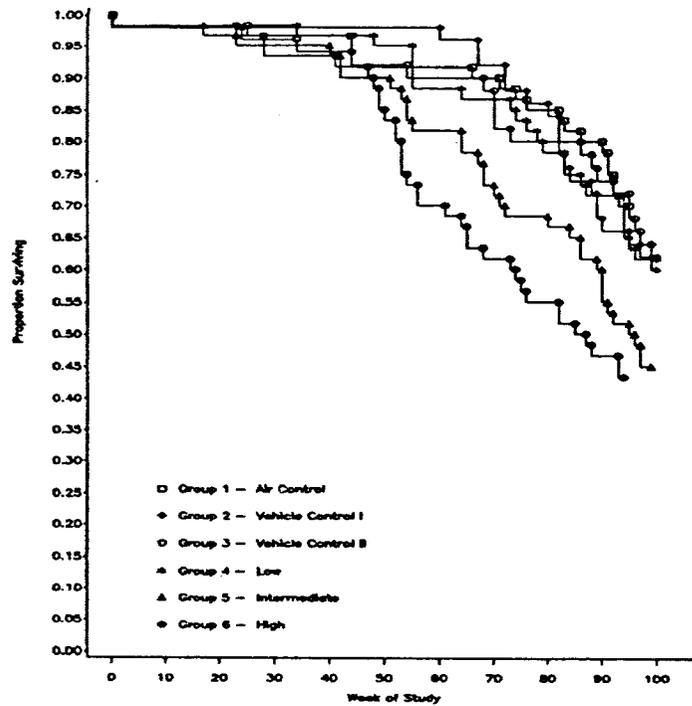


Figure 6.5 Kaplan-Meier survival curve of the 101-week inhalation carcinogenicity study of tiotropium in male mice.

Clinical signs: No treatment-related effects were observed.

Palpable masses: No treatment-related effects were observed

Body weight: There was no treatment-related change in body weight in the female mice.

Figure 6.6 presents the animal body weight as a function of treatment time. There was no apparent difference in body weight between treatment and control groups.

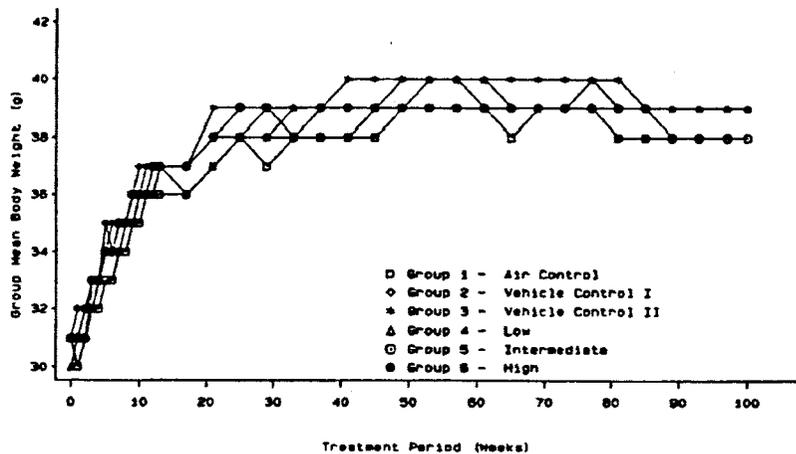


Figure 6.6 Body weight as a function of time in the 101-week carcinogenicity study in Male mice.

Food consumption: The high dose group showed statistically significant reduction in food consumption when compared to the vehicle control group 2. The reduction became apparent starting at week 10 and lasted until the termination of the animal. The average daily food consumption in Week 93 was 52, 39, 37, 51, 37 and 33 gram/animal for the air, vehicle 1, vehicle 2, low dose, mid and high dose groups, respectively.

Water consumption: No treatment-related effects were observed.

Ophthalmology: No treatment-related effects were observed.

Hematology: No remarkable changes were observed (Table 6.28). The changes in neutrophil and lymphocyte numbers were statistically significant for the vehicle 2 and high dose groups. These changes are not considered treatment-related.

**Table 6.28 Hematological Findings
of the 101-Week Inhalation Carcinogenicity Study in Male Mice**

Group	1	2	3	6
Treatment	Air Control	Vehicle 1	Vehicle 2	High Dose
Neutrophil				
Week 55	44.1 ± 14.4	46.8 ± 14.6	37.4 ± 9.9*	36.5 ± 12.3*
Week 79	39.2 ± 12.5	38.8 ± 11.0	47.9 ± 13.6*	47.4 ± 10.6*
Lymphocytes				
Week 55	51.3 ± 14.8	49.9 ± 14.6	58.5 ± 11.3*	59.8 ± 12.1*
Week 79	56.4 ± 12.3	56.7 ± 11.1	47.2 ± 13.1*	48.1 ± 10.4*

P < 0.05 when compared to group 2.

Salivation: Tiotropium produced a dose-dependent inhibition of the pilocarpine-induced salivation. Figure 6.7 shows the results of the salivation test (measured by the wet surface area from salivation). The high dose tiotropium completely blocked saliva production in male mice.

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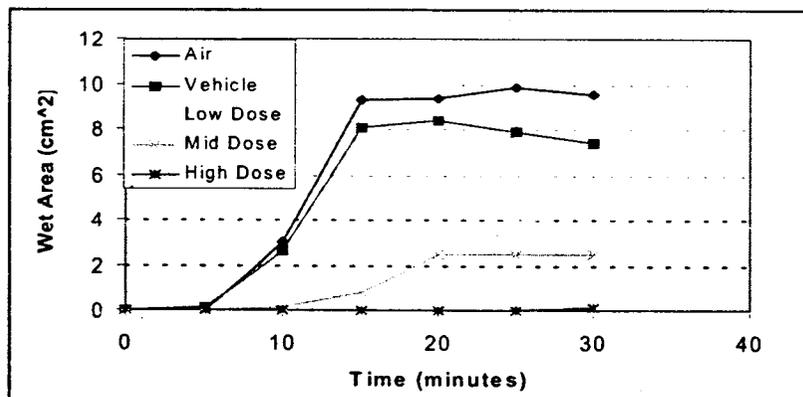


Figure 6.7 Inhibition of pilocarpine-induced salivation by Tiotropium in female mice (Mean Wet Area in cm², week 52). The figure was drawn based on data in Table 11 of the study report (vol. 1.68, p106).

Gross pathology: Increased incidences of distended intestine and urinary bladder, and discolored Hardarian gland were seen in the mid and high dose groups (Table 6.29).

Table 6.29. Gross Pathology Findings (incidence) in the 101-Week Inhalation Toxicity Study in Male Mice

Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle		Tiotropium (mg/kg/day)		
				0.02	0.07	0.31
Distended intestine						
Caecum	0	0	0	0	2	11
Colon	0	0	0	0	3	12
jejunum	0	0	0	0	2	0
Rectum	0	0	0	0	3	18
Abdomen	0	0	1	0	1	3
Harderian glands						
Discolored	0	0	0	0	0	13
Dark	0	0	2	0	3	5
Speckled/dark foci (1 or both)	7	1	4	5	11	0
Urinary bladder distension	8	6	3	8	12	11

Histopathology:

Non-neoplastic Changes:

Non-neoplastic changes associated with tiotropium treatment in male mice were distended lumen of the intestine, liver cell necrosis, and some other minor changes. Table 6.30 summarizes most remarkable non-neoplastic changes in the study.

**Table 6.30 Non-Neoplastic Findings (incidence) of
the 101-Week Inhalation Toxicity Study in Female Mice**

Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle		Tiotropium (mg/kg/day)		
				0.02	0.07	0.31
Number of animals examined	60	50	50	60	60	60
Nasal cavity:						
Cyst(s), (level 1)	1	5	4	7	11	3
Amyliodosis (level 3)	4	1	6	10	7	6
Lung: congestion	7	4	3	7	16	7
Intestine: Distended lumen						
Jejunum	1		1		2	2
Ileum	1	1	1		1	6
Colon	1		1		3	17
Rectum	1		1		3	23
Inflammation/ rectum		1				5
Mucosal hyperplasia/ rectum		1			3	3
Urinary bladder: distended lumen	7	8	4	9	17	14
Testes: Leydig cell hyperplasia	13	4	6	16	23	14
Sublingual gland: amyloidosis	9	4	13	6	19	14
Lymph node:						
Amyloidosis	1		2	3	7	
lymphoid hyperplasia	1	1	3	2	4	4
lymphoid hyperplasia/messent.	8	7	12	18	11	20
Hardarian gland:						
Lymphoid cell filtration	11	13	10	15	21	17
Porphyrin-associated chronic inflammation/granuloma	13	7	4	14	23	19

Neoplastic Findings:

No tiotropium-associated tumors were observed in the 101-week inhalation carcinogenicity study in male mice. Table 6.31 lists all tumors observed in the study. There are no remarkable increases in tumor incidences in the tiotropium treatment groups when compared to the control groups.

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Table 6.31 Neoplastic Findings (incidence) of the 101-Week Inhalation Toxicity Study in Female Mice						
Groups	I	II	III	IV	V	VI
Treatment	Air	Vehicle		Tiotropium (mg/kg/day)		
				0.02	0.07	0.31
Number of animals examined	60	50	50	60	60	60
Liver: Hemangioma						1
Hemangiosarcoma	1	1				
Hepatocellular carcinoma	5	6	7	5	4	1
Hepatocellular adenoma	7	6	6	7	4	5
Lung: alveolar/bronchiolar adenoma	13	9	3	3	9	
Alveolar/bronchiolar carcinoma	9	7	3	4	7	2
Kidney: Tubular carcinoma				1		
Pancreas: islet cell adenoma						1
Testes: Leydig cell adenoma	3	4	4	4	2	3
Adenoma (rete testis)	1					
hemangioma					1	
Epididymides: Leydig cell adenoma			1			
Seminal vesicle: adenoma			1		1	
Spleen: Hemangioma				1		
Pituitary gland: adenoma			1		1	
Thyroid gland: follicle cell adenoma	2			1		
Adrenal cortices: adenoma					1	2
Adrenal medullas: medullary tumors (benign)				2	1	
Hemolymphoretical system: thymus lymphoma	9	11	6	8	3	4
Non-thymus lymphoma		2	1	2	2	
Malignant lymphoma				1		
Histiocytic sarcoma				1	1	
Myeloid leukemia	1			1		
Mass cell tumor			1			
Mesenteric lymph node: hemangiosarcoma	1					
Harderian gland: adenoma	8	1	3	6	5	4
Adenocarcinoma		1			1	
Sciatic nerves: malignant schwannoma			1			
Skin/subcutis: Malignant schwannoma	1					1
Squamous cell carcinoma	1					
Sarcoma			1			
Sternum: Hemangiosarcoma					1	

Study Summary: Sixty male CD-1 mice/dose were by nose-only inhalation tiotropium bromide at time-weight average doses of 0.02, 0.07, 0.31 $\mu\text{g}/\text{kg}/\text{day}$ (pulmonary deposited doses) for 101 weeks. The controls were one air control of 60 mice and two vehicle control groups of 50 mice each. The vehicle did not contain BAC. Additional 30 mice/dose were included for the evaluation of inhibition of salivation by tiotropium.

A dose-related increase in mortality was observed in the tiotropium treatment groups. The mid and high dose groups showed statistically significantly higher mortality rates than the controls and low dose groups. Due to the high mortality, the high dose group was thus terminated at week

94. The low and mid dose groups were allowed to continue with the study until week 101 when all the animals were terminated. The survival rate at week 101 was 70%, 62%, 64%, 63% and 45% for the air, vehicles 1 and 2, low and mid dose groups. Tiotropium treatment did not have remarkable effect in body weight, nor were there effects on food and water consumption, ophthalmology or hematology examinations. Tiotropium exhibited a dose-related inhibition of pilocarpine-induced salivation. The high dose tiotropium completely blocked the salivation. Distended intestine and urinary bladder were observed in the mid and high dose groups. There were no significant differences in tumor rate among the treatment and control groups.

VI.2 Summary and Evaluation of Carcinogenicity Studies:

Three carcinogenicity bio-assays were conducted to evaluate the carcinogenic potential of tiotropium bromide. These studies are a 24-month inhalation study in rats (both males and females), a 19-month inhalation study in female mice, and a 23-month inhalation study in male mice. These studies revealed no evidence of tiotropium carcinogenicity. The CDER Executive Carcinogenicity Assessment Committee reviewed and concurred with the following evaluation on June 25, 2002. The Executive CAC report is attached as an addendum in Section VI.5.1.

VI.2.A Rats (Study 98-2727)

Fifty Wistar rats/sex/dose were given by nose-only inhalation 0.6, 1.5 and 5.3 $\mu\text{g}/\text{kg}/\text{day}$ (pulmonary deposited doses) of tiotropium bromide for 104 weeks. The control was divided into three subgroups (50 rats/sex each subgroup): air, vehicle without benzalkonium chloride (BAC) and vehicle with BAC. Mean plasma tiotropium concentrations were 0.15, 0.26 and 1.09 ng/ml at month 23 for the low, mid and high dose groups, respectively. There were dose-related decreases in body weights in the tiotropium treatment groups. The body weight decrease was 19% and 13% for the high dose males the females at the end of the study. There were no significant differences in the survival rates among the groups. Inflammation of the urinary bladder and proctitis were observed in all tiotropium treatment groups. Mild irritation of the upper respiratory tract was seen in groups treated with either BAC alone or BAC in combination with tiotropium bromide. There was no difference in the incidences of tumors between the control and tiotropium treatment groups.

Adequacy of the carcinogenicity study and appropriateness of the test model: This study is adequate. The Wistar rats is a frequently used and accepted animal model in evaluating carcinogenic potential of pharmaceuticals. The dose selection of the study is acceptable.

The dose selection of rat study is appropriate. The selection was based on a 13-week dose ranging study and a one-year toxicity study in the same strain in which tiotropium caused a dose-related decrease in body weights. In the 13-week study (Study U91-0493), the respective decrease in body weight gains for the low dose (5 $\mu\text{g}/\text{kg}/\text{day}$), mid dose (42 $\mu\text{g}/\text{kg}/\text{day}$) and high dose rats (350 $\mu\text{g}/\text{kg}/\text{day}$) was 40%, 67% and 78% in males and 28%, 48% and 71% in females. In the one-year study (Study U93-0945), the respective decrease in the absolute body weights for the low dose (1 $\mu\text{g}/\text{kg}/\text{day}$), mid dose (7 $\mu\text{g}/\text{kg}/\text{day}$) and high

dose (45 µg/kg/day) rats was 9%, 21% and 29% in the male and 3%, 13% and 16% in the female. The above data justify the selection of 5 µg/kg/day as the top dose of the 2-year carcinogenicity study (U98-2727). The acceptability of the dose selection is further supported by the achievement of MTD in the carcinogenicity study. The achievement of the MTD is indicated by the decrease in body weights in the treatment groups. Compared to the controls, the respective body weight decrease was 5%, 15% and 19% in the males and 9%, 12% and 13% in the females for the low, mid and high dose groups. The proper selections of species and dose render adequate and valid.

Evaluation of tumor findings: Tiotropium is not carcinogenic in rats as the study did not reveal any treatment-related tumors at the MTD of tiotropium.

VI.2.B Mice

Female Mice: 60 female CD-1 mice/dose were given by nose-only inhalation 0, 2.7, 4.8, 9.1 µg/kg/day (pulmonary deposited doses) of tiotropium bromide for 83 weeks (Study 98-2726). The control was divided into two subgroups groups: air (60) and vehicle (100). The vehicle control was further divided into two identical groups of 50 mice each. The vehicle lacked BAC that was present in the previous rat study. Mean plasma tiotropium concentrations (determined from satellite groups) were 16.4, 32.1 and 32.2 ng/ml for the low, mid and high dose groups, respectively. There were no differences in body weights between the control and the tiotropium treatment groups. However, the high dose group showed statistically higher mortality, starting at week 60 and onward. The mortality rate at the termination of the study (week 83) was 18%, 30%, 32%, 30%, 40% and 59% for the air, vehicles 1 and 2, low, mid and high dose groups, respectively. Histological evaluation at terminal sacrifice showed that following in the mid and high dose groups: distended intestine lumen, rectum mucosal hyperplasia, diminished size or atrophy of the spleen, and multicellular necrosis in the liver. There were not significant differences in tumor rate among the treatment and control groups.

Adequacy of the carcinogenicity study and appropriateness of the test model: This study is also adequate. The CD-1 mice is a frequently used and accepted species in evaluating carcinogenic potential of pharmaceuticals. The dose selection of the study is acceptable.

The study has achieved or exceeded the MTD. Thirteen-week dose ranging studies in the same strain of animals show that the 9 µg/kg/day is at least the MTD: a 40% decrease in body weight gain and mortality occurs at higher doses. The high mortality rate and an early termination of the 84-week carcinogenicity study further demonstrate that the study has achieved and exceeded the MTD. The mortality rate at the termination of the study (week 83) was 18%, 30%, 32%, 30%, 40% and 59% for the air, vehicles 1 and 2, low, mid and high dose groups, respectively. Consequently, 49, 35, 34, 42, 36 and 24 live mice are available for evaluation of tumors at the time of termination. Thus, both species and dose selections are acceptable.

A potential short-coming of the study is its short test duration. Animals are treated for 83 weeks (approximately 19 months), slightly short of the preferred 2 years. The duration of

treatment is no longer an issue as the Division has determined that such a test duration was acceptable in this species. The Division has informed the decision via a telephone conversation on 31-AUG-1995. The Division's decision was based on the high mortality rates in the males and the Agency's policy then considering the test duration of 18 months to be adequate in evaluating the carcinogenicity of pharmaceuticals in mice.

Evaluation of tumor findings: Tiotropium is not carcinogenic in female mice as the study did not reveal any treatment-related tumors at or above the MTD of tiotropium.

Male mice: 60 male CD-1 mice/dose were given by nose-only inhalation tiotropium bromide at time-weight average doses of 0.02, 0.07, 0.31 $\mu\text{g}/\text{kg}/\text{day}$ (pulmonary deposited doses) for 101 weeks (Study U99-1464). These dose were derived from the respective actual mean tiotropium dose levels of 0.02, 0.1, 0.42 $\mu\text{g}/\text{kg}/\text{day}$ for the first 62 weeks and 0.015, 0.035 and 0.15 $\mu\text{g}/\text{kg}/\text{day}$ for the remaining period of time (week 62 – 101) for the low, mid and high dose groups. The high mortality in the tiotropium treatment groups prompted the dose reduction in the second half of the experiment. The design of the control groups was identical to the previous female study: one air control group of 60 mice and two vehicle control groups of 50 mice each. The vehicle did not contain BAC. Additional 30 mice/dose were included for the evaluation of inhibition of salivation by tiotropium.

A dose-related increase in mortality was observed in the tiotropium treatment groups. The mid and high dose groups showed statistically significantly higher mortality rates than the controls and low dose groups. Due to the high mortality, the high dose group was thus terminated at week 94. The low and mid dose groups were allowed to continue with the study until week 101 when all the animals were terminated. The survival rate at week 101 was 70%, 62%, 64%, 63% and 45% for the air, vehicles 1 and 2, low and mid dose groups. Tiotropium treatment did not have remarkable effect in body weight, nor were there effects on food and water consumption, ophthalmology or hematology examinations. Tiotropium exhibited a dose-related inhibition of pilocarpine-induced salivation. The high dose tiotropium completely blocked the salivation. Distended intestine and urinary bladder were observed in the mid and high dose groups. There were no significant differences in tumor rate among the treatment and control groups.

Adequacy of the carcinogenicity study and appropriateness of the test model: This study is also adequate. As discussed previously, the CD-1 mice is a frequently used and accepted species in evaluating carcinogenic potential of pharmaceuticals. The dose selection of the study is acceptable as it also achieves and exceeds the MTD.

The dose selection of the male mice is acceptable. The dose selection was based on the previously aborted carcinogenicity study in the same strain (U98-2726) in which even the lowest dose (2.7 $\mu\text{g}/\text{kg}/\text{day}$) caused excessive mortality by week 52 of the treatment. The dose selection of Study U98-2726 was based on previous 13-week dose ranging studies. Apparently, the 13-week dose ranging study is not appropriate in predicting response of male mice to tiotropium. Similarly, even the one-year study is not a good predictor of the response of male mice after a longer term exposure. The dose adjustment of the second carcinogenicity study illustrates the point well. The initial high dose of the this

carcinogenicity study, 0.83 µg/kg/day that is lower than the previous low dose of 2.7 µg/kg/day, has to be reduced to 0.29 µg/kg/day. Even the newly adjusted dose still causes excessive mortality and the high dose group animals have to be sacrificed pre-terminally at week 94. The survival rate at week 94 was 72%, 53% and 43% for the low, mid and high dose groups, respectively; while the rate of the control groups was between 68 – 74%. Finally, tiotropium is a cholinergic antagonist that inhibits the saliva secretion and interferes with the bowl movement. The high dose group shows a complete block of salivation. The mid and high dose groups show distensions of intestine lumen and urinary bladder. The increase mortality and exhibition of secondary pharmacological effects of the drug indicate that the MTD has been achieved and exceeded.

Evaluation of tumor findings: Tiotropium is not carcinogenic in male mice as the study did not reveal any treatment-related tumors at or above the MTD of tiotropium.

VI.3 Overall Summary of Carcinogenicity Studies:

The carcinogenic potential of tiotropium bromide has been evaluated in traditional inhalation (nose-only) bioassays in rats and mice. The test duration range from 83 weeks to 104 weeks (19 – 24 months). Table 6.32 summarizes iotropium doses in these studies. Each study reaches or exceeds the MTD. No evidence of tiotropium tumorogenicity was found in any studies.

Table 6.32 Tiotropium Doses in Carcinogenicity Studies

Study #	Species	Sex	Test duration (weeks)	Tiotropium (µg/kg/day) ¹		
				Low dose	Mid Dose	High Dose
U98-2727	Rat	Male	104	0.6	1.5	5.3
		Female	104	0.6	1.5	5.3
U99-1464	Mouse	Male	101	0.02	0.07	0.31
U9802726		Female	83	2.7	4.8	9.1

1. Estimated pulmonary depositions.

VI.4 Carcinogenicity Conclusion:

No evidence of tiotropium tumorogenicity was found in 19 to 24-month inhalation carcinogenicity studies in mice and rats.

Recommendations for further analysis: None.

VI.5 Labeling Recommendations (Carcinogenesis)

VI.5.1 Recommended Labeling for Carcinogenesis

No evidence of tumorigenicity was observed in a 104-week inhalation study in rats at tiotropium doses up to [redacted], a 83-week inhalation study in female mice at doses up to 9.1 [redacted] and a 101-week inhalation study in male mice at doses up to [redacted]. These doses correspond to approximately [redacted] on a mg/m^2 basis, respectively.

VI.5.2 The Sponsor's Proposed Labeling

VI.5.3 Estimating Dose Ratios

The exposure (dose) ratios in the sponsor's proposed labeling need revision. The sponsor proposes [redacted]

As with other inhalation studies in the application, respective pulmonary deposition factors (DF) of 5% and 7% in mice and rats should be used to calculate the actual exposure. The application of the appropriate DFs results in revised doses up to 0.31, 9.1 and 5.3 $\mu\text{g}/\text{kg}/\text{day}$ in male mice, female mice and rats. Further incorporation of appropriate conversion factors for body surface areas yields final doses of 0.93, 27.3 and 31.8 $\mu\text{g}/\text{m}^2/\text{day}$ in these animals. Table 6.33 presents the parameters used in deriving the dose (mg/m^2) ratios between animals and humans. The newly derived dose ratios (≤ 2) are significantly smaller than the ones (≤ 94) proposed by the sponsor.

It may not be proper to use the available plasma tiotropium levels of the carcinogenicity studies to determine the exposure ratios of the drug between animals and humans. Table 6.34 also presents dose ratios based plasma tiotropium levels. These ratios are based on snap shots of plasma levels in animals because they are levels 15 minutes post dosing. The steady-state concentration is, therefore, unknown. It could be much lower than the reported values because of the short half-life of the drug in animals. In addition, no apparent dose-concentration response was seen in the rat carcinogenicity study. Furthermore, that the plasma drug level is not available in male mice (Study U99-1464). The available data do not properly reflect the pharmacokinetic characteristics of tiotropium in animals. The unreliability of the plasma level and the lack of AUC data render the use of plasma level ratios unacceptable. In addition, ratios based on mg/m^2 are smaller and more conservative. Thus, plasma level should not be used for estimate dose ratios between animals and humans.

**Table 6.33 Dose Ratio Calculations
for the Carcinogenesis Section of the Tiotropium Labeling**

Species	Conversion Factor	Study No	Dose		Ratio (animal/human)	
			$\mu\text{g}/\text{kg}$	$\mu\text{g}/\text{m}^2$	Calculated	Rounded to
Human	37	Labeling	0.36	13.3	-	
Mice, M	3	U99-1464	0.31	0.93	0.07	1/15
Mice, F	3	U98-2726	9.1	27.3	2.05	2
Rat	6	U98-2727	5.3	31.8	2.39	2
				C_{plasma} (ng/ml)		
Human	-	Labeling	0.36	0.019		
Mice, F	-	U98-2726	9.1	35.3 ^a	1,858	1,900
Rat,	-	U98-2727	5.3	0.958 ^b	50.4	50

a. Average of plasma tiotropium levels at weeks 57 (38.4 ng/ml) and 83 (32.2 ng/ml).

b. Average of male and female plasma tiotropium levels at months 12 and 23.

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VI.5 Addendum/appendix listing for Carcinogenicity Studies:**VI.5.1 CAC Report:****Executive CAC****Date of Meeting: June 25, 2002**

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Dave Morse, Ph.D., HFD-150, Alternate Member
Chuck Resnick, Ph.D., HFD-110, Alternate Member
Joseph Sun, Ph.D., HFD-570, Supervisory Pharmacologist

Presenting Reviewer and Author of Draft: Luqi Pei, Ph.D., HFD-570

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

NDA # 21-395

Drug Name: Tiotropium Bromide

Sponsor: Boehringer Ingelheim

Background

Three inhalation studies were completed to evaluate carcinogenicity of tiotropium in mice and rats. Tiotropium is a muscarinic cholinergic receptor antagonist and is being developed as inhalation therapy for bronchospasm and dyspnea. Metabolic pathways for the drug in animals and humans are similar. Toxicity studies up to one year in treatment duration have identified the following as the target organs of tiotropium toxicity: the respiratory and gastrointestinal tracts, eyes, heart and urinary bladder. Tiotropium is non-genotoxic in five *in vitro* and *in vivo* assays.

In mice, separate carcinogenicity studies were completed in the male and female because of a remarkable difference in mortality between the sexes. The first study was started in both males and females; however, the male section was aborted at treatment week 52 because of excessive mortality. Thus, the study evaluated the tumor incidence in the females only. The early termination of the males in the first study prompted a second study at lower doses in males only. For both species, dose-selection was based on the results of three-month dose-ranging studies. None of the carcinogenicity study protocols had the Agency's concurrence prior to commencement of the experiment.

Rat Carcinogenicity Study

Wistar rats (50 /sex/dose) were given via nose-only inhalation 0.6, 1.5 and 5.3 $\mu\text{g}/\text{kg}/\text{day}$ (pulmonary deposited doses) of tiotropium bromide for 104 weeks. Three additional groups (50/sex/group) received air, vehicle without benzalkonium chloride (BAC) or vehicle with BAC and served as controls. There were no significant differences in the survival rates among the groups. Dose-related decreases in body weight gain were observed in the tiotropium treatment groups. Body weights at the termination of the study were 19% and 13% lower than control for the high dose males and females, respectively. Inflammation of the urinary bladder and prostatitis were observed in all tiotropium treatment groups. Mild irritation of the upper respiratory tract was seen in groups treated with either BAC alone or BAC in combination with tiotropium bromide. There was no difference in the incidences of tumors between the control and tiotropium treatment groups.

Mouse Carcinogenicity Study

Female Mice: Female CD-1 mice (60/dose) were given via nose-only inhalation 0, 2.7, 4.8, 9.1 $\mu\text{g}/\text{kg}/\text{day}$ (pulmonary deposited doses) of tiotropium bromide for 83 weeks. Two additional groups received either air (60 mice) or vehicle (100 mice) to serve as controls. The vehicle control group was further divided into two subgroups of 50 mice each. The vehicle lacked BAC that was present in the rat study. There were no differences in body weights between the control and the tiotropium treatment groups. However, the high dose group showed statistically higher mortality (59%). Histological evaluation showed following in the mid and high dose groups: distended intestine lumen, rectum mucosal hyperplasia, diminished size or atrophy of the spleen, and multicellular necrosis in the liver. There were no significant differences in tumor rates among the treatment and control groups.

Male mice: Sixty male CD-1 mice/dose were given via nose-only inhalation tiotropium bromide at time-weight average doses of 0.02, 0.07, 0.31 $\mu\text{g}/\text{kg}/\text{day}$ (also pulmonary deposited doses) for 101 weeks. These dose were derived from the respective actual mean tiotropium dose levels of 0.02, 0.1, 0.42 $\mu\text{g}/\text{kg}/\text{day}$ for the first 62 weeks; and 0.015, 0.035 and 0.15 $\mu\text{g}/\text{kg}/\text{day}$ for the remaining period (week 62 – 101) for the low, mid and high dose groups. The high mortality in the tiotropium treatment groups prompted the dose reduction. The design of the control groups was identical to that used for the female study: one air control group of 60 mice and two identical vehicle control groups of 50 mice each. An additional 30 mice/dose were included for the evaluation of inhibition of salivation by tiotropium.

The mid and high dose groups showed statistically significantly higher mortality rates than the controls and low dose groups. Due to the high mortality, the high dose group was terminated at week 94. (The remaining groups were terminated at week 101.) Tiotropium exhibited a dose-related inhibition of pilocarpine-induced salivation; the high dose of tiotropium blocked the salivation completely. Distended intestine and urinary bladder were observed in the mid and

high dose groups. There were no significant differences in tumor rates among the treatment and control groups.

Executive CAC Recommendations and Conclusions

1. The studies are acceptable. The MTD was attained based on the treatment-related increase in mortality for mice and the decrease in body weight gain for rats.
2. Tiotropium bromide treatment produced no evidence of tumorigenicity in either species.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\n
/Division File, HFD -570
/Joseph Sun, HFD-570
/Luqi Pei, HFD-570
/Anthony Zeccola/CSO/PM, HFD-570
/ASeifried, HFD-024

VI.5.2 List of organs and tissues examined:**Histopathology Inventory for NDA No. 21-395 (Carcinogenicity studies)**

Study	U98-2727	U98-2726	U99-1464
Species	Rats	Female Mice	Male Mice
Adrenals	x	x	x
Aorta	x	x	x
Blood Smear		x	x
Bone Marrow smear	x	x	x
Bone (femur)			x
Brain	x	x	x
Cecum	x	x	x
Cervix			
Colon	x	x	x
Duodenum	x	x	x
Epididymis			x
Esophagus	x	x	x
Eye	x	x	x
Fallopian tube			
Gall bladder		x	x
Gross lesions		x	x
Harderian gland	x	x	x
Heart	x	x	x
Ileum	x	x	x
Injection site	x		
Jejunum	x	x	x
Kidneys	x		
Lachrymal gland	x	x	x
Larynx	x	x	x
Liver	x	x	x
Lungs	x	x	x
Lymph nodes, cervical	x		
Lymph nodes, mandibular		x	x
Lymph nodes, mesenteric	x	x	x
Mammary Gland	x	x	x
Nasal cavity	x	x	x
Optic nerves	x	x	x
Ovaries	x	x	
Pancreas	x	x	x
Parathyroid	x	x	x
Peripheral nerve			
Pharynx			
Pituitary	x	x	x
Prostate	x	x	x
Rectum	x	x	x
Salivary gland	x	x	x
Sciatic nerve	x	x	x
Seminal vesicles	x	x	x
Skeletal muscle		x	x
Skin	x	x	x

Spinal cord	x	x	x
Spleen	x	x	x
Sternum	x	x	x
Stomach	x	x	x
Testes	x		x
Thymus	x	x	x
Thyroid	x	x	x
Tongue	x	x	x
Trachea	x	x	x
Urinary bladder	x	x	x
Uterus	x	x	
Vagina		x	
Zymbal gland			
Standard List			

X, histopathology performed

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VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

VII.1 Reproductive and developmental toxicology summary

Reproductive toxicity studies in rats and rabbits have revealed no evidence of teratogenicity; nor did the drug affect fertility index. However, fetal resorption, post implantation loss and litter loss, decreases in the number of corpora lutea and implants were associated with inhalation of tiotropium at doses of $\geq 7 \mu\text{g}/\text{kg}$. The results indicate that tiotropium is not teratogenic but embryofetocidal.

A total of six definitive reproductive toxicity studies of tiotropium have been completed (Table 7.1). They consist of four inhalation studies and two oral studies. The inhalation studies include three studies in rats (Segments I, II and III each) and a Segment II study in rabbits. The formulation of the inhalation studies is aqueous aerosols that contained 0.01% benzalkonium chloride and 0.01% EDTA. The oral formulation is 0.5% tylose was given by gavage.

Table 7.1 Definitive Reproductive Toxicity Studies of Tiotropium

Study #	Study Design	Species	Formulation	Treatment	Dose ^a ($\mu\text{g}/\text{kg}/\text{day}$)
U93-0239	Seg. I	Rat	IH	9/2 wks pre-mating day to 21 post partum	0.75, 7, 139
U93-0622	Seg. II			GD 6 - 17 ^b	
U96-2493	Seg. III			GD 17 - weaning	
U92-0623	Seg. II	Rabbit	IH	GD 6 - 18	0.9, 11, 50
U90-0687	Seg. II	Rat	PO	GD 6 - 17	1, 25, 500 (mg/kg/d)
U91-0340	Seg. II	Rabbit		GD 6-18	1, 10, 100 (mg/kg/d)

- Estimated pulmonary deposition for inhalation studies. See Table 7.2 for details in dose conversion.
- GD = gestation days.

Tiotropium doses of inhalation studies in Table 7.1 are the estimated amount deposited in the pulmonary region. They differ from those cited in the Non-clinical Summary section and the study reports of the submission. The difference is attributed to the definition of estimated exposure for inhalation studies. The Division regards pulmonary deposits as the estimated exposure. The submission, however, uses the total inhaled doses and pulmonary deposit for the reproductive and general toxicity studies, respectively. Using the pulmonary deposits for the reproductive studies ensures consistency in dose estimation in all inhalation toxicity studies.

The selective use of the regional deposits in the respiratory system results in a discrepancy in the estimated animal exposures of the inhalation studies. Table 7.2 presents a comparison of the inhalation doses between the study report, the submission and the current review. The original study reports and previous reviews use the target doses. The Summary section of the submission also uses total body burden (i.e., the total inhaled dose or total achieved dosage). Consequently, the doses in the Summary section differ slightly from the original study. The current review uses the estimated pulmonary deposition, by applying the same deposition factor used in the general

inhalation toxicity studies. The review also uses averages of dosing to simplify the discussion. The reason is that the dose distribution between the studies in rats is rather similar.

Table 7.2 Tiotropium Exposures in Inhalation Reproductive Toxicity Studies

Species	Study No.	Dose ($\mu\text{g}/\text{kg}/\text{day}$)				
		Group	Study Reports	Submission	This Review ^a	
					Individual	Average
Rat	U93-0239	LD	10	11	0.8	0.8
	U93-0622					
	U96-2493			10	0.7	
	U93-0239	MD	100	97 – 100	7	7
	U93-0622					
	U96-2493					
	U93-0622	HD	2,000	1,838	129	139
U93-0239	1,980			139		
U96-2493	2,110			148		
Rabbit ^b	U93-0623	LD	10	9	0.9	-
		MD	100	110	11	-
		HD	500	500	50	-

a. The dose in this review column equals to the achieved dose (the submission column) multiplied by deposition factors of 0.07 and 0.10 in rats and rabbits, respectively.

b. The deposition fraction of particles with MMAD of μm in rabbits is not well understood. It is believed that a 10% is a reasonable estimate as the deposition factor of 5%, 7% and 10% for mice, rats and dogs, respectively.

Table 7.3 summarizes noticeable findings in the reproductive toxicity studies of tiotropium. No evidence of teratogenicity is revealed, neither the evidence of effect on fertility index; however, the studies do show that tiotropium is not only embryocidal and fetocidal but also affects post natal development of the offsprings.

VII.1.1 Embryo and fetal effect

As shown in Table 7.3, litter loss, fetal resorption, and the increase in post implantation loss and minor skeletal variations are associated with tiotropium treatment. Total litter loss occurred at inhalation tiotropium doses of $\leq 7 \mu\text{g}/\text{kg}/\text{day}$ in rats (U96-2493). Increased post plantation loss /total resorption occurred in rats (U93-0239) and rabbits (U92-0623) at respective inhalation tiotropium doses $\leq 7 \mu\text{g}/\text{kg}/\text{day}$ and $50 \mu\text{g}/\text{kg}/\text{day}$ in the absence of significant maternal toxicity. Minor skeletal variations [i.e., short 13th rib at oral doses of $\leq 25 \mu\text{g}/\text{kg}/\text{day}$ in rats (Study UU90-0687) and extra ribs] occur at inhalation doses of $\leq 11 \mu\text{g}/\text{kg}/\text{day}$ in rabbit. No such effects were noted at $0.8 \mu\text{g}/\text{kg}/\text{day}$ in rats and $11 \mu\text{g}/\text{kg}/\text{day}$ in rabbits, respectively. The tiotropium labeling should include fetal resorption, and the increase in post implantation loss and litter loss. It may not include

VII.1.2 Effect of Fertility

Tiotropium did not affect the fertility index in rats, but it caused decreases in the number of corpora lutea, implants and live birth in rats (Table 7.3, Study U93-0239). The mid (7 µg/kg/day) and high dose groups (139 µg/kg/day) showed, respectively, decreases of 16% and 13% in the number of corpora lutea, 20% and 15% in the number of implants, and 21% and 22% in the number of live pups at birth. The high dose was maternally toxic while the mid dose was not. The findings in the mid dose group should be considered a treatment-related effect. The decrease in the number of corpora lutea and the implants suggest that tiotropium might interfere with the ovulation process. Since inhibition of ovulation in humans may lead to decrease in fertility, this finding should be included in the labeling.

Table 7.3 Noticeable Findings in Reproductive Toxicity Studies of Tiotropium

Species	Segment	Study No.	Observation	IH Tiotropium (µg/kg/day)		
				0.8	7	139
Rat	I	U93-0239	Parental toxicity: death, ♂	0/30	2/30	4/30
			♀	0/30	0/30	2/30 ^a
			↓ # of corpora lutea (%)	0	16**	13**
			↓ implants (%)	0	20**	15**
			# of live pups at birth	14.7	11.6*	11.4**
			↑ post implantation loss			13%
	Fetal: total resorption	0/30	1/30	2/30		
	Delayed sexual maturation		x	x		
	II	U92-0622	Maternal toxicity: (↓ bdwt gain)		8%	17%
	Pups: impaired passive avoidance behavior		+/-	x		
Delayed sexual maturation	x	x	x			
III	U96-2493	Maternal toxicity (↓ bdwt gain)	22%	61%	98%	
Mean pup weight (g)	43.9	39.6*	31.5**			
Total litter loss	1/25	3/25	10/25			
Fetal: delayed sexual maturation ♀	x	x	x			
Rabbit	II	U92-0623	Maternal toxicity (↓ food consumption)		11	50
			Fetal: ↑ post implantation loss		36%	53%
			Extra rib (% of fetus)	5	30	23
				Oral Tiotropium		
Rat	II	U90-0687	Tiotropium Dose (mg/kg/day)	1	25	500
			Maternal toxicity (↓ bdwt gain)			10%
			Fetal: Short 13 th rib (% fetus)	8	28	86
Rabbit	II	U91-0340	Tiotropium Dose (mg/kg/day)	1	10	100
			Maternal toxicity: coprostasis (↓ food consumption)		1/15	15/18
			Fetal effect: none.		47%	63%

* p < 0.05; ** P < 0.01.

a. The two female deaths occurred on day 12 of the paring and day 20 post partum each.

VII.1.3 Pre- and Post- Natal Development.

Rat pups exposed to tiotropium maternally showed a delay in sexual maturation and a signal for effect on neurological development. The sexual maturation was measured by vaginal opening in the female and occurrence of balanopreputal skinfold in the males. Table 7.4 summarizes the effect of sexual maturation. The delay in sexual maturation occurred in all three rat inhalation studies (one each for Segments I, II and III studies), but a dose-response relationship was not strong. It was unclear whether the maximum response had been achieved. That the inhalation study in rabbits and two oral studies in both rats and rabbits did not show such a finding suggested that the delay in sexual maturation might occur only in rats at high systemic exposure. This was because the bioavailability of oral tiotropium was very low (< 1%). The relevance of the finding of delayed sexual maturation in rats to the safety assessment of the drug in humans is unclear at present. Nonetheless, it should be mentioned in the labeling.

Table 7.4 Developmental Findings in the Offspring (F₁)

Study#	Species /Study	Formulation	Treatment	Dose	Sex	Delay in Sexual Maturation (days) ^a		
						LD	MMD	HD
U96-2493	Rat, Seg. III	IH	G17 - weaning	0.8, 7, 139 µg/kg/day	M	0.1	1.6*	3.2*
					F	0.1	0.9*	2.5*
U93-0239	Rat, Seg. I	IH	9/2 wks pre-mating To weaning		M	0.2	0.1	1.1
					F	1.5*	1.5*	1.2*
U92-0622	Rat, Seg. II	IH	G6 - 17		M	2.6*	1.9*	2.0*
					F	1.1	3.4*	2.0*
U92-0623	Rabbit, S II	IH	G6 - 18		0.01, 0.1, 0.5 mg/kg/day	No treatment-related effect was observed in either sex.		

a. Delayed vaginal opening and occurrence of cleavage of balanopreputal skinfold.

* p < 0.05.

The effect of tiotropium on the impairment of passive avoidance behavior in pups was less certain. The Segment II inhalation toxicity study (U93-0622) showed a possible and definite impairment of the passive avoidance behavior at 7 µg/kg/day (MD) and at 139 µg/kg/day (HD, Table 7.5), respectively. This was indicated by the statistically significant decrease in both the time to the first entry to the safety chamber and the time spent in the safety chamber on both days 2 and 22 of the high dose group pups. The mid dose pups showed a non-statistically significant decrease in the time to first entry safety chamber on day 22 only. No impairment of the passive avoidance behavior occurred at 0.8 µg/kg/day.

The above findings prompted a further review of the possible behavioral impairment in pups of other reproductive studies. The review revealed the following statements in the Segments I and III study reports in rats:

- 1) "...[T]here was a tendency for animals derived from parents treated at 2.0 mg/kg/day [*i.e.*, 139 µg/kg/day pulmonary dose] to show slightly impaired performance compared to the controls, evident as quicker initial times and a reduction in the amount of time spent in the

non-chock compartment, none of the difference attained statistical significance ($P > 0.05$)” (Study U93-0239, vol. 86, p 44).

- 2) “Females derived from F0 females treated at 2.0 mg/kg/day day (*i.e.*, 139 $\mu\text{g}/\text{kg}/\text{day}$ pulmonary dose) had significantly quicker head entry time compared with controls... Differences did not generally attain statistical significance and were not considered to be of toxicologically importance.” (U96-2493, Vol. 89, p 34).

Table 7.5 Effect of Tiotropium on Pup Passive Avoidance Behavior in Rats (Study U92-0622)

Tiotro-pium ($\mu\text{g}/\text{kg}/\text{day}$)	Rat # showing head entry		Time to 1 st entry (sec)		Time in safe chamber (sec)		rat# w/ max. avoid. time	
	M	F	M	F	M	F	M	F
Day 2								
0			180	180	180	180	9	8
0.8			180	180	180	180	7	8
7			180	180	180	180	7	10
139			119*	47*	139*	163*	4	3
Day 22								
0	2	6	180	29	180	99	7	1
0.8	8	9	180	39	180	92	8	2
7	8	8	70	36	139	146	3	1
139	9	7	30*	16	73*	78	0	0

These statements apparently reflected occasional findings in the certain monitored parameters. Table 7.6 summarizes the noticeable findings of the behavioral impairment. These findings were inconsistent among three studies. The Segment I study showed no evidence of behavior impairment in pups. The Segment III study showed a statistically significant decrease in the time to first entry to the safe chamber at the high dose of. The segment II study also showed impairment at high doses. Overall, the impairment generally occurred at a significant maternally toxic dose only. Surprisingly, the behavioral impairment occurred only in the Segment II study but not the Segment I study that had a much longer duration of exposure. Yet, behavioral testing usually was not performed in the Segment II studies. Since the results of this behavioral test were variable in nature, it was unclear whether tiotropium truly caused the behavioral impairment in pups. Thus, the labeling may

VII. 2 Reproductive and developmental toxicology conclusions:

Tiotropium was not teratogenic but embro/fetocidal in animals at inhalation doses of $\leq 7 \mu\text{g}/\text{kg}/\text{day}$. Tiotropium caused total litter loss and decreases in the number of corpora lutea, implants, live births and mea pup weights at $7 \mu\text{g}/\text{kg}/\text{day}$ in rats. The drug also delayed sexual maturation in rat pups exposed to it maternally at the same dose.

Table 7.6 Summary of the Noticeable Behavioral Changes in Pups

Sex			Time (sec, mean)							
			Male				Female			
Tiotropium (ug/kg/day)			0	0.8	7	139	0	0.8	7	139
Day 2 (ret)	Seg.	n								
U90-0622	II	10	180 ^a	180	180	119*	180	180	180	47*
U93-0239	I	12	180 ^a	180	180	180	180	180	180	180
U96-2493	III	20	59 ^b	51	180	61	180	159	84	180
Day 22										
U90-0622	II	10	180 ^a	180	70	30*	29	39	36	16
U93-0239	I	12	155 ^c	142	180	86	115	131	147	180
U96-2493	III	20	56 ^b	79	70	44	72	77	33	29*

- a. Time to first full body entry
- b. Time to First head entry
- c. Total time in the safe chamber

VII. 3 Labeling recommendations:

VII.3.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

In rats, decreases in the number of corpora lutea and the percentage of implants were noted at inhalation tiotropium doses of _____ or greater (approximately _____ on a mg/m² basis). No such effects were observed at _____ on a mg/m² basis). The fertility index _____

VII.3.2 Pregnancy

Pregnancy Category C. No evidence of structural alterations was observed in rats and rabbits at inhalation tiotropium doses of up to _____ respectively. These doses correspond to approximately _____ times the rr _____

However, in rats, fetal resorption, litter loss, and decreases in the number of live pups at birth and the mean pup weights were observed at inhalation tiotropium doses of _____ (approximately _____ a mg/m² basis).

In rabbits, an increase in post implantation loss was observed in at an inhalation dose of _____ (approximately _____ on a mg/m² basis). Such effects were not observed at inhalation doses of _____ in rat and rabbits, respectively. These doses correspond to _____