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RESEARCH**

APPLICATION NUMBER:

21-395

PHARMACOLOGY REVIEW(S)

REVIEW OF PHARMACOLOGY AND TOXICOLOGY DATA

Addendum to Review No. 2

Application Information

NDA number: 21-395
Drug Name: Spiriva HandiHaler DPI (Tiotropium bromide)
Sponsor and/or agent: Boehringer Ingelheim
Date of submission: July 31, and December 30, 2003
Submission Content: Labeling revision

Reviewer Information

Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Drug Products
Division Code: HFD-570
Review Completion Date: January 7, 2003

This addendum revises a word in Dr. Pei's nonclinical labeling review of Spiriva application dated December 23, 2003. The word "deposited" in the qualifying sentence following dose multiples of the Newly Suggested Labeling (Section 3) needs to be replaced with "deposited". The sentence now should read as "These dose multiples may be overestimated due to difficulties in measuring deposited doses in animal inhalation studies."

There were discrepancies in the text between Section 3 (the clean section of the suggested labeling) and Section 4 (the annotated section) in Dr. Pei's labeling review. Section 3 states

Section 4 states "These dose multiples may be overestimated due to difficulties in measuring deposited doses in animal inhalation studies". Section 4 should supercede Section 3.

The sponsor's December 30, 2003 submission includes the following variations of text in the "Carcinogenesis, Mutagenesis, Impairment of Fertility", "Pregnancy" and "OVERDOSAGE" sections:

" or "
Both sentences need to be revised as "These dose multiples may be overestimated due to difficulties in measuring deposited doses in animal inhalation studies".

Recommendation

Replace the sentence " in the sections of carcinogenesis (Lines 256 – 257 and Lines 269 - 271), pregnancy (Lines 284 – 285), and Overdosage (Lines 380 – 381) of the proposed labeling dated December 30, 2003 with "These dose multiples may be overestimated due to difficulties in measuring deposited doses in animal inhalation studies".

Luqi Pei, Ph.D.
Pharmacologist

Timothy McGovern, Ph.D.
Supervisory Pharmacologist

**APPEARS THIS WAY
ON ORIGINAL**

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/s/

Luqi Pei
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PHARMACOLOGIST

Timothy McGovern
1/7/04 01:28:41 PM
PHARMACOLOGIST
I concur.

REVIEW OF PHARMACOLOGY AND TOXICOLOGY DATA

Review No. 2

Application Information

NDA number: 21-395
Drug Name: Spiriva HandiHaler DPI (Tiotropium bromide)
Sponsor and/or agent: Boehringer Ingelheim
Date of submission: July 31, 2003
Submission Content: Labeling revision

Reviewer Information

Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Drug Products
Division Code: HFD-570
Review Completion Date: December 23, 2003

Summary: This review evaluates the sponsor's revisions of the nonclinical sections of the labeling of Spiriva HandiHaler[®] in the 31-JUL-2003 submission. The revisions are in concordance with the agreement made between BI and the Division on April 1, 2003. The revisions primarily modify tiotropium dose ratios between animals and humans in the previous submissions and are generally acceptable. Minor text revisions are also included. This review suggests a revised version of labeling.

REVIEW

NDA 21-395 was recommended for approval in the initial nonclinical review for this application dated September 17, 2002 (electronic signature date of September 20, 2002) pending recommended revisions to the product label. Overall, the application was considered to be Approvable (letter dated December 20, 2002). Of note, safety qualification of various drug product impurities are being handled via consults with the CMC review team. Discussions with the sponsor regarding the nonclinical aspects of the product label took place on October 21, 2002 and April 1, 2003 (see minutes of teleconferences).

This review evaluates the sponsor's revisions of the nonclinical sections of the labeling of Spiriva HandiHaler[®] in the 31-JUL-2003 submission. The revisions primarily modify tiotropium dose ratios between animals and humans in the previous submissions and are generally acceptable. The review also recommends minor text revisions.

1. Dose Ratio Revisions

Labeling review of the Spiriva HandiHaler has been ongoing since the original submission. The Division and BIPI have generally agreed on the text portion of the nonclinical sections

of the labeling.¹ However, there were significant differences (approximately 15 fold) in animal to human dose ratio calculations between the sponsor and the Division. These differences prompted discussions between the Division and BIPI as documented in telephone conferences on October 21, 2002 and April 1, 2003. An agreement on how to calculate the dose ratios was finally reached on April 1, 2003.² The agreement included using the inhaled dose of tiotropium base as the actual level of exposure in both animals and in humans.

This review calculates the dose ratios according to the agreement. These new dose ratios between animals and humans differ from the previous review and the sponsor's proposal in the original submission. Using rat carcinogenicity study (Study No. 98-2727) as an example (Table 1), tiotropium ratios for the same group of rats in Dr. Pei's previous review and BIPI's estimates were 2 and 30, respectively, while the current review derives a dose ratio of 25. The difference is attributed to two factors: the deposition factor and the form of tiotropium (salt vs. base) in animal studies.

Table 1. Tiotropium Dose Ratios between Rats and Human in Study U98-2727

Tiotropium	Document		
	Original submission	Pei's Review (9-17-03)	Current review
Dose ($\mu\text{g}/\text{kg}/\text{day}$)	74.1	5.3	59.3
Dose ratio, calculated ^a	33	2.4	27
, rounded to	30	2	25

a. On a mg/m^2 basis. Obtained from multiplying the $\mu\text{g}/\text{kg}/\text{day}$ dose by a conversion factor of 6 in animals and dividing that product by the human dose of $13.32 \text{ mcg}/\text{m}^2$ ($18 \text{ mcg}/\text{day}$ for a patient of 50 kilograms). Each clinical actuation delivers 18 mcg tiotropium base, corresponding to 22.5 mcg tiotropium bromide.

a. Deposition Factor

Given the lack of quality plasma exposure data, the use of appropriate deposition factors plays an important role in determining the drug exposure levels in inhalation toxicity studies in animals. The Division has traditionally (although not exclusively) been using the pulmonary deposition as the actual animal exposure. The reason is that only a small fraction (about 7% for inhaled particles with a MMAD of μm) of the inhaled drug under the usual experimental conditions is deposited in the pulmonary region. Dr. Luqi Pei's review dated September 17, 2002 used this approach to estimate tiotropium deposition in animals. In contrast, BIPI, in their original submission, used total inhaled dose as the actual exposure in animals. This resulted in a 15-fold difference in tiotropium doses in animals between the estimates by Dr. Pei's and the sponsor. Using the same rat carcinogenicity study (Study No. 98-2727) as an example (Table 1), Dr.

¹ The 02-Oct-2002 submission incorporated the Division's recommendation on the text portion of the labeling. The Division's recommendation was based on Dr. Luqi Pei's review dated September 17, 2002.

² The minutes state: "BI and the Division agreed to calculate the ratios between animals and humans using the following parameters: the delivered (or achieved) dose in the inhalation toxicity studies in animals and 18 mcg tiotropium/actuation in humans (i.e., $13.32 \text{ mcg}/\text{m}^2$). The delivered dose in animals will be based on the same form (e.g., free base or bromide salt) as what is present in the clinical formulations. The label will include a statement indicating that this exposure of tiotropium in animals is likely overestimated."

Pei's and BIPI's estimated tiotropium high dose was 5.3 and 74.1 $\mu\text{g}/\text{kg}/\text{day}$, respectively.

The significant difference in tiotropium exposure level in animals prompted internal discussions about the acceptable dosing estimates in inhalation toxicity studies. On March 27, 2003, the pharmacology and toxicology discipline decided that, for labeling purposes, it was acceptable to use the total inhaled dose as the actual exposure levels in animals. The minutes of the group discussion can be found in Appendix 1. This review uses the total inhaled doses of tiotropium as the actual exposure in animals.

b. Tiotropium Chemical Form

Another contributing factor to the large dose ratios by the sponsor is the inconsistent use of tiotropium salt or base in estimating the exposure levels in animals and humans. The molecular weight of tiotropium salt and base are 392.2 and 490.4, respectively. This results in a molecular weight ratio of 1.25. The sponsor previously used tiotropium salt as the amount of animal exposure and the base for human exposures, resulting in a 25% increase in the animal to human dose ratio.

In the telephone conference of April 1, 2003, the Division and BIPI agreed to use the amount of tiotropium base as the exposure level in animals and humans. Again, using the rat carcinogenicity study (Study No. 98-2727) as an example, the estimated tiotropium dose for the high dose group was decreased from 74.1 and 59.3 $\mu\text{g}/\text{kg}/\text{day}$ (Table 1).

The effect of the above two factors was present in each animal to human tiotropium dose ratio of the labeling. This review will not discuss each dose ratio separately. It rather summarizes the newly revised dose ratios between animals and humans based on this new approach (Table 2, next page). The "Rounded to" column (far right) represents the currently suggested numbers. The "Calculated" column represents the sponsor's newly proposed numbers in the 30-JUL-2003 submission. These ratios are calculated based on surface area (mg/m^2). The revised doses are acceptable.

The newly proposed labeling also adds the following text: _____

The review recommends revising the sentence to "These dose multiples may be overestimated due to difficulties in measuring deposited doses in animal inhalation studies". This change will avoid potential confusion in data interpretation. It is also recommended that the labeling for other inhalation drug products contain a similar statement.

Table 2. Tiotropium Dose Ratios (Animal / Human) in Spiriva Labeling

Study #	Species	Section in Labeling	Achieved Dose			Dose Ratio ^d	
			(mg/kg/day)		(mg/m ³) ^c	(animal/human)	
			Original Subm. ^a	30-JUL-03 Subm. ^b	30-JUL-03 subm.	Calculated	Round to
U98-2727	Rat	Carcinogenesis	0.0741	0.0593	0.356	26.7	25
U98-2726	Mouse, (F)		0.181	0.1445	0.434	33	35
U98-1464	(M)		0.005	0.002	0.006	0.45	one-half
U93-0239	Rat	Fertility	0.097	0.078	0.468	35	35
U93-0239			0.011	0.009	0.054	4.1	4
U96-2493			2.11	1.689	10.13	760	760
U96-2493	Rat	Pregnancy	1.838	1.471	8.83	660	660
U92-0622			0.011	0.009	0.054	4.1	4
U93-0239			0.011	0.009	0.054	4.1	4
U90-0687			500	400	2,400	180,000	180,000
U92-0623	Rabbit	Pregnancy	0.5	0.4	4.8	360	360
U92-0623			0.011	0.088	1.056	79	80
U92-0623			0.009	0.007	0.084	6.3	6
U91-0340			100	80	960	72,000	72,000
U92-0652	Mouse	Overdose	23.24	32.357	97.1	7,287	7,300
U90-0517	Rat		334.5	267.71	16.6	120,600	120,000
U93-0729	Dog		0.704	0.563	11.3	845	850
U90-0494	Mouse		1336	1068.8	3,206	241,000	240,000
U90-0493	Rat		4750	3800	22,800	1,712,000	1,700,000

- Doses (tiotropium bromide) in study reports and labeling of the original submission.
- Doses (tiotropium base) in the 31-Jul-2003 submission. The molecular weight ratio between tiotropium bromide and tiotropium is 1.25.
- Obtained from multiplying mg/kg/day dose of the 30-JUL-03 submission by an appropriate conversion factor. The conversion factor was 3, 6, 12 and 20 for mice, rats, rabbits and dogs, respectively.
- On a mg/m³ basis. The tiotropium dose in humans is 13.32 mcg/m³ (18 mcg/day for a patient of 50 kilograms).

2. Other issues of the labeling

This section addresses the labeling-related comments of Dr. David Morse, Associate Director of Office of Drug Evaluation II, in his memorandum to Dr. Robert Meyer on October 18, 2002.

- Include in the pregnancy section of the product label the adverse effects (i.e., decrease in numbers of corpora lutea and implants, an increase in fetal resorptions, and an increase in the numbers of dead pups at birth, etc.) observed in animals with tiotropium bromide.³ Dr. Pei's review dated September 17, 2002 for the original NDA had

³ Text of the comment (2): "Review of the reproductive toxicity data for tiotropium bromide in rats, suggests a significant dose response related decrease in numbers of corpora lutea and implants, an increase in fetal resorptions, and an increase in the numbers of dead pups at birth. Further, it induced lower pup weights, increased numbers of litter losses and delayed sexual maturation in rats. Post-implantation losses were increased in rabbits. Tiotropium bromide administration (via inhalation or oral administration) did not cause any teratogenic effects in rats and rabbits. Delayed sexual maturation and post implantation losses were not observed in the oral embryo-fetal development studies in either species. Since these adverse reproductive effects were observed in the absence of significant maternal toxicity (or paternal toxicity when applicable), it is recommended that these study findings be included in the product labeling. The pregnancy section of the product label should be revised to reflect the adverse effects observed in animals treated with tiotropium bromide."

considered this comment. The proposed labeling of the 02-OCT-2002 and 31-JUL-2003 submissions has incorporated all the findings of interest. This comment is considered adequately addressed.

- b. Interspecies comparison of exposure (Comment 4). The current proposed labeling uses body surface area (mg/m^2) for inter-species comparison. Dr. Morse considers this approach acceptable although an approach based pharmacokinetic data is preferred. This application does not use the pharmacokinetic approach because it contains the insufficient animal pharmacokinetic data. This issue is considered resolved.

c. Rephrasing text.

- 1) Replacing the phrase "_____ with "the human dose on a body surface area basis" (Comment 4, Bullet 2). The term "the human dose" is in compliance with the CRF (21 CFR201.57), although it deviates from the terminology used by the Division for other similar products. The Division has been using the term "on a mg/m^2 basis" for all other similar drug products. This review, therefore, recommends replacing the phrase "the _____ recommended human daily dose on a mg/m^2 basis" with "the recommended human daily dose on a mg/m^2 basis". The term "_____ should be deleted since only one dose is proposed for human use.

- 2) Replacing the word "_____ of the phrase "_____ under the heading of "Carcinogenesis, Mutagenesis and Impairment of Fertility" with "_____ (Comment 4, Bullet 3). This comment is not applicable as the newly proposed text (below) is considered acceptable.

"Tiotropium bromide demonstrated no evidence of mutagenicity or clastogenicity in the following assays: the bacterial gene mutation assay, the V79 Chinese hamster cell mutagenesis assay, the chromosomal aberration assays in human lymphocytes *in vitro* and mouse micronucleus formation *in vivo*, and the unscheduled DNA synthesis in primary rat hepatocytes *in vitro*."

- 3) Deleting the _____ s in the Pregnancy and Overdose Sections (Comment 4, Bullet 4). Dr. Morse reasons that deleting the _____ would "avoid potential confusion regarding the positive findings in the inhalation reproductive toxicity studies and the lack of findings in the oral exposure reproductive studies". Dr. Morse cites the same reason for dropping the _____. The review concurs with Dr. Morse's recommendation. The sponsor needs to provide pharmacokinetic data (e.g., plasma AUCs or C_{max}) in animals should the sponsor insist on including these oral studies in the product label.

- 4) Including _____ s in the "Overdose" section (Comment 4, Bullet 5). This review recommends against this revision. Adverse reactions of tiotropium in humans are well described in the labeling. Signs and symptoms of anticholinergic agents including tiotropium in humans are well understood. Signs and symptom of tiotropium intoxication in animals differ from that in humans. Adding _____

prolongs the labeling without adding significant benefits to patient and doctors.

3. Newly Suggested Labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of tumorigenicity was observed in a 104-week inhalation study in rats at tiotropium doses up to 0.059 mg/kg/day, in an 83-week inhalation study in female mice at doses up to 0.145 mg/kg/day, and in a 101-week inhalation study in male mice at doses up to 0.002 mg/kg/day. These doses correspond to 25, 35 and 0.5 times the recommended human daily dose (RHDD) on a mg/m² basis, respectively. These dose multiples may be overestimated due to difficulties in measuring — in animal inhalation studies.⁴

Tiotropium bromide demonstrated no evidence of mutagenicity or clastogenicity in the following assays: the bacterial gene mutation assay, the V79 Chinese hamster cell mutagenesis assay, the chromosomal aberration assays in human lymphocytes *in vitro* and mouse micronucleus formation *in vivo*, and the unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

In rats, decreases in the number of corpora lutea and the percentage of implants were noted at inhalation tiotropium doses of 0.078 mg/kg/day or greater (approximately 35 times the RHDD on a mg/m² basis). No such effects were observed at an inhalation dose of 0.009 mg/kg/day (approximately 4 times the RHDD on a mg/m² basis). The fertility index, however, was not affected at inhalation doses up to 1.689 mg/kg/day (approximately 760 times the RHDD on a mg/m² basis). These dose multiples may be overestimated due to difficulties in measuring — in animal inhalation studies.

Pregnancy

Pregnancy Category C

No evidence of structural alterations was observed in rats and rabbits at inhalation tiotropium doses of up to 1.471 and 0.007 mg/kg/day, respectively. These doses correspond to approximately 660 and 6 times the RHDD on a mg/m² basis. However, in rats, fetal resorption, litter loss, decreases in the number of live pups at birth and the mean pup weights, and a delay in pup's sexual maturation were observed at inhalation tiotropium doses of \geq 0.078 mg/kg (approximately 35 times the RHDD on a mg/m² basis). In rabbits, an increase in post implantation loss was observed at an inhalation dose of 0.4 mg/kg/day (approximately 360 times the RHDD on a mg/m² basis). Such effects were not observed at inhalation doses of 0.009 and up to 0.088 mg/kg/day in rats and rabbits, respectively. These doses correspond to approximately 4 and 80 times the RHDD on a mg/m² basis,

⁴ Dr. Linda Hu in her memorandum dated November 3, 2003 commented that such a statement is not present in the labeling of other similar drug products. The review considers it has adequately addressed Dr. Hu's comment, given the modification of the text and the decision to use inhaled doses from inhalation toxicity studies in animals.

respectively. These dose multiples may be overestimated due to difficulties in measuring in animal inhalation studies.

There are no adequate or well-controlled studies in pregnant women. SPIRIVA should be used during pregnancy only if the potential benefit justifies the risk to the fetus.

OVERDOSAGE

...

No mortality was observed at inhalation tiotropium doses up to 32.4 mg/kg in mice, 267.7 mg/kg in rats and 0.6 mg/kg in dogs. These doses correspond to 7,300, 120,000 and 850 times the recommended human daily dose on a mg/m² basis, respectively. These dose multiples may be overestimated due to difficulties in measuring lung doses in animal inhalation studies.

4. Annotated Labeling

This section is an annotated version of the labeling review that provides a comparison between the Division's newly suggested versions and the sponsor's most recently proposed version.

Carcinogenesis, Mutagenesis, Impairment of Fertility

[

]

____|____ Draft Labeling Page(s) Withheld

Recommendation

1. As indicated in the initial NDA review, this application is recommended for approval from a nonclinical perspective.
2. The suggested revisions to the labeling (Section 3) should be conveyed to the sponsor.

Luqi Pei, Ph.D.
Pharmacologist

Timothy McGovern, Ph.D.
Supervisory Pharmacologist

APPENDIX 1.**Meeting Minutes**

Date: March 27, 2003

Subject: Assessing systemic drug exposures in inhalation toxicity studies in animals for labeling purposes

Preparer: Luqi Pei, Ph.D.

The pharmacology and toxicology group of DPADP discussed the best approach for assessing systemic drug exposure of inhalation studies in animals. By majority opinion, we decided the following: for the labeling purpose, we will use the total inhaled dose and state that the animal dose may be overestimated. For general toxicity studies, we continue using the pulmonary deposit as actual exposure.

Recent labeling reviews of tiotropium (Spiriva, NDA 21-395, sponsored by BI) prompted the discussion. BI and DPADP disagree on the dose ratios of tiotropium between animals and humans. The disagreement was attributed primarily to difference in the estimated animal doses. BI uses the total inhaled dose in animals while DPADP uses the estimated pulmonary deposit. The use of inhaled dose results in larger dose ratios that give BI a better comfort level. Our discussions concentrated on the accuracy of dose estimation of inhalation toxicity studies and some regulatory implications. The following summarizes deliberation points.

Estimating systemic exposure of drugs in inhalation toxicity studies in animals is a complex process. The pulmonary region and the respiratory and digestive tracts all contribute to the systemic exposure. AUCs (plasma or serum) are the best and most reliable indicator of the systemic drug exposure. In the absence of AUCs, estimates can be derived from aerosol distribution patterns and bioavailability (F) of the drug at the pulmonary region and respiratory and digestive tracts. An accurate estimate requires a good characterization of both the aerosol distribution pattern and the bioavailability. Although the distribution pattern of aerosols of given diameter is relatively constant, Fs vary remarkably among drugs. Systemic exposure can be calculated if Fs are known. Unfortunately, Fs are not available in most cases.

The lack of Fs complicates the selection of the best dose estimate. It is unclear which of the following of the choices is best: the pulmonary deposit, the respiratory system deposit and the total body burden. The pulmonary deposit alone is overly conservative. It underestimates the systemic exposure because it ignores the contribution of the respiratory tract and digestive system for drugs with high nasal and oral Fs. The deposits of the respiratory system or the total inhaled may not improve the precision of dose estimation. The deposit in the respiratory system could be more accurate if the F for the respiratory tract is large and the oral F is small. However, it could overestimate the systemic exposure if both Fs are low. For same reasons, the use of total inhaled dose could be also imprecise.

Given the uncertainties of the dose estimation, using the total inhaled dose can eliminate some uncertainties. Thus, the total inhaled dose appears to be the best choice.

Using the total inhaled dose may also encourage the sponsor conduct inhalation toxicity studies. The pulmonary deposit only is the most conservative approach, but it may reduce sponsor's incentive to conduct the preferred but costly inhalation carcinogenicity studies. We routinely accept and describe the oral carcinogenicity studies that evaluate the inhalation drug products in the labeling. Many inhalation drug products on the market use dose ratios derived from oral carcinogenicity studies in the labeling. These ratios significantly exaggerate the difference for drugs with the low oral and respiratory F_s . Given the same nominal dose, an inhalation carcinogenicity study would have smaller dose ratio than the oral studies although the former actually achieved higher systemic exposure than the latter. Although the labeling explicitly states that the ratio are derived from a different route of administration (oral), the key difference could be easily overlooked by average users who rush through the labeling and look only for numbers. This puts the sponsor of inhalation toxicity studies into a disadvantageous position even though they have done a better job to characterize the carcinogenicity potential of the drug. Such a sponsor would be reluctant to conduct inhalation carcinogenicity studies in the future when it figures out that oral studies would also support approval of the intended clinical inhalation use.

Dose ratios play a minimal role in determining the approvability of a drug. This Division would not approval a true or suspect human carcinogen for drugs intended for chronic use even though they may have extremely large dose ratios. On the other hand, the Division has approved drugs with dose ratio of less than one in their carcinogenicity studies. Examples are glucocorticosteroids. Thus, the dose ratio is not critical in the approvability of a drug.

It has been problematic to determine the actual animal exposure in the labeling of the marketed drug products, even if one traces the labeling review of approved drugs. It is probably ideal to describe the exposure conditions as the reports of NTP inhalation toxicity studies. This, however, would result in an information overload in the labeling because it will produce a longer labeling. Also, a detailed description of the exposure condition of the inhalation carcinogenicity studies may create confusions among users because the remarkable difference in the protocols between the clinical use and the laboratory exposure. A simple presentation of the inhaled dose in animals will eliminate the necessity for a detailed description of the exposure condition and avoid the confusion.

Estimating actual systemic exposure of a drug in animals from inhalation toxicity studies is a challenging task. It involves many hypothesis and uncertainties. Ratios based on AUCs between animals and humans are the most accurate and preferred. In the absence of AUC data, the ratios that are based on the total inhaled dose in animals are acceptable given their inheritant shortcomings. The dose estimation in the general toxicity studies, especially early phase of drug development, however, should use pulmonary deposit only. This will ensure that the testing subject is adequately protected.

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/s/

Luqi Pei
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PHARMACOLOGIST

Timothy McGovern
12/23/03 02:00:48 PM
PHARMACOLOGIST
I concur.

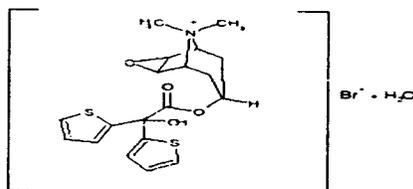
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-395
Review number: 1
Sequence number/date/type of submission: 000/December 12, 2001/Original submission
Information to sponsor: Yes (), No ()
Sponsor and/or agent: Beohringer Ingelheim Pharmaceutical Inc.
Manufacturer for drug substance: The same as above

Reviewer Name: Luqi Pei, Ph.D.
Division Name: Pulmonary and Allergy Drug Products
Division Code: HFD-570
Review Completion Date: September 17, 2002

Drug:

Trade Name: Tiotropium bromide inhalation dry powder
Generic Name: Spiriva®
Code Name: Ba 679 BR
Chemical Name: (1 α , 2 β , 4 β , 5 α , 7 β)-[(hydroxydi-2-thienylacetyl)-9,9-dimethyl-3-oxa-9-azoniatcyclo[3.3.1.0^{2,4}]nonane bromide monohydrate
CAS Registry Number: N/A
Molecular Formula/Weight: C₁₉H₂₂NO₄S₂Br.H₂O/ 490.3
Structure:



Manufacturer for the Drug: Beohringer Ingelheim Pharmaceutical Inc.
Relevant INDs/NDAs/DMFs: IND 46,687
Drug Class: Muscarinic cholinergic receptor antagonist
Indication: Bronchospasm and dyspnea associated with chronic obstructive pulmonary disease (COPD)
Clinical formulation: Capsules containing 18 μ g tiotropium (22.5 μ g tiotropium bromide) blended with lactose monohydrate as the carrier
Route of Administration: Oral inhalation
Proposed use: Use one capsule (18 μ g) a day with the HandiHaler inhalation device

Executive Summary

I. Recommendations

A. Recommendation on Approvability:

Approval of tiotropium bromide is recommended from the non-clinical viewpoint. This application has evaluated the general toxicity, genetic toxicity, carcinogenicity and reproductive toxicity of tiotropium. Inhalation, the intended clinical route of administration, is the route of exposure in most animal toxicity studies. The application has adequately characterized the toxicology profile of tiotropium and approval of the drug is recommended.

B. Recommendation for Nonclinical Studies: None.

C. Recommendations on Labeling:

1. Carcinogenesis, mutagenesis and fertility

No evidence of tumorigenicity was observed in a 104-week inhalation study in rats at tiotropium doses up to _____ 83-week inhalation study in female mice at doses up to _____, and a 101-week inhalation study in male mice at doses up to _____. These doses correspond to approximately _____ recommended human daily dose (MRHD) on a mg/m^2 basis, respectively.

Tiotropium bromide demonstrated no evidence of mutagenicity or clastogenicity in the following assays: the bacterial gene mutation assay, the V79 Chinese hamster cell mutagenesis assay, the chromosomal aberration assays in human lymphocytes *in vitro* and mouse micronucleus formation *in vivo*, and the unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

In rats, decreases in the number of corpora lutea and the percentage of implants were noted at inhalation tiotropium doses of _____ day or greater (approximately _____ on a mg/m^2 basis). No such effects were observed at _____ (approximately _____ basis). The fertility index, however, was not affected at inhalation doses up to 139 $\mu\text{g}/\text{kg}/\text{day}$ (approximately _____ basis).

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 _____

However, in rats, fetal resorption, litter loss, decreases in the number of live pups at birth and the mean pup weights, and a delay in pup's sexual maturation were observed at inhalation tiotropium doses of \geq _____ times the _____ on a mg/m^2 basis).

In rabbits, an increase in post implantation loss was observed in at an inhalation dose of _____ (approximately _____ on a mg/m^2 basis). Such effects were not observed at inhalation doses of _____ in rat and rabbits, respectively. These doses correspond to approximately _____ on a mg/m^2 basis, respectively.

3. Overdose

Overdosage: No mortality was observed at inhalation tiotropium doses up to _____ in mice, _____ in rats and _____ in dogs. These doses correspond to _____ times the _____ on a mg/m^2 basis, respectively.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

No evidence of genotoxicity, carcinogenicity and teratogenicity of tiotropium bromide was found although the drug may be embryo/fetocidal when rats and rabbits were exposed to it during pregnancy.

General toxicity studies showed that the gastrointestinal tract and secretory glands were the primary target organs of toxicity. Other organs included the eye, respiratory tract, heart and urinary bladder. Tiotropium inhibited gastrointestinal motility and production of tear and saliva. It irritated the respiratory tract and disrupted the balance between sympathetic/parasympathetic system resulting in an increase in the heart rate at high doses. It caused urinary proteinaceous deposits due to the atony of the urinary bladder and cataracts in rats. Keratoconjunctivitis sicca occurred in dogs. The inhalation NOAEL value of tiotropium was ≤ 7 and $0.4 \mu\text{g}/\text{kg}/\text{day}$ in rats and dogs, respectively.

Inhalation reproductive toxicity studies in rats and rabbits revealed no evidence of structural alterations; however, embryo and fetal toxicity of the drug was observed in both species. In rats, a pre- and post-natal development study showed a total litter loss and a decrease in mean pup weights at tiotropium doses of $\geq 7 \mu\text{g}/\text{kg}$. A fertility study showed fetal resorption and decreases in the number of corpora lutea, the

percentage of implants, and the number of live pups at the same doses. The fertility index, however, was not affected. These two studies, along with a teratogenicity study, showed a delay in sexual maturation (1-3.5 days) in pups exposed to the drug maternally at inhalation doses of $\geq 7 \mu\text{g}/\text{kg}/\text{day}$. The sexual maturation is measured by vaginal opening in the female and occurrence of balanopreputal skinfold in the male. In rabbits, an increase in post implantation loss was observed at an inhalation dose of $50 \mu\text{g}/\text{kg}/\text{day}$. No such effects were noted at inhalation doses of 0.8 and $11 \mu\text{g}/\text{kg}/\text{day}$ in rats and rabbits, respectively.

Two oral reproductive studies showed no evidence of fetal development effect at tiotropium doses up to 500 and $100 \text{mg}/\text{kg}/\text{day}$ in rats and rabbits; however, oral bioavailability of the drug was poor in these species while the inhaled drug is readily absorbed.

No evidence of genotoxicity and tumorigenicity was observed. Five genetic toxicity assays of tiotropium have revealed no evidence of genotoxicity. These assays were Bacterial mutation in *S. typhimurium* and *E. coli*, V79 CHO mammalian gene mutation *in vitro*, human lymphocyte chromosomal aberration *in vitro*, unscheduled DNA synthesis in primary rat hepatocytes *in vitro* and mouse micronucleus formation *in vivo*. Three 19- to 24-month inhalation carcinogenicity studies in mice and rats have revealed no evidence of tiotropium tumorigenicity. These studies are a 104-week study in rats, a 83-week study in female mice, and a 101-week study in male mice. Their respective tiotropium are up to 5.3, 9.1 and $0.31 \mu\text{g}/\text{kg}/\text{day}$. Each of the studies achieved the maximum tolerated dose of the drug.

B. Pharmacologic Activity

Tiotropium is a long-acting muscarinic cholinergic antagonist. It selectively binds to the $m_{3.5}$ receptors ($K_d = 9 \text{pM}$) and blocks the action of acetylcholine at the receptor site. The blockade of m_3 receptor located in the respiratory tree results in bronchodilation. Tiotropium is quaternary amine that does not cross the blood-brain barrier readily. Tiotropium's affinity to m_1 and m_2 receptors is slightly lower: $K_d = 32 - 151 \text{pM}$.

C. Nonclinical Safety Issues Relevant to Clinical Use

None. Reproductive toxicity studies in rats and rabbits revealed that tiotropium was embryocidal and fetocidal, interfered with the ovulation process in rats, and delayed sexual maturation in rat pups exposed to the drug maternally, although there was no evidence of teratogenicity or affecting fertility index. The findings have been addressed in labeling review.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

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PHARMACOLOGY/TOXICOLOGY REVIEW

Background: Tiotropium bromide (Ba 679 BR) has been previously filed with the Division under IND 46, 687. Most non-clinical studies, with exception of the carcinogenicity studies of the drug and studies evaluating tiotropium impurities, in the current NDA submission have been previously submitted under the IND. Consequently, the Division has reviewed the studies. See Satish Tripathi's reviews dated 28-AUG-1996, 17-SEP-1997, 10-DEC-1997 and 08-JAN-1998, and Dr. Timothy McGovern's review dated 02-NOV-2001. The Previously Reviewed Studies section of the current review refer the previous reviews as Review No. 1, 2, 3 4 and 5, respectively. Review No. 6 is a review dated August 28, 2002 in NDA 21-395 for a Chemistry Consult Request evaluating the safety of tiotropium impurities by Dr. Luqi Pei. This review evaluates the newly submitted studies and conducts an overall non-clinical safety evaluation of the current application.

I. PHARMACOLOGY:

Previously Reviewed Studies

Study Description	Report #	Vol.	Review #
Biochemical characterization of Ba 679 BR in vitro	U90-0561	15	1
Bronchospasmolytic and cardiovascular effects in anesthetized dogs after local administration of an over therapeutic dose of a nebulized aqueous solution	U91-0408	16	1
Report on the bronchospasmolytic effect of the anticholinergic agent Ba 679 BR	U91-0455	15	1
Bronchospasmolytic and cardiovascular effects after inhalation of a nebulized aqueous solution of Ba 679 BR with a nebulizer device in anaesthetized dogs	U92-0621	16	1
Tiotropium bromide and tiotropium bromide , new long-acting anticholinergics exhibiting very slow dissociation kinetics from human muscarinic receptor subtypes	U93-0225	15	1
Affinities of Ba 679 BR decomposition products for human muscarinic receptor subtypes Hm1 to Hm5	U93-0507	16	1
Bronchoprotective effect and antagonism against Pilocarpine induced salivation of Ba 679 BR after single and repeated treatment in guinea pigs	U94-0005	16	1
Protective effect of inhaled Ba 679 BR against acetylcholine induced bronchospasms in Wistar and Fischer rats	U94-0069	15	1
Protective effect of ipratropium bromide, Ba 679 BR and tiotropium bromide after inhalation against acetylcholine induced collapse in conscious guinea pigs	U94-0070	16	1
Estimation of the administered of Ba 679 BR in an inhalation with guinea pigs (reported in U91-0455)	U94-0231	15	1
Investigation of Ba 679 BR in isolated rectum from guinea pig for antagonism against the spastic actions of carbachol, histamine and BaCl ₂	U98-2850	16	5

Studies Reviewed in this Review

Study Description	Report #	Vol./p
New mathematical model for the determination of the slow dissociation kinetics of the long acting antimuscarinics tiotropium bromide and tiotropium bromide in comparison ipratropium bromide from human muscarinic receptors	U99-1004	15

Synopsis April 1994 of the Pharmacology of Ba 679 BR (INN: tiotropium bromide)	U94-0277	15
Influence of repeated once daily inhalation of tiotropium bromide on lung function, lacrimation and heart rate in conscious dogs	U99-1413	16

I. 1 Primary pharmacodynamics:

Study Title: *Synopsis April 1994 of the Pharmacology of Ba 679 BR (INN: tiotropium bromide, Study U94-0277)*

This is a review article summarizing pharmacology of tiotropium as April 1994. Studies referenced in the article have been reviewed previously and no additional review is needed.

I.2 Mechanism of action:

Study Title: *New mathematical model for the determination of the slow dissociation kinetics of the long acting antimuscarinic tiotropium bromide and — in comparison ipratropium bromide from human muscarinic receptors (Study U99-1004)*

A new mathematical model was developed to estimate the receptor affinity of tiotropium and other cholinergic antagonists. The estimates were based on binding data of tritium-labeled tiotropium iodide, ipratropium iodide and — to human muscarinic receptor expressed in Chinese hamster ovary cells. Table 1.1 shows dissociation constant and half-lives of tiotropium-receptor complex.

Table 1.1 Dissociation Constant and Half-lives of Three Cholinergic Antagonists

Receptor Type	hm1	Hm2	Hm3	Hm4	Hm5
Kd (pmol/l)					
Tiotropium	151	32	9	8	9
—	37	55	35	17	65
Ipratropium	240	291	281	105	741
Dissociation $t_{1/2}$ (hr)					
Tiotropium	11.1	3.6	27.1	19.9	141.6
—	4.7	0.8	6.1	4.4	13.2
Ipratropium	0.1	0.04	0.2	0.1	0.2

I.3 Drug activity related to proposed indication:

Study Title: *Influence of repeated once daily inhalation of tiotropium bromide on lung function, lacrimation and heart rate in conscious dogs (Study No. U99-1413)*

In conscious dogs, inhalation of tiotropium bromide (2.3 µg/day/dog) inhibited acetylcholine-induced bronchospasms (60%) on days 14 through 28. This inhibition subsided completely

within 14 days after cessation of the treatment. No inhibition of bronchospasm was observed on day 1 of the treatment. The dogs were anaesthetized when the bronchospasmolytic effect was measure. No effect on lacrimation and heart rate was observed.

I.4 Secondary pharmacodynamics: Not applicable.

I.5 Pharmacology summary:

Tiotropium is a long-acting muscarinic cholinergic receptor antagonist. It binds reversibly to muscarinic cholinergic receptors located in the airways, blocks the bronchoconstriction of acetylcholine, and results in bronchodilation. Tiotropium has a high affinity to m_3 , m_4 and m_5 receptors ($K_d = 9$ pM) and a relatively low affinity to m_2 and m_1 receptors ($K_d = 32 - 151$ pM). The dissociation half-life of tiotropium- m_3 receptor complex at 23°C is 27 hours. The pA_2 value of tiotropium is approximately 9.5 in isolated-tracheal tissues from guinea pigs and humans. *In vivo* studies show that the median effective inhalation dose (ED_{50}) of tiotropium against acetylcholine-induced bronchoconstriction is approximately one $\mu\text{g}/\text{kg}$ in guinea pigs, rabbits and dogs. The maintenance dose of the drug, however, is approximately 3 – 10 fold lower than the ED_{50} . The to-be-marketed clinical dose of tiotropium in COPD patients is 18 μg once a day (*i.e.*, 0.36 $\mu\text{g}/\text{kg}/\text{day}$).

The secondary pharmacological effect of tiotropium may include tachycardia and inhibition of salivation, and lacrimation and bowel movement. The inhibition of salivation occurs at concentrations five times lower than the bronchodilation in guinea pigs, suggesting that the salivation be probably the earliest indicator of tiotropium side effects,

Tiotropium has a low affinity to H_1 histamine receptor ($IC_{50} = 0.09$ μM). Its affinity to receptors of H_2 histamine, noradrenaline, serotonin, dopamine, adenosine, nicotine, benzodiazapine and MK801 is negligible ($IC_{50} = \geq 10$ μM).

I.6 Pharmacology conclusions:

Tiotropium is a long-acting muscarinic cholinergic receptor antagonist.

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II. SAFETY PHARMACOLOGY:

Previously Reviewed Studies

Study Description	Report #	Vol.	Review #
Antimicrobial and antisalivatory effect of Ba 679 Br in the conscious dog (i.v. administration)	U90-0668	17	1
Antimicrobial and antisalivatory effect of Ba 679 Br in the conscious dog (oral administration)	U90-0669	17	1
Mydriatic and antimicrobial effects of Ba 679 Br in the rat	U90-0670	17	1
Antisalivatory activity of Ba 679 BR, atropine-sulfate and ipratropium bromide in rats	U91-0322	17	1
Bronchospasmolytic and cardiovascular effects in anesthetized dogs after local administration of an over therapeutic dose of a nebulized aqueous solution	U91-0408	16	1
Effects on salivary secretion in mice after p.o. and i.v. doses of Ba 679	U91-0409	17	1
Effects of oral Ba 679 BR on intestinal passage in mice	U91-0410	17	1
Report on the bronchospasmolytic effect of the anticholinergic agent Ba 679 BR	U91-0455	16	1
Effect of subcutaneous Ba 679 BR on nocturnal locomotor activity of mice	U91-0466	16	1
Effects on gastric secretion of subcutaneous Ba 679 BR in rats	U91-0467	17	1
Effects of oral Ba 679 BR on diuresis in rats	U91-0468	17	1
EEG experiments with Ba 679 BR administered i.v. to rabbits	U91-0469	16	1
Bronchospasmolytic and cardiovascular effects after inhalation of a nebulized aqueous solution of Ba 679 BR with a Respimat device in anaesthetized dogs	U92-0621	16	1
Comparison of the bronchospasmolytic and cardiovascular effects in anaesthetized dogs after inhalation of a nebulized aqueous solution of Ba 679 BR, salbutamol or a combination of both compounds	U93-0657	17	1
The effects of Ba 679 BR and on the pupil size in male mice	U93-0710	17	1
Mydriatic effect of Ba 679 BR, ipratropium bromide and atropine in conscious beagles and the antidotal or antagonistic effect of muscarinic agonists and β -receptor blockers	U93-0910	17	1
Effects of Tiotropium bromide (Ba 679 BR, 0.1, 1.0, and 10 mg/kg/day, subcutaneously administered for 1 - 12 days, on gastrointestinal transit in male mice	U94-0368	17	2
Effects of Tiotropium bromide (Ba 679 BR, 0.5, 1.5 and 5 mcg(k ⁻¹ day)), subcutaneously administered for 1 - 12 days, on pilocarpine induced salivation in male NMRI mice	U94-0369	17	2
Ba 679 BR - Investigation of Ba 679 BR antagonism against Pilocarpine induced salivation following treatment by inhalation	U95-0222	16	2
Ba 679 BR - Validation of Ba 679 BR antagonism against Pilocarpine induced salivation following treatment by inhalation	U95-0221	17	2
Inhaled Ba 679 BR and inhaled + i.v. corticosteroids in dogs	U97-2730	18	5
Pentylentetrazole seizure in mice after intravenous pretreatment with Ba 679 BR	U98-2879	16	5
Effects of Ba 679 BR (tiotropium bromide) on body temperature in conscious mice after intravenous administration	U98-2851	16	5
Investigation of intravenously administered tiotropium bromide (Ba 679 BR) in mice after i.p. injection of phenyl-p-benzoquinone in the writhing test	U98-2292	16	5
Investigation of Ba 679 BR by intravenous administration in anaesthetized dogs for cardiovascular and respiratory effects	U98-2386	16	5

Studies Reviewed in This Review

Study Description	Report #	Vol./p
Inhaled Ba 679 BR and intravenous theophylline in dogs	U98-2010	18
Dose-dependency of tachycardia induced by intravenous tiotropium bromide and its	U99-1400	18

antagonism by intravenous verapamil

II.1 Neurological effects: N/A.

II.2 Cardiovascular effects: N/A.

II.3 Pulmonary effects: N/A.

II.4 Renal effects: N/A.

II.5 Gastrointestinal effects: N/A.

II.6 Abuse liability: N/A.

II.7 Other:

II.7.A Study Title: Dose-dependency of tachycardia induced by intravenous tiotropium bromide and its antagonism by intravenous verapamil

Verapamil antagonized tiotropium bromide-induced tachycardia in conscious dogs. Intravenous administration of tiotropium (5 µg/kg, IV) resulted in a marked increase in heart rate (Figure 2.1). Intravenous infusion of verapamil (0.3 mg/kg over a period of 15 minutes) attenuated the increase in the heart rate. This was a non-GLP study conducted at BI Pharma KG, Binger Straße 173, D-55216 Ingelheim am Rhein.

II.7.B Study Title: Inhaled Ba 679 BR and intravenous theophylline in dogs (Study U98-2010)

Tiotropium and theophylline combination was studied on their cardiovascular effects in anaesthetized female dogs (mixed bred, n = 5). Tiotropium (2.5 µg/dog) was given by aerosol. Acetylcholine (10 – 30 µg/kg every 5 – 10 min.) was given intravenously (bolus injection) one minute after administration of tiotropium. Theophylline (10 mg/kg, IV) was given 12 – 14 min after the commencement of acetylcholine treatment. Heart rate was monitored with EKG. Aortic pressure, the maximum rate of rise of left ventricular pressure was measured via a pressure transducer catheter. Tiotropium decreased transpulmonary pressure, but had no effect on the cardiovascular parameters. Theophylline decreased aortic pressure and increase the rising rate of ventricular pressure. Tiotropium attenuated the cardiovascular effect of Theophylline (Table 2.1), but had no effect on the transpulmonary pressure.

Table 2.1 Cardiovascular Effect of Tiotropium and Theophylline (% change from Controls)

Treatment	Tiotropium	Theophylline	Tiotropium & Theophylline
Transpulmonary pressure	-42	0	-50
Heart rate	-3	67	-2
Max. rising rate of l. ventricular pressure	3	105	2
Systolic aortic pressure	-0.5	-22	0.3
Diastolic aortic pressure	-4	-38	-0.8

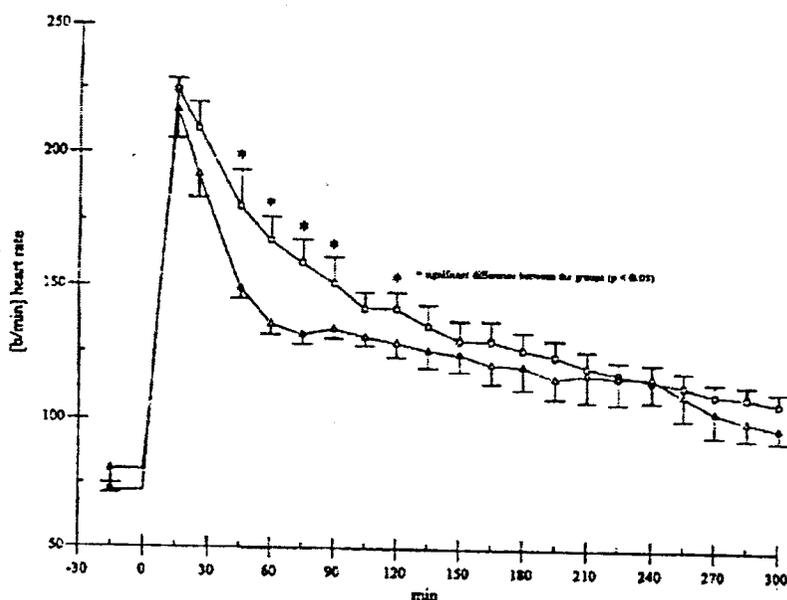


Figure 2.1 Influence of tiotropium alone (- -) or in combination with verapamil (-Δ-) on heart rate in conscious beagle dogs (n = 4).

II.8 Safety Pharmacology Summary:

Safety pharmacology of tiotropium has been studied in mice, rats, rabbits and dogs. Evaluated parameters included neurological, cardiovascular, respiratory, gastrointestinal and ocular effects of the drug. The route of administration included intravenous, subcutaneous, oral and inhalation. Tiotropium doses in the safety pharmacology studies exceeded the to-be-marketed clinical dose.

CNS effect:

Tiotropium at a SC dose of 2.5 mg/kg did not affect nocturnal locomotion in mice, neither did it affect the number of writhing symptoms associated with IP injections of phenylbenzoquinone, or the seizure threshold associated with intravenous infusion of pentylenetetrazole (10 µg/kg, IV). Tiotropium produced a minimum decrease in body temperature (-0.5°C), dry oronasal mucosa and inability to keep hold on the decline plate in modified Irwine test at 100 µg/kg (IV). Tiotropium (up to 1.0 mg/kg, IV) did not affect ECG patterns in conscious rabbits.

Cardiovascular effect:

In conscious dogs, bolus IV tiotropium elevated the heart rate by 4 fold (from about 60 bpm to 220 bpm) at 5 µg/kg, but not at ≥ 2 µg/kg, nor at an inhalation dose of 3 µg/kg. The cardiovascular effect of tiotropium was much less pronounced in anesthetized dogs. The maximum increase in heart rate (about 22%) occurred at 10 µg/kg (IV), although the highest

tested dose is 30 µg/kg. The drug did not affect cardiac output, renal and femoral blood flow, systolic arterial and left ventricular pressure.

Respiratory effect:

Tiotropium inhibited acetylcholine-induced bronchospasms, the intended pharmacological effect of the drug. In anesthetized dogs, tiotropium at doses up to 30 µg/kg (IV) did not affect respiratory volume, transpulmonary pressure, lung compliance, resistance and respiratory minute volume.

Gastrointestinal Effect:

Tiotropium inhibited pilocarpine-induced salivation in anesthetized mice. The ED50 was 4.2 µg/kg and 8.2 mg/kg for intravenous and oral administration, respectively. Tiotropium also inhibited gastric secretion in rats with an ED50 of 3.2 µg/kg (SC).

Ocular Effect:

Tiotropium caused mydriasis and inhibition of lacrimation at IV doses of 1.7 µg/kg in conscious rats and dogs. Mydriasis lasted up to 7 day after a topical administration of 5 µg in the eye in dogs.

Renal Effects:

Tiotropium did not affect renal function in conscious rats following oral administration of 0.3-10 mg/kg.

Other: - Drug Interaction

Tiotropium (2.5 µg/kg, inhalation) abolished theophylline-induced increases in the heart rate, the rising rate of the left ventricular pressure, and systolic and diastolic aortic pressures. Verapamil (0.3 mg/kg infused intravenously over a period of 15 minutes) attenuated tiotropium-induced tachycardia in dogs. Corticosteroid pretreatment (2 mg/kg prednisolone, IV for 2 days or 1 mg/kg beclomethasone given 1 hr prior to tiotropium) did not affect the bronchodilatory effect of inhalation tiotropium in dogs. In isolated Guinea pig rectum, tiotropium was a partial antagonist of histamine, shifting the concentration-response curve of histamine to the right. Co-administration of tiotropium and Cimetidine led to a 36% increase in the tiotropium steady-state plasma levels.

II.9 Safety pharmacology conclusions:

Tiotropium at therapeutic plasma concentrations may increase heart rate and inhibit salivation and gastrointestinal motility. These effects, however, are secondary pharmacological activity of typical muscarinic antagonists.

III. PHARMACOKINETICS/TOXICOKINETICS:

Previously Reviewed Studies

Study Description	Report #	Vol.	Review #
Absorption			
Biochemical investigations with Ba 679 BR in the mouse	U91-0704	18	1
Biochemical investigations with Ba 679 BR in the rat	U91-0236	19	1
Satellite biochemical studies to the 4-week intravenous toxicity study of Ba 679 BR in rats	U92-0380	20	1
Biochemical investigations with Ba 679 BR in the dog	U92-0476	21	1
Plasma protein binding of Ba 679 BR (species comparison)	U92-0728	21	1
Plasma levels of Ba 679 BR in the inhalation range finding study in mature male and female rats and their offspring (Accompanying toxicokinetics study U92-0679)	U94-0030	19	1
Absorption and distribution of [¹⁴ C] Ba 679 BR in rats after intratracheal administration	U99-0205	20	5
Tiotropium bromide: Pharmacokinetics in male and female rats after intravenous, oral and intratracheal administration of 10 mg/kg Ba 679 BR	U99-1322	18	5
Tiotropium bromide: Pharmacokinetics in female rabbits after intravenous and oral administration of 1.0 mg/kg Ba 679 BR	U99-1336	20	5
Tiotropium bromide: Excretion balance and renal clearance in male and female rats after intravenous and intratracheal administration of 10 mg/kg Ba 679 BR	U99-1347	19	5
Tiotropium bromide: Pharmacokinetics in male and female mice after intravenous and oral administration of 10 mg/kg Ba 679 BR	U99-1357	18	5
Tiotropium bromide: Pharmacokinetics in male and female beagle dogs after intravenous (0.1 mg/kg) and oral (1.0 mg/kg) administration of Ba 679 BR	U99-1358	20	5
Tissue Distribution			
Whole-body autoradiographical investigations with Ba 679 BR in rats after intravenous, intratracheal and oral administration	U90-0448	21	1
Placental transfer of C-Ba 679 BR after intravenous administration to pregnant rats	U99-0167	21	5
Excretion of ¹⁴ C-Ba 679 BR into milk after intravenous administration to lactating rats	U99-0166	21	5
Enzyme Induction			
Investigation on the cytochrome P-450 induction potential of Ba 679 BR in the male rat	U91-0117	22	1

Studies Reviewed in This Review

Study Description	Report #	Vol./p
Absorption		
ADME studies with Ba 679 BR in rabbits	U96-2322	20
Tiotropium bromide: Dose dependency in rats after intravenous administration of 5 mcg/kg to 1000 mcg/kg Ba 679 BR	U99-1359	19
Tissue Distribution		
The interaction of tiotropium bromide (Ba 679) with biological membrane as determined by the membrane partition coefficient	U98-2540	21
Tissue distribution of ¹⁴ C- Ba 679 BR after intravenous administration to male rats	U99-0210	21
Protein binding of [³ H] Tiotropium in human plasma	U99-1707	22
Whole-body autoradiography in pigmented rats after intravenous administration of ¹⁴ C-Ba 679 BR	U00-0051	21
Whole body autoradiography after a single intravenous administration of 8 mg/kg [¹⁴ C]Tiotropium in male albino and male pigmented rats – comparison of	U00-1189	21

<i>radioluminography with tissue dissection techniques</i>		
Study on radiolabeled metabolites in milk after intravenous administration of ¹⁴ C-Ba 679 BR to lactating rats	U00-0101	21
Investigation of the in vitro permeability of tiotropium bromide (Ba 679 BR) through Caco-2 cell monolayers	U00-1350	22
<i>Metabolism</i>		
ADME studies with Ba 679 BR in rabbits	U96-2322	20
<i>Excretion:</i>		
Excretion balance of [¹⁴ C] Ba 679 BR after intravenous administration to male and female rats	U99-0216	22
Half-life and excretion rate of drug related radioactivity in urine after intravenous administration of 500 mcg/kg [³ H] Ba 679 BR to rats	U00-1050	23
ADME Method validation Studies		23
Determination of tiotropium in dog plasma and dog urine by LC-MS/MS	U00-1206	23
Determination of tiotropium in rat plasma and rat urine by LC-MS/MS	U00-1451	23
<i>Others</i>		
Effect of cimetidine on the renal excretion of Tiotropium bromide in Dogs	U00-1339	22

III. 1 PK parameters:

III.1.A Absorption:

III.1.A.1 Study Title: ADME studies with Ba 679 BR in rabbits (Study U96-2322)

Four female rabbits were given a bolus IV or oral dose of 1 mg/kg of ¹⁴C-labelled tiotropium bromide. The label was either at the methylscopine or dithienylglycolic acid. Radioactivity of plasma, whole blood, feces and urine was measured at various times after the dosing. Figure 3.1 presents the tiotropium concentration-time course in the plasma. Little radioactivity was seen in the plasma after oral administration although the report stated that about 13% of oral tiotropium was absorbed.

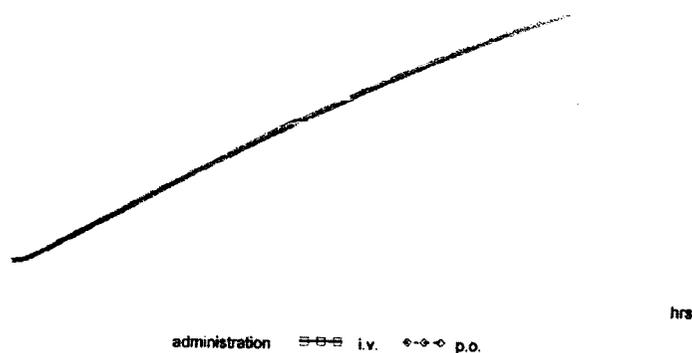


Figure 3.1 Plasma tiotropium concentrations-time course in female rabbits (Study U96-2322)

III.1.A.2 Study Title: Tiotropium bromide: Dose dependency in rats after intravenous administration of 5 $\mu\text{g/kg}$ to 1000 $\mu\text{g/kg}$ Ba 679 BR (Study U99-1539).

Plasma tiotropium concentrations were determined with HPLC-MS/MS in male rats. Tiotropium doses were 4, 12, 48, 200 or 800 $\mu\text{g/kg}$ (i.e., 5 – 1000 $\mu\text{g/kg}$ of Ba 679 BR). Plasma tiotropium concentration rose proportionally with dose (Figure 3.2). Table 3.1 presents the C_{max} and AUC of the tiotropium.

Table 3.1 Plasma Tiotropium Concentrations in Rats

Tiotropium ($\mu\text{g/kg}$, IV)	4	12	48	200	1000
C _{max} (ng/ml)	2.1	8.5	29.6	98.2	510.5
AUC _{0-∞} (ng.hml)	0.4	1.7	7.3	22.4	106.3

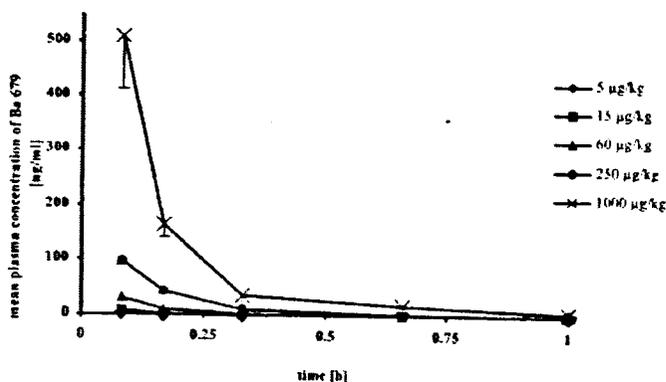


Figure 3.2 Mean plasma tiotropium concentration in male rats (n = 4/dose). Tiotropium was administered as Ba 679 BR.H₂O.

III.1.B Distribution:

Tissue distribution of tiotropium was studied in rats and rabbits. In rabbits, two females were given 2 mg/kg of the drug intravenously (Study U96-2322). Tissue drug concentrations were measured 5 and 30 minutes post dosing. Table 3.2 shows the results. The kidney and bile had the highest drug concentrations.

Table 3.2 Tissue Tiotropium Concentrations (μg tiotropium/g tissue)

	Blood	Plasma	Liver	Heart	Lungs	Kidney	Bile
5 min.	1.37	2.40	8.52	0.84	1.93	43.0	10.1
30 min.	0.49	0.84	7.26	0.29	0.82	3.81	11.5

In rats, four male Sprague-Dawley rats were given intravenously 8 mg/kg of ¹⁴C-labelled tiotropium (U99-0210). Tissue tiotropium levels were determined radioscintigraphically at 0.25,

4, 48 and 72 hr post dosing. The kidney, liver and intestinal contents had the highest concentration of the drug (Table 3.3).

Table 3.3 Tissue Tiotropium Concentration and $t_{1/2}$ in male rats.

Tissue	Concentration (ng eq / g or ml)					$t_{1/2}$ (hr)
	0.25 hr	4 hr	24 hr	48 hr	72 hr	
Blood	1190.70 ± 147.95	161.37 ± 15.05	25.33 ± 2.08	10.16 ± 2.05	6.97 ± 0.91	25.8
Plasma	2050.19 ± 305.56	117.03 ± 16.12	22.65 ± 1.00	12.33 ± 2.55	7.47 ± 1.17	30.0
Whole brain	98.72 ± 10.88	40.52 ± 10.16	6.94 ± 8.02	N.D.	11.89 ± 0.88	-
Hypophysis	1413.26 ± 141.64	819.60 ± 172.19	123.54 ± 84.89	N.D.	69.52 ± 51.53	-
Eyeballs	634.90 ± 116.17	100.72 ± 10.80	25.33 ± 5.29	14.60 ± 1.88	10.81 ± 1.02	39.1
Salivary gland	3380.85 ± 620.51	1872.84 ± 205.14	192.08 ± 23.69	46.42 ± 8.25	26.68 ± 1.98	16.9
Thyroid gland	1310.36 ± 83.56	540.70 ± 176.18	174.21 ± 48.89	116.63 ± 18.44	94.95 ± 23.95	54.8
Trachea	2492.17 ± 361.89	418.23 ± 109.65	75.97 ± 19.81	36.63 ± 2.68	18.09 ± 13.03	23.2
Thymus	1915.72 ± 494.76	1245.02 ± 188.07	95.03 ± 31.32	27.16 ± 3.93	18.37 ± 1.78	20.2
Heart	1202.44 ± 81.62	675.85 ± 173.15	330.53 ± 59.02	149.11 ± 34.57	64.09 ± 5.28	20.3
Lung	2442.84 ± 138.85	691.71 ± 116.63	105.53 ± 17.92	45.18 ± 6.17	26.77 ± 2.82	24.3
Pancreas	4553.22 ± 359.80	2355.39 ± 897.67	351.56 ± 121.64	106.65 ± 24.71	30.40 ± 9.41	13.6
Spleen	989.16 ± 84.10	196.54 ± 23.43	61.42 ± 7.48	38.43 ± 4.42	31.47 ± 3.97	49.8
Adrenal gland	1232.77 ± 123.65	570.59 ± 99.08	171.75 ± 45.94	92.17 ± 10.84	46.04 ± 4.49	25.3
Kidney	47198.03 ± 10848.23	3157.73 ± 885.99	750.65 ± 197.27	393.17 ± 127.25	312.84 ± 122.38	38.0
Liver	32279.40 ± 4080.07	6150.07 ± 396.24	1280.24 ± 75.85	776.82 ± 124.82	447.30 ± 45.03	31.6
Prostate	2993.56 ± 2733.04	344.85 ± 7.94*	105.51 ± 48.73	43.41 ± 15.45	17.46 ± 2.71	18.5
Testis	622.11 ± 118.28	77.55 ± 11.73	34.05 ± 9.29	25.95 ± 2.11	18.42 ± 2.53	54.2
Epididymis	1105.87 ± 143.37	192.08 ± 23.54	51.04 ± 9.26	31.08 ± 6.38	19.99 ± 0.81	35.5
Adipose tissue	738.40 ± 319.28	127.22 ± 37.17	21.92 ± 21.05	8.68 ± 10.53	4.87 ± 3.64	22.1
Skeletal muscle	357.66 ± 61.66	66.78 ± 5.07	43.37 ± 5.59	40.09 ± 6.24	27.16 ± 2.26	71.1
Brown fat	3343.97 ± 727.43	2979.39 ± 1364.83	535.73 ± 276.30	82.94 ± 36.91	16.89 ± 12.75	9.6
Stomach with contents	30354.06 ± 29688.75	6015.31 ± 5002.18	1455.74 ± 1260.81	2378.82 ± 1578.01	223.13 ± 223.33	-
Small intestine with contents	61603.30 ± 13352.43	25671.94 ± 5963.16	575.84 ± 227.08	2327.32 ± 2696.88	190.19 ± 56.86	-
Large intestine with contents	2223.09 ± 513.74	91328.60 ± 21323.64	75944.27 ± 9174.88	9562.05 ± 5578.70	1396.63 ± 531.32	-

The effect of skin color on tiotropium distribution was studied in rats. Study U98-1189 compared the tissue distribution of tiotropium between albino (SD) and pigmented (Norway Brown) rats. The study design was identical with Study U99-0210 for each rat strain. The results showed that the albino and pigmented rats had the same pattern of tissue distribution of tiotropium. The results in the albino rats were also similar to study U99-0210. Another study in male Brown Norway rats (Study U00-0051, 8 mg/kg) showed that pigmented (melanin-containing) tissues contained very little tiotropium, suggesting that tiotropium have no affinity to melanin. Tissue tiotropium concentrations were determined by autoradiogram.

Tiotropium concentration in the milk was studied in rats (Study U00-0101). Two lactating SD rats (Lactation day 13) were given intravenously 8 mg/kg of ^{14}C -labelled tiotropium. Plasma and milk were determined at 0.25, 4, 24 and 48 hr post dosing. A portion of samples was also extracted with 1 N hydrochloric acid for the determination of metabolites. The metabolites were separated with thin layer chromatography and quantified scintigraphically. Results showed that tiotropium concentrations in the milk were 1.7 – 6.6 times that in the plasma (Figure 3.3).

Note: The study report claims it determines the tiotropium metabolites but it lacks method validation. Given the knowledge that tiotropium hydrolyzes during sample collection process, it is questionable these are true "metabolites" when the analysis involves a rather harsh condition (acidification, extraction and evaporation etc.).

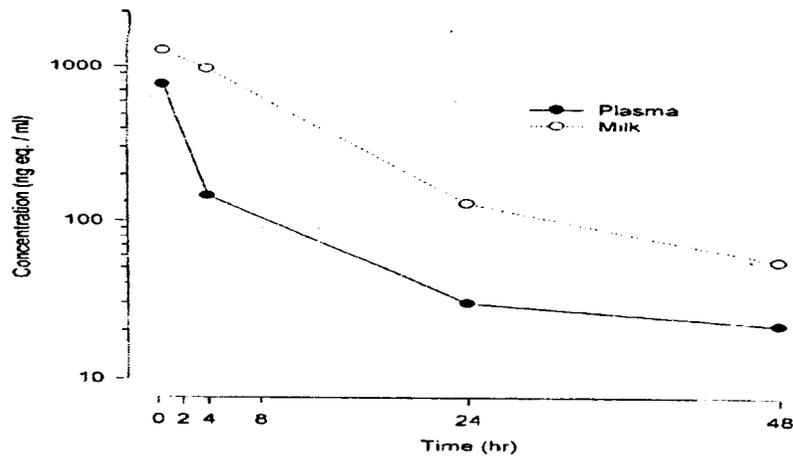


Figure 3.3 Tiotropium concentrations in plasma and milk of lactating rats.

In vitro studies were performed to evaluate the membrane permeability of tiotropium. A monolayer of cells mimicking human intestine membrane was constructed (Study U00-1350). The cell was derived from a human colon adenocarcinoma cell line. Tiotropium was administered as apical-to-basolateral flux. The permeability was determined by the amount of the drug present in the basolateral-to-apical efflux. The study showed that about 10-20% of oral tiotropium would be absorbed in humans and the absorption involved no active transport mechanism. Another study was performed to determine the membrane partition coefficient of tiotropium in an artificial membrane model (Study U98-2540). The membrane was prepared as microsomes derived from egg phosphatidylcholine. The study showed that tiotropium readily crosses the membrane.

Approximately 72% tiotropium in plasma was protein-bound in humans (Study U99-1707)

III.1.C Metabolism:

Metabolism of tiotropium occurred in rabbits. Figure 3.4 shows the metabolic pattern of a urine fraction (Study U96-2322). However, this may not be true metabolism as suggested by the study report: "...[T]he ester bond of the substance [tiotropium] is split during the collection of the corresponding samples from these animals (vol. 20, p. 13)."

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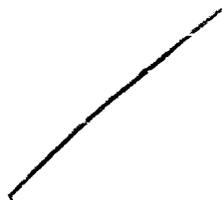


Figure 3.4 HPLC recording of tiotropium metabolic profile in the urine in rabbits (Study U96-2322). The small peak is tiotropium and the main peak is a hydrolysis product of tiotropium.

III.1.D Excretion:

Tiotropium excretion was studied in rats and rabbits. The route of administration affected the tiotropium excretion patterns. Table 3.4 shows that the percentage of tiotropium excreted in feces and urine after oral and intravenous administration in female rabbits (Study U96-2322). Following oral administration, only a small fraction of radioactivity was recovered in the urine, while about two-thirds of the radioactivity was recovered following the intravenous administration.

Table 3.4 Percent of Excreted Tiotropium (1 mg/kg) within 8 days in Rabbits

	Renal	Fecal	Wash Water	Total
Intravenous	63.1	30.0	3.4	96.5
Oral	8.2	68.7	1.2	78.0

The tiotropium excretion patterns in rats were similar to that in rabbits. Sprague-Dawley rats (4/sex) were given an intravenous bolus of 8 mg/kg of radio-labeled tiotropium (Study U99-0216). Urine and feces were collected for 240 hr post dosing. Radioactivity of the sample was determined. About 55% and 43% of radioactivity was recovered in the urine and feces, respectively (Figure 3.5). Most radioactivity was recovered by 96 hr post dosing. No sex difference in elimination was found.

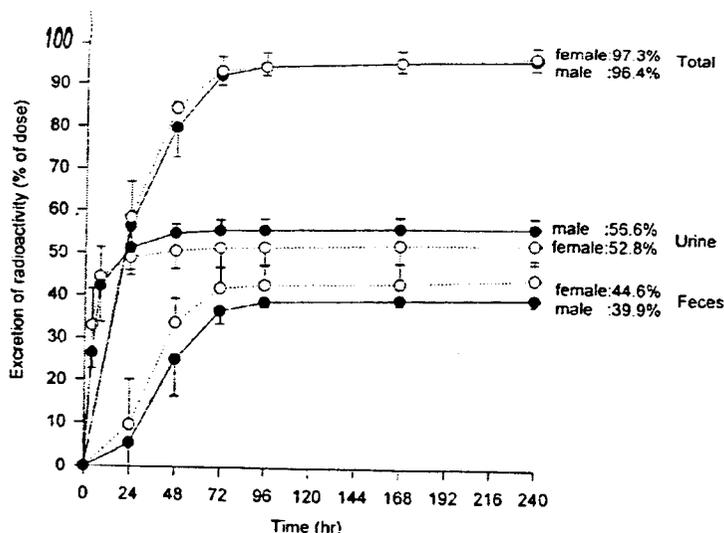


Figure 3.5 Cumulative urinary and fecal excretion of tiotropium in rats.

Tiotropium-pretreatment did not affect its urinary excretion rate (Study U00-1050). Male Chbb:THOM rats pretreated with unlabeled tiotropium (0.5 mg/kg 24 hr prior to the treatment) were given intravenously 0.4 mg/kg of tritium-labeled tiotropium. A control group did not receive the pretreatment. Urinary radioactivity was determined for up to 192 hr post dosing of the radio labeled drug. Terminal half-life of the drug was about 58 hr. The results showed that tiotropium pretreatment did not affect the terminal half life.

Renal excretion of tiotropium was determined in dogs (Study U00-1339). Cimetidine was used to determine the role of active secretion in tiotropium renal excretion. Both drugs were given by intravenous infusion and the infusion rate was 125 and 1,000 $\mu\text{g}/\text{kg}/\text{h}$ for tiotropium and cimetidine, respectively. The steady state tiotropium concentration was 50 ng/ml. The renal clearance of tiotropium was 3.9 ml/min/kg (vs. 42 ml/min/kg for ipratropium). Most drug was excreted by glomerular filtration, while about 20% could be eliminated by active secretion.

III.1.E Other studies:

The validation of LC-MS/MS determination of tiotropium in plasma and urine samples in rats and dogs was described (Studies U00-1206 and U1451). The method was similar to the human sample analysis. Tiotropium was extracted from the sample prior to LC-MS/MS analysis. The method appears to be valid. This method, however, differed remarkably from the TLC method employed to determine metabolites in rats (Study U00-0101).

III.2 Toxicokinetic Parameters: N/A.

III.3 PK/TK summary:

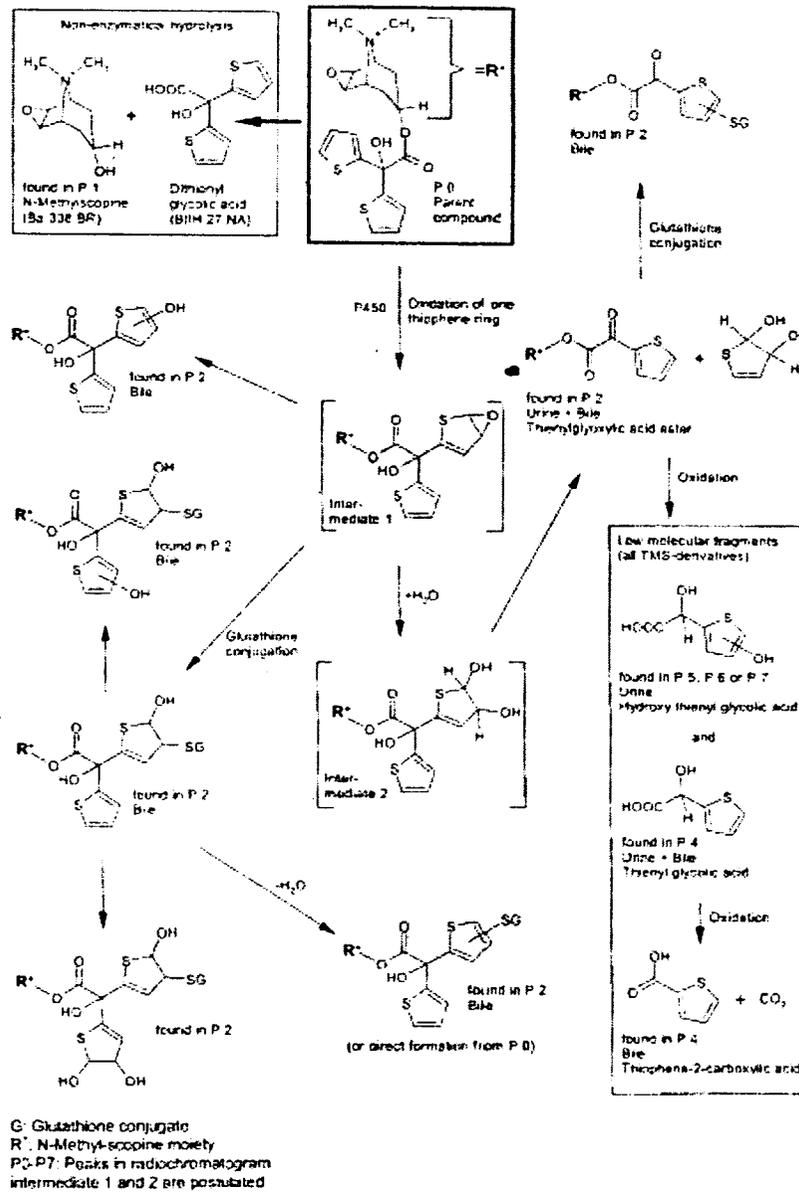
III.3.A PK Summary

Bioavailability of tiotropium varied with the route of administration. A significant amount of inhaled tiotropium was absorbed while only a small fraction of orally administered drug was absorbed. About 20% inhaled tiotropium was absorbed in humans while 96% of intratracheally-instilled drug was readily absorbed in rats. Oral bioavailability of the drug was 0.02, 0.5, < 1.0, 6.3 and 2.4 % in mice, rats, rabbits, dogs humans, respectively. Peak plasma concentration was reached 15 minutes after intratracheal administration in rats. Following oral administration, the peak plasma drug concentration was reached within an hour in mice and rabbits and within 6 – 16 hours in rats and dogs.

Once absorbed, tiotropium was rapidly distributed into blood rich organs. Plasma tiotropium levels declined to less than 5% within one hour after intravenous injection. This decline was attributed to both rapid tissue distribution and high renal clearance. The volume of distribution was about 26 l/kg in animals and 32 l/kg humans (vs. ≤ 0.8 l/kg body water). The terminal half-lives of the drug was 21 – 58 and 132 hrs in rats and humans, respectively. Tissue drug concentrations were in the following descending order (rats): small intestine content > kidney > bile > liver > Plasma > lung > heart. The brain contained the lowest drug concentration. Skin pigmentation did not affect tissue distribution of the drug. Tiotropium was not readily partitioning into erythrocytes (partition coefficient of 0.5-0.9). Tiotropium was secreted into milk readily. Rat milk contained 1.7 – 6.6 times higher tiotropium than the plasma. The percentage of protein binding in humans (65.5%) was three times that in animals (15.5 – 21.7% in mice, rats, rabbits and dogs).

Tiotropium was metabolized in the liver. Figure 3.6 presents hypothesized metabolic pathways for tiotropium in humans. Glutathione conjugation was the dominant phase II pathway. *In vitro* studies with liver microsomes showed that CYP 3A4 and 2D6 contribute to the tiotropium metabolism in humans. Tiotropium metabolic patterns varied with the route of administration. Tiotropium metabolic patterns between animals and humans are qualitatively similar with dogs resembling humans most. After IV administration, the parent compound dominates in the urine in mice and rats, N-methylscopine dominated in rabbits while the two compounds were comparable in dogs. After oral administration, parent compound was undetectable in the urine in all animal species. In the bile the parent compound was absent following IV administration in mice and rats.

Majority of tiotropium was excreted in the urine in all species. Following IV administration, the recovery rate in the urine was approximately 75%, 70%, $\leq 67\%$, $\leq 55\%$ and $\leq 56\%$ for dogs, humans, mice, rats and rabbits. The remaining portion is excreted through the bile.



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Figure 3.6 Hypothesized Metabolic Pathways for Tiotropium

Note: The sponsor identifies N-methylscopine and dithienylglycolic acid as major metabolites of tiotropium. Breakage of the ester bond of tiotropium results in N-methylscopine and dithienylglycolic acid. This is in fact a hydrolysis process. The hydrolysis rate is temperature- and pH- dependent and does not require the presence of any enzymes. Such a definition may be questionable because the process requires no enzymes. Thus, there is no conclusive evidence to demonstrate that two compounds are metabolites.

III.3.B TK Summary

Toxicokinetics of inhalation tiotropium was evaluated in mice, rats and dogs. Accumulation apparently occurred in rats, but not in mice and dogs. Table 3.5 summarizes plasma tiotropium levels in the subchronic and chronic inhalation toxicity studies. In rats, plasma tiotropium level rose as the treatment was prolonged. It could take as long as three months to reach a steady state. The drug accumulation is not surprising given the long terminal half-life of the drug (21-58 hr in animals and 132 hr in humans). However, in mice, no definitive conclusion can be drawn. In two 13-week studies that monitored plasma tiotropium level in mice, one study (U92-0717) showed significant drug accumulation while the other (U92-0271) showed no accumulation at all. In dogs, no pattern of drug accumulation was apparent.

Table 3.5 Plasma Tiotropium Levels in Subchronic and Chronic Inhalation Toxicity Studies

Study#	Species/ duration	Formu- lation	Dose (µg/kgday)	Time (weeks)	Plasma Tiotropium (ng/ml)							
					Male				Female			
					LD	ML D	MH D	HD	LD	ML D	MH D	HD
U93- 0944	Rat, 13wks	DP ^a	6, 42, 392	1	0.2	-	4.1	14.1	1.0	-	3.8	22.2
				6	2.0	-	8.0	18.3	3.2	-	12.2	21.5
				13	2.5	-	9.8	20.5	2.8	-	14.2	30.8
U92- 0295	Rat, 13 wks	AA	5, 22, 65, 320	2	0.7	2.1	2.2	55.2	0.4	1.8	4.7	13.1
				13	2.6	3.5	22.4	45.7	2.7	5.6	36.0	54.8
U98- 2727	Rat, 104 wks	AA	0.9, 2.2, 7.5,	Mo. 12	0.12		0.24	0.80	0.14		0.25	0.85
				Mo. 23	0.15		0.26	1.12	0.15		0.26	1.06
					Average of Male and Female							
					LD	MLD		MHD	HD			
U93- 0946	Rat ^b 52 wks	AA	0.9, 6.7, 45	Weeks	12	0.02		0.25	4.5			
				32	0.1		1.1	14.3				
				52	0.04		0.57	9.99				
U92- 0717	Mouse 13 wks	AA	1.6, 8.8, 45, 210	2	1.5	6.3		26.4	110.4			
				12	0.5	4.7		43.6	402.1			
U92- 0271	Mouse 13 wks	AA	5.2, 19.2, 57.8, 145	2	3.3	11.4		37.3	107.4			
				12	2.9	8.7		25.3	65.6			
U93- 0942	Dog ^{b,c} 13 wks	DP	1.2, 14.2, 133	1				12.8	22.1			
				7				0.11	23.0			
				13				0.63	2.6			
U93- 938	Dog ^{b,c} 52 wks	AA	0.5, 4.5, 45	1				0.13	5.52			
				7				0.26	2.37			
				13				0.28	2.42			
				27				0.52	4.20			
				51				0.57	1.73			

a. DP = dry powder, AA = aqueous aerosol.

b. Means of both sexes.

c. Values at one minute post-dose in dogs.

III.4 PK/TK conclusions:

Inhalation Tiotropium was readily bioavailable in animals. Tiotropium had a large volume of distribution and a long terminal half-life. Metabolism of tiotropium occurred in the liver. Glucuronate conjugates were primary metabolites in the liver. Non-enzymatic hydrolysis occurred in the plasma. The drug was primarily excreted in the urine in unchanged form. Drug accumulation occurred in the rat and a steady state was reached within three months.

IV. GENERAL TOXICOLOGY:**Previously Reviewed Studies**

Study Description	Report #	Vol.	Review #
Acute Toxicity:			
Acute oral and intravenous toxicity studies in mice	U90-0494	24	1
Respirable dust acute toxicity study by inhalation in rats	U90-0517	24	1
Acute oral and intravenous toxicity studies in rats	U90-0493	24	1
Single dose inhalation toxicity study in mice	U91-0812	23	1
Comparative acute intravenous toxicity study in Wistar and Fischer rats	U95-0485	24	2
Single dose inhalation study in dogs	U91-0224	24	1
Inhalation feasibility study in dogs	U93-0729	24	1
Repeat-Dose Toxicity:			
1. Inhalation Toxicity Studies:			
a. Dry Powder Studies:			
2 week inhalation toxicity study in the rat	U93-0943	26	1
14 day inhalation toxicity study in dogs (powder with lactose) – Toxicokinetics to U93-0941	U93-0941	39	1
	U93-0933	39	1
4-week inhalation tolerability study in dogs (lactose formulation)	U93-0766	40	1
13 week inhalation toxicity study in the rat	U93-0944	28	1
13 week inhalation toxicity study in dogs - powder Toxicokinetics to U93-0942	U93-0942	47	1
	U93-0948	48	1
b. Aqueous Aerosol Studies:			
4 week preliminary inhalation toxicity study in rats	U90-0691	27	1
13 week inhalation toxicity study comparing FW 49 and F 344 rats Toxicokinetics to U93-0934	U93-0934	27	1
	U93-0905	28	1
13-weeks inhalation toxicity study in rats Toxicokinetics to U91-0493	U91-0493	30	1
	U93-0059	31	1
4-week oral range-finding study in the rat Toxicokinetics to U92-0477	U92-0477	35	1
	U91-0482	35	1
Preliminary one month inhalation toxicity study in dogs Toxicokinetics to U91-0306	U91-0306	41	1
	U92-0716	41	1
3 month inhalation toxicity study in dogs Toxicokinetics to U91-0511	U91-0511	45	1
	U92-0784	46	1
3 month inhalation toxicity study in dogs Toxicokinetics to U91-0511	U91-0511	45	1
	U92-0784	46	1
52 week inhalation toxicity study in the rat	U93-0945	32	1
12-month inhalation toxicity study in dogs Toxicokinetics to U93-0938	U93-0938	49	1
	U94-0086	50	1

2. Intravenous Toxicity Studies			
3-week intravenous range finding study in the rat	U93-0632	36	1
4 week intravenous toxicity study in the rat	U93-0808	37	1
4 week intravenous toxicity study in rats - Satellite biochemical studies	U92-0380	20	1
4 week intravenous toxicity study in dogs	U91-0494	42	1
3. Oral Toxicity Studies			
Preliminary oral and intravenous toxicity study in dogs	U90-0614	41	1
Toxicokinetics to U90-0614	U91-0491	41	1
3-month oral toxicity study in rat	U91-0492	43	1
Toxicokinetics to U91-0492	U93-0740	44	1
13 week oral toxicity study in dogs	U91-0510	50	1
Toxicokinetics to U91-0510	U93-0747	52	1
4. Studies of Impurities/Degradation Products			
Acute intravenous toxicity study in mice	U91-0845	52	1
Acute intravenous toxicity study in mice	U91-0860	52	1
Acute intravenous toxicity study in mice	U91-0844	52	1
Acute intravenous toxicity study in mice	U92-0680	52	1
unscheduled DNA synthesis test (UDS) in rat hepatocytes <i>in vitro</i>	U91-0636	57	6
point mutation testing in Salmonella typhimurium and Escherichia coli assay	U92-0474	56	6
Mouse bone marrow micronucleus test (IV)	U98-2246	57	6
P Testing for point-mutagenic activity with salmonella typhimurium	U92-0498	56	6
Point-mutagenicity study in Salmonella typhimurium of	U92-0074	56	6
Micronucleus assay of	U99-1477	56	6
Micronucleus assay of	U99-1478	56	6
Micronucleus assay of after repeated inhalation	U99-1565	56	6
Mutagenicity study with in the <i>S. typhimurium</i> / mammalian microsome assay (Ames test)	U99-1650	56	6
Chromosomal aberrations in human lymphocytes with <i>in vitro</i>	U99-1651	56	6
Acute oral and intravenous toxicity studies in mice	U92-0584	54	6
(aqueous solution) 13 week inhalation toxicity study in rats	U97-2187	54	6
4 week inhalation toxicity study of tiotropium bromide and degradation products in rats	U00-1104	53	6

Studies Reviewed in This Review

Study Description	Report #	Vol./p
Preliminary inhalation tolerance study in mice	U91-0813	25
Preliminary whole body inhalation tolerance study in mice (single dose)	U92-0652	24
Preliminary 28 day whole body inhalation toxicity study in mice	U93-0207	25
Pilot Study on the effect of tiotropium by inhalation in rabbits	U92-0294	88

IV.1 General Toxicity Study Review

Study Title: Preliminary Inhalation Tolerance Study in Mice (Study U91-0813).

CD-1 mice (10/sex/dose) were exposed nose-only to 0, 7.5, 47.5, 265 and 400 µg/kg/day of tiotropium (pulmonary dose) for seven days. Clinical signs and clinical pathology parameters were monitored. Neither necropsy nor histopathology was conducted. Decreases in body weight

and WBC counts, and increases in ALT, AST, BUN and LDH were observed in tiotropium treatment groups.

Study Title: Preliminary Acute Whole Body Inhalation Tolerance Study in Mice (Study U91-0652).

Five CD-1 mice per sex were exposed to 5 mg/kg/day of tiotropium (pulmonary deposition from 1 hr whole body exposure) and observed for 14 days post exposure. The following parameters were monitored: clinical sign, body weights, feed consumption and necropsy. Body weight decreased following the exposure but recovered later on. There was no apparent change in feed consumption, neither were there abnormal necropsy findings.

Study Title: Preliminary 28 day Whole Body Inhalation Tolerance Study in Mice (Study U91-0207).

CD-1 mice (20/sex/dose) were exposed to 2.2 and 0.35 mg/kg/day of tiotropium (pulmonary deposition from whole body exposure, 1 hr/day) for 8 and 28 days respectively. Another mice (18/sex/dose) were used as satellite animals for determination of plasma drug levels. There was no control in the study. The high dose animals were terminated on day 9 because of the high mortality (10/38 for males and 8/38 for females in the high dose group and 3/38 for males and 2/38 for the females in the low dose group). Hunched posture, closed/partially closed eyes, piloerection, decreases in body weights and feed consumption were observed in both groups. Necropsy examination showed the following: a slight increase in size of colon and rectum in high dose mice while a distension of the urinary bladder in the low dose group. Histologic evaluation revealed centrolobular hepatocytic hypertrophy (6/15) and cytoplasmic rarefaction (5/15) in the high dose males.

Study title: A pilot study on the effect of Ba 679 BR by inhalation on the rabbit (Study No. U92-0294), Volume 88.

This study was submitted under the reproductive toxicity section because the sponsor used the study as a dose-range study for Segment I toxicity studies. Six non-pregnant female rabbits were given 0.5, 2 and 5 mg/kg/day of tiotropium (total body deposition, via a mask) for 10 – 13 days. Clinical and macroscopic observations were made. The mid and high dose animals showed marked decrease in body weights and fecal output, also observed were lack of pupil reflex and inappetence. The changes in the low dose group were minimal.

IV.2 General Toxicology Summary:

Dr. Satish Tripathi has reviewed and summarized general toxicity studies of tiotropium in his reviews dated 28-AUG-1996, 17-SEP-1997, 10-DEC-1997 under IND 46,687. The newly submitted studies evaluated preliminary tolerance of tiotropium mice, rats and rabbits. These

studies provide no new and significant information relevant to the preclinical safety evaluation of tiotropium. Another comprehensive summary is not warranted. The following is a brief summary of general toxicity of tiotropium.

IV.2.A Review of Tiotropium Doses

It is necessary to review the tiotropium doses of inhalation toxicity studies of this NDA submission prior to the brief summary. The reason is that the submission is inconsistent in reporting tiotropium doses in its inhalation studies. The Non-clinical Summary Section reports the estimated pulmonary exposure (deposits) for most inhalation studies. The individual study reports, however, use the total inhaled doses, not the estimated pulmonary exposure, as the exposure. Consequently, previous reviews of corresponding studies under IND 46,687 also use the total inhaled dose as the actual exposure. To avoid further confusion, Table 4.1 Lists the newly estimated pulmonary doses for the pivotal general inhalation toxicity studies of the drug.

Table 4.1 Principle Repeat Dose Toxicity Studies of Tiotropium

Species	Duration of Treatment; Recovery (wks)	Route of Administration	Formulation	Dose ^c (µg/kg/day)	Study No.
Mice	13; 0	IH ^a	Aqueous	5.2, 19.2, 57.8, 145	U92-0271
CD-1	13; 0	IH		1.6, 8.8, 45, 210	U92-0717
Rat,	13; 6	IH	Powder	6, 42, 392	U93-0944
Wistar	13; 8	IH	Aqueous	4.9, 42, 350	U91-0493
	52; 0	IH	Aqueous	0.9, 6.7, 45	U93-0945
	13; 8	PO	Oral gavage	100, 5000, 300,000	U91-0492
	4; 6	IV		10, 400, 16,000	U93-0808
Fisher	13	IH	Aqueous	5, 22, 65, 320	U92-0295
	13	IH	Aqueous	3.5, 16.2, 300	U92-0765 ^b
	13	IH	Aqueous	5.4	U93-0934 ^b
	16	IH	Aqueous	0.6, 1, 5.1	U93-0946 ^b
Dogs,	13; 4	IH	powder	1.2, 14.2, 133	U93-0942
Beagle	13; 4	IH	Aqueous	1.0, 11.2, 131	U91-0511
	52; 0	IH	Aqueous	0.5, 4.5, 45	U93-0938
	13; 8	PO	Gelatin capsule	5, 30, 200, 1000	U93-0510
	4; 0	IV		4, 20, 100	U91-0494

a. IH = inhalation; PO = oral gavage, IV = intravenous.

b. Not submitted but referred in the Non-clinical Summary section.

c. Doses for inhalation studies are the estimated pulmonary dose based on DFs of 5%, 7% and 10% for mice, rats and dogs, respectively. Notice that the doses in the individual study reports are the total inhaled doses. Major source of the dose information was Table 3.6.6.2.2:1 of the NDA submission (Vol. 1, Item 3, page 75).

IV.2.B General Toxicity of Tiotropium

General toxicity of tiotropium has been evaluated in mice, rats and dogs for the duration of up to one year. The route of administration included intravenous, inhalation and oral gavage. Acute toxicity of tiotropium was studied in mice, rats, and dogs by IV, PO and inhalation administration. Table 4.2 lists the approximate LD50 value of tiotropium in animal species. Signs of tiotropium toxicity included reduced motility, tremor, dyspnea, tachycardia, hunched posture, coprostitis, convulsion, and eventually deaths.

Table 4.2. Approximate LD50 Value (mg/kg) of Tiotropium in Laboratory Animals

Route of Administration	Approximate LD50 Value (mg/kg)		
	Mice	Rats	Dogs
Intravenous	15.6	19.5	-
Oral	1,216	3,648	-
Inhalation ^a	> 6.5	> 21	> 30

a. Estimated pulmonary dose based on a DF of 5%, 7% and 10% in mice, rats and dogs.

Repeat-dose toxicity of tiotropium was evaluated following inhalation, intravenous and oral administration for up to one year in treatment duration in mice, rats and dogs. Inhalation was the route of administration for most toxicity studies. The majority of inhalation studies used aqueous aerosols as the formulation while some studies used lactose powder. The duration of treatment was up to three months in mice and one year in rats and dogs. Table 4.1 lists pivotal toxicity studies supporting tiotropium safety. The highest inhalation dose was 210, 392 and 133 µg/kg/day in mice, rats and dogs, respectively. The studies identified the following as the target organ of tiotropium toxicity: eye, gastrointestinal tract, respiratory tract, liver, urinary bladder, salivary glands and heart. Changes mostly were results of exaggerated pharmacological reaction of tiotropium or known changes associated with inhalation studies. Examples were the increase in heart rate, the inhibition of salivary production and other secretory glands, the inhibition of gastrointestinal motility, urinary retention and urinary proteinaceous deposition in rats, and the slight irritation to the respiratory tract in inhalation studies. The NOAEL value decreased as the treatment was prolonged (Table 4.3).

Table 4.3 Tiotropium NOAELs in Laboratory Animals

Species	Route	NOAEL (µg/kg/day)			
		2 weeks	4 weeks	13 weeks	52 weeks
Mice	Inhalation, aqueous solution			4	
Rat, Wistar	Inhalation, aqueous solution		161	5	<7
	Oral gavage		100		
	Intravenous		10		
Dog	Inhalation, dry powder	4		1	
	Inhalation, aqueous solution		20	1	0.4
	Oral gavage			5	
	Intravenous		< 4		

In the eye, repeat exposure of tiotropium caused cataracts in mice and rats and keratoconjunctivitis sicca (KCS) in dogs, and mydriasis in all species. With the exception of mydriasis, relevance of these findings is unknown. Cataract in rats and mice occurred only with inhalation exposure of 13 weeks or longer inhalation. The severity appeared dependent upon inhalation dose, but not the level of systemic exposure. In Studies U93-0944 and U93-0945 (13 weeks, dry powder or aqueous solution), rats with a mean plasma tiotropium level as low as 0.2 – 3.2 ng/ml developed cataracts. However, the same strain of rats with a mean plasma level of 18 ng/ml of an oral gavage study (U91-0492) did not show the lesion. This findings suggest that cataract is probably a result of high local exposure of the eye in the inhalation studies that is unlikely in the clinical situations.

Tiotropium causes keratoconjunctivitis sicca in dogs. The lesion occurred independent of the route of administration. Table 4.2 summarizes the dose-response relationship of this finding. The sponsor interprets the lesion as a result of decrease in tear production, which subsequently leads to damage of the corneal and conjunctival epithelium and induces an inflammatory response. Also, decreased tear production is a pharmacodynamic effect of anticholinergic agents. Furthermore, dogs seem to be more sensitive to the anticholinergic induced KCS. Finally, KCS is not observed in the clinical trials (up to one year in treatment duration), although “bilateral conjunctivitis in addition to dry mouth was seen in healthy volunteers following repeat once daily inhalation of 141 µg” (the Overdosage of Labeling). Overall, the finding of conjunctivitis in dogs is relevant in the safety evaluation of tiotropium, but the lesion is monitorable clinically.

Mydriasis that occurred in both rats and dogs was a functional and reversible change.

Table 4.2 Keratoconjunctivitis Sicca in Dogs.

Study #	Duration (weeks)	Route/ Formulat'n	Dosing Parameter	Dose Level			Onset (wk)
				Low	Mid	High	
U93- 0766	4	IH, DP	µg/kg/day	2.8*		16.1*	1/2
			C _{plasma} (ng/ml)	1.4		4.24	
U93- 0942	13	IH, DP	µg/kg/day	1.2	14.2*	133*	1
			C _{plasma} (ng/ml)		0.1-12.8	2.6-23.0	
U91- 0511	13	IH, AA	µg/kg/day	1.0	11.2*	131*	1
			C _{plasma} (ng/ml)	0.2-0.6	0.7-2.5	18.9-33.0	
U93- 0938	52	IH, AA	µg/kg/day	0.5	4.5	44.8*	1
			C _{plasma} (ng/ml)		0.2-0.6	1.8-5.5	
U91- 0510	13	PO	µg/kg/day	5	30*	1000*	2
			C _{plasma} (ng/ml)		0.2-0.5	1.1-3.8	
U91- 0494	4	IV	µg/kg/day	4	20*	100*	1/2
			C _{plasma} (ng/ml)	N/A	N/A	N/A	
U00- 3029	2, human	IH	µg/kg/day	0.3			
			C _{plasma} (ng/ml)	0.019			

* Dose at which keratoconjunctivitis sicca occurred.

IV.3 General Toxicology Conclusion

Tiotropium possesses a toxicity profile typical of a muscarinic anticholinergic agent. The target organ of tiotropium toxicity includes the eye, gastrointestinal tract, respiratory tract, urinary bladder, salivary glands, heart and respiratory tract. The NOAEL value decreases as the treatment is prolonged. The 52-week NOAEL value in inhalation studies is < 7 and 0.4 µg/kg/day in Wistar rats and dogs, respectively.

IV.4 Labeling Review

IV.4.1 Recommended Labeling:

Overdosage: No mortality was observed at inhalation tiotropium doses up to — mg/kg in mice, mg/kg in rats and — mg/kg in dogs. These doses correspond to times the _____) on a mg/m² basis, respectively.

High doses of tiotropium may lead to anticholinergic signs and symptoms. However, there were no systemic anticholinergic effects following a single inhaled dose of up to 282 µg tiotropium in healthy volunteers.

IV.4.1 Review of Dose Ratios in the Labeling:

Table 4.4 summarizes the dose calculations in the Overdosage section of the tiotropium labeling.

Table 4.4 Dose Ratio Calculations in the Overdosage Section of the Tiotropium Labeling

Species	Conversion Factor	Dose		Ratio (animal/human)	
		mg/kg	mg/m ²	Calculated	Rounded to
Human	37	0.00036 (IH)	0.01332	-	-
Mice	3	6.6 (IH)	19.8	1486	1,500
		1,216 (PO)	3648	273,873	270,000
Rat	6	21 (IH)	126	9459	9,500
		3,550 (PO)	21,300	1,599,099	1,600,000
Dog	20	30 (PO)	600	45,045	45,000

V. GENETIC TOXICOLOGY:

Not applicable. Dr. Satish Tripathi has reviewed and summarized genetic toxicity studies of tiotropium under IND 46,687 (see reviews dated 28-AUG-1996, 17-SEP-1997, 10-DEC-1997.) Tiotropium tests negative in the following assays:

- Bacterial gene mutation in *S. typhimurium* and *E. coli*
- Gene mutation in V79 CHO cell line *in vitro*
- Chromosomal aberrations in human lymphocytes *in vitro*
- The unscheduled DNA synthesis in primary rat hepatocytes *in vitro*
- Micronucleus formation in mice *in vivo*

Genetic Toxicity Labeling Review:

The following is recommended for the Tiotropium Labeling:

Tiotropium bromide demonstrated no evidence of mutagenicity or clastogenicity in the following assays: the bacterial gene mutation assay, the V79 Chinese hamster cell mutagenesis assay, the chromosomal aberration assays in human lymphocytes *in vitro* and mouse micronucleus formation *in vivo*, and the unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

The Sponsor's Proposed Labeling:

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APPEARS THIS WAY
ON ORIGINAL

VI. CARCINOGENICITY:**VI.1 Individual Study Review****VI.1.A Study Title: Ba 679 BR (Tiotropium) 24-Month Inhalation Carcinogenicity Study in Wistar Rats.**

Key findings: Tiotropium is non-carcinogenic in rats under the test conditions.

Study no: U98-2727
Study type (if not reflected in title): Nose-only inhalation
Volume #, and page #: Volume 78 - 85
Conducting laboratory and location:
Date of study initiation: January 11, 1994
Date of Study Completion: February 15, 1996
Study Report Date: 23-AUG-2000; June 26, 1999
GLP compliance: In compliance with OECD GLP
QA reports: yes (x), no ()
Drug, lot #, radiolabel, and % purity: Batch II; 98.9% calculated with respect to the dried substance and 99.8% peak area in HPLC assay

CAC concurrence: None

Study Type (2 year bioassay, alternative model etc.): 2-year bioassay
Species/strain: Wistar/Chbb:THOM
Number/sex/group; age at start of study: 50; 9 - 10 weeks of age
Animal weight at start of exposure: Male: *ca* 190 g; female: *ca* 150 g.
Animal housing: 20 ± 2°C; 12 hr light cycle; 2/cage
Feed and water: Rat and Mouse (modified) No. 1 diet SQC expanded; feed and water *ad libitum*

Formulation/vehicle (Table 6.1):

Ingredient	Formulation		Vehicle	
	I	II	I	II
Tiotropium (mg)	10.38	103.8	-	-
Benzalkonium chloride (mg)	10.0	10.0	-	10.0
Hydrochloric acid (0.1 N, ml)	0.8	0.8	0.8	0.8
Water for injection (ml)	100	100	100	100

Drug stability/homogeneity: Expiration date: July 1996; 99% purity; Batch No. II

Design: Table 6.2 shows the overall design of the 24-month inhalation carcinogenicity study of tiotropium in rats.