

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**21-518**

**PHARMACOLOGY REVIEW(S)**



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

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-518  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: Various submissions between 7/28/03 and 10/3/03  
PRODUCT: solifenacin succinate  
INTENDED CLINICAL POPULATION: adults with urinary urge incontinence  
SPONSOR: Yamanouchi Pharmaceutical Co., Ltd.  
DOCUMENTS REVIEWED: N000 BP (6/26/03; 7/28/03; 8/25/03; 8/29/03; 9/22/03;  
10/3/03)  
N000 BZ (8/25/03)  
REVIEW DIVISION: Division of Reproductive and Urologic Drug  
Products (HFD-580)  
PHARM/TOX REVIEWER: Lynnda Reid, Ph.D., Supervisory Pharmacologist  
DIVISION DIRECTOR: Daniel Shames, M.D.  
PROJECT MANAGER: Jean Makie

Date of review submission to Division File System (DFS): October 13, 2004

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ON ORIGINAL**

***EXECUTIVE SUMMARY***

**I. Recommendations**

- A. Recommendation on approvability: Approval
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling:

1 Draft Labeling Page(s) Withheld

## II. Summary of nonclinical findings

**A. Brief overview of nonclinical findings:** Animal deaths observed following administration of solifenacin succinate were usually accompanied by signs of CNS toxicity, i.e., underactivity, ataxia, tremors, convulsions, prostration, hunched posture, piloerection and abnormal respiration. The most common clinical signs were mydriasis and salivation. These findings are consistent with class effects associated with other muscarinic receptor antagonists.

ECG effects consisting of prolongation of P-wave, PR interval, QRS duration and QT interval were observed in dogs. However, QT interval values were within the normal range, accompanied by decreases in heart rate between 4 and 8 beats/minute, and C<sub>max</sub> values were 24 to 87-fold higher than at the proposed therapeutic dose of 10 mg/day in humans. Similar effects have been observed with other muscarinic 3M receptor antagonists, e.g., dofetilide, tolterodine and oxybutynin.

Solifenacin succinate was not mutagenic or genotoxic in the standard battery of *in vitro* and *in vivo* assays. Two-year carcinogenicity studies in mice and rats did not demonstrate any increase in tumor incidences associated with oral administration of solifenacin succinate.

There were no effects on fertility or early reproductive parameters following administration of solifenacin succinate to male and female mice or rats. There were no teratogenic effects observed in rats or rabbits. However, administration of solifenacin succinate to mice during the period of major organogenesis resulted in an increased incidence of cleft palate at doses that resulted in decreased maternal weight gain. Reduced fetal and pup weights, and delayed development were also observed in F1 generation mice. No

reproductive or developmental effects were observed at doses approximately 2 fold that of the maximum recommended human dose (MRHD) in mice and rabbits and at the highest tolerated dose in rats (<1 the MRHD).

Solifenacin succinate caused minor skin irritation and severe, potentially irreversible, ocular mucosal damage in rabbits.

Solifenacin was not antigenic in the guinea pig.

B. Pharmacologic activity: M3 muscarinic antagonist

C. Nonclinical safety issues relevant to clinical use:

- 1) Solifenacin succinate has been shown to potentially reduce heart rate and induce prolongation of the P-wave and PR interval and the QRS duration and QT interval. *In vitro*, solifenacin succinate was shown to inhibit the HERG potassium current at a concentration of 0.27  $\mu\text{M}$ .
- 2) A relationship between cleft palate in mice and *in utero* exposures to solifenacin succinate could not be ruled out.
- 3) *In utero* and lactational exposures resulted in reduced fetal and pre-weaning pup weights, peripartum and postpartum mortalities, and delayed development.
- 4) Severe and seemingly irreversible ocular mucosal damage, especially opacity and edema to the cornea and falling of the nictating membrane was observed in rabbits with 10 and 100 mg/eye (unrinsed). Rinsing appeared to ameliorate the effect. Effects were reversible over time at 1 mg (unrinsed) and following rinsing 10-30 seconds after instillation at higher concentrations.

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## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### INTRODUCTION AND DRUG HISTORY

**NDA number:** 21-518

**Review number:** 2

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

**N000 BP (6/26/03; 7/28/03; 8/25/03; 8/29/03; 9/22/03; 10/3/03)**

**N000 BZ (8/25/03)**

**Information to sponsor:** labeling recommendations

**Sponsor and/or agent:** Yamanouchi Pharma America, Inc., Paramus, NJ

**Manufacturer for drug substance:** Yamanouchi Pharmaceutical Co., Ltd., Japan

**Reviewer name:** Lynnda Reid, Ph.D.

**Division name:** Reproductive and Urologic Drugs

**HFD #:** 580

**Review completion date:** October 12, 2004

#### Drug:

**Trade name (proposed):** VESICARE™

**Generic name (list alphabetically):** solifenacin succinate

**Code name:** YM905

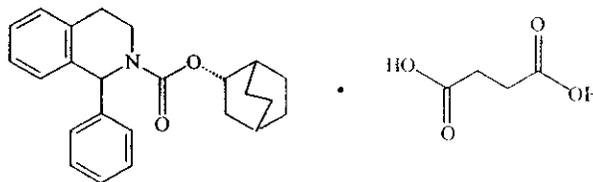
**Chemical name:** (+)-(1S,3'R)-3'-quinuclidin-3'-yl 1-phenyl-1,2,3,4-tetrahydro-2-isoquinoline-2-carboxylate monosuccinate

**CAS registry number:** ?

**Molecular formula:** C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>·C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>

**Molecular weight:** 480.56

**Structure:** Solifenacin succinate contains two chiral centers, therefore enantiomeric and diastereomeric isomers are possible.



**Relevant INDs/NDAs/DMFs:** IND 58,135

**Drug class:** muscarinic M3 receptor antagonist

**Intended populations:** adults with urinary urge incontinence

**Clinical formulation:** Tablets: 2.5 mg, 5 mg and 10 mg

**Route of administration:** Oral

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

**Studies reviewed within this submission:** Addendum 2-Year Rodent Carcinogenicity Reports

- 1) 104-Week Carcinogenicity Study in CD-1 Mice: Summary Tables of the Incidence of Neoplastic Lesions in All Study Dose Groups (3, 10, 20 mg/kg/day in males and 2, 7.5, 15 mg/kg/day in females)
- 2) 104-Week Carcinogenicity Study in F-344 Rats: Summary Tables of the Incidence of Neoplastic Lesions in All Study Dose Groups (0, 10, 30, 200 mg/kg/day)

**Studies not reviewed within this submission:** For a detailed review of the nonclinical pharmacology and toxicology studies reviewed in support of the safety of solifenacin succinate, please refer to the original NDA review dated September 11, 2003. Only a brief summary of the overall toxicology findings and the updated carcinogenicity study results will be presented in this review.

**TOXICOLOGY****Overall toxicology summary:**General toxicology:

*Mice:* In the 13-week study, twelve deaths occurred: one female at 250 mg/kg, and five males and six females at 400 mg/kg. Signs of toxicity at 250 and 400 mg/kg/day included underactivity, ataxia, tremors, convulsions, prostration, hunched posture, piloerection and abnormal respiration. Depressed weight gain was also seen in males at 400 mg/kg/day. There were no significant microscopic findings that correlated with any changes in clinical chemistry parameters or organ weights. The NOAEL was considered 100 mg/kg/day.

Chronic (26-weeks) administration of 100 and 200 mg/kg/day solifenacin succinate in mice resulted in erosion of the GI tract mucosa and inflammation of the lower GI tract, with the most sensitive area being the duodenum. A dose-related exacerbation of porphyrin deposits in the Harderian glands was also noted. The NOAEL in mice following 26 weeks of exposure was 30 mg/kg/day resulting in solifenacin succinate AUC<sub>24</sub> values of 745 and 566 ng.h/ml in males and females, respectively.

*Rats:* Administration of solifenacin succinate to rats for 4 weeks at doses of 5, 10, 25 or 50 mg/kg/day resulted in the deaths of 1 male at 25 mg/kg, and 2 males and 7 females at 50 mg/kg/day. Convulsions prior to death were noted in the 25 mg/kg male and in 3 of the 50 mg/kg females. The most common clinical signs were mydriasis and salivation observed in males at • 10 mg/kg and in females at • 25 mg/kg. Abnormal respiratory sounds were noted in both sexes at • 10 mg/kg/day. The NOAEL was 5 mg/kg/day.

Chronic (26-week) administration of solifenacin succinate at doses of 60 mg/kg/day in female and 100 mg/kg/day in male rats exceeded the MTD. There were 5 deaths in females after week 15 in the 30 mg/kg/day group; 6 deaths in the 60 mg/kg/day group and a further 9 deaths after the dosage was reduced to 45 mg/kg/day. One male treated with 100 mg/kg/day died during week 8. There were no significant gross or histopathological changes that would account for these deaths.

Doses of 30 mg/kg/day and greater resulted in low body weight gains and signs of distress immediately following dosing. As in the 4-week study, animals treated with 10 mg/kg evinced mydriasis, salivation and abnormal respiration following dosing. Changes in various organ weights, i.e., adrenals, thymus, liver, kidney and spleen, were not associated with any histopathology. The slightly higher liver weights in males and changes in transaminase activity suggest a potential effect upon liver metabolism. Changes in urinary output, electrolyte levels and relative weight also suggest that the kidneys may be a sensitive target tissue. Changes in clinical pathology and organ weights had either reversed or improved by the end of a 10-week recovery period. Follicular degeneration of the ovaries and/or uterine atrophy were evident in 2 females dosed at 30 mg/kg/day and in 13 animals given 60/45 mg/kg/day. The NOAEL is considered to be 3 mg/kg/day, a dose resulting in solifenacin succinate AUC<sub>24</sub> values of 19 and 48 ng.h/ml and BY-348C (M1 active metabolite) AUC<sub>24</sub> values of 5042 and 8928 ng.h/ml in males and females, respectively.

*Dogs:* Administration of solifenacin to dogs at 1, 3, 10 or 30 mg/kg/day for 4 weeks or at 3, 6, 12 or 25/18 mg/kg/day for 13 weeks resulted in excessive salivation and vomiting at the highest doses. Tremors, ataxia, convulsions, prostration and lower body weights were also seen in high-dose animals. Minor changes in erythrocyte parameters were also seen (not evident in the 52-week study). ECG effects were observed in the 4-week and 13-week toxicology studies. In the 4-week study at a dose of 30 mg/kg/day there was a prolongation of the P-wave, the PR interval and the QRS duration and QT interval in some dogs. However, QT interval values in all animals were within the normal range and were usually accompanied by slower heart rates. In the 13-week study the only changes observed were prolongation of the P-wave and PR interval at 25/18 mg/kg/day which produced C<sub>max</sub> of 24-32-fold higher than the proposed maximum human therapeutic dose. Organ weight changes and microscopic lesions were observed in the kidney and thymus in the 4-week study but not in the 13-week (or 52-week) study. The NOAEL was 3 mg/kg in the 4-week study and 12 mg/kg in the 13-week study.

Chronic (52-week) administration of solifenacin succinate at doses up to 20 mg/kg/day to dogs was in general, clinically unremarkable. Group mean heart rates for males receiving 12 or 20 mg/kg/day and females receiving 6, 12 or 20 mg/kg/day were lower in comparison with the predose values on most occasions during the dosing period. The largest reduction was observed in males dosed at 20 mg/kg/day during week 38. Changes in the P, PR, QRS, ST and QT intervals were in general related to the concurrent fluctuations in the heart rates and were generally within the expected ranges.

There were also dose-related changes in mean absolute and relative spleen weights. In males, spleen weights decreased with increasing dose, statistically and biologically significant only at 20 mg/kg/day. In females, there was a decrease in the mean absolute and relative spleen weights at doses of 12 mg/kg/day, however, at 20 mg/kg/day, spleen weights were significantly higher relative to controls. Females dosed at 20 mg/kg/day also presented with an increased incidence and degree of perivascular lymphoid accumulation in the urinary bladders. The higher spleen weights could be secondary to this accumulation of urinary lymphoid cells.

The NOAEL was 6 mg/kg/day in males and 3 mg/kg/day in females. These doses resulted in mean AUC<sub>24</sub> values between 395-497 ng.h/ml in males and 47-165 ng.h/ml in females. AUC levels actually decreased between weeks 26 and 52.

*General toxicology conclusions:* Solifenacin does not readily cross the blood brain barrier, however at the highest doses tested in mice, rats and dogs, morbidity appeared to be the result of CNS toxicity. The brain is the most sensitive organ. The CNS effects are consistent with class effects noted in other muscarinic receptor antagonists.

In rats, changes in urinary output, electrolyte levels and relative weight also suggest that the kidneys may be a sensitive target tissue.

Cardio-toxicity was observed in dogs as evinced by prolongation of the P-wave, PR interval, QRS duration and QT interval. The potential for solifenacin to block potassium channels was confirmed *in vitro* in the HERG assay.

Genetic toxicology: Based on a valid standard test battery consisting of bacterial mutagenicity, chromosome aberrations (*in vitro*) and rat micronucleus assay, solifenacin succinate shows no evidence of genotoxicity.

Carcinogenicity: Based on valid 2-year bioassays in mice and rats, solifenacin succinate shows no evidence of tumorigenicity.

Reproductive toxicology:

*Fertility:* Solifenacin succinate had no effect on fertility, reproductive functions or early embryonic development when administered to mice and rats at systemic exposure levels 5 and 10 fold greater in male and female mice, respectively, and <1 and 5 fold greater in male and female rats when compared to the highest mean AUC values achieved in humans. These values represent the highest doses tested in mouse and rat reproductive and developmental studies.

*Embryo-fetal toxicity:* Mouse fetal body weights were significantly reduced following *in utero* exposures to solifenacin succinate at • 100 mg/kg/day. There were no effects on embryo-fetal development in mice, rats or rabbits. Exposures (based on comparison of mean AUC<sub>24</sub> values) were approximately 2 fold greater in mice, approximately 1/5<sup>th</sup> in rats and 1.8 fold greater in rabbits than expected exposure in humans.

Cleft palate was observed only in mice at the highest dose tested. This dose was maternally toxic and approximately 10 fold higher than expected exposures in female patients. Exposures which did not result in any fetuses with cleft palate resulted in systemic exposures 5 fold higher than expected in female patients.

*Developmental effects:* Effects observed in F1 mouse offspring exposed from gestation day 6 through lactation included increase in pup peripartum mortality, decreased pup weight and weight gain, and delayed development at maternal doses • 100 mg/kg, and an increase in pup mortality postpartum at a maternal dose of 250 mg/kg. Vaginal patency was also delayed in F1 female offspring at • 100 mg/kg. There were no effects on fertility or reproductive parameters in the F1 offspring, and with the exception of a potential increase in the percent of male fetuses, there were no developmental effects in the F2 offspring. Doses which did not result in any observable developmental effects 1<sup>st</sup> generation offspring mice resulted in systemic exposures approximately 2 fold greater than expected in female patients.

**Special toxicology:** Care should be exercised in keeping the product away from the eyes and directions to rinse eyes immediately if exposed to solifenacin.

**Carcinogenicity:**

The original 2-year mouse and rat study reports were presented to the ExecCAC on May 27, 2003. At that time, the committee agreed that the study doses used in both rat and mouse studies were adequate. However, the committee felt that they could not concur with the reviewer on the absence of drug-related neoplasms in either species due to low survival and greater than 10% body weight deferences between high-dose and control animals. The Sponsor was asked to perform a complete analysis of all tissues from the low- and mid-dose groups.

In addition, the committee wanted to see more information on infections, SDA (sialodacryoadenitis) in rats and 'respiratory infections' in mice, which occurred early and appeared to correlate with the increased mortality observed during the first 12 months in both studies.

**104-Week Carcinogenicity Study in CD-1 Mice: Summary Tables of the Incidence of Neoplastic Lesions in All Study Dose Groups (3, 10, 20 mg/kg/day in males and 2, 7.5, 15 mg/kg/day in females)**

**Key study findings:** no drug-associated increase in neoplastic lesions

**Study number:** R905-TX-023 (20021105)  
**Volume #, and page #:** EDR (N000 BP, 7/28/03)  
**Conducting laboratory and location:** —  
**Date of study initiation:** August 16, 1999  
**GLP compliance:** Yes  
**QA report:** yes ( x ) no ( )  
**Drug, lot #, and % purity:** YM905 Batch No. K9059804, —  
**CAC concurrence:** Yes (See Appendix 1)

STUDY DURATION:	Std 2 year bioassay (104 weeks)	
LABORATORY:	—	
STUDY STARTING DATE:	August 16, 1999	
STUDY ENDING DATE:	September 28, 2001	
CARCINOGENICITY STUDY REPORT DATE:	September 17, 2002	
MOUSE STRAIN:	CD-1 mice	
ROUTE:	oral gavage (10 ml/kg)	
NUMBER OF MICE:	<u>Main Study</u>	<u>Toxicokinetic Study</u>
- Control (C):	70/sex	-
- Low Dose (LD):	70/sex	20/sex
- Middle Dose (MD):	70/sex	20/sex
- High Dose-1 (HD1):	70/sex	20/sex
- High Dose-2 (HD2):	70/sex	20/sex

**MOUSE DOSE LEVELS (mg/kg/day):**

- Low Dose: 10
- Middle Dose: 30
- High Dose-1: 100
- High Dose-2: 200

**BASIS FOR DOSES SELECTED:** Doses were selected based on mortalities and adverse clinical behavior in the 13-week dose-range finding study where mice were administered doses of 30, 100, 250 or 400 mg/kg/day. Mortalities were observed in both sexes at 400 mg/kg/day, with one death (female) and overt behavioral toxicity (male) at 250 mg/kg.

**PRIOR FDA DOSE CONCURRENCE (CAC):** Yes; July 27, 1999

**MOUSE CARCINOGENICITY:** Negative

**MOUSE TUMOR FINDINGS (details):** In the original report, only animals with gross lesions were examined from the low- and mid-dose groups. At the request of the ExecCAC, all tissues from low- and mid-dose animals were read by the same pathologist who read the original study slides. The Sponsor indicated that over 80% of the tissues were looked at in the original study report. The following is a comparison of the original report with those submitted after reading all slides for all groups. Any values that have changed have been highlighted in Tables M-1 and M-2 (comparisons of the tumor incidences in the original report to the amended full report).

Tumor incidence was unaffected by treatment and were of the types normally encountered CD-1 mice at

**Table M-1: Histopathology - group distribution of neoplastic findings for all male mice in 2-year carcinogenicity study.**

Males	Original Report (80%)					Amended Report (100%)				
	0	10	30	100	200	0	10	30	100	200
Adrenals:										
Cortical Adenoma	7	6	3	8	2	7	13	4	8	2
Phaeochromocytoma	-	-	-	1	1	-	-	-	1	1
Malignant Phaeochromocytoma	0	1	-	-	-	0	1	-	-	-
Bone:										
Osteoma	-	1	-	1	-	-	1	-	1	-
Brain:										
Meningioma (maningeal sarcoma)	-	-	-	-	-	1	-	-	-	-
Harderian Glands:										
Adenoma	7	5	7	2	2	7	8	7	2	2
Adenocarcinoma	3	3	4	-	-	3	3	4	-	-
Hemopoietic:										
Malignant Lymphoma	13	12	7	9	5	13	12	7	9	5
Histoicytic sarcoma	2	6	5	-	3	2	6	5	-	3
Myeloind Leukemia	1	-	-	2	-	1	-	-	2	-
GI Tract:										
Cecum - Adenocarcinoma	1	-	-	-	-	1	-	-	-	-
Colon - Adenoma	-	-	1	1	-	-	-	1	1	-

Adenocarcinoma	-	-	-	1	1	-	-	-	1	1
Duodenum - Adenoma	-	-	1	-	-	-	-	1	-	-
Stomach - Squamous cell papilloma	-	1	-	-	-	-	1	-	-	-
Adenoma	-	-	1	-	-	-	-	1	-	-
Tongue - Adenoma	1	-	-	-	-	1	-	-	-	-
Kidneys:										
Adenoma	1	-	-	1	-	1	-	-	1	-
Liver:										
Hepatocellular adenoma	6	9	10	4	7	6	12	10	4	7
Hepatocellular carcinoma	4	7	4	1	1	4	7	4	1	1
Hemangioma	1	-	-	-	-	1	-	-	-	-
Hemangiosarcoma	1	4	2	-	-	1	4	2	-	-
Cholangioma (M) / Ito cell tumor (F)	-	1	-	-	-	-	1	-	-	-
Lungs:										
Bronchioloalveolar Adenoma	22	16	17	20	11	22	18	17	20	11
Bronchioloalveolar Adneocarcinoma	6	5	8	2	1	6	5	8	2	1
Pancreas:										
Islet cell adenoma	1	1	-	2	-	1	1	-	2	-
Pituitary:										
Adenoma - Pars Distalis	-	-	-	1	-	-	-	1	1	-
Adenoma - Pars Intermedia	-	-	-	-	-	-	-	-	1	-
Salivary Gland:										
Squamous cell carcinoma	-	-	-	1	-	-	-	-	1	-
Testes:										
Interstitial (Leydig) cell adenoma	5	1	4	3	-	5	1	4	3	-
Adenoma	-	-	-	-	1	-	-	-	-	1
Thymus:										
Thyoma (Lymphoid)	-	1	1	-	-	-	1	1	-	-
Thyroids:										
Follicular cell adenoma	1	-	-	1	-	1	-	1	1	-
C-cell adenoma	-	-	-	-	-	-	1	-	-	-
Hemangiomas:										
Mesenteric lymph node	1	-	-	-	-	1	-	-	-	-
Prostate	-	1	-	-	-	-	1	-	-	-
Spleen	2	-	-	1	-	2	1	-	1	-

**Table M-1: Histopathology - group distribution of neoplastic findings for all female mice in 2-year carcinogenicity study.**

Females	Original Report (80%)					Amended Report (100%)				
	0	10	30	100	200	0	10	30	100	200
Adrenals:										
Phaeochromocytoma	1	-	-	-	1	1	-	-	-	1
Bone:										
Osteoma	-	1	1	-	-	-	1	1	-	-
Osteosarcoma	-	1	-	-	-	-	1	-	-	-
Chondroma	-	1	-	-	1	-	1	-	-	1

<b>Brain:</b>										
Meningioma	-	1	-	-	-	-	1	-	-	-
<b>Harderian Glands:</b>										
Adenoma	7	2	-	1	2	7	3	3	2	1
Adenocarcinoma	-	2	-	1	2	-	2	-	1	2
<b>Hemopoietic:</b>										
Malignant Lymphoma	13	12	13	6	9	13	12	14	6	9
Histiocytic sarcoma	3	4	6	3	4	3	4	6	3	4
<b>GI Tract:</b>										
Colon - Adenocarcinoma	-	-	1	-	-	-	-	1	-	-
Jejunum - Adenocarcinoma	-	-	-	-	1	-	-	-	-	1
Stomach - Adenoma	-	1	-	-	-	-	1	-	-	-
Rectum - Squamous cell papilloma	-	1	-	-	-	-	1	-	-	-
<b>Liver:</b>										
Hepatocellular adenoma	2	3	2	-	-	2	3	2	-	-
Hemangiosarcoma	-	-	2	-	-	-	-	2	-	-
Cholangioma (M) / Ito cell tumor (F)	-	-	1	-	-	-	-	1	-	-
<b>Lungs:</b>										
Bronchioloalveolar Adenoma	15	15	11	7	5	15	15	12	7	5
Bronchioloalveolar Adneocarcinoma	4	4	-	-	1	4	4	-	-	1
<b>Mammary:</b>										
Adenocarcinoma	1	1	2	1	-	1	1	2	1	-
<b>Ovaries:</b>										
Schwannoma	-	1	-	-	-	-	1	-	-	-
Granulosa Cell Tumor	1	2	1	1	0	1	2	1	1	0
Luteoma	1	3	3	3	1	1	3	3	3	1
Cystadenoma	-	-	1	-	-	-	-	1	-	-
Leiomyoma	-	-	-	-	1	-	-	-	-	1
Sertoli cell adenoma	1	-	-	-	-	1	-	-	-	-
Tubulostromal adenoma	-	-	1	1	0	-	-	1	1	0
Undifferentiated stromal tumor	-	-	0	1	0	-	-	0	1	0
<b>Pancreas:</b>										
Islet cell adenoma	-	1	-	-	-	-	1	-	-	-
<b>Pituitary:</b>										
Adenoma	3	3	-	-	-	3	3	-	-	-
<b>Spleen:</b>										
Hemangioma	-	1	-	-	-	-	1	-	-	-
Hemangiosarcoma	1	-	-	-	-	1	-	-	-	-
<b>Thymus:</b>										
Thyoma (Lymphoid)	6	4	9	4	2	6	4	9	4	2
Thyoma (Epithelial)	-	-	1	-	-	-	-	1	-	-
<b>Uterine / Cervix:</b>										
Endometrial Polyp	1	1	-	1	-	1	1	-	1	-
Leiomyoma	1	-	-	2	1	1	-	-	2	-
Leiomyosarcoma	1	1	1	-	1	1	1	1	-	1
Malignant schwannoma	-	-	-	1	-	-	-	-	1	-
<b>Uterus:</b>										

Endometrial Polyp	4	9	6	3	2	4	9	6	3	2
Leiomyoma	3	1	-	-	-	3	1	-	-	-
Leiomyosarcoma	-	-	-	1	-	-	-	-	1	-
Endometrial adenoma	-	2	-	-	-	-	2	-	-	-
Endometrial stromal cell sarcoma	-	-	2	-	2	-	-	2	-	2
Histiocytic sarcoma	-	1	1	-	-	-	1	1	-	-
Vagina:										
Histiocytic sarcoma	2	-	-	-	-	2	-	-	-	-
Fibroma	1	-	-	-	-	1	-	-	-	-
Hemangiomas:										
Uterus	1	1	2	-	1	1	1	2	-	1
Hemangiosarcoma:										
Uterus	-	2	-	-	-	-	2	-	-	-

The final percentage rates for adrenal adenomas are 10, 18.6, 5.7, 11.4 and 2.9% for the 0, 10, 30, 100 and 200 mg/kg groups, respectively. These rates are higher than previously reported by Charles River, even for the control group (See Tables M-3 and M-4). However, there is no consistent dose-response pattern and is not statistically significant (Sponsor analyses).

**Table M-3: Charles River Historical Data in CD-1 Male Mice (March 2000)**

Location & Tumor	# Studies (# Organs)	# Lesions	Percent	Minimum %	Maximum %
<b>Adrenals:</b>	46 (2526)				
Cortical Adenoma		30	1.19	1.56	7.14
Phaeochromocytoma		11	0.44	1.11	5.00
Malignant Phaeochromocytoma		-	-	-	-
<b>Harderian Glands:</b>	46 (2565)				
Adenoma		120	4.73	1.67	14.00
Adenocarcinoma		11	0.43	1.43	8.33
<b>Liver:</b>	46 (2571)				
Hepatocellular adenoma		269	10.46	2.86	28.00
Hepatocellular carcinoma		136	5.29	1.54	16.00
Hemangioma		9	0.35	1.54	4.00
Hemangiosarcoma		29	1.13	1.11	5.00
<b>Lungs:</b>	46 (2575)				
Bronchioloalveolar Adenoma		368	14.29	2.00	42.00
Bronchioloalveolar adneocarcinoma		177	6.87	1.43	26.00

**M-4: Charles River Historical Data in CD-1 Female Mice (March 2000)**

Location & Tumor	# Studies (# Organs)	# Lesions	Percent	Minimum %	Maximum %
<b>Hemopoietic:</b>	48 (2822)				
Malignant Lymphoma		274	9.71	1.67	50.00
Histiocytic sarcoma		111	3.93	1.67	18.33

Lungs:	48 (2773)				
Bronchioloalveolar Adenoma		236	8.51	1.67	26.67
Bronchioloalveolar adneocarcinoma		113	4.08	0.77	18.37
Uterus:	48 (2812)				
Endometrial Polyp		146	5.19	1.67	17.14
Leiomyoma		40	1.42	1.43	7.50
Leiomyosarcoma		36	1.28	0.86	6.00
Endometrial adenoma		3	0.11	1.54	2.00
Endometrial stromal cell sarcoma		33	1.17	1.43	8.00
Hemangioma		15	0.53	1.25	4.62
Hemangiosarcoma		14	0.50	0.77	4.08

MOUSE STUDY COMMENTS: The only significant pathological finding which appears to be treatment/dose-related is the rate of deaths attributed to respiratory disease during the first 52 weeks of the study. Mortality was increased in both males and females receiving 100 mg/kg/day and in males receiving 200 mg/kg/day. The increases in mortalities at the higher dose levels were apparent during the first year of the study. After week 53, mortality in each dose group was almost comparable to the lower doses.

Bodyweight gain was unaffected in males and females dosed at 10 mg/kg/day and in males at 30 mg/kg/day. Overall, bodyweight gain reductions in males receiving 100 or 200 mg/kg were 19 and 20%, respectively. Those in females receiving 30, 100 or 200 mg/kg/day were 17, 23 and 36%, respectively. Statistically significant decreases in high-dose animals were observed beginning between weeks 4-5 in males and weeks 10-12 in females.

Beginning week 20, overall food consumption was reduced by approximately 5% in males and females dosed at 200 mg/kg/day. Overall food intake in animals receiving 10, 30 or 100 mg/kg/day was unaffected by treatment.

Respiratory 'Disease' revisited: In response to the ExecCAC's request 'to more fully characterize the respiratory infections' reported in the initial study report, the Sponsor performed a reanalysis of the pathology data. In the male mice, deaths during the first 50 weeks of the study were 2.9%, 5.7%, 11.4%, 18.6% and 34.3% in the 0, 10, 30, 100 and 200 mg/kg dose groups, respectively. During the final 54 weeks of the study the percent deaths by dose group calculated from the numbers of mice surviving at week 50 were 43%, 37%, 46%, 44% and 29%, respectively. Thus over the final 54 weeks of the study the mortality rate in the males administered solifenacin succinate approximated the mortality rate in the controls. After an initial review of the microscopic descriptions for individual male mice in the high dose group (200 mg/kg), it was their opinion that the description of "respiratory disease - present" may not have been correct. The diagnosis was not associated with a consistent or specific morphologic change in any organ or tissue but was based on signs of labored breathing, serous fluid in nasal passages and inflammatory cells in the nasal passages. (Note: Findings of serous fluid in nasal passages and inflammatory cells in the nasal passages were also observed in animals across groups, including controls, at terminal sacrifice.)

When Yamanouchi became aware of the unexpected "mortality" in the study, the company sent a representative to Europe to review what was occurring in the laboratory. It was their conclusion that the data suggested that some mice were experiencing respiratory distress secondary to

gaseous distention of the intestinal tract. In response to their observations at the laboratory, Yamanouchi requested that the laboratory assign additional technicians to the study to reduce the number of mice being dosed/technician to allow more time and care for the dosing procedure. This significantly reduced the incidence of clinical signs of respiratory distress and the subsequent sacrifice of the mice. Mice were not treated with any for of supportive or preventive medications.

**104-Week Carcinogenicity Study in F-344 Rats: Summary Tables of the Incidence of Neoplastic Lesions in All Study Dose Groups (0, 10, 30, 200 mg/kg/day)**

**Key study findings:** no drug-associated increase in neoplastic lesions

**Study number:** R905-TX-024 (20020924)  
**Volume #, and page #:** EDR (N000 BP, 8/29/03 and 9/22/03)  
**Conducting laboratory and location:**  
**Date of study initiation:** August 31, 1999  
**GLP compliance:** Yes  
**QA report:** Yes  
**Drug, lot #, and % purity:** YM905 Batch No. K9059804. —  
**CAC concurrence:** YES [See Appendix I]

STUDY DURATION: Std 2 year bioassay (104 weeks)  
 LABORATORY:  
 STUDY STARTING DATE: August 31, 1999  
 STUDY ENDING DATE: September 25, 2001  
 CARCINOGENICITY STUDY REPORT DATE: October 31, 2002  
 RAT STRAIN: CDF® (F-344) — BR rats —  
 ROUTE: oral gavage (5 ml/kg)  
 DOSING COMMENTS: standard oral study

NUMBER OF RATS:	<u>Main Study</u>	<u>Toxicokinetic Study</u>
- Control (C):	60/sex	-
- Low Dose (LD):	60/sex	12/sex
- Middle Dose (MD)	60/sex	12/sex
- High Dose (HD):	60/sex	12/sex

RAT DOSE LEVELS* (mg/kg/day):	<u>Males</u>	<u>Females</u>
- Low Dose:	3	3
- Middle Dose:	10	7.5
- High Dose:	20	15

**BASIS FOR DOSES SELECTED:** Doses were selected based on mortalities and adverse clinical behavior in the 26-week chronic toxicology study where rats were administered doses of 3, 10, 30, 60 or 100 mg/kg/day. For the female rats, the Exec CAC recommended doses of 3, 7.5 and 15 mg/kg/day based on mortalities at 30 and 60 mg/kg/day. For male rats, 20 mg/kg/day was selected as the high dose based on one death at 100 mg/kg and decreases in final body weight relative to control of 21% at 100• 75 mg/kg and 11% at 30 mg/kg. 3 mg/kg/day was considered the NOAEL in both sexes.

**PRIOR FDA DOSE CONCURRENCE (CAC):** Yes; July 27, 1999

**RAT CARCINOGENICITY: Negative**

**RAT TUMOR FINDINGS (details):** At the request of the ExccCAC, all tissues from low- and mid-dose animals were read by the same pathologist who read the original study slides. The Sponsor indicated that over 60% of the tissues were looked at in the original study report. The following is a comparison of the original report with those submitted after reading all slides for all groups. Any values that have changed have been highlighted in Tables R-1 and R-2 (comparisons of the tumor incidences in the original report to the amended full report).

There were no statistically significant (trend or pairwise) increases in tumor incidence or onset in drug-treated animals in any dose group when compared to concurrent controls. The most common neoplastic findings were pituitary adenomas in both sexes, benign interstitial cell tumor of the testes in all males, and large granular lymphocytic leukemia primarily in males but also in females across groups. There was also a high incidence of islet cell carcinoma in the pancreas of controls males, but not in any of the treatment groups.

**Table R-1: Rat Histopathology - group distribution of neoplastic findings for male rats in 2-year carcinogenicity study (R905-TX-024)**

<b>Males Rats (n=60/group)</b>	<b>Original Report (60%)</b>				<b>Amended Report (100%)</b>			
	<b>0</b>	<b>30</b>	<b>10</b>	<b>20</b>	<b>0</b>	<b>30</b>	<b>10</b>	<b>20</b>
<b>Adrenal Cortex:</b>								
Adenoma	1	0	0	0	1	0	0	0
Osteosarcoma	1	0	0	1	1	0	0	1
<b>Mesothelioma</b>	0	0	0	0	0	0	1	0
<b>Adrenal Medulla:</b>								
Pheochromocytoma	5	0	0	0	5	0	0	0
Complex Pheochromocytoma	0	1	0	0	0	1	0	0
Malignant Pheochromocytoma	1	0	0	1	1	0	0	1
<b>Bone (sternum):</b>								
<b>Osteosarcoma</b>	1	0	0	0	0	0	0	1
<b>Brain/spinal cord:</b>								
Multicentric glial neoplasm	0	1	0	0	0	1	0	0
<b>Epididymis:</b>								
<b>Mesothelioma</b>	3	0	0	1	3	0	2	1
<b>Harderian Glands:</b>								
Squamous cell carcinoma	0	0	0	1	0	0	0	1
<b>Heart:</b>								
Mesothelioma	0	0	1	0	0	0	1	0
<b>Hemopoietic:</b>								
Malignant Lymphoma	1	0	0	0	1	0	0	0
<b>Myeloid Leukemia</b>	21	21	21	16	21	25	23	16
<b>GI Tract:</b>								
Cecum - Mesothelioma	1	0	0	0	1	0	0	0
Colon - Mesothelioma					0	0	1	0
<b>Duodenum - Mesothelioma</b>	0	0	0	0	0	0	1	0
- Leiomyosarcoma	0	0	0	1	0	0	0	1
Stomach - Adenomatous polyp					0	0	1	0
<b>Kidneys:</b>								
<b>Mesothelioma</b>	1	0	0	0	1	0	1	0
<b>Papilloma - transitional cell</b>	0	0	0	0	0	1	0	0
<b>Liver:</b>								
<b>Hepatocellular adenoma</b>	4	1	2	5	4	4	3	5

Mesothelioma	1	0	1	0	1	0	1	0
<b>Lungs:</b>								
Mesothelioma	0	0	1	0	0	0	1	0
Osteosarcoma (metastatic)	1	0	0	1	-	-	-	-
<b>Mammary Gland:</b>								
Fibroadenoma	1	0	0	1	1	0	0	1
<b>Pancreas:</b>								
Mesothelioma	0	0	0	0	0	0	1	0
Adenoma - Islet cell	3	1	0	1	3	1	1	1
Carcinoma - Islet cell	1	0	0	0	1	0	0	0
<b>Pituitary:</b>								
Adenoma	24	22	15	14	24	28	20	14
Craniopharyngioma	0	0	0	0	0	1	0	0
<b>Prostate:</b>								
Mesothelioma	1	0	0	0	1	0	1	0
<b>Salivary Gland:</b>								
Sarcoma	0	0	2	1	0	0	2	1
<b>Seminal Vesicle:</b>								
Mesothelioma	1	0	0	0	1	0	0	0
<b>Skeletal Muscle:</b>								
Carcinoma - basal cell	0	1	0	0	0	1	0	0
<b>Skin:</b>								
Squamous cell papilloma	0	2	0	1	0	3	1	1
Basal cell adenoma	1	0	1	0	1	0	1	0
Keratoacanthoma	3	0	1	3	3	0	2	3
Trichoepithelioma	0	1	0	0	0	1	0	0
Preputial gland adenoma	0	1	0	0	0	1	0	0
Basal cell carcinoma	0	1	0	0	0	1	0	0
Squamous cell carcinoma	0	0	0	1	0	0	0	1
Fibrosarcoma	1	0	0	0	1	0	0	0
<b>Subcutaneous Tissue:</b>								
Fibroma	4	1	0	0	4	1	0	0
Leiomyoma	1	0	0	0	1	0	0	0
Fibrosarcoma	1	0	0	0	1	0	0	0
Myxosarcoma	1	0	0	1	1	0	0	1
Osteosarcoma	1	0	0	0	1	0	0	0
Sarcoma	0	0	0	1	0	0	0	1
Sarcoma (associated w/implant)	0	0	0	1	0	0	0	1
<b>Testes:</b>								
Interstitial (Leydig) cell adenoma	49	39	40	43	49	41	45	43
Mesothelioma	4	0	0	1	4	0	1	1
<b>Thyroids:</b>								
Follicular cell adenoma	1	0	0	0	1	0	0	0
Follicular cell carcinoma	1	0	1	0	1	1	1	0
C-cell adenoma	1	1	1	1	1	1	3	1
C-cell carcinoma	2	1	0	3	2	3	3	3
<b>Urinary bladder:</b>								
Mesothelioma	2	0	0	0	2	0	1	0
Carcinoma - transitional cell	1	0	0	0	1	0	0	0
<b>Zymbal's gland:</b>								
Adenoma	0	0	0	0	0	0	1	0
Carcinoma	0	2	0	0	0	2	0	0
<b>Hemangiosarcomas:</b>								
Mesenteric lymph node	1	0	0	0	1	0	0	0

**Table R-1: Rat Histopathology - group distribution of neoplastic findings for female rats in 2-year carcinogenicity study (R905-TX-024)**

<b>Females Rats (n=60/group)</b>	<b>Original Report (60%)</b>				<b>Amended Report (100%)</b>			
	<b>0</b>	<b>30</b>	<b>10</b>	<b>20</b>	<b>0</b>	<b>30</b>	<b>10</b>	<b>20</b>
<b>Adrenal Cortex:</b>								
<b>Adenoma</b>	1	0	0	1	1	0	1	1
<b>Adrenal Medulla:</b>								
<b>Pheochromocytoma</b>	1	0	0	0	1	1	0	0
<b>Malignant Pheochromocytoma</b>	2	0	0	0	2	0	0	0
<b>Brain/spinal cord:</b>								
Mixed glioma	1	0	0	0	1	0	0	0
<b>Cervix:</b>								
<b>Endometrial stromal polyp</b>	0	0	1	1	0	2	3	1
<b>Carcinoma</b>	0	1	0	0	0	1	0	0
<b>Leiomyosarcoma</b>	1	0	0	0	1	0	1	0
<b>Endometrial stromal sarcoma</b>	0	0	1	0	0	0	0	0
<b>Heart:</b>								
<b>Endocardial Schwannoma</b>	0	0	0	0	0	1	1	0
<b>Hemopoietic:</b>								
<b>Myeloid Leukemia</b>	9	13	8	8	9	13	11	8
<b>Kidneys:</b>								
<b>Papilloma - transitional cell</b>	1	0	0	0	1	0	0	0
<b>Carcinoma - transitional cell</b>	1	0	0	1	1	0	0	1
<b>Nephroblastoma</b>	0	0	0	0	0	0	1	0
<b>Liver:</b>								
<b>Hepatocellular adenoma</b>	0	0	0	0	0	1	0	0
<b>Mammary Gland:</b>								
<b>Fibroadenoma</b>	2	4	3	1	2	4	3	1
<b>Ovary:</b>								
<b>Carcinoma -invasive transitional cell</b>	1	0	0	0	1	0	0	0
<b>Pancreas:</b>								
<b>Adenoma - Islet cell</b>	1	1	0	0	1	1	0	0
<b>Pituitary:</b>								
<b>Adenoma</b>	22	16	18	17	22	19	23	17
<b>Carcinoma</b>	1	0	0	0	1	0	0	0
<b>Skin:</b>								
<b>Squamous cell papilloma</b>	0	0	1	1	0	0	1	1
<b>basal cell adenoma</b>	0	1	0	0	0	1	0	0
<b>Subcutaneous Tissue:</b>								
<b>Fibroma</b>	0	0	1	0	0	0	1	0
<b>Thyroids:</b>								
<b>Follicular cell adenoma</b>	0	0	0	0	0	0	1	0
<b>Follicular cell carcinoma</b>	2	0	0	1	2	0	0	1
<b>C-cell adenoma</b>	2	0	0	2	2	0	2	2
<b>C-cell carcinoma</b>	3	0	0	0	3	2	1	0
<b>Urinary bladder:</b>								
<b>Papilloma - transitional cell</b>	0	0	0	0	0	1	0	0
<b>Uterus:</b>								
<b>Endometrial stromal polyp</b>	8	5	7	4	8	7	6	4
<b>Endometrial stromal sarcoma</b>	0	1	0	1	0	0	0	1
<b>Zymbal's gland:</b>								
<b>Adenoma</b>	0	0	1	0	0	0	1	0

**RAT STUDY COMMENTS:**

- 1) Mean body weights were significantly lower for both the high-dose males and females over the course of the entire study.
- 2) SDA occurred at about 6 months into the study. There was a particularly high mortality rate in females dosed at 7.5 and 15 mg/kg between weeks 23 and 26, the same time SDA was showing up. Histologically, this infection presented differently according to where in the course of the disease a rat was at the time of death.

Additional information on SDA infections: Infections were self-limiting with no medical intervention.

Timeline	Events
1/28/00	announced that clinical signs consistent with exposure to SDAV were detected in rats housed in their surgical unit and infected rodents may have been shipped
2/18/00	reported that they had detected SDAV in rats housed in their facility in
2/21/00	No significant signs consistent with SDA was observed in rats in the Yamanouchi study (Study Week 22)
2/29/00	reported clinical signs consistent with SDA (swelling around the neck and decreased body weights) were detected in the Yamanouchi study (Week 23)
3/01/00	First deaths reported in Yamanouchi study (Week 24)
3/07/00	"Swelling around neck" disappeared in all rats in Yamanouchi study (Week 25)

EXECUTIVE CAC: Based on the additional information supplied, the Committee has concurred with the Division and concluded that both the rat and mouse studies are negative for carcinogenicity.

**APPEARS THIS WAY  
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APPENDIX 1

EXECUTIVE CARCINOGENICITY ASSESSMENT COMMITTEE MINUTES

Executive CAC

Date of Meeting: October 5, 2004

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair  
Joseph Contrera, Ph.D., HFD-901, Member  
Abby Jacobs, Ph.D., HFD-024, Member  
Tim McGovern, Ph.D., HFD-570, Alternate Member  
Lynnda Reid, Ph.D., HFD-580, Team Leader

Author of Draft: Lynnda Reid

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

NDA # 21-518

Drug Name: solifenacin succinate

Sponsor: Yamanouchi Pharma America, Inc.

Your Background: The original 2-year mouse and rat study results were presented to the ExecCAC on May 27, 2003. At that time, the committee felt that they could not concur with the reviewer on the absence of drug-related neoplasms in either species due to low survival and greater than 10% body weight differences between high-dose and control animals. The Sponsor was asked to perform a complete analysis of all tissues from the low- and mid-dose groups. The committee also wanted to see more information on infections, SDA (sialodacryoadenitis) in rats and 'respiratory infections' in mice, which appeared to correlate with the increased mortality observed during the first 12 months in both studies. Complete analysis of all animals in both the rat and mouse studies were subsequently performed and submitted for review.

Rat Carcinogenicity Study: Negative

At the request of the ExecCAC, all tissues from low- and mid-dose animals were read by the same pathologist who read the original study slides. The Sponsor indicated that over 60% of the tissues were looked at in the original study report.

RAT TUMOR FINDINGS (details): There were no statistically significant (trend or pairwise) increases in tumor incidence or onset in drug-treated animals in any dose group when compared to concurrent controls. The most common neoplastic findings were pituitary adenomas in both sexes, benign interstitial cell tumor of the testes in all males, and large granular lymphocytic leukemia primarily in males but also in females across groups. There

was also a high incidence of islet cell carcinoma in the pancreas of controls males, but not in any of the treatment groups.

SDA: On January 28, 2000, \_\_\_\_\_, the supplier of the rats used in this study, announced that clinical signs consistent with exposure to SDAV were detected in rats housed in their surgical unit and infected rodents may have been shipped. On February 29, 2000, Week 23 of the study \_\_\_\_\_ reported clinical signs consistent with SDA (swelling around the neck and decreased body weights) were detected in the Yamanouchi study. The first deaths occurred in the Yamanouchi study during week 23. Findings consistent with SDA, i.e., swelling around neck, disappeared in all rats in the Yamanouchi study by week 25.

#### Mouse Carcinogenicity Study: Negative

At the request of the ExecCAC, all tissues from low- and mid-dose animals were read by the same pathologist who read the original study slides. The Sponsor indicated that over 80% of the tissues were looked at in the original study report.

**MOUSE TUMOR FINDINGS (details):** Tumor incidence was unaffected by treatment and were of the types normally encountered CD-1 mice at \_\_\_\_\_. The final percentage rates for adrenal adenomas in male mice were 10, 18.6, 5.7, 11.4 and 2.9% for the 0, 10, 30, 100 and 200 mg/kg groups, respectively. These rates are higher than previously reported by Charles River, even for the control group (See Tables M-3 and M-4). However, there is no consistent dose-response pattern and is not statistically significant (Sponsor analyses).

Respiratory infections: After an initial review of the microscopic descriptions for individual male mice in the high dose group (200 mg/kg), it was the opinion that the description of "respiratory disease - present" may not have been correct. The diagnosis was not associated with a consistent or specific morphologic change in any organ or tissue but was based on signs of labored breathing, serous fluid in nasal passages and inflammatory cells in the nasal passages. (Note: Findings of serous fluid in nasal passages and inflammatory cells in the nasal passages were also observed in animals across groups, including controls, at terminal sacrifice.)

When Yamanouchi became aware of the unexpected "mortality" in the study, the company sent a representative to Europe to review what was occurring in the laboratory. It was their conclusion that the data suggested that some mice were experiencing respiratory distress secondary to gaseous distention of the intestinal tract. In response to their observations at the laboratory, Yamanouchi requested that the laboratory assign additional technicians to the study to reduce the number of mice being dosed/technician to allow more time and care for the dosing procedure. This significantly reduced the incidence of clinical signs of respiratory distress and the subsequent sacrifice of the mice. Mice were not treated with any form of supportive or preventive medications.

#### Executive CAC Recommendations and Conclusions:

Rats:

- The Committee previously concluded that the study was adequate.
- Based on the additional information supplied, the Committee has concluded that the study is negative for carcinogenicity.

Mice:

- The Committee previously concluded that the study was adequate.
- Based on the additional information supplied, the Committee has concluded that the study is negative for carcinogenicity.

David Jacobson-Kram, Ph.D.  
Chair, Executive CAC

cc:\  
NDA 21-518/Division File, HFD-580  
Reidl/Team leader, HFD-580  
Makiej/CSO/PM, HFD-580  
/ASeifried, HFD-024

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

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Lynnda Reid  
10/13/04 03:18:32 PM  
PHARMACOLOGIST

To: Florence Houn  
Director ODE III

From: John Leighton  
Associate Director for Pharmacology/Toxicology, ODE III

Subject: Solifenacin succinate (Vesicare)  
NDA 21-518

Date: October 17, 2003

#### Introduction

— is an anti-muscarinic compound developed for the treatment of overactive bladder. A complete set of pharmacology and toxicology studies were submitted and reviewed for the NDA.

#### Comments

The Division's pharmacology/toxicology team has adequately addressed the issues related to the approvability of solifenacin succinate. I concur with the Division's recommendation. Labeling issues remain to be addressed.

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/s/

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John Leighton  
10/17/03 09:14:23 AM  
PHARMACOLOGIST

**PHARMACOLOGY/TOXICOLOGY COVER SHEET**

NDA number: 21-518

Review number: 1

Sequence number/date/type of submission: N000 dated December 19, 2002

Information to sponsor: Yes ( ) No ( x )

Sponsor and/or agent: Yamanouchi Pharma America, Inc., Paramus, NJ

Manufacturer for drug substance: Yamanouchi Pharmaceutical Co., Ltd., Japan

Reviewer name: Lynnda Reid

Division name: Division of Reproductive and Urologic Drug Products

HFD #: 580

Review completion date: September 11, 2003

**Drug:**

Trade name: VESICARE™

Generic name (list alphabetically): solifenacin succinate

Code name: YM905

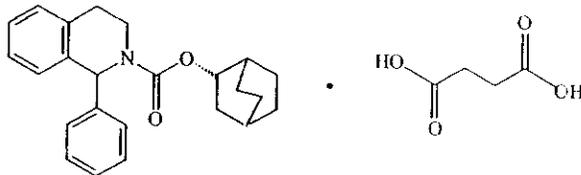
Chemical name: (+)-(1S,3'R)-3'-quinuclidin-3'-yl 1-phenyl-1,2,3,4-tetrahydro-2-isoquinoline-2-carboxylate monosuccinate

CAS registry number: ?

Molecular formula: C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>·C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>

Molecular weight: 480.56

Structure: YM905 contains two chiral centers, therefore enantiomeric and diastereomeric isomers are possible.



Relevant INDs/NDAs/DMFs: IND 58,135

Drug class: muscarinic M3 receptor antagonist

Indication: Urinary urge incontinence

Clinical formulation: Tablets: 2.5 mg, 5 mg and 10 mg

Route of administration: Oral

Proposed use: Clinical studies were performed at either 5-mg or 10-mg solifenacin succinate once a day. The recommended starting dose of VESICARE ® is 5 mg daily. The dose may be increased to 10 mg daily if needed. For patients with moderate or severe liver or severe kidney disease, the

maximum recommended dose of VESICARE ® is 5 mg daily. VESICARE ® should be taken once a day with liquids and swallowed whole. It can be taken with or without food.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: Solifenacin (YM905) is a new molecular entity shown to have antagonistic action on muscarinic M3 receptors. Under IND 58,135, opened on April 2, 1999, it was being investigated for the treatment of \_\_\_\_\_ During the EOP2 meeting held on September 19, 2000, the Sponsor indicated that the proposed indication for Phase III trials would be for the \_\_\_\_\_

\_\_\_\_\_ A Pre-NDA meeting was held on July 1, 2002

Studies reviewed within this submission:

Pharmacology:

- 1) primary pharmacology
  - effects on bladder volume and pressure in anaesthetized rats
  - effects on carbachol-induced bladder contractions and salivary secretion in mice
  - effects of distension-evoked visceral sensory response in the anaesthetized rat
- 2) secondary pharmacology
- 3) selectivity as assessed in receptor-binding studies

Safety Pharmacology:

- 4) general safety pharmacology of YM-64250
  - central nervous system
  - respiratory and circulatory system
  - digestive system
  - urinary excretion
  - local anaesthetic effects
- 5) effect of solifenacin and metabolites on dog isolated cardiac Purkinje fibers
- 6) effect of solifenacin, its metabolites, and other antimuscarinics on the human HERG channel
- 7) affinity of solifenacin and metabolites for various neurotransmitter-related receptors and ion channels
- 8) pharmacodynamic drug interactions

General Toxicology:

- 9) 26-week oral toxicity studies in mice
- 10) 26-week oral gavage study in F344 rats with a 10 week recovery period
- 11) 4-week oral toxicity study in beagle dogs
- 12) 13-week oral toxicity study in beagle dogs
- 13) 52-week oral toxicity studies in beagle dogs
- 14) 14-day intravenous toxicity study in beagle dogs

Carcinogenicity:

- 15) 2-year bioassay in mice

16) 2-year bioassay in rats

Reproductive and Developmental Toxicology:

- 17) fertility and early embryonic development to implantation in mice
- 18) embryo-fetal development in mice
- 19) investigation of fetal cleft palate in mice
- 20) prenatal and postnatal development in mice
- 21) fertility and early embryonic development to implantation in male rats
- 22) fertility and early embryonic development to implantation in male rats
- 23) embryo-fetal development in rats
- 24) embryo-fetal development in rabbits
- 25) toxicokinetic study in pregnant rabbits

Studies not reviewed within this submission: The following studies were reviewed under IND 58,135. Any relevant information from these studies has been incorporated in to the appropriate summary sections of this NDA.

ADME:

- 1) *in vitro* metabolic profiles for various species
- 2) quantitative analysis of metabolites in urine and bile
- 3) <sup>14</sup>C-YM905 pharmacokinetic studies in mice, rats and dogs
- 4) tissue distribution studies in mice and rats including whole body radiography
- 5) quantitative tissue distribution studies in mice and rats
- 6) enzyme induction after repeat-dosing in rats
- 7) single-dose i.v. studies in rats and dogs
- 8) PK study in rats and dogs following i.v. administration
- 9) 8-day repeat-dose followed by 1 week washout period in dogs
- 10) 15-day repeat-dose oral studies in mice and dogs
- 11) effect of food on absorption in dogs

General Toxicology:

- 12) 4-days i.v. studies in mice
- 13) 2-week i.v. studies in mice
- 14) 2-week oral toxicity studies in mice
- 15) 13-week oral toxicity studies in mice
- 16) acute oral study in rats
- 17) 4-week oral toxicity study in rats
- 18) acute oral study in dogs
- 19) acute i.v. study in dogs

Genetic Toxicology:

- 20) *in vitro* reverse mutation test in bacteria
- 21) *in vitro* chromosome aberration test in mammalian cells
- 22) *in vivo* micronucleus test in rats

Reproductive and Developmental Toxicology:

- 23) embryo-fetal development in mice (oral dose range-finding study)

- 24) embryo-fetal development in rats (oral dose range-finding study)
- 25) embryo-fetal development in rabbits (oral dose range-finding study)

Special Toxicology Studies:

- 26) local tolerance
- 27) intravenous, perivenous and intraarterial tolerance study in rabbits
- 28) skin irritation study in rabbits
- 29) eye irritation study in rabbits
- 30) delayed type skin reaction study in guinea pigs
- 31) hemolytic study

**APPEARS THIS WAY  
ON ORIGINAL**

*Executive Summary*

I. Recommendations

A. Recommendation on Approvability: Approval

B. Recommendation for Nonclinical Studies: Final analysis of mouse and rat carcinogenicity studies has been submitted electronically for review and statistical analysis. New data will be presented to the ExecCAC as soon as the statistical review is completed. Revised labeling will be recommended if needed following ExecCAC review.

C. Recommendations on Labeling: revised labeling for the following sections:

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7

                     Draft Labeling Page(s) Withheld

## II. Summary of Nonclinical Findings

**Brief Overview of Nonclinical Findings:** Animal deaths observed following administration of solifenacin succinate were usually accompanied by signs of CNS toxicity, i.e., underactivity, ataxia, tremors, convulsions, prostration, hunched posture, piloerection and abnormal respiration. The most common clinical signs were mydriasis and salivation. These findings are consistent with class effects associated with other muscarinic receptor antagonists.

ECG effects consisting of prolongation of P-wave, PR interval, QRS duration and QT interval were observed in dogs. However, QT interval values were within the normal range, accompanied by decreases in heart rate between 4 and 8 beats/minute, and C<sub>max</sub> values were 24 to 87-fold higher than at the proposed therapeutic dose of 10 mg/day in humans. Similar effects have been observed with other muscarinic 3M receptor antagonists, e.g., dofetilide, tolterodine, darifenacin and oxybutynin.

Solifenacin succinate was not mutagenic or genotoxic in the standard battery of *in vitro* and *in vivo* assays. Preliminary results do not show any increase in tumor incidences following administration of solifenacin succinate for two years in mice and rats.

There were no effects on fertility or early reproductive parameters following administration of solifenacin succinate to male and female mice or rats. There were no teratogenic effects observed in rats or rabbits. However, administration of solifenacin succinate to mice during the period of major organogenesis resulted in an increased incidence of cleft palate at doses that resulted in decreased maternal weight gain. Reduced fetal and pup weights, and delayed development were also observed in F1 generation mice. No reproductive or developmental effects were observed at doses approximately 2 fold that of the maximum recommended human dose (MRHD) in mice and rabbits and at the highest tolerated dose in rats (<1 the MRHD).

Solifenacin succinate caused minor skin irritation and severe and potentially irreversible ocular mucosal damage in rabbits. It was not antigenic in the guinea pig.

Pharmacologic Activity: M3 muscarinic antagonist

### Nonclinical Safety Issues Relevant to Clinical Use:

- 1) Solifenacin succinate has been shown to potentially reduce heart rate and induce prolongation of the P-wave and PR interval and the QRS duration and QT interval. *In vitro*, solifenacin succinate was shown to inhibit the HERG potassium current at a concentration of 0.27  $\mu$ M.
- 2) A relationship between cleft palate in mice and *in utero* exposures to solifenacin succinate could not be ruled out.
- 3) *In utero* and lactational exposures resulted in reduced fetal and pre-weaning pup weights, peripartum and postpartum mortalities, and delayed development.
- 4) Severe and seemingly irreversible ocular mucosal damage, especially opacity and edema to the cornea and falling of the nictating membrane was observed in rabbits with 10 and 100 mg/eye (unrinsed). Rinsing appeared to ameliorate the effect. Effects were reversible over time at 1 mg (unrinsed) and following rinsing 10-30 seconds after instillation at higher concentrations.

The Sponsor is conducting a clinical QT study to evaluate the clinical relevance of drug-related cardiovascular effects observed in dogs. Potential drug related reproductive and developmental effects will be discussed in the label with a recommended pregnancy category of C.

III. Administrative

A. Reviewer signature: \_\_\_\_\_

B. Supervisor signature:      Concurrence - \_\_\_\_\_

Non-Concurrence - \_\_\_\_\_

(see memo attached)

C. cc: list:

NDA 21-518  
HFD-580  
HFD-580/Pharm/Reid  
HFD-580/Pharm/Thornton  
HFD-580/CSO/King  
HFD-580/MO/Guodong

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**PHARMACOLOGY/TOXICOLOGY REVIEW****I. PHARMACOLOGY:**

Primary pharmacodynamics: Solifenacin succinate (here after referred to as solifenacin or YM905) is a muscarinic antagonist with high affinity for M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> receptor subtypes. The sponsor interprets this as "M<sub>3</sub>-selective", but these affinities are all relatively similar to each other. The active metabolite of YM905 (BY-348C) has a similar binding profile with somewhat less affinity for each subtype than the parent compound.

Compound	Muscarinic Receptor Affinity (nM)		
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
YM905	2.2	6.4	1.4
BY-348C	55	390	21

Mechanism of action: inhibition of smooth muscle of bladder via M<sub>3</sub> receptor antagonism

Drug activity related to proposed indication: Antimuscarinic drugs are used for the treatment of urinary disorders due to their ability to inhibit contraction of smooth muscle in the urinary bladder.

Secondary pharmacodynamics: Inhibition of muscarinic M<sub>3</sub> receptors also reduces salivary secretion and contraction of intestinal and ciliary smooth muscles.

**II. SAFETY PHARMACOLOGY:**

**Neurological effects:** The affinity of solifenacin for various neurotransmitter-related receptors and ion channels was evaluated in the radioligand binding assay. Solifenacin (10 µM) did not show inhibitory effects • 50% on radioligand binding to the following receptors and ion channels: nicotine (ganglionic and neuronal sites), histamine (H<sub>1</sub> and H<sub>2</sub>), adrenaline (alpha<sub>1</sub>, alpha<sub>2</sub> and beta), dopamine, serotonin, opioid, benzodiazepine, clozapine and purine receptors, and calcium channels (type L and N), potassium channels [adenosine triphosphate (ATP)-sensitive, voltage-insensitive and sensitive] and sodium channel site 1. Solifenacin showed an inhibitory effect of approximately 50% on histamine H<sub>3</sub>, and inhibitory effects of 80% or greater on the sodium channel site 2 and the sigma receptors.

**Cardiovascular effects:** In electrophysiological studies, solifenacin did not affect action potential durations in *in vitro* models of dog Purkinje fibers and papillary muscles from guinea pig myocardium but inhibited the HERG potassium current, with an IC<sub>50</sub> value of 0.27 µM.

In repeat nonclinical toxicology studies in dogs, oral administration of solifenacin was associated with ECG changes, e.g., A-V block, prolonged P-wave and prolonged PR, QRS and QT intervals.

**Effect on potassium current in cells expressing the HERG channel** (Table 1): The effect of solifenacin on potassium current in Chinese hamster ovary (CHO) cells stably expressing the HERG

channel was assessed using the whole-cell patch clamp technique. Solifenacin inhibited the HERG potassium current at an IC<sub>50</sub> value of 0.27  $\mu\text{M}$ . In the same study, dofetilide, a representative IKr blocker, tolterodine and DD 01, an active metabolite of tolterodine, inhibited the HERG potassium current with IC<sub>50</sub> values of 0.025, 0.0089 and 0.31  $\mu\text{M}$ , respectively. When comparing IC<sub>50</sub> values, the inhibitory potency of solifenacin on the HERG current was about 1/11 and 1/30-fold that of dofetilide and tolterodine, respectively, and was equipotent to that of DD 01, indicating that solifenacin is less potent than dofetilide and tolterodine, and is similar in potency to DD 01 (Figure 1).

The effect of solifenacin, oxybutynin, tolterodine, DD 01, darifenacin and dofetilide on the HERG channel was evaluated in another functional model, the <sup>86</sup>Rb efflux assay. All the drugs tested showed a concentration-dependent inhibitory effect on the HERG channel and the rank order, according to the IC<sub>50</sub> values, was dofetilide = tolterodine > solifenacin darifenacin = DD 01 > oxybutynin (Figure 2). Compared with IC<sub>50</sub> values of the antimuscarinics tested, the sensitivity of <sup>86</sup>Rb efflux assay was 4.8- to 16-fold lower than that of the patch clamp assay. However, the rank order of potency deduced by the <sup>86</sup>Rb efflux assay was almost identical to that derived from the patch clamp assay, supporting the HERG data.

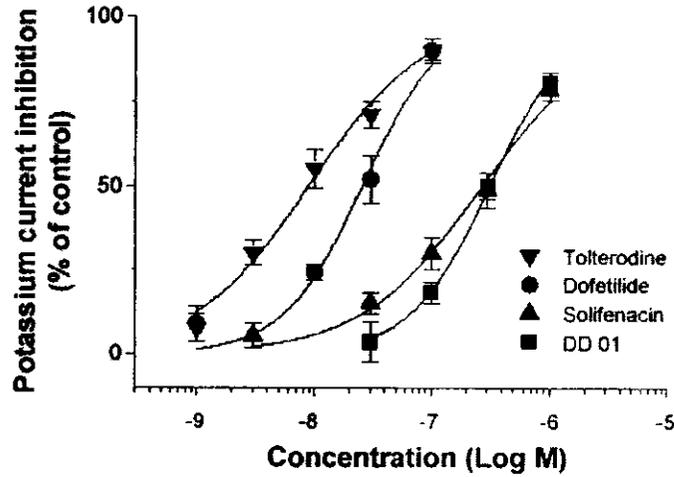
Table 1: Inhibitory effects of solifenacin, dofetilide and various antimuscarinics on potassium current and <sup>86</sup>Rb efflux in CHO cells expressing the HERG potassium channel

Compounds	Potassium current		<sup>86</sup> Rb efflux		Ratio [R/K]
	IC <sub>50</sub> values ( $\mu\text{M}$ ) [K]	Relative potency	IC <sub>50</sub> values ( $\mu\text{M}$ ) [R]	Relative potency	
Dofetilide	0.025	11	0.12	18	4.8
Tolterodine	0.0089	30	0.14	15	16
Solifenacin	0.27	1.0	2.1	1.0	7.8
Darifenacin	-	-	4.7	0.45	-
DD 01	0.31	0.87	4.9	0.43	16
Oxybutynin	-	-	29	0.072	-

IC<sub>50</sub> values of potassium current study were estimated by the non-linear regression analysis from 5 independent determinations and those of <sup>86</sup>Rb efflux assay were from 4 independent determinations

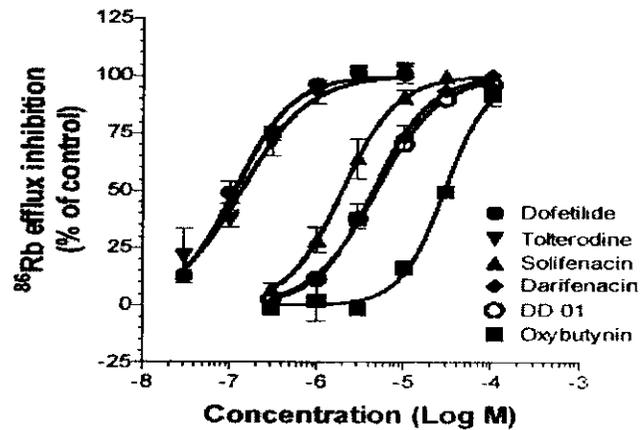
**Effect on action potential parameters in dog Purkinje fibers and guinea pig papillary muscles:** The effect of solifenacin (0.003, 0.03 and 0.3  $\mu\text{M}$ ) on intracellularly recorded action potential parameters was evaluated in the isolated dog Purkinje fiber preparation electrically paced at 1 and 0.5 Hz. Under the conditions of this assay, *in vitro* exposure to solifenacin at concentrations of 0.003, 0.03 and 0.3  $\mu\text{M}$  had no effect on resting membrane potential, upstroke amplitude, and maximum rate of depolarization or action potential duration. Similarly, in papillary muscles isolated from guinea pig myocardium, solifenacin had no significant effects on the action potential parameters at the same concentration range as in the dog Purkinje fibers study.

Figure 1: Inhibitory effect of solifenacin, dofetilide, tolterodine and DD 01 on potassium currents in CHO cells stably expressing the HERG potassium channel.



Inhibitory effects were evaluated from the tail current and each point represents the mean + SEM of 5 independent determinations.

Figure 2: Inhibitory effect of solifenacin, dofetilide and other antimuscarinics on <sup>86</sup>Rb efflux from CHO cells stably expressing the HERG potassium channel.



Each point represents the mean + SEM of 4 independent determinations

**Cardio/Pulmonary effects:** In pentobarbital-anesthetized dogs, solifenacin succinate administered i.v. at doses up to 0.3 mg/kg did not affect respiration rate, blood pressure, left ventricular pressure, heart rate, maximum rise rate of left ventricular pressure (max dp/dt), common carotid arterial blood flow, femoral arterial blood flow or ECG recordings (lead II). Solifenacin succinate increased respiration rate, decreased blood pressure and left ventricular pressure and prolonged PR interval at doses of 1 mg/kg, and decreased common carotid arterial blood flow at dose of 3 mg/kg. At a dose of 10 mg/kg, complete atrioventricular block was observed in 4 of 5 animals, resulting in the death of one of the 4 animals.

Similarly, i.v. administration of oxybutynin at doses up to 1 mg/kg did not affect respiratory and cardiovascular parameters in pentobarbital-anesthetized dogs. Oxybutynin increased respiration rate and decreased blood pressure, left ventricular pressure, common carotid arterial blood flow and femoral arterial blood flow at doses of 3 and 10 mg/kg iv, and decreased max dp/dt, prolonged PR interval and shortened QRS interval at a dose of 10 mg/kg iv. At a dose of 30 mg/kg iv, 3 of 5 animals died of respiratory arrest.

**Renal effects:** Solifenacin had no effects on urine volume or electrolyte excretion in rats.

**Gastrointestinal effects:** In isolated guinea pig ileum, solifenacin did not inhibit histamine- and BaCl<sub>2</sub>-induced contractions but inhibited contractions induced by acetylcholine and serotonin, which are considered to be related to the muscarinic receptor antagonistic effect of solifenacin. Solifenacin showed emetogenicity in dogs, but had neither an effect on gastrointestinal transit in mice nor an irritative action on the gastric mucosa of rats.

**Abuse liability:** not addressed

**Other:**

- Intradermal administration of solifenacin produced infiltration anesthesia in guinea pigs (similar to effects produced by oxybutynin and lidocaine).
- Solifenacin induced mydriasis in mice and a tendency to induce mydriasis in rabbits, an effect mediated by muscarinic M<sub>3</sub> receptor antagonism.
- Instillation of solifenacin in rabbit eyes showed no significant changes in the intraocular pressure.

**Safety pharmacology conclusions:** The pharmacologic profile for solifenacin was similar to that of other antimuscarinic drugs, e.g., oxybutynin, tolterodine and darifenacin.

Solifenacin induced effects have been observed on the cardiovascular system in *in vitro* assays and *in vivo* toxicology studies. In the HERG assay, solifenacin inhibited the potassium current at an IC<sub>50</sub> value of 0.27 μM. However, at a similar concentration, there was no effect on resting membrane potential, upstroke amplitude, and maximum rate of depolarization or action potential duration in isolated dog Purkinje fiber and guinea pig myocardium papillary muscle preparations.

In pentobarbital-anesthetized dogs, a single i.v. dose of 1 mg/kg solifenacin succinate increased respiration rate, decreased blood pressure and left ventricular pressure, and prolonged PR interval. At 3 mg/kg common carotid arterial blood flow was decreased, and at 10 mg/kg complete atrioventricular block was observed in 4 of 5 animals and one of the 4 animals died. The NOAEL following a single i.v. dose in anesthetized dogs was 0.3 mg/kg.

*Note: In vivo*, repeat nonclinical toxicology studies in dogs, oral administration of solifenacin was associated with ECG changes consistent with reduced heart rates, e.g., A-V block, prolonged P-wave and prolonged PR, QRS and QT intervals.

The maximum total plasma concentration of solifenacin achieved at the maximum recommended human dose (MRHD) of 10 mg/day at steady state was 62.9 ng/ml. The plasma protein binding of solifenacin in humans is 98% *in vivo*, thus the unbound plasma concentration ( $C_{max,u}$ ) is estimated to be about 1.26 ng/ml. On the other hand, solifenacin inhibited the HERG potassium current, with an  $IC_{50}$  value of 0.27  $\mu$ M, and did not affect action potential durations at the highest concentration of 0.3  $\mu$ M. These concentrations correspond to free, non-protein bound plasma concentration of solifenacin of approximately 97.9 and 109 ng/ml, respectively. The concentration ratios between 0.27  $\mu$ M and 0.3  $\mu$ M and mean steady state  $C_{max,u}$  values in humans at the MRHD of 10 mg are 78 and 87, respectively.

### III. PHARMACOKINETICS/TOXICOKINETICS:

**PK parameters:** The dose-dependence of the *in vivo* pharmacokinetics varies between species.  $C_{max}$  and AUC values for solifenacin in mice (3-30 mg/kg), rats (3-30 mg/kg) and dogs (2-10 mg/kg) after a single oral dose of solifenacin succinate increase more than proportionally with increasing doses, especially in dogs, indicative of non-linear pharmacokinetics. The nonlinear pharmacokinetics in animals may be due to saturation of either hepatic or intestinal first-pass metabolism, or to saturation of the elimination processes. In humans, plasma concentrations approach linearity over the range of doses between 5 and 100 mg.

**Mice:** After repeated oral administration of 100 mg/kg of YM905 to male mice for 15 days, plasma concentrations of both the unchanged drug and the metabolite YM-64250 (corresponding to the major metabolite peak in humans) were at steady-state on day 8. Plasma concentrations of YM-64250 were detectable within 15 minutes of dosing and were comparable with those of the unchanged drug on days 1, 8 and 15.

PK Table 1: Plasma concentrations of YM905 and major metabolite in male mice following repeat dosing with 100 mg/kg/day YM905.

Analyte	Day	$C_{max}$ (ng/ml)	$T_{max}$ (hr)	$AUC_{0-24}$ (ng.hr/ml)	$t_{1/2}$ (h)
YM905 (free base)	1	1509 $\pm$ 696	0.25	2879.2	1.9
	8	1003 $\pm$ 322	0.25	2154.1	1.7
	15	968 $\pm$ 133	0.25-0.5	2708.2	2.1
M2 (YM-64250)	1	1561 $\pm$ 530	0.25-0.5	3803.8	1.7
	8	1267 $\pm$ 265	0.25-0.5	2430.0	1.3
	15	1516 $\pm$ 168	0.5	3176.4	1.5

In mice and dogs, steady state plasma concentrations were reached by day 8 of dosing. In contrast, accumulation ratios in rats, determined from  $C_{max}$  and AUC data collected between day 1 and 28 of

dosing, increased with increasing dose. At 50 mg/kg, the accumulation ratio was 1.3 in males, in contrast to an accumulation ratio of 5.52 in females.

PK Table 2: Plasma concentrations of YM905 in dogs following repeat dosing with 30 mg/kg/day YM905.

Analyte	Day	Males			Females		
		Cmax (ng/ml)	Tmax (h)	AUC <sub>0-24</sub> (ng.hr/ml)	Cmax (ng/ml)	Tmax (h)	AUC <sub>0-24</sub> (ng.hr/ml)
YM905 (Free base)	1						
	29		2				

PK Table 3: Mean plasma concentrations of YM905 and BY-348C (M1) in rats following repeat dosing with 50 mg/kg/day YM905.

Analyte	Day	Males		Females	
		Cmax (ng/ml)	AUC <sub>0-24</sub> (ng.hr/ml)	Cmax (ng/ml)	AUC <sub>0-24</sub> (ng.hr/ml)
YM905 (Free base)	1	28.82	212	42.83	345
	28	27.10	275	226.84	1904
BY-348C	1	623.07	5690	870.27	10.637
	28	448.56	5758	873.99	12,975

**Absorption:** Solifenacin succinate was readily absorbed following oral administration: 82.9% in rats, 84.7% in dogs and 88.0% in humans. However, absolute bioavailability was low due to high hepatic first-pass metabolism: 13.3-31.4% in mice (3-30 mg/kg), 0.2-1.8% in rats (3-30 mg/kg) and 3.0-25.1% in dogs (2-10 mg/kg).

In dogs, oral administration of solifenacin (5 mg/kg) with food resulted in a 50% decrease in Cmax and AUC<sub>0-∞</sub> values compared to those under fasted conditions. It appeared that the presence of food decreases the extent of absorption as well as the rate of absorption.

An *in situ* study in rats was performed using <sup>14</sup>C-solifenacin succinate to determine the site of absorption in the gastrointestinal tract for solifenacin. The extent of absorption of radioactivity was highest from the ileum (90.8%), followed by the colon (85.4%), the jejunum (67.7%) and the duodenum (59.6%) with minimal absorption of radioactivity from the stomach (0.3%). These results indicate that solifenacin absorption would be expected to occur throughout the digestive tract with maximal absorption occurring in the ileum and little if any absorption occurring in the stomach.

**Distribution:** In all species studied, solifenacin was extensively protein-bound over plasma concentrations ranging between — μg/ml. The fraction bound varied between species (91.2% to 93.7% for mice, 84.3% to 91.2% for rats, 86.2% to 88.9% for dogs) and is highest in human plasma (92.7% to 96.1% *in vitro* and approximately 98% *in vivo*). The extent of binding decreases as the plasma concentration increases, especially in rats. The main binding protein in human plasma is α1-acid glycoprotein.

After intravenous administration of solifenacin to mice, rats and dogs, the volume of distribution was high (6.8, 6.7 and 10.1 L/kg, respectively). Administration of  $^{14}\text{C}$ -solifenacin in mice and rats demonstrated rapid distribution to all tissues. Distribution was highest in the liver and kidney and lowest in the brain. Clearance from eyes in mice and testes in rats was slower than for other tissues. In the eyes of pigmented mice  $C_{\text{max}}$  values occurred at 4 hours and decreased slowly ( $t_{1/2} = 134$  hours). *In vitro* studies demonstrated that solifenacin was highly, but reversibly, bound to melanin (bound fraction = 37.5 to 73.2%). In rats, clearance from the testes was slower than for other tissues, with 85% of the radioactivity present at 24 hours. The levels of radioactivity in the brains of mice and rats were much lower than the plasma levels.

In pregnant mice, solifenacin crosses the placenta and enters the fetus. Radioactivity was detected in the milk of the lactating mouse, in some neonatal tissues, and in clotted milk in the neonatal stomach.

The maximum total plasma concentration of solifenacin achieved at the maximum recommended human dose (MRHD) of 10 mg/day at steady state was 62.9 ng/ml. The plasma protein binding of solifenacin in humans is 98% *in vivo*, the unbound plasma concentration ( $C_{\text{max,u}}$ ) is estimated to be about 1.26 ng/ml.

**Metabolism:** The major metabolite (M2) produced by human microsomes from solifenacin is also produced by microsomes from all laboratory species tested, though to a lesser extent in rats. M1, an active major metabolite in rats and rabbits was not detected *in vitro* or *in vivo* in humans.

- M1 - BY-348C, YM-80268 (specific to rats and rabbits)
- M2 - *N*-oxide of quinuclidine ring of solifenacin (YM-64250)
- M3 - 4*R*-hydroxy solifenacin (YM-80264)
- M4 - *N*-oxide of 4*R*-hydroxy solifenacin (YM-270293)
- M5 - *N*-glucuronide of quinuclidine ring of solifenacin (YM-277743)

Rat M1 showed about 2- to 3-fold lower affinity for the muscarinic receptors than solifenacin and similar subtype selectivity to solifenacin, with  $\text{pK}_i$  values of 55 nM for the muscarinic  $M_1$  receptor, 390 nM for the muscarinic  $M_2$  receptor and 21 nM for muscarinic  $M_3$  receptor. Three additional metabolic products were observed only in dogs: M4-*O*-glucuronide, M9 (YM-211089) and M10 (YM-211582).

Metabolites M1 and M3 demonstrated anti-muscarinic properties with a 2-3 fold lower binding affinity for the rat and human muscarinic  $M_3$  receptors, respectively. Metabolites M2, M4 and M5 showed no binding affinity for muscarinic  $M_3$  receptors *in vitro* at concentrations up to 10  $\mu\text{M}$ .

The following table provides a comparison of drug and metabolite concentrations ( $C_{\text{max}}$ ) and systemic exposures ( $\text{AUC}_{0-24}$ ) at steady state levels. Both the mouse and dog concentrations represent the NOAEL dose.

Species	Mouse	Dog	Human
Dose	100 mg/kg - 15 days	12 mg/kg - 15 days	10 mg - 14 days

Analyte	C <sub>max</sub> (ng/ml)	AUC <sub>0-24</sub> (ng.hr/ml)	C <sub>max</sub> (ng/ml)	AUC <sub>0-24</sub> (ng.hr/ml)	C <sub>max</sub> (ng/ml)	AUC <sub>0-24</sub> (ng.hr/ml)
YM905	967.7	2708	186.7	1418	63.9	1214
M2	1516	3176	406.7	2345	12.7	239
M3	-	-	11.4	183	4.7	95
M4	-	-	62.9	437	6.2	121
M5	-	-	3.9	15	1.7	29

Metabolism is slow in human microsomes when compared to metabolism by microsomes from the mouse, rat, hamster, dog and monkey. The slow rate of *in vitro* metabolism by human microsomes is consistent with the drug's long half-life (~50 hours) *in vivo*.

Note: *In vitro*, the human metabolite M3 showed slight (about 10%), non-significant inhibition of the HERG potassium current at a concentration of 0.3  $\mu$ M and inhibited the <sup>86</sup>Rb efflux in a concentration-dependent manner, with an IC<sub>50</sub> value of 23  $\mu$ M. In rats, M3 did not affect cardiovascular and respiratory parameters evaluated following a single i.v. dose at 0.1 mg/kg.

**Excretion:** Following oral administration of <sup>14</sup>C-solifenacin to mice, rats and dogs, 58.0%, 74.7% and 81.7%, respectively, of the total administered radioactivity was excreted in the feces, and 37.0%, 23.6% and 8.6%, respectively, was excreted in the urine by 168 hours post-dosing. Across the three species, the primary route of excretion was fecal, probably via biliary excretion. However, in humans urinary excretion is the principal route of elimination.

A decrease in clearance with repeated oral dosing was observed in male and female dogs and female rats but not in male rats, mice or humans.

**PK/TK summary:** *In vitro* metabolism and *in vivo* pharmacokinetic studies demonstrated qualitatively, but not quantitatively similar metabolic patterns in rats, mice, dogs and humans. The formation of the metabolite M1 was specific to rats and rabbits. Metabolites M1 and M3 demonstrated anti-muscarinic properties with a 2-3 fold lower binding affinity for the rat and human muscarinic M<sub>3</sub> receptors, respectively.

**PK/TK conclusions:** Based on ADME parameters, the mouse, rat and dog represent appropriate species for conducting nonclinical safety assessments for solifenacin succinate. The major human metabolites were found in these models and were adequately tested.

**IV. GENERAL TOXICOLOGY:****YM905: 26-Week Repeated Oral Dose Toxicity Study of YM905 in Mice**

**Key study findings:** NOAEL = 30 mg/kg/day. The most sensitive tissues in mice appear to be the GI tract, liver and kidney. Erosion of the mucosa and inflammation of the lower GI tract, with the most sensitive area being the duodenum, was observed in males and females treated at 100 and 200 mg/kg/day.

**Study no:** R905-TX-026

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** —

**Date of study initiation:** July 15, 1999

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, radiolabel, and % purity:** K9059804, —, 100% pure

**Formulation/vehicle:** 0.5% aqueous methylcellulose

**Methods:** Animals were treated once daily by oral gavage for 26 weeks.

**Dosing:**

Species/strain: —: CD-1(ICR) mice (SPF) —

#/sex/group or time point (main study): 15/sex/group

Satellite groups used for toxicokinetics: 20/sex/UM905 group

Age: ~6 weeks

Weight: males - 27.1-32.8 g; females - 19.6-25.7 g

Doses in administered units: 0, 10, 30, 100 and 200 mg/kg/day

Route, form, volume, and infusion rate: oral gavage, 0.1 ml/10 g body weight

**Observations and times:**

Clinical signs: before dosing and 1 and 5 hours after dosing

Body weights: weekly

Food consumption: weekly

Ophthalmoscopy: weeks -1, 13 and 26

Clinical Pathology: Clinical Pathology: Blood samples were collected from the abdominal aorta for routine hematology and clinical chemistry evaluations on fasted, anesthetized animals the day after the final dosing.

Gross pathology: A complete necropsy was performed on all main study that died prematurely or at scheduled termination.

Organs weighed: adrenals, brain, heart, kidneys, liver, lungs, mandibular and submandibular glands, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thymus, thyroid, uterus

Histopathology: All tissues were preserved, however, only the following tissues were examined microscopically:

- all gross lesions
- all animals in the control and high-dose groups
- organs and tissues in lower dose groups when abnormalities were detected in the highest dose group

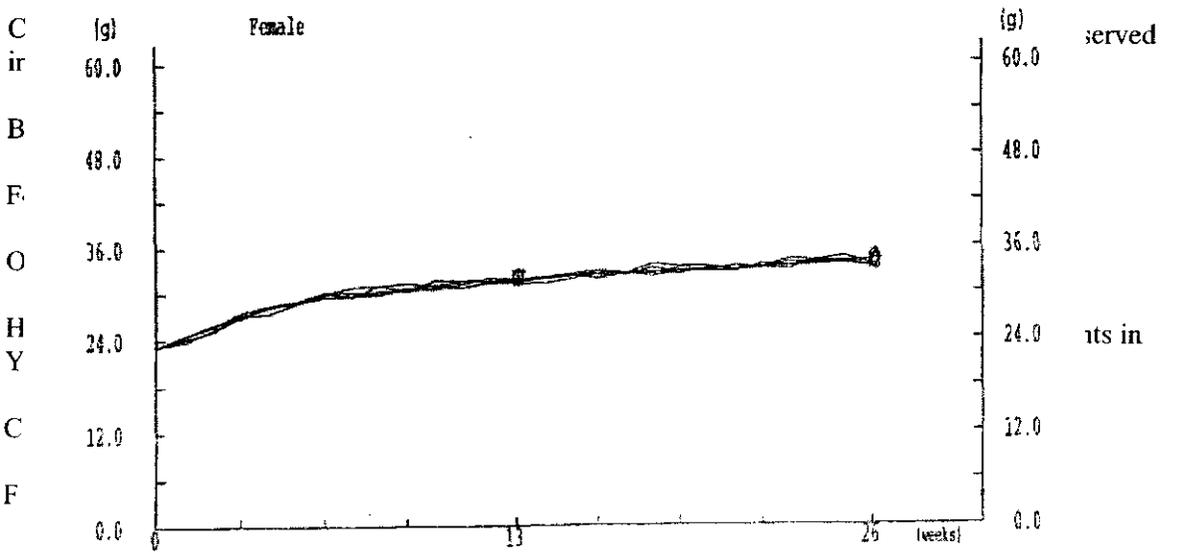
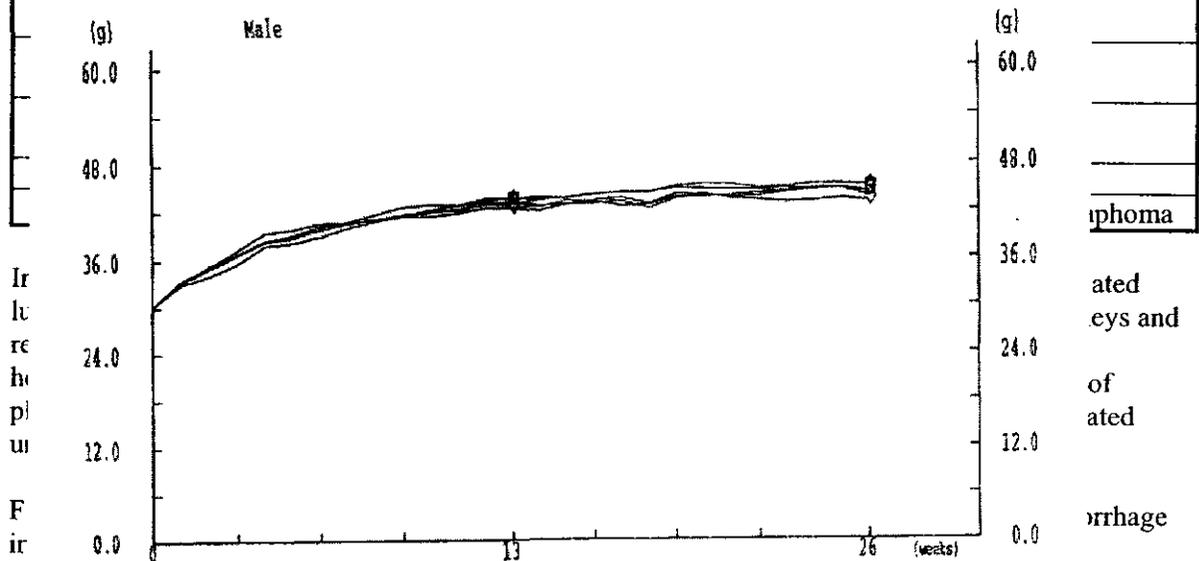
In addition, electron microscopy was performed on samples of the kidney and liver from 2 main study male and female animals in the two highest dosage groups  
 Toxicokinetics: During week 26, blood samples were collected from toxicokinetic animals at 1, 2, 4, 8 and 24 hours from 2-4 males and 4 females/time point. Analysis was performed using HPLC.

**Results:**

Mortality: Six males and one female died or were terminated during the treatment period.

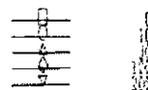
Animal	Dose	Week	Clinical Findings	Cause of death
M-1202	30	10	ulcers and dirty hair around genital organs	?
M-1111	10	11	ulcers and dirty hair around genital organs, prolapsed penis	?
M-1303	100	13	ulcers and dirty hair around genital organs	?

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ON ORIGINAL



Exp. No. 4490(159-022)

Dose level (mg/kg)



Gross pathology: The only notable macroscopic findings were blackened Harderian glands in 2, 6, 6, 12 and 13 males and 5, 8, 7, 6 and 12 females receiving 0, 10, 30, 100 or 200 mg/kg, respectively.

Organ weights: unremarkable

Histopathology: At 200 mg/kg, the following effects were observed in the GI tract:

- regeneration of the duodenum was observed in • 50% of the males (7/14) and females (8/15)
- ulceration and inflammation of the jejunum in one male
- lymphoid tissue hyperplasia in the cecum, colon and/or rectum in 3 males and 2 females
- Kupffer cell mobilization in 6 males

Moderate deposit of pigment in the Harderian glands accounted for the blackened coloration observed at necropsy. The pigmentation was localized in the acini and was considered to be due to the deposit of porphyrins. No abnormalities of the epithelial cells were reported.

Toxicokinetics: There was a dose-proportional increase in YM905 (free base) exposure for both AUC and Cmax values. The increase in AUC was greater than that in Cmax.

Dose (mg/kg/day)	Males			Females		
	Cmax (ng/ml)	Tmax (h)	AUC <sub>24</sub> (ng.h/ml)	Cmax (ng/ml)	Tmax (h)	AUC <sub>24</sub> (ng.h/ml)
10	75.8	1-2	235.0	51.2	1	104.5
30	417.1	1	744.5	189.1	1	566.0
100	528.4	1	4197.7	442.5	1	2928.2
200	1078.4	1	7235.8	511.4	2-8	7424.1

**Summary of individual study findings:** The primary findings in mice were erosion of the mucosa and inflammation of the GI tract, with the most sensitive area being the duodenum in animals treated at 100 and 200 mg/kg/day. Similar effects were seen in the jejunum and ileum, but at a much lower incidence. The inflammation occurred focally from the lamina propria and the submucosa to the serosa but was not accompanied by epithelial changes.

The Sponsor has concluded that the pre-term deaths observed in this study were due to irritation caused by the aluminum cages and exacerbated by soiled cage conditions. There is no evidence from either a dose-response or a time-response for genital ulcerations which would suggest a direct effect from YM905.

The NOAEL appears to be 30 mg/kg/day in both males and females.

**YM905: Toxicity Study by Oral Gavage Administration to F344 Rats for 26 Weeks Followed by a 10 Week Recovery Period**

**Key study findings:** NOAEL = 3 mg/kg/day. Sensitive tissues appear to be the female reproductive organs, liver and kidney.

**Study no:** R905-TX-005

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

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

**Date of study initiation:** April 13, 1998

**GLP compliance:** yes

**QA report:** yes

**Drug, lot #, radiolabel, and % purity:** K9059702. — pure

**Formulation/vehicle:** 0.5% aqueous methylcellulose

**Methods:** Animals were treated once daily by oral gavage for at least 26 weeks, with the recovery phase animals being held for a further 10 week period without treatment.

**Dosing:**

Species/strain: F344 Rats

#/sex/group or time point (main study): 15-18/sex/group (see dosing schedule)

Satellite groups used for toxicokinetics and recovery: 6-12/sex/group (see dosing schedule)

Age: 26 to 30 days

Weight: males - 91-122 g; females - 82-108 g

Doses in administered units:

Group	Dose (mg/kg)		Number of Animals		
	Weeks 1-13	Weeks 14-26	Main Study	Recovery	Toxicokinetics
1	0	0	15/sex	6/sex	-
2	3	3	15/sex	-	12/sex
3	10	10	15/sex	-	12/sex
4	30	30	18/sex	6/sex	12/sex
5	60	45	18 females	6 females	12 females
6	100	75	18 males	6 males	12 males

Note: Due to the number of deaths that occurred in Groups 4, 5 and 6, some main study animals were reassigned to the recovery phase in order to have sufficient animals in both phases for a meaningful assessment.

Route, form, volume, and infusion rate: oral gavage, 5 ml/kg

**Observations and times:**

Clinical signs: Cage side observations were made twice daily during treatment and once daily during recovery. Physical examinations were performed weekly.

Body weights: weekly

Food consumption: weekly

Ophthalmoscopy: weeks -1, 12 and 25

Clinical Pathology: Blood samples were collected for routine hematology and clinical chemistry evaluations during week 26 of treatment and week 10 of recovery.

Urinalysis: Overnight (17 hr) urine samples were collected during weeks 12 and 25.

**Gross pathology:** A complete necropsy was performed on all main study and recovery animals which died prematurely or at scheduled termination.

**Organs weighed:** adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands (submandibular), seminal vesicles, spleen, testes, thymus, thyroid w/parathyroids, uterus w/cervix

**Histopathology:** All tissues were preserved, however, only the following tissues were examined microscopically:

- all gross lesions
- groups 1, 4, 5 and 6: adrenals, brain, femur, heart, kidneys, liver, lungs, mammary area w/overlying skin, spinal cord, stomach, thyroid w/parathyroids, uterus
- group 2 and 3 females that died prematurely: uterus, vagina and ovaries
- recovery animals: uterus, vagina, ovaries and tissues with gross abnormalities

In addition, electron microscopy was performed on samples of the kidney and liver from 2 main study male and female animals from groups 1, 4, 5 and 6.

**Toxicokinetics:** During week 26, blood samples were collected from toxicokinetic animals at 0, 0.5, 2, 4, 8 and 24 hours. Each animal provided blood at 2-3 of the specified sampling time-points.

**Other:** pinna reflex (hearing) was evaluated on weeks -1 and 25

### **Results:**

**Mortality:** One male treated with 100 mg/kg/day died during week 8. In females, there were 5 deaths after week 15 in the 30 mg/kg/day group; 6 deaths in the 60 mg/kg/day group and a further 9 deaths after the dosage was reduced to 45 mg/kg/day. There were no significant gross or histopathological changes that would account for these deaths. Follicular degeneration of the ovaries and/or uterine atrophy were evident in 2 females dosed at 30 mg/kg/day and in 13 animals given 60/45 mg/kg/day.

**Clinical signs:** The following clinical signs were observed:

- salivation after dose administration in males and females at • 30 mg/kg/day
- pupillary dilations in females at • 10 mg/kg/day and males at • 30 mg/kg/day
- increased incidence of wet/yellow staining in the perigenital region in males and especially females at • 10 mg/kg/day
- increased respiratory noise after dosing in a few females at • 30 mg/kg/day and a few males at 100/75 mg/kg/day
- the majority of females treated at 60 mg/kg/day showed piloerection during the 1<sup>st</sup> week of treatment

**Recovery:** None of these treatment-related clinical signs were evident during the recovery period.

**Body weights:** Overall weight gains of males and females receiving • 30 mg/kg/day were significantly lower than those of the controls ( $p < 0.01$ ).

**Food consumption:** Statistically significant decreases in food consumption were observed in high-dose males throughout the study period and for high-dose females beginning week 8.

**Ophthalmoscopy:** unremarkable

**Pinna reflex:** unremarkable

**Hematology:** The only remarkable findings were slight increases in leukocyte (PMNs and lymphocytes) and platelet counts in females • 30 mg/kg/day observed at the end of the treatment period. At the end of the recovery period, all groups were similar.

**Clinical chemistry:** Changes (>10%) were observed in the following clinical chemistry parameters:

- increased alkaline phosphatase activity in females at • 10 mg/kg/day and in males • 30 mg/kg/day
- decreased ALT activity in females at • 10 mg/kg/day and in males at • 30 mg/kg/day
- decreased AST activity in females at • 30 mg/kg/day and in males at • 100/75 mg/kg/day
- decreased plasma glucose in females at • 60/45 mg/kg/day ( $p < 0.05$ )
- decreased cholesterol, triglyceride and phospholipid in males receiving 100/75 mg/kg/day ( $p < 0.01$ ); decreased cholesterol and phospholipid in females at 30 mg/kg/day and 10 mg/kg/day, respectively
- slightly decreased albumin levels and total protein in females at 60/45 mg/kg/day
- slightly increased phosphorous levels in females at • 30 mg/kg/day and males at 100/75 mg/kg/day

At the end of the recovery period transaminase activity remained low while the alkaline phosphatase activity remained slightly higher in females in the 60/45 mg/kg/day group; cholesterol and phospholipid levels remained low in females; total protein levels were slightly improved in females; and phosphorus levels were still reduced in females in the 60/56 mg/kg/day group.

**Urinalysis:** In males, low volume and low potassium were observed at 100/75 mg/kg/day. In females, urinary pH, sodium and chloride levels were elevated at 60/45 mg/kg/day. At the end of the recovery period, the effects observed in electrolyte output (•K in males and •Na and Cl in females) were still present.

**Gross pathology:** unremarkable after 26 weeks treatment and 10 weeks recovery

**Organ weights:** The following effects were observed on organ weights:

- increased relative adrenal weights in males and females at • 30 mg/kg/day
- slightly low absolute thymus weights in females at 60/45 mg/kg/day
- increased relative liver weights in males at • 10 mg/kg/day
- increased relative kidney weights in males at • 30 mg/kg/day
- decreased relative spleen weights in males at 100/75 mg/kg/day

Recovery: No effects on organ weights were observed.

**Histopathology:** Unremarkable in animals terminated following 26 weeks of treatment with or without 10 weeks of recovery.

*[Review Note: Follicular degeneration of the ovaries and/or uterine atrophy were evident in females which died prematurely during treatment (2 females dosed at 30 mg/kg/day and 13 at 60/45 mg/kg/day).]*

**Toxicokinetics:** The rate and extent of systemic exposure of rats to YM905, characterized by  $C_{max}$  and  $AUC_{24}$ , increased with increasing dose over the range of doses in both males and females. These increases were, however, greater than the proportionate dose increment and there was statistically significant evidence of non-proportionality ( $p < 0.020$ ). Overall, the  $C_{max}$  and  $AUC$  values at 45 and

75 mg/kg/day in females and males, respectively, were approximately 1.6-fold higher in males and approximately 37 to 4.7-fold higher in females than those values predicted from a linear relationship.

C<sub>max</sub> and AUC values for YM905 (free base) in week 26 are presented in the following table. T<sub>max</sub> occurred at approximately 0.5 hours and plasma concentrations of YM905 were quantifiable in all samples taken at 24 hours from animals receiving • 30 mg/kg/day.

Dose (mg/kg/day)	YM905			
	C <sub>max</sub> (ng/ml)		AUC <sub>24</sub> (ng.h/ml)	
	Males	Females	Males	Females
3	3.05	7.71	19	48
10	15.49	42.75	82	123
30	47.83	161.93	325	844
45	-	430.46	-	3402
75	126.19	-	748	-

The metabolite ratios were generally high (range 4.7 to 23.5), indicating extensive metabolism of YM905 to BY-348. C<sub>max</sub> and AUC values for metabolite BY-348C in week 26 are presented in the following table. T<sub>max</sub> for BY348C generally occurred at 0.5 hours, but also occurred at 2 or 8 hours post-dose at the higher dosages.

Dose (mg/kg/day)	BY-348C (M1)			
	C <sub>max</sub> (ng/ml)		AUC <sub>24</sub> (ng.h/ml)	
	Males	Females	Males	Females
3	96.7	149.75	469	889
10	232.5	388.35	1896	3015
30	400.7	615.71	5042	8928
45	-	985.63	-	16,726
75	846.99	-	10,022	-

The extent of systemic exposure (AUC) of rats to BY-438C increased in approximate proportion with increasing dose of YM905 over the range 3 to 30 mg/kg/day. At 45 mg/kg/day in females, the AUC values were approximately 1.3-fold higher than would be predicted, while at 75 mg/kg/day in males, the AUC values were approximately 14% lower than would be predicted from a linear relationship.

**Summary of individual study findings:** Doses of 60 mg/kg/day in females and 100 mg/kg/day in males exceeded the MTD. Doses of 30 mg/kg/day and greater resulted in low weight gains and signs of distress immediately following dosing. Changes in clinical chemistry and organ weights were not associated with any histopathology. The slightly higher liver weights in males and changes in transaminase activity suggest a potential effect upon liver metabolism. Changes in urinary output, electrolyte levels and relative weight also suggest that the kidneys may be a sensitive target tissue. Changes in clinical pathology and organ weights were either reversed or improved by the end of the 10-week recovery period. The NOAEL was 6 mg/kg/day in males and 3 mg/kg/day in females.

**Four-Week Oral Toxicity Study of YM905 in Beagle Dogs:**

**Key study findings:** Adverse findings included thymic involution in 30 mg/kg males and females, eosinophilic swelling of the epithelial cells in the straight portion of the renal proximal tubule in 30 mg/kg/ males, and decreased heart rates resulting in slight changes in P, PR, QRS, ST and QT intervals at 10 and 30 mg/kg/day. The NOAEL was 3 mg/kg/day in males and females.

**Study no:** R905-TX-006 (R905-TX-047 Statistical Report)

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** Yamanouchi, Tokyo

**Date of study initiation:** October 1, 1996

**GLP compliance:** yes

**QA report:** yes

**Drug, lot #, radiolabel, and % purity:** Lot No. 9051Z, . —

**Formulation/vehicle:** 10% mixture of YM905 in lactose

**Dosing:**

Species/strain: Beagle dogs —

#/sex/group or time point (main study): 3/sex/group

Age: ~14 months

Weight: males - 9.3 - 13.1 kg; females 8.2 - 11.0 kg

Doses in administered units: 1, 3, 10 and 30 mg/kg/day

Route, form, volume, and infusion rate: oral, gelatin capsules

**Observations and times:**

Clinical signs: daily

Body weights: weekly and prior to necropsy

Food consumption: daily

Ophthalmoscopy: pre-dose, week 3

EKG: prior to treatment initiation, 4 hours after first dose, prior to and 4 hours after dose on day 23

Clinical pathology: pre-dose, weeks 2 and 4

Urinalysis: pre-dose, weeks 2 and 4

Gross pathology: Animals were killed following an overnight fast by intravenous overdose of pentobarbitone followed by rapid exsanguination. All animals were subjected to a full macroscopic examination of tissues.

Organs weighed: adrenals, brain, heart, kidneys, liver, lungs ovaries, pancreas, pituitary, prostate, salivary glands, spleen, testes with epididymides, thymus, and thyroids w/parathyroids

Histopathology: The standard list tissues from all animals were examined microscopically.

Electron microscopy was performed on sections of liver and kidney (cortex, outer and inner medulla) from all animals.

Toxicokinetics: Blood samples were collected from all dogs on day 1 and after administration on day 29 at 1, 2, 4, 8 and 24 hours post-dosing. Samples were analyzed by — method by Yamanouchi.

Other: Fecal occult blood test (\*\* — - Pre-dose, weeks 2 and 4

**Results:**

Mortality: none

Clinical signs: All animals in the 30 mg/kg group had increased incidences of vomiting and mydriasis, and some animals showed decreased locomotor activity. Tremors were observed in all 30 mg/kg males during weeks 3-4.

Body weights: There were decreases in body weight (~10%) at 30 mg/kg.

Food consumption: In the 30 mg/kg groups, one male and two females showed decreased food consumption throughout the treatment period.

Ophthalmoscopy: unremarkable

Fecal occult blood: unremarkable

Hematology: One female had an increase WBC count due to an increase in neutrophils at week 4.

Clinical chemistry: unremarkable

Urinalysis: unremarkable

Gross pathology: Gastric fundic mucosa was slightly thickened in one 30 mg/kg male.

Electrocardiography (See Table): The electrocardiogram recorded 4 hours after administration in week 4 of treatment showed a tendency of prolonged P-R intervals in all animals, two of which showed first -degree atrioventricular block. In addition, tall P waves, extended P and QRS widths, and prolonged QT intervals were noted in some animals. Of these, the degree of QT interval prolongation was a little larger than the variation of QT width of control animals, but the value of prolongation was within the normal values for dogs. ECG readings obtained immediately before administration on the same day were limited only to slightly extended P or QRS widths in some animals.

Organ weights: In the 30 mg/kg group, all animals showed a slight decrease in absolute and relative thymus weights. Females in this group also exhibited a slight decrease in absolute heart weight.

Histopathology: Eosinophilic swelling (frequently with deeply eosinophilic droplets) of the epithelium and slightly unclear brush border in about half of the distal side of the straight portion of the renal proximal tubule was observed in one control male, one 3 mg/kg male, one 10 mg/kg male, and all 30 mg/kg males. Electron microscopic analysis showed a diffuse increase in sER in cytoplasm in the straight portion of the renal proximal tubule in one 30 mg/kg male.

All animals dosed at 30 mg/kg showed thymic involution due to a decrease in cortical lymphocytes (correlated with decreased thymic weights).

## ECG results following 4-week oral administration of YM905 to Beagle dogs:

Males (n=3):	Control	YM905			
		1.0 mg/kg	3.0 mg/kg	10.0 mg/kg	30 mg/kg
Day 1, Pre-Dose					
Heart Rate	96 ± 6	108 ± 24	94 ± 23	114 ± 16	90 ± 10
QTc	195-193-237	274-254-235	234-264-235	239-247-211	252-264-260
Day 1, 4 hrs Post-Dose					
Heart Rate	102 ± 12	92 ± 9	108 ± 10	104 ± 15	102 ± 16
QTc	231-229-259	236-247-254	233-264-243	252-244-208	290-283-240
Plasma level (ng/ml)	0	<3.0	<3.0	20	262.9
Day 1, 24 hrs Post-Dose					
Heart Rate	104 ± 7	90 ± 12	94 ± 28	100 ± 18	88 ± 15
QTc	321-213-246	-	-	243-246-214	264-273-270
Wk 4, Pre-Dose					
Heart Rate	92 ± 9	96 ± 12	100 ± 40	106 ± 13	76 ± 17
QTc	238-210-251	259-251-267	255-251-264	260-237-230	259-306-268
Wk 4, 4 hrs Post-Dose					
Heart Rate	110 ± 9	102 ± 22	114 ± 18	116 ± 9	102 ± 10
QTc	235-192-259	231-225-234	237-232-248	291-249-241	314-321-320
Plasma level (ng/ml)	0	<3.0	8.3	133.5	1202.9

Females (n=3):	Control	YM905			
		1.0 mg/kg	3.0 mg/kg	10.0 mg/kg	30 mg/kg
Pre-Dose					
Heart Rate	92 ± 4	108 ± 28	96 ± 18	94 ± 13	106 ± 23
QTc	241-216-241	245-239-227	240-227-241	266-243-264	249-230-259
Wk 1, 4 hrs Post-Dose					
Heart Rate	94 ± 7	116 ± 24	100 ± 4	94 ± 13	92 ± 9
QTc	239-240-231	248-239-230	257-234-212	241-269-271	271-248-292
Cmax (ng/ml)	0	<3.0	<3.0	14.1	182.5
Wk 1, 24 hrs Post-Dose					
Heart Rate	94 ± 9	102 ± 12	96 ± 6	106 ± 5	98 ± 14
QTc	243-243-254	-	-	257-271-254	260-268-244
Wk 4, Pre-Dose					
Heart Rate	94 ± 4	120 ± 18	98 ± 9	98 ± 17	98 ± 13
QTc	237-233-233	258-256-266	241-228-254	251-252-277	287-278-247
Wk 4, 4 hrs Post-Dose					
Heart Rate	92 ± 4	120 ± 18	108 ± 22	94 ± 13	102 ± 16
QTc	228-235-224	244-232-256	265-240-237	263-270-281	276-307-318
Plasma level (ng/ml)	0	<3.0	3.7	112.2	744.3

Toxicokinetics: Inter-individual variation in plasma YM905 concentrations was high, with coefficients of variation generally being greater than 50%, and in the range of 10.7% to 200%. The rate and extent of systemic exposure of rats to YM905, characterized by C<sub>max</sub> and AUC<sub>24</sub>, increased with increasing dose over the range of doses in both males and females.

Dose (mg/kg/day)	YM905 (free base) in Males					
	Single Dose			repeat dosing for 28 days		
	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (h)	AUC <sub>24</sub> (ng·hr/ml)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (h)	AUC <sub>24</sub> (ng·hr/ml)
3						
10						
30						

Dose (mg/kg/day)	YM905 (free base) in Females					
	Single Dose			repeat dosing for 28 days		
	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (h)	AUC <sub>24</sub> (ng·hr/ml)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (h)	AUC <sub>24</sub> (ng·hr/ml)
3						
10						
30						

**Summary of individual study findings:**

- NOAEL = 3 mg/kg/day
- dose-related changes in QT interval at 10 and 30 mg/kg/day
- thymic involution and decreased thymus weights in males at 30 mg/kg/day
- accumulation of YM905 following repeated oral administration
- eosinophilic swelling of the epithelial cells in the straight portion of the renal proximal tubule in males.

**YM905: Toxicity Study by Oral Capsule Administration to Beagle Dogs for 13 Weeks**

**Key study findings:** Adverse findings included convulsions at 25 mg/kg/day, decreased heart rates resulting in slight changes in P, PR, QRS, ST and QT intervals. The NOAEL was 12 mg/kg/day in males and females.

**Study no:** R905-TX-007 (VVE 002/983306)

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:**

**Date of study initiation:** March 19, 1998

**GLP compliance:** yes

**QA report:** yes

**Drug, lot #, radiolabel, and % purity:** Lot CC9052Z,

**Formulation/vehicle:** 10% mixture of YM905 in lactose

**Dosing:**

Species/strain: Beagle dogs

#/sex/group or time point (main study): 3/sex/group

Age: 33 -38weeks

Weight: males - 9.4 - 11.8 kg; females 9.3-11.6 kg

Doses in administered units: 3, 6, 12 and 25 (reduced to 18 during week 7) mg/kg/day

Route, form, volume, and infusion rate: oral, gelatin capsules

**Observations and times:**

Clinical signs: daily

Body weights: weekly and prior to necropsy

Food consumption: daily

Ophthalmoscopy: pre-dose and week 12

EKG: pre-dose, weeks 6, 11(males), 12 (females)

Clinical pathology: pre-dose, weeks 7 and 12

Gross pathology: Animals were killed following an overnight fast by intravenous overdose of pentobarbitone followed by rapid exsanguination. All animals were subjected to a full macroscopic examination of tissues.

Organs weighed: adrenals, brain, heart, kidneys, liver, lungs ovaries, pituitary, prostate, salivary glands, spleen, testes, thymus, thyroids w/parathyroids and uterus w/cervix

Histopathology: The standard list tissues from all animals were examined microscopically.

Electron microscopy was performed on sections of liver and kidney (cortex, outer and inner medulla) from all animals in groups 1, 4 and 5.

Toxicokinetics: Blood samples were collected from all dogs on day 1 and during week 13 at 1, 2, 4, 8 and 24 hours post-dosing. Samples were analyzed by  $^{\text{—}}$  using HPLC.

Other: Pinna reflex was evaluated prior to dosing and during week 11.

**Results:**

Mortality: One control female was euthanized during week 1 due to idiopathic febrile narcotizing arteritis syndrome. This animal was replaced.

Clinical signs: Significant clinical observations were limited to the high dose group. One male was suspected to have had a convulsion at approximately 2 hours post-dosing during week 4. Signs included nervousness, body tremors, marked underactivity, prostration, pale gums, marked ataxia, extreme anxiety, weak pulse and high heart rate. The animals coat was also markedly wet, ungroomed and covered in sawdust. A further high dose male had a number of convulsive episodes on the 5<sup>th</sup> and 7<sup>th</sup> day of week 6, while one high dose female had several convulsions on the 7<sup>th</sup> day of week 6.

Body tremors were observed in all high dose females during week 1, in all high dose males during week 2, and in the majority of all high dose animals through week 7. Isolated incidences of body tremor were occasionally observed during the first 6 weeks of treatment in a few animals dosed at 3 or 12 mg/kg/day.

As a consequence of the marked signs of toxicity, the highest dose was reduced from 25 to 18 mg/kg/day from the 2<sup>nd</sup> day of week 7. Following reduction of the dose, no further convulsive episodes were observed, though one female showed underactivity, ataxia, prostration and had an abnormal gait and increased and labored respiration in week 11.

Other signs attributed to treatment included emesis, salivation and active resistance to dosing.

Body weights: Slight losses of body weight or decreased body weight gain were noted at 25 mg/kg/day. Following reduction of the high dose to 18 mg/kg/day, bodyweight gain was similar to controls.

Food consumption: Food consumption was reduced at 25 mg/kg/day during the first six weeks of treatment. Following reduction of the dose to 18 mg/kg/day, food consumption showed a return to normal.

Ophthalmoscopy: unremarkable  
 Pinna reflex: unremarkable  
 Hematology: unremarkable  
 Clinical chemistry: unremarkable  
 Urinalysis: unremarkable

Electrocardiography (See Tables): Evaluation of ECG traces revealed slightly prolonged P-wave interval and slightly prolonged PR interval in high-dose males and females. None of these changes were evident 24 hours after dose administration.

ECG results following oral administration of YM905 for 13-weeks to Beagle dogs:

Males (n=3):	Control	YM905			
		3.0 mg/kg	6.0 mg/kg	12.0 mg/kg	25/18 mg/kg
Pre-Dose					
Heart Rate	137 ± 2	94 ± 24	112 ± 17	89 ± 17	119 ± 17
QT	185 ± 6	203 ± 21	188 ± 12	203 ± 13	188 ± 3
QTc	279 ± 8	250 ± 12 *	255 ± 12 *	245 ± 7 *	264 ± 17
Wk 6, 4 hrs Post-Dose					
Heart Rate	118 ± 31	101 ± 17	120 ± 24	122 ± 32	130 ± 6
QT	189 ± 15	188 ± 14	181 ± 2	185 ± 15	190 ± 13
QTc	262 ± 24	243 ± 2	256 ± 24	260 ± 14	279 ± 14
Wk 6, 24 hrs Post-Dose					
Heart Rate	113 ± 7	136 ± 16	147 ± 5 *	113 ± 24	118 ± 5
QT	185 ± 4	179 ± 5	171 ± 6	185 ± 11	197 ± 13
QTc	254 ± 6	268 ± 10	267 ± 14	252 ± 16	275 ± 19
Wk 11/12, 4 hrs Post-Dose					
Heart Rate	126 ± 20	98 ± 3	114 ± 19	103 ± 23	118 ± 11
QT	185 ± 9	203 ± 7	195 ± 11	204 ± 17	204 ± 11
QTc	267 ± 13	260 ± 10	268 ± 16	265 ± 25	286 ± 18
Wk 11/12, 24 hrs Post-Dose					
Heart Rate	134 ± 33	117 ± 31	115 ± 21	119 ± 35	101 ± 17
QT	177 ± 4	188 ± 21	191 ± 5	189 ± 13	198 ± 14
QTc	262 ± 30	258 ± 9	264 ± 25	263 ± 23	254 ± 9

Females (n=3):	Control	YM905			
		3.0 mg/kg	6.0 mg/kg	12.0 mg/kg	25/18 mg/kg
Pre-Dose					
Heart Rate	92 ± 15	122 ± 11	99 ± 15	120 ± 6	111 ± 38
QT	205 ± 14	186 ± 9 *	203 ± 13	190 ± 13 *	190 ± 18 *
QTc	253 ± 14	264 ± 6	260 ± 4	268 ± 5	253 ± 24
Wk 6, 4 hrs Post-Dose					
Heart Rate	123 ± 25	123 ± 13	117 ± 32	141 ± 5	136 ± 24
QT	183 ± 15	184 ± 7	184 ± 15	177 ± 6	199 ± 20
QTc	260 ± 4	263 ± 9	254 ± 15	272 ± 14	296 ± 16 *
Wk 6, 24 hrs Post-Dose					
Heart Rate	125 ± 21	136 ± 21	133 ± 25	132 ± 5	128 ± 11
QT	175 ± 18	173 ± 12	175 ± 16	179 ± 4	190 ± 4
QTc	252 ± 14	259 ± 9	259 ± 19	265 ± 8	277 ± 13 *
Wk 11/12, 4 hrs Post-Dose					
Heart Rate	129 ± 19	122 ± 21	99 ± 27	115 ± 16	126 ± 37
QT	188 ± 4	187 ± 10	205 ± 23	201 ± 3	190 ± 15
QTc	275 ± 23	266 ± 8	259 ± 11	277 ± 18	270 ± 30
Wk 11/12, 24 hrs Post-Dose					
Heart Rate	118 ± 11	141 ± 6	107 ± 42	140 ± 10	115 ± 25
QT	190 ± 14	175 ± 5	196 ± 33	181 ± 5	199 ± 8
QTc	265 ± 10	267 ± 8	252 ± 19	277 ± 9	274 ± 31

Toxicokinetics: The rate and extent of systemic exposure of YM905 in dogs, characterized by C<sub>max</sub> and AUC, generally increased with increasing dose at a greater than dose proportional increments. Mean accumulations ratios ranged between 2 and 10. The times at which the maximum plasma concentrations occurred were generally one to two hours dosing and all were in the range of one to eight hours.

Mean C<sub>max</sub> and AUC following oral administration of YM905 for 13 weeks.

Dose (mg/kg/day)	YM905 (free base) in Males			
	Single Dose		repeat dosing for 13 weeks	
	C <sub>max</sub> (ng/ml)	AUC <sub>24</sub> (ng·hr/ml)	C <sub>max</sub> (ng/ml)	AUC <sub>24</sub> (ng·hr/ml)
3	0.8 ± 1.4	-	7.2 ± 2.3	46 ± 21
2	5.8 ± 3.4	40 ± 25	41.4 ± 10.0	306 ± 117
12	24.9 ± 20.1	152 ± 82	158.9 ± 10.6	1156 ± 505
18/25	154.2 ± 81.8	1137 ± 554	439.6 ± 291.2	4029 ± 1717

Dose (mg/kg/day)	YM905 (free base) in Females			
	Single Dose		repeat dosing for 13 weeks	
	C <sub>max</sub> (ng/ml)	AUC <sub>24</sub> (ng·hr/ml)	C <sub>max</sub> (ng/ml)	AUC <sub>24</sub> (ng·hr/ml)
3	2.4 ± 3.7	-	5.9 ± 1.6	52 ± 3
6	6.9 ± 0.2	36 ± 22	63.7 ± 9.7	353 ± 104
12	70.9 ± 41.8	535 ± 412	280.9 ± 197.9	2470 ± 1856
18/25	61.7 ± 22.1	593 ± 443	593.0 ± 222.3	5133 ± 2412

Gross pathology: unremarkable

Organ weights: Absolute and relative spleen and testes weights were slightly increased in males though there was no dose-response relationship. In addition, mean uterine weights were low in females receiving 12 or 25/18 mg/kg/day, and there was a trend towards low ovarian weights in these animals.

Histopathology: Microscopic evaluation of the uterus in high-dose females revealed uterine immaturity.

**Summary of individual study findings:**

- convulsions and tremors at 25 mg/kg/day; symptoms resolved after lowering dose to 18 mg/kg/day
- lower heart rates following YM905 administration and concurrent changes in P, PR, QRS, ST and QT intervals
- NOAEL = 12 mg/kg/day

**YM905: Toxicity Study by Oral Capsule Administration to Beagle Dogs for 52 Weeks**

**Key study findings:** Adverse findings included perivascular lymphoid accumulation in the urinary bladder, decreased heart rates resulting in slight changes in P, PR, QRS, ST and QT intervals. The NOAEL was 3 mg/kg/day in males and females.

**Study no:** R905-TX-008 (VVH 018/993353)

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

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

**Date of study initiation:** November 10, 1998

**GLP compliance:** yes

**QA report:** yes

**Drug, lot #, radiolabel, and % purity:** Batch no. 90501, —

**Formulation/vehicle:** 10% mixture of YM905 in lactose

**Dosing:**

Species/strain: Beagle dogs

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

Age: 32-43 weeks

Weight: males - 10.5-13.8 kg; females 10.0-13.2 kg

Doses in administered units: 3, 6, 12 and 20 mg/kg/day

Route, form, volume, and infusion rate: oral, gelatin capsules

**Observations and times:**

Clinical signs: daily

Body weights: weekly and prior to necropsy

Food consumption: daily

Ophthalmoscopy: pre-dose, weeks 25 and 51

EKG: pre-dose, weeks 12, 25, 38 and 51

Clinical pathology: pre-dose, weeks 13, 26, 39 and 52

Urinalysis: pre-dose, weeks 13, 26, 39 and 52

Gross pathology: Animals were killed following an overnight fast by intravenous overdose of pentobarbitone followed by rapid exsanguination. All animals were subjected to a full macroscopic examination of tissues.

Organs weighed: adrenals, brain, heart, kidneys, liver, lungs ovaries, pituitary, prostate, salivary glands, spleen, testes, thymus, thyroids w/parathyroids and uterus w/cervix

Histopathology: The standard list tissues from all animals were examined microscopically (See Histopathology Inventory). Electron microscopy was performed on sections of liver and kidney (cortex, outer and inner medulla) from all animals in groups 1, 4 and 5.

Toxicokinetics: Blood samples were collected from all dogs on day 1 and during weeks 26 and 52 at 1, 2, 4, 8 and 24 hours post-dosing. Samples were analyzed by  using HPLC.

Other: Pinna reflex - Pre-dose, weeks 25 and 51

**Results:**

Mortality: none

Clinical signs:

- Salivation was observed immediately before, during and/or just after administration primarily in the mid- and high-dose groups.
- Two male dogs were diagnosed with beagle pain syndrome (polyarteritis), one dosed at 6 mg/kg/day (week 25) and the other at 12 mg/kg/day (week 35).
- With the exception of one female control, there was evidence of estral activity in all females at least once during the study duration.

Body weights: unremarkable

Food consumption: unremarkable

Ophthalmoscopy: unremarkable

Pinna reflex: unremarkable

Hematology: unremarkable

Clinical chemistry: unremarkable

Urinalysis: unremarkable

Electrocardiography (See Tables): Group mean heart rate for males receiving 12 or 20 mg/kg/day and females receiving 6, 12 or 20 mg/kg/day was lower in comparison with the predose values on most occasions during the dosing period. The largest reduction was observed in males dosed at 20 mg/kg/day during week 38. Changes in the P, PR, QRS, ST and QT intervals were in general related to the concurrent fluctuations in the heart rates and were in general within the expected ranges.

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<b>Females (n=3):</b>	<b>Control</b>	<b>3.0 mg/kg</b>	<b>6.0 mg/kg</b>	<b>12.0 mg/kg</b>	<b>20 mg/kg</b>
<b>Wk -2, Pre-Dose</b>					
Heart Rate	102	124	142	153	126
QT	194	194	183	178	183
QTc	252	275	278	262	260
<b>Wk 12, 4 hrs Post-Dose</b>					
Heart Rate	90	123	131	107	101
QT	206	185	184	202	212
QTc	250	159	270	264	269
<b>Wk 12, 24 hrs Post-Dose</b>					
Heart Rate	93	99	130	103	92
QT	203	203	174	205	209
QTc	250	244	255	263	253
<b>Wk 25, 4 hrs Post-Dose</b>					
Heart Rate	102	134	144	106	102
QT	196	176	181	190	195
QTc	255	255	277	248	246
<b>Wk 25, 24 hrs Post-Dose</b>					
Heart Rate	109	126	124	103	94
QT	192	191	183	199	217
QTc	255	266	260	259	265
<b>Wk 38, 4 hrs Post-Dose</b>					
Heart Rate	104	143	133	103	100
QT	188	174	182	204	206
QTc	246	266	270	255	264
<b>Wk 38, 24 hrs Post-Dose</b>					
Heart Rate	101	117	118	82	79
QT	188	188	185	211	224 *
QTc	239	253	257	243	254
<b>Wk 51, 4 hrs Post-Dose</b>					
Heart Rate	104	137	140	134	110
QT	188	178	175	192	205
QTc	246	262	264	275 *	274 *
<b>Wk 51, 24 hrs Post-Dose</b>					
Heart Rate	112	133	143	123	92
QT	193	182	177	189	212
QTc	261	260	267	267	256
<b>Cmax (ng/ml) Day 1</b>					
		2 ± 1	8 ± 5	34 ± 33	137 ± 105
<b>Cmax (ng/ml) Wk 26</b>					
		10 ± 6	54 ± 26	229 ± 110	494 ± 309
<b>Cmax (ng/ml) Wk 52</b>					
		5 ± 1	65 ± 29	190 ± 78	624 ± 84

Gross pathology: unremarkable

Organ weights: There were dose-related changes in mean absolute and relative spleen weights. In males, spleen weights decreased with increasing dose and were statistically and biologically significant at 20 mg/kg/day. In females, there was a decrease in the mean absolute and relative spleen weights at doses • 12 mg/kg/day, however, at 20 mg/kg/day, spleen weights were significantly higher relative to controls.

Dose (mg/kg/day)	Mean Absolute and Relative Spleen Weights:			
	Males		Females	
	Absolute	Spleen:Brain Ratio	Absolute	Spleen:Brain Ratio
0	106.6	1.26	96.8	1.25
3	99.0	1.19	91.4	1.17
6	101.9	1.14	81.9	1.01
12	96.4	1.14	86.4	1.08
20	72.4	0.87	102.9	1.37

The effect in females could be a secondary effect related to the lymphoid accumulation observed in the urinary bladder mucosa (see histopathology).

Histopathology: There was an increased incidence and degree of perivascular lymphoid accumulation in the urinary bladders of females dosed at 20 mg/kg/day.

Perivascular lymphoid accumulation in female beagles dosed with YM095 for 52 weeks:					
	0 mg/kg/day	3 mg/kg/day	6 mg/kg/day	12 mg/kg/day	20 mg/kg/day
Minimal	1	0	0	1	0
Slight	0	0	0	0	1
Moderate	0	0	0	0	2
Total	1/4	0/4	0/4	1/4	3/4

Submucosal or submucosal/muscle layer edema was also seen in 2 of the affected females receiving 20 mg/kg/day. In addition, one affected animal showed slight transitional cell hyperplasia and vacuolation associated with this submucosal lesion. Urinary perivascular lymphoid accumulation was not seen in males.

Toxicokinetics: Inter-individual variation in plasma YM905 concentrations was high, with coefficients of variation generally being greater than 50%, and in the range of 10.7% to 200%. The rate and extent of systemic exposure of rats to YM905, characterized by C<sub>max</sub> and AUC<sub>24</sub>, increased with increasing dose over the range of doses in both males and females. However, these increases were greater than the proportionate dose increment and there was statistically significant evidence of non-proportionality (p<0.001). Overall, the C<sub>max</sub> and AUC values at the highest dose (20 mg/kg/day) were approximately 5.6 to 22.6-fold than those values predicted from a linear relationship.

C<sub>max</sub> and AUC values for YM905 (free base) following a single dose and during weeks 26 and 52 are presented in the following tables. T<sub>max</sub> generally occurred at approximately 2 hours (outliers between 1 to 8 hours) and plasma concentrations of YM905 were quantifiable in samples taken at 24 hours during weeks 26 and 52 from animals receiving • 6 mg/kg/day.

Dose (mg/kg/day)	C <sub>max</sub> (ng/ml) in Dogs for YM905 (free base)					
	Day 1		Week 26		Week 52	
	Male	Female	Male	Female	Male	Female
3	3.1 ± 1.1	1.7 ± 1.3	11.1 ± 8.7	10.1 ± 5.5	10.9 ± 5.5	5.1 ± 1.1
6	9.2 ± 9.7	8.0 ± 5.2	54.2 ± 22.3	54.0 ± 25.6	42.1 ± 13.4	64.5 ± 29.5
12	71.1 ± 47.6	34.4 ± 33.1	362.7 ± 135.6	229.4 ± 109.7	399.5 ± 62.2	190.3 ± 78.2
20	118.8 ± 33.0	136.6 ± 104.6	754.4 ± 217.2	494.0 ± 308.7	743.7 ± 328.6	624.2 ± 84.4

Dose (mg/kg/day)	AUC <sub>24</sub> (ng·hr/ml) in Dogs for YM905 (free base)					
	Day 1		Week 26		Week 52	
	Male	Female	Male	Female	Male	Female
3	13 ± 0	10 *	176 ± 46	165 ± 35	91 ± 43	47 ± 22
6	36 ± 20	35 ± 14	497 ± 91	425 ± 131	395 ± 101	485 ± 230
12	371 ± 166	406 ± 491	3141 ± 1227	1893 ± 551	3173 ± 586	1807 ± 468
20	1140 ± 539	757 ± 282	7555 ± 2555	6114 ± 2406	7425 ± 2517	7101 ± 835

\* n=1

Terminal half-lives were in the range of 1.3 to 7.1 hours, and tended to be longer following repeated dosing compared to a single dose. The mean accumulation ratios were greater than one at all doses, indicating that accumulation occurs after repeated oral administration of YM905.

Dose (mg/kg/day)	Accumulation Ratios based on AUC in Dogs for YM905 (free base)			
	Week 26		Week 52	
	Male	Female	Male	Female
3	6.9	6.9*	6.0	7.3*
6	15.8	12.5	12.4	13.7
12	8.6	7.2	8.2	6.9
20	7.4	8.1	7.3	9.8

\*n=1

#### Summary of individual study findings:

- lower heart rates following YM905 administration and concurrent changes in P, PR, QRS, ST and QT intervals
- decreased spleen weights in males and increased spleen weights in females at 20 mg/kg/day
- increased incidence and severity of perivascular lymphoid accumulation in the urinary bladders of females dogs at 20 mg/kg/day
- slight accumulation of YM905 following repeated oral administration

#### 14-Day Intravenous Toxicity Study with YM905 in Dogs

**Key study findings:** There were no dose-related effects on ECG parameters at 3 mg/kg/day, the highest dose administered.

**Study no:** R905-TX-036 (D200101838-01.00)

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** '-----'

**Date of study initiation:** March 21, 2000

**GLP compliance:** yes

**QA report:** yes

**Drug, lot #, radiolabel, and % purity:** Lot No. K9059804, —

**Formulation/vehicle:** 0.9% Sodium Chloride for Injection (USP)

**Dosing:**

Species/strain: Beagle dogs — )

#/sex/group or time point: 3/sex/group

Age: 7-8 months

Weight: males - 8.9-12.38 kg; females 6.4-8.9 kg

Doses in administered units: 0, 0.3, 1 or 3 mg/kg/day

Route, form, volume, and infusion rate: bolus i.v. Injection, 2 mg/kg

**Observations and times:**

Clinical signs: daily

Body weights: days 1, 5, 8, 12 and 15

Food consumption: daily

EKG: pre-dose, weeks 12, 25, 38 and 51

Clinical pathology: twice pre-dose (days -13 and -9) and on day 14

Gross pathology: Animals were killed randomly following an overnight fast by intravenous overdose of pentobarbitone followed by rapid exsanguination. All animals were subjected to a full macroscopic examination of tissues.

Organs weighed: adrenals, brain, heart, kidneys, liver w/gall bladder, lungs ovaries, pituitary, prostate, salivary glands, spleen, testes w/epididymides, thymus, thyroids w/parathyroids and uterus

Histopathology: The standard list tissues from all animals were examined microscopically.

Toxicokinetics: Blood samples were collected from all dogs on days 1 and 14 at 1, 2, 4, 8 and 24 hours post-dosing. Samples were analyzed by — using HPLC.

**Results:**

Mortality: none

**Clinical signs:** Adverse clinical signs were observed only at the 3 mg/kg/day level and included vomitus, excessive salivation, thin appearance, tremors (only on day 13 immediately post-dosing), and hypoactivity. These findings were noted at a higher incidence among the males when compared with the females administered the same dose.

**Body weights:** One male and one female dosed at 3 mg/kg/day lost 0.6 and 0.4 kg, respectively, over the course of the study.

**Food consumption:** The male and female with weight loss also had thin appearance and lower food consumption than that of the other animals in the same group.

**Electrocardiography:** There were no drug-related effects on the ECG parameters measured (See Table).

**Hematology:** unremarkable

**Clinical chemistry:** unremarkable

**Urinalysis:** unremarkable

Gross pathology: unremarkable

Organ weights: unremarkable

Histopathology: unremarkable

Toxicokinetics: Plasma concentrations of YM905 at 24 hours postdose were not quantifiable in any of the animals. The terminal half-lives in individual dogs on days 1 and 14 were in the range of 1.3 to 2.1 hours, and appeared to be independent of dose and sex.

ECG results in male dogs following i.v. administration of YM905 for 14 days:

Males (n=3):	Control	YM905		
		0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Wk -2, Pre-Dose				
Heart Rate	119 ± 26	108 ± 29	117 ± 7	109 ± 36
QT	187 ± 12	200 ± 36	207 ± 12	193 ± 31
QTc	261 ± 22	266 ± 36	289 ± 10	254 ± 18
Day 1, Pre-Dose				
Heart Rate	120 ± 23	134 ± 36	121 ± 20	100 ± 24
QT	200 ± 20	200 ± 0	210 ± 10	213 ± 31
QTc	281 ± 21	297 ± 39	298 ± 38	271 ± 10
Day 1, 10 min Post-Dose				
Heart Rate	134 ± 39	108 ± 23	114 ± 22	120 ± 30
QT	193 ± 12	203 ± 21	207 ± 12	193 ± 12
QTc	285 ± 29	270 ± 19	283 ± 18	271 ± 22
Cmax (ng/ml) single dose		184 ± 152	499 ± 215	1755 ± 1197
Day 12, Pre-Dose				
Heart Rate	124 ± 7	114 ± 22	133 ± 17	108 ± 22
QT	197 ± 15	203 ± 25	193 ± 9	203 ± 32
QTc	282 ± 24	278 ± 21	286 ± 9	269 ± 13
Day 12, 10 min Post-Dose				
Heart Rate	123 ± 14	136 ± 39	128 ± 12	126 ± 15
QT	200 ± 0	200 ± 20	200 ± 0	203 ± 15
QTc	286 ± 17	297 ± 15	292 ± 14	294 ± 9
Cmax (ng/ml) 14 doses		139 ± 60	398 ± 146	1631 ± 695

ECG results in female dogs following i.v. administration of YM905 for 14 days:

Females (n=3):	Control	YM905		
		0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Wk -2, Pre-Dose				
Heart Rate	117 ± 25	88 ± 52	118 ± 19	126 ± 24
QT	193 ± 12	200 ± 20	200 ± 0	187 ± 12
QTc	270 ± 40	231 ± 78	280 ± 22	270 ± 31
Day 1, Pre-Dose				
Heart Rate	134 ± 44	115 ± 32	119 ± 22	117 ± 44
QT	200 ± 0	207 ± 6	207 ± 12	173 ± 74
QTc	296 ± 49	283 ± 41	290 ± 21	230 ± 74
Day 1, 10 min Post-Dose				
Heart Rate	115 ± 8	105 ± 11	121 ± 15	140 ± 22
QT	203 ± 6	207 ± 12	207 ± 6	207 ± 12
QTc	281 ± 11	274 ± 27	293 ± 20	314 ± 9
Cmax (ng/ml) single dose		57 ± 31	328 ± 226	1242 ± 851
Day 12, Pre-Dose				
Heart Rate	138 ± 20	110 ± 14	122 ± 4	124 ± 23
QT	203 ± 6	190 ± 10	200 ± 0	193 ± 11
QTc	308 ± 21	256 ± 7	286 ± 5	276 ± 8
Day 12, 10 min Post-Dose				
Heart Rate	131 ± 16	113 ± 21	102 ± 6	135 ± 26
QT	197 ± 6	193 ± 12	203 ± 6	203 ± 6
QTc	291 ± 24	263 ± 15	265 ± 5	303 ± 23
Cmax (ng/ml) 14 doses		82 ± 15	487 ± 140	1090 ± 849

**Summary of individual study findings:** Dose-related findings were noted at 3 mg/kg/day and included vomitus, excessive salivation, thin appearance (related to weight loss and decreased appetite), tremors and hypoactivity. There were no dose-related effects on ECG parameters, clinical pathology parameters, differences in organ weights, or macroscopic or microscopic observations. The NOAEL was 1 mg/kg/day.

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**TOXICOLOGY SUMMARY:****Single oral dose toxicity:**

**Rats:** At doses up to 2000 mg/kg in male rats and 1000 mg/kg in female rats, signs of toxicity included mydriasis, decreased locomotor activity, prone or lateral position, ocular discharge, twitching, salivation and clonic convulsions. The approximate lethal doses were 1000 and 500 mg/kg in male and female rats, respectively.

**Dogs:** One female dog administered 60 mg/kg died following vomiting and retching and signs of toxicity similar to those observed in rats: twitching, mydriasis, abnormal gait, tonic convulsions and urinary incontinence.

**Repeat oral dose toxicity:**

**Mice:** In the 13-week study, twelve deaths occurred: one female at 250 mg/kg, and five males and six females at 400 mg/kg. Signs of toxicity at 250 and 400 mg/kg/day included underactivity, ataxia, tremors, convulsions, prostration, hunched posture, piloerection and abnormal respiration. Depressed weight gain was also seen in males at 400 mg/kg/day. There were no significant microscopic findings that correlated with any changes in clinical chemistry parameters or organ weights. The NOAEL was considered 100 mg/kg/day.

Chronic (26-weeks) administration of 100 and 200 mg/kg/day YM905 in mice resulted in erosion of the GI tract mucosa and inflammation of the lower GI tract, with the most sensitive area being the duodenum. A dose-related exacerbation of porphyrin deposits in the Harderian glands was also noted. The NOAEL in mice following 26 weeks of exposure was 30 mg/kg/day resulting in YM905 AUC<sub>24</sub> values of 745 and 566 ng.h/ml in males and females, respectively.

**Rats:** Administration of YM905 to rats for 4 weeks at doses of 5, 10, 25 or 50 mg/kg/day resulted in the deaths of 1 male at 25 mg/kg, and 2 males and 7 females at 50 mg/kg/day. Convulsions prior to death were noted in the 25 mg/kg male and in 3 of the 50 mg/kg females. The most common clinical signs were mydriasis and salivation observed in males at • 10 mg/kg and in females at • 25 mg/kg. Abnormal respiratory sounds were noted in both sexes at • 10 mg/kg/day. The NOAEL was 5 mg/kg/day.

Chronic (26-week) administration of YM905 at doses of 60 mg/kg/day in female and 100 mg/kg/day in male rats exceeded the MTD. There were 5 deaths in females after week 15 in the 30 mg/kg/day group; 6 deaths in the 60 mg/kg/day group and a further 9 deaths after the dosage was reduced to 45 mg/kg/day. One male treated with 100 mg/kg/day died during week 8. There were no significant gross or histopathological changes that would account for these deaths.

Doses of 30 mg/kg/day and greater resulted in low body weight gains and signs of distress immediately following dosing. As in the 4-week study, animals treated with • 10 mg/kg evinced mydriasis, salivation and abnormal respiration following dosing. Changes in various organ weights, i.e., adrenals, thymus, liver, kidney and spleen, were not associated with any histopathology. The slightly higher liver weights in males and changes in transaminase activity suggest a potential effect upon liver metabolism. Changes in urinary output, electrolyte levels and relative weight also suggest that the kidneys may be a sensitive target tissue. Changes in clinical pathology and organ weights had either reversed or improved by the end of a 10-week recovery period. Follicular degeneration of the ovaries and/or uterine atrophy were evident in 2 females dosed at 30 mg/kg/day and in 13

animals given 60/45 mg/kg/day. The NOAEL is considered to be 3 mg/kg/day, a dose resulting in YM905 AUC<sub>24</sub> values of 19 and 48 ng.h/ml and BY-348C (M1 active metabolite) AUC<sub>24</sub> values of 5042 and 8928 ng.h/ml in males and females, respectively.

Dogs: Administration of solifenacin to dogs at 1, 3, 10 or 30 mg/kg/day for 4 weeks or at 3, 6, 12 or 25/18 mg/kg/day for 13 weeks resulted in excessive salivation and vomiting at the highest doses. Tremors, ataxia, convulsions, prostration and lower body weights were also seen in high-dose animals. Minor changes in erythrocyte parameters were also seen (not evident in the 52-week study). ECG effects were observed in the 4-week and 13-week toxicology studies. In the 4-week study at a dose of 30 mg/kg/day there was a prolongation of the P-wave, the PR interval and the QRS duration and QT interval in some dogs. However, QT interval values in all animals were within the normal range and were usually accompanied by slower heart rates. In the 13-week study the only changes observed were prolongation of the P-wave and PR interval at 25/18 mg/kg/day which produced C<sub>max</sub> of 24-32-fold higher than the proposed maximum human therapeutic dose. Organ weight changes and microscopic lesions were observed in the kidney and thymus in the 4-week study but not in the 13-week (or 52-week) study. The NOAEL was 3 mg/kg in the 4-week study and 12 mg/kg in the 13-week study.

Chronic (52-week) administration of YM905 at doses up to 20 mg/kg/day to dogs was in general, clinically unremarkable. Group mean heart rates for males receiving 12 or 20 mg/kg/day and females receiving 6, 12 or 20 mg/kg/day were lower in comparison with the predose values on most occasions during the dosing period. The largest reduction was observed in males dosed at 20 mg/kg/day during week 38. Changes in the P, PR, QRS, ST and QT intervals were in general related to the concurrent fluctuations in the heart rates and were generally within the expected ranges.

There were also dose-related changes in mean absolute and relative spleen weights. In males, spleen weights decreased with increasing dose, statistically and biologically significant only at 20 mg/kg/day. In females, there was a decrease in the mean absolute and relative spleen weights at doses • 12 mg/kg/day, however, at 20 mg/kg/day, spleen weights were significantly higher relative to controls. Females dosed at 20 mg/kg/day also presented with an increased incidence and degree of perivascular lymphoid accumulation in the urinary bladders. The higher spleen weights could be secondary to this accumulation of urinary lymphoid cells.

The NOAEL was 6 mg/kg/day in males and 3 mg/kg/day in females. These doses resulted in mean AUC<sub>24</sub> values between 395-497 ng.h/ml in males and 47-165 ng.h/ml in females. AUC levels actually decreased between weeks 26 and 52.

#### **General Toxicology Issues:**

Animal deaths observed following administration of solifenacin were usually accompanied by signs of CNS toxicity, i.e., underactivity, ataxia, tremors, convulsions, prostration, hunched posture, piloerection and abnormal respiration. The most common clinical signs were mydriasis and salivation.

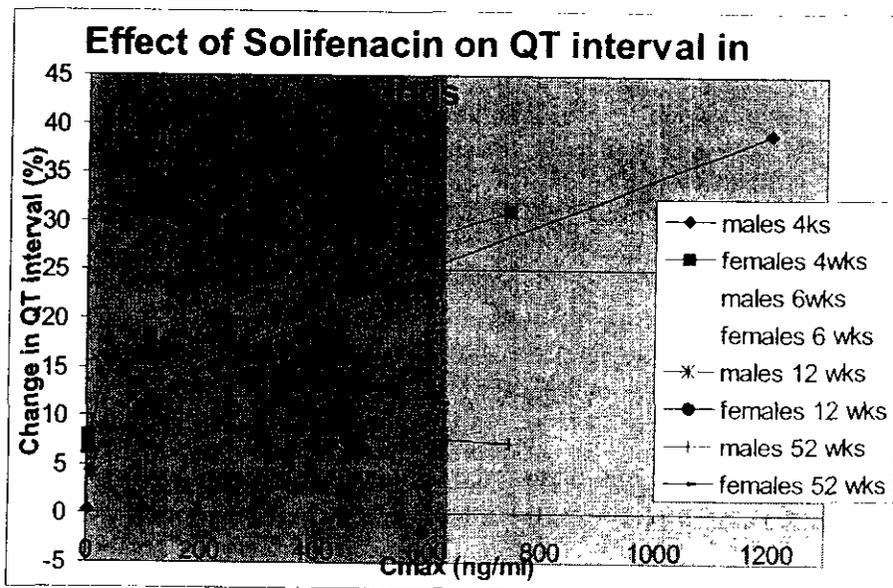
Cardiac Effects in Dogs: ECG effects consisting of prolongation of the P-wave, PR interval, QRS duration and QT interval were observed in some dogs treated at 30 mg/kg/day for 4 weeks. However, QT interval values in all animals were within the normal range, accompanied by decreases

in heart rate between 4 and 8 beats/minute, and C<sub>max</sub> was 54 to 87-fold higher than at the therapeutic dose of 10 mg/day in humans. In the 13-week study the only changes observed were prolongation of the P-wave and PR interval at 25/18 mg/kg/day which produced C<sub>max</sub> of 24-32-fold higher than the therapeutic dose. There were no significant cardiovascular or ECG effects observed in the 52-week toxicity study at doses up to 20 mg/kg/day and C<sub>max</sub> values of 45 to 54-fold higher than the proposed therapeutic dose.

Solifenacin plasma levels (ng/ml) and %QTc change compared to control in dogs 4 hours post oral administration:

Dosing Duration	Males		Females	
	ng/ml	% QTc change	ng/ml	% QTc change
4 weeks	<3	0.4	<3	6.6
	8.3	4.4	3.7	8
	133.5	13.7	112.2	18.5
	1202.9	39.0	744.3	31.1
6 weeks	439.6	6.5	593	13.8
12 weeks	439.6	7.1	593	-1.8
52 weeks	11	3.8	5	6.5
	42	5.3	65	7.3
	400	8	190	11.7
	744	7.2	624	11.4

C<sub>max</sub> ranges in clinical studies following multiple doses of 10 mg/day: 62.6 ± 25.8 ng/ml in males and 63.2 ± 20.6 ng/ml in females



Following observations of ventricular repolarization effects in dogs, Yamanouchi performed *in vitro* studies to assess the effect of YM905 on action potential parameters in isolated dog cardiac Purkinje

fibers and the effect of YM905 on HERG-mediated potassium current. In Purkinje fibers, YM905 showed no increase but rather a slight decrease in the duration of the action potential at concentrations up to 0.3  $\mu\text{M}$  with stimulation of 0.5 and 1.0 Hz. The Sponsor attributed this decrease to potential effects on sodium channels. CHO cells with stable expression of the HERG-K channel showed evidence of inhibition of the potassium current with an approximate  $\text{IC}_{50}$  of 0.3  $\mu\text{M}$ . This concentration is ~80-fold higher than the mean steady state maximum unbound plasma concentration of YM905 in humans at the therapeutic dose of 10 mg per day.

In pentobarbital anesthetized dogs a single intravenously administered dose of YM905 produced a dose-dependent prolongation of the PR interval at 1 and 3 mg/kg and complete AV blockage at 10 mg/kg. However, there was no effect on QTc intervals at doses up to 10 mg/kg. In a 2-week repeat i.v. study performed at doses up to 3 mg/kg/day, there were no significant effects on ECG parameters.

**Toxicology conclusions:** Solifenacin does not readily cross the blood brain barrier, however at the highest doses tested in mice, rats and dogs, morbidity appeared to be the result of CNS toxicity. The brain is the most sensitive organ. The CNS effects are consistent with class effects noted in other muscarinic receptor antagonists.

In rats, changes in urinary output, electrolyte levels and relative weight also suggest that the kidneys may be a sensitive target tissue.

Cardio-toxicity was observed in dogs as evinced by prolongation of the P-wave, PR interval, QRS duration and QT interval. The potential for solifenacin to block potassium channels was confirmed *in vitro* in the HERG assay.

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## Histopathology Inventory for IND # 58,135:

Study:	R905-TX-0	R905-TX-005	R905-TX-008
Species:	CD-1 Mice	CD Rat	Beagle Dog
Duration:	26 weeks	26 weeks	52 weeks
Adrenals	X*	X*	X*
Aorta	X		X
Bone Marrow smear	X		
Bone (femur)	X	X*	X*
Brain	X*	X*	X*
Cecum	X		
Cervix			
Colon	X		
Duodenum	X		
Epididymis	X		
Esophagus	X		
Eye	X		X
Fallopian tube			
Gall bladder	X		
Gross lesions	X	X	X
Harderian gland	X		X
Heart	X*	X*	X*
Ileum	X		X
Injection site			
Jejunum	X		X
Kidneys	X*	X*	X*
Lachrymal gland	X		X
Larynx			
Liver	X*	X*	X*
Lungs	X*	X*	X*
Lymph nodes, cervical			
Lymph nodes mandibular	X		X
Lymph nodes, mesenteric	X		X
Mammary Gland		X*	X
Nasal cavity			
Optic nerves	X		X
Ovaries	X*	X	X*
Pancreas	X		X
Parathyroid	X		X
Peripheral nerve			
Pharynx			
Pituitary	X*	*	X*
Prostate	X*	*	X*
Rectum	X		X
Salivary gland	X*	*	X*
Sciatic nerve	X		X

Seminal vesicles	X*	*	
Skeletal muscle	X		X
Skin	X		
Spinal cord	X	X	X
Spleen	X*	*	X*
Sternum			X
Stomach	X	X	X
Testes	X*	*	X*
Thymus	X*	*	X*
Thyroid	X*	X*	X*
Tongue	X		X
Trachea	X		X
Urinary bladder	X		X
Uterus	X*	X*	X*
Vagina	X	X	X
Zymbal gland			
Standard List			
Other	Parotid		

X, histopathology performed

\*, organ weight obtained

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## V. GENETIC TOXICOLOGY:

**Genetic toxicology summary:** The standard battery of mutagenicity and genotoxicity studies were performed with YM905 (IND 58,135 (N000), Review No. 1, 8/16/99):

Assay	Study No.	Dose/Concentration	Results
Standard Ames Assay	905-TX-017	5 to 1250 µg/plate	negative
Chromosome Aberrations in Human Lymphocytes	905-TX-018	8.6 - 4806 µg/ml	negative
Micronucleus Test in Rats	905-TX-019	250, 500, 1000 mg/kg	negative

**Genetic toxicology conclusions:** Based on a valid standard test battery consisting of bacterial mutagenicity, chromosome aberrations (in vitro) and rat micronucleus assay, YM905 shows no evidence of genotoxicity.

**Labeling recommendations:** The Sponsor's proposed labeling is acceptable with the following modification:

Solifenacin succinate was not mutagenic in the *in vitro* *Salmonella typhimurium* or *Escherichia coli* microbial mutagenicity test

← chromosomal aberration test in human peripheral blood lymphocytes with or without metabolic activation or in the *in vivo* ← micronucleus test in rats.

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**VI. CARCINOGENICITY:**

**Study title: YM905 - Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice for 104 weeks**

**Key study findings:** preliminary findings were negative

**Study number:** R905-TX-023 (20021105)

**Volume #, and page #:** EDR

**Conducting laboratory and location:**

**Date of study initiation:** August 16, 1999

**GLP compliance:** Yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** YM905 Batch No. K9059804, —

**CAC concurrence:** No (See Appendix 1)

**Study Type:** 2 year bioassay (104 weeks)

**Species/strain:** CD-1 mice

**Number/sex/group; age at start of study:** 70/sex/group

**Animal housing:** one animal/cage randomly distributed in study room with 12 hour light cycles; mice were identified by tail tattoo and cages were color coded by treatment group

**Formulation/vehicle:** 0.5% aqueous methylcellulose

**Drug stability/homogeneity:** documented in study Appendix 1

**Methods:**

Doses: 0, 10, 30, 100 or 200 mg/kg/day

Basis of dose selection: Doses were selected based on mortalities and adverse clinical behavior in the 13-week dose-range finding study where mice were administered doses of 30, 100, 250 or 400 mg/kg/day (Report No. VVE007/983657). Mortalities were observed in both sexes at 400 mg/kg/day, with one death (female) and overt behavioral toxicity (male) at 250 mg/kg.

Restriction paradigm for dietary restriction studies: none

Route of administration: oral gavage, 10 ml/kg

Frequency of drug administration: once daily

Dual controls employed: no

Interim sacrifices: none

Satellite PK or special study group(s): 20/sex/YM905 group (treated for 104 weeks)

Deviations from original study protocol: There were no significant deviations from protocol that would adversely impact the validity of the assay.

Statistical methods: Data was independently reviewed by CDER biostatistician. (See Appendix 2)

**Observations and times:**

Clinical signs: Animals and cages were inspected at least twice daily for evidence of reaction to treatment or morbidity. More detailed examinations including palpitations were performed weekly. Animals judged to be *in extremis* were euthanized.

**Body weights:** Each animal was weighed before treatment commenced, at weekly intervals (with the exception of week 15), and prior to necropsy. Group mean weight changes were calculated from the weight changes of individual animals surviving the specified period.

**Food consumption:** recorded weekly

**Hematology:** blood smears from tail veins were prepared during week 104 and retained pending requested examination

**Clinical chemistry:** na

**Organ weights:** na

**Gross pathology:** Animals were euthanized by carbon dioxide inhalation. Full necropsies performed on all animals including the satellite animals at the time of scheduled or unscheduled termination. The necropsy procedure included a review of the history of each animal and a detailed examination of the external features and orifices, the neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and their viscera.

**Histopathology:** The following tissues were collected from all main study mice:

adrenal (2)	kidney (2)	sciatic nerve
aorta	lacrimal gland	seminal vesicle (2)
brain	liver	skeletal muscle (thigh)
cecum	lung w/bronchi	skin
cervix	lymph nodes	spinal cord (3)
colon	- mandibular	spleen
duodenum	- mesenteric	sternum w/marrow
epididymis (2)	mammary gland w/skin	stomach
esophagus	masses w/lymph nodes	testes (2)
eye w/optic nerve (2)	ovary (2)	thymus
femur w/marrow	pancreas	thyroid w/parathyroid (2)
gall bladder	pituitary	tongue
gross lesions	prostate	trachea
Harderian gland (2)	rectum	urinary bladder
head (nasal cavity x4)	salivary glands	uterus
heart	- mandibular (2)	vagina
ileum	- parotid	
jejunum		

All tissues from each main study rat in the control and high-dose groups and from each main study rat that died or was sacrificed at an unscheduled interval from the low- and mid-dose groups were examined microscopically. In addition, the cecum, colon, duodenum, head, ileum, jejunum, ovaries, rectum, stomach and thymus were all examined from all animals. Macroscopic lesions and/or masses were examined microscopically from all animals. Suspected target organs noted necropsy or at the high dose were examined microscopically from each rat. Histopathological peer review was performed by

**Toxicokinetics:** Blood samples from the retro-orbital sinus were obtained from unfasted satellite animals (2/sex/time point) during week 36. Samples were collected at 1, 2, 4, 8 and 24 hours post dosing and analyzed using HPLC.

## Results:

**Mortality (Figures M1 and M2):** Mortality was increased in both males and females receiving 100 mg/kg/day and in males receiving 200 mg/kg/day. The increases in mortalities at the higher dose

levels were apparent during the first year of the study. After week 53, mortality in each dose group was almost comparable to the lower doses.

Dose (mg/kg/day)	Males					Females				
	0	10	30	100	200	0	10	30	100	200
No. animals/sex/group	70	70	70	70	70	70	70	70	70	70
Unscheduled Deaths:										
Weeks 1 - 13	0	1	1	3	7	0	0	1	14	5
14 - 26	0	1	4	3	8	0	1	4	8	6
27 - 52	2	2	4	7	10	3	4	5	8	7
53 - 78	13	11	12	12	10	13	6	17	7	9
79 - 91	5	11	9	7	7	7	13	6	3	4
92 - 104	12	4	10	12	2	16	9	13	9	10
Total Mortality	32	30	40	44	44	39	33	46	49	41
% Mortality	46	43	57	63	63	56	47	66	70	59
Total Survival	48	40	30	26	26	31	37	24	21	29
% Survival	54	57	43	37	37	44	53	34	30	41

**Clinical signs:** Adverse signs related to treatment occurred primarily in animals that died or were killed prematurely during the treatment period. These signs comprised distension of the abdomen, firm ventral abdomen and irregularities of respiration, including rales and slow, deep or gasping respiration. These signs were also observed at a lower frequency at the lower dosages and in the controls, particularly during the early part of the treatment period. Other signs included piloerection and, less frequently, hunched posture and underactivity.

**Body weights (Figures M3 and M4):** Bodyweight gain was unaffected in males and females dosed at 10 mg/kg/day and in males at 30 mg/kg/day. Overall, bodyweight gain reductions in males receiving 100 or 200 mg/kg were 19 and 20%, respectively. Those in females receiving 30, 100 or 200 mg/kg/day were 17, 23 and 36%, respectively. Statistically significant decreases in high-dose animals were observed beginning between weeks 4-5 in males and weeks 10-12 in females.

**Food consumption:** Beginning week 20, overall food consumption was reduced by approximately 5% in males and females dosed at 200 mg/kg/day. Overall food intake in animals receiving 10, 30 or 100 mg/kg/day was unaffected by treatment.

Figure M1: Percentage survival versus period of treatment - Males

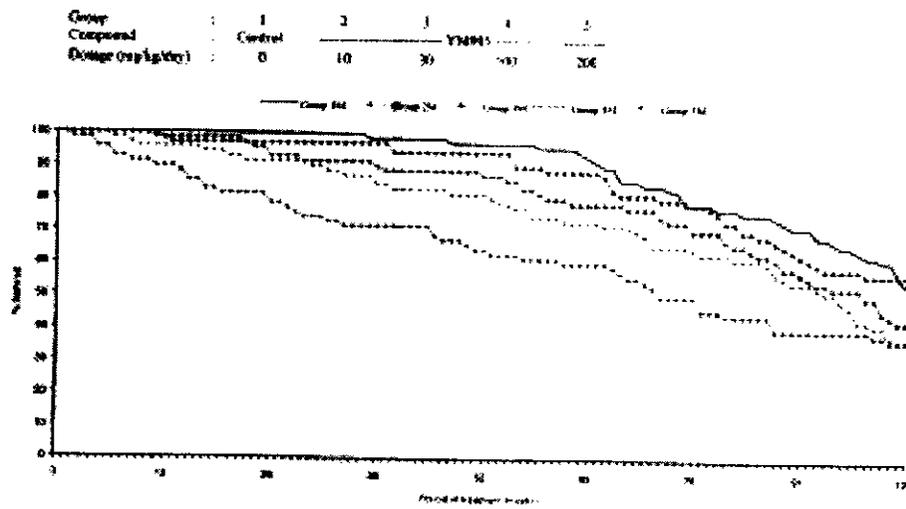


Figure M2: Percentage survival versus period of treatment - Females

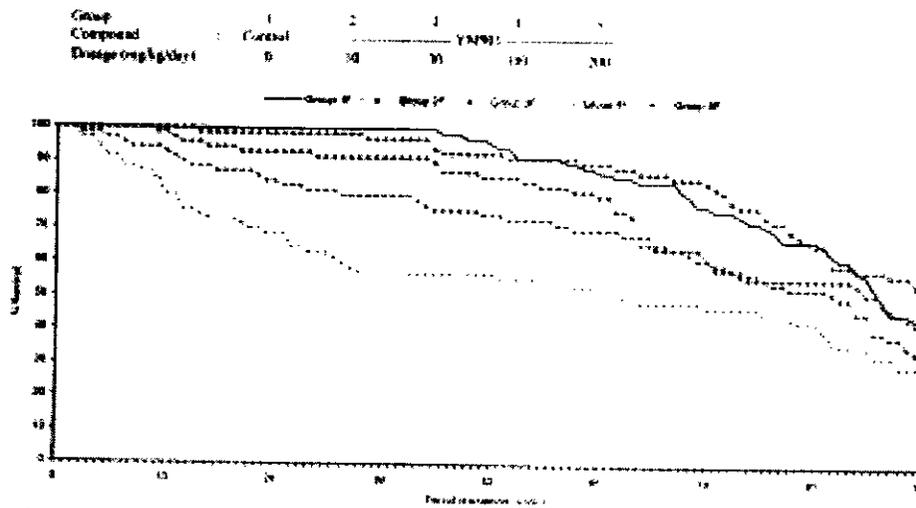


Figure M3: Group mean body weight versus period of treatment - Males

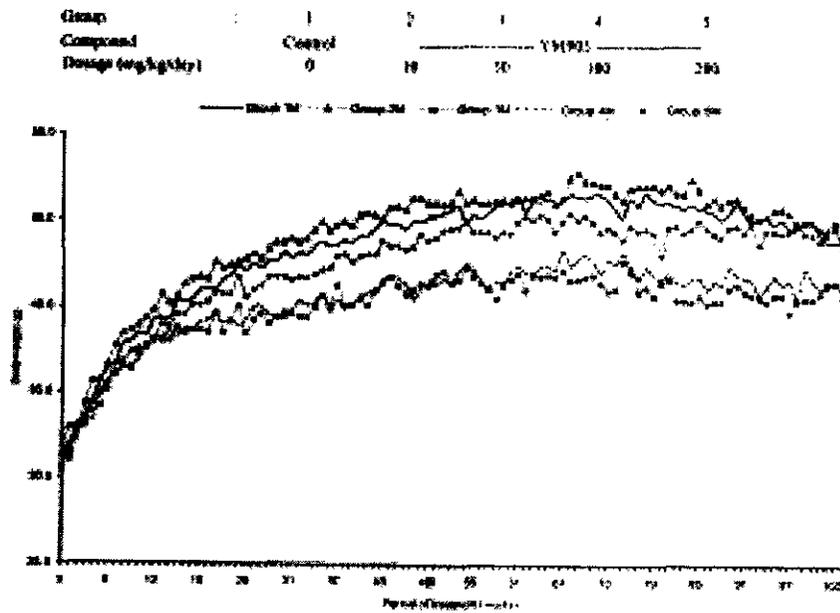
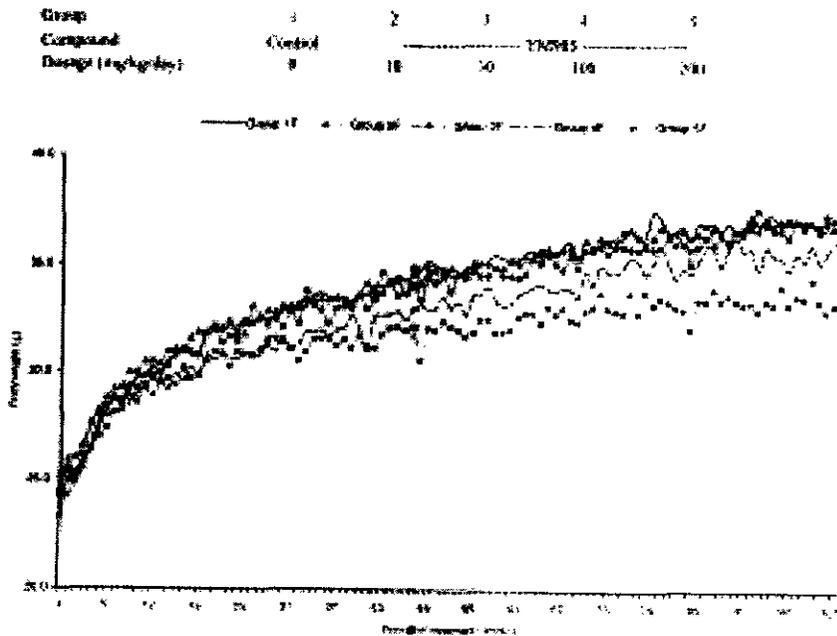


Figure M4: Group mean body weight versus period of treatment - Females



Toxicokinetics: Mean YM905 C<sub>max</sub> and AUC values estimated up to 24 hours post-dose during Week 36, derived from a 5-point profile, are summarized below. Plasma concentrations are expressed in terms of YM905 free base.

Dose (mg/kg/day)	C <sub>max</sub> (ng/ml)		AUC <sub>24</sub> (ng.h/ml)	
	Males	Females	Males	Females
10	83.63	107.16	268	343
30	259.59	215.17	1245	2051
100	509.91	489.39	5184	5053
200	750.34	904.23	6220	11,133

Gross pathology: The primary finding in animals killed or found dead during the treatment period was GI tract (e.g., esophagus, stomach, duodenum, ileum, jejunum, cecum, colon and/or rectum) distension with and without abdominal distension or abnormal (gaseous) GI tract contents. Distension of the stomach, duodenum, ileum, jejunum and/or cecum were common. Distension of the esophagus was observed in only one female dosed at 200 mg/kg, while distension of the rectum was observed in only two males dosed at 100 mg/kg and seven males at 200 mg/kg.

Distended gall bladders were also observed, occurring earlier in females dosed at 100 and 200 mg/kg. At the end of the study, there were no significant differences in the total number of animals with distended gall bladders between control and treated animals.

	Males					Females				
	0	10	30	100	200	0	10	30	100	200
Weeks 1-13: (# examined)	0	1	1	3	7	0	0	1	14	5
Abdominal distension	0	0	0	1	4	0	0	0	0	0
Abnormal GI contents	0	0	0	1	6	0	0	0	0	0
Gall bladder distension	0	0	0	1	0	0	0	0	4	1
Weeks 14-26: (# examined)	0	1	4	3	8	0	1	4	8	5
Abdominal distension	0	0	3	0	5	0	0	2	3	2
Abnormal GI contents	0	0	2	2	8	0	0	3	7	3
Gall bladder distension	0	0	1	0	0	0	0	1	1	1
Weeks 27-52: (# examined)	2	2	4	7	10	3	4	5	8	7
Abdominal distension	1	0	1	3	6	0	0	2	5	2
Abnormal GI contents	1	3	6	5	5	3	1	2	0	0
Gall bladder distension	2	3	2	4	3	4	2	7	2	2
Weeks 53-78: (# examined)	13	11	12	12	10	13	6	17	7	9
Abdominal distension	4	0	1	5	1	4	2	6	2	3

Abnormal GI contents	0	0	2	4	8	0	1	1	5	0
Gall bladder distension	1	0	0	2	1	1	0	0	1	1
Weeks 79-91: (# examined)	5	11	9	7	7	7	13	6	3	4
Abdominal distension	1	3	3	2	2	5	5	1	0	0
Abnormal GI contents	1	3	1	5	2	2	3	1	0	0
Gall bladder distension	2	1	9	7	7	7	13	6	3	4
Weeks 92-104: (# examined)	12	4	10	12	2	16	9	13	9	10
Abdominal distension	2	2	1	1	0	9	4	5	4	3
Abnormal GI contents	1	0	2	1	1	0	1	2	0	1
Gall bladder distension	0	0	0	3	2	4	4	4	5	5

Gross observations in animals surviving for the entire 104-week period of treatment were of the types normally encountered in aged CD-1 mice and occurred at expected in-house frequencies.

**Histopathology:**

Non-neoplastic: In addition to the GI and gall bladder findings in animals killed or dying during the treatment period, the predominant treatment-related non-neoplastic histology findings were serous fluid with and without inflammatory cells in the nasal passages. Epithelial debris, sloughing and necrosis was also observed in the nasal passages, primarily only in the 100 and 200 mg/kg groups. These effects were only seen in YM905 treated animals through week 52. Thereafter, similar changes were observed in the nasal passages of control and treated animals in a dose-related manner.

	Males					Females				
	0	10	30	10	20	0	10	30	10	20
Weeks 1-13: (# examined)	0	1	1	3	7	0	0	1	14	5
Serous fluid in nasal passage	0	0	0	1	7	0	0	1	12	4
Inflammatory cells in nasal passage	0	0	0	1	3	0	0	1	6	3
Epithelial debris in nasal passage	0	0	0	0	3	0	0	0	3	4
Thymic atrophy	0	1	0	1	3	0	0	1	7	3
Weeks 14-26: (# examined)	0	1	4	3	8	0	1	4	8	6
Serous fluid in nasal passage	0	0	2	3	8	0	0	3	8	4
Inflammatory cells in nasal passage	0	0	2	3	3	0	0	2	4	2
Epithelial debris in nasal passage	0	0	0	0	2	0	0	0	4	2
Weeks 27-52: (# examined)	2	2	4	7	10	3	4	5	8	7
Serous fluid in nasal passage	0	1	2	7	7	1	1	2	7	6
Inflammatory cells in nasal passage	0	0	2	6	8	0	1	1	4	4
Epithelial debris in nasal passage	0	0	0	2	1	0	0	0	1	1

Epithelial necrosis/sloughing	0	0	0	0	1	0	0	0	0	1
Thymic lymphoid atrophy	0	0	1	4	3	0	1	2	4	2
Weeks 53-78: (# examined)	13	11	12	12	10	13	6	17	7	9
Serous fluid in nasal passage	0	3	5	8	5	0	0	7	2	7
Inflammatory cells in nasal passage	1	4	4	8	7	1	1	3	2	3
Epithelial debris in nasal passage	0	0	0	1	0	0	0	0	0	0
Weeks 79-91: (# examined)	5	11	9	7	7	7	13	6	3	4
Serous fluid in nasal passage	2	4	1	7	7	1	5	1	0	3
Inflammatory cells in nasal passage	1	1	1	4	6	0	0	0	0	2
Weeks 92-104: (# examined)	12	4	10	12	2	16	9	13	9	10
Serous fluid in nasal passage	4	1	3	6	2	2	1	4	5	10
Inflammatory cells in nasal passage	2	1	2	4	2	4	0	2	4	8
Epithelial debris in nasal passage	0	0	0	0	0	0	0	0	1	0
Epithelial necrosis/sloughing	0	0	1	1	0	0	0	0	0	0
Terminal Sacrifices Wk 104: (# examined)	38	40	30	26	26	31	37	24	21	29
Serous fluid in nasal passage	15	16	16	11	20	2	8	5	12	23
Inflammatory cells in nasal passage	7	5	4	7	16	1	2	2	9	27

Thymic atrophy (purportedly due to stress) was reported in animals killed or dying only during the treatment periods between weeks 1-13 and weeks 27-52:

	Males					Females				
	0	10	30	100	200	0	10	30	100	200
Thymic lymphoid atrophy:										
Weeks 1-13:	0	1/1	0/1	1/3	3/7	0	0	1/1	7/14	3/5
Weeks 27-52:	0/2	0/2	1/4	4/7	3/10	0/3	1/4	2/5	4/8	2/7

Major factors (neoplastic and non-neoplastic) contributing to premature deaths were reported as follows:

	Males					Females				
	0	10	30	10	20	0	10	30	10	20
Weeks 1-13: (# examined)	0	1	1	3	7	0	0	1	14	5
Respiratory Disease	-	0	0	1	6	-	-	1	9	4
Skin Disease	-	1	1	0	0	-	-	0	0	0
Weeks 14-26: (# examined)	0	1	1	3	8	0	1	2	8	5
Respiratory Disease	-	0	2	3	8	-	0	3	8	4
Skin Disease	-	0	0	0	0	-	1	1	0	0
<b>Hemopoietic neoplasia</b>	-	1	1	0	0	-	0	0	0	0

<b>Weeks 27-52: (# examined)</b>	2	2	4	7	10	3	4	5	8	7
Respiratory Disease	0	0	2	5	6	0	0	2	7	4
Skin Disease	0	0	0	0	0	1	0	3	0	1
Genito-urinary disease	0	0	0	1	0	0	3	0	0	0
Gastro-intestinal disease	0	0	0	0	2	0	0	0	0	0
<b>Hemopoietic neoplasia</b>	2	1	1	1	2	2	1	0	1	2
<b>Weeks 53-78: (# examined)</b>	13	11	12	12	10	13	6	17	7	9
Respiratory Disease	0	2	2	5	2	0	1	3	1	1
<b>Pulmonary neoplasia</b>	0	1	1	0	0	1	0	0	0	0
Skin Disease	2	0	1	1	0	2	0	1	2	0
Genito-urinary disease	1	1	2	0	0	3	3	5	1	3
Gastro-intestinal disease	0	1	2	1	2	0	1	0	0	0
CNS disease	0	0	0	0	0	0	0	0	0	1
<b>Hemopoietic neoplasia</b>	8	3	1	2	1	4	1	4	2	3
<b>Hepatic neoplasia</b>	0	0	1	0	0	0	0	0	0	0
<b>Hardarian Gland neoplasia</b>	1	0	1	0	1	0	0	0	0	0
<b>Salivary Gland neoplasia</b>	0	0	0	1	0	0	0	0	0	0
<b>Muscle neoplasia</b>	0	0	1	0	0	0	0	0	0	0
<b>Subcutaneous neoplasia</b>	0	0	0	0	0	0	0	1	0	0
<b>Weeks 79-91: (# examined)</b>	5	11	9	7	7	7	13	6	3	4
Respiratory Disease	1	0	0	0	2	0	1	0	0	0
<b>Pulmonary neoplasia</b>	0	0	2	0	0	0	1	0	0	0
Skin Disease	1	0	0	0	0	0	0	1	0	0
<b>Skin neoplasia</b>	0	0	0	0	0	1	0	0	0	0
Genito-urinary disease	0	1	2	2	0	3	4	3	0	0
<b>Genito-urinary neoplasia</b>	0	0	0	0	0	0	0	0	1	0
Gastro-intestinal disease	0	0	0	1	0	0	0	0	0	0
<b>Gastro-intestinal neoplasia</b>	0	0	0	0	0	0	1	0	0	0
Hemorrhage	0	0	0	1	0	0	0	0	0	0
<b>Hemopoietic neoplasia</b>	0	6	4	2	3	2	3	2	2	2
<b>Vascular neoplasia</b>	1	1	0	0	0	0	3	0	0	0
<b>Hepatic neoplasia</b>	1	2	0	0	0	0	0	0	0	0
<b>Abdominal neoplasia</b>	1	0	0	0	0	0	0	0	0	0
<b>Weeks 92-104: (# examined)</b>	12	4	10	12	2	16	9	13	9	10
Respiratory Disease	0	1	0	0	0	0	0	0	0	0
<b>Pulmonary neoplasia</b>	2	0	0	1	0	1	0	0	0	0
Skin Disease	1	0	1	0	0	2	1	0	1	1
<b>Skin neoplasia</b>	0	0	0	0	0	1	0	0	0	0
Genito-urinary disease	0	0	2	2	0	5	4	2	4	1
<b>Genito-urinary neoplasia</b>	0	0	0	0	0	0	0	1	1	1
Gastro-intestinal disease	0	0	1	2	0	0	0	0	0	0
<b>Gastro-intestinal neoplasia</b>	0	0	0	1	0	0	0	0	0	1
<b>Hemopoietic neoplasia</b>	3	2	3	5	1	5	2	6	1	4
<b>Vascular neoplasia</b>	1	0	0	0	0	0	0	2	0	0

<b>Hepatic neoplasia</b>	2	0	0	0	0	0	0	0	0	0
<b>Subcutaneous neoplasia</b>	1	0	0	0	0	1	0	2	0	0
<b>Muscle neoplasia</b>	0	0	0	0	0	0	0	0	0	1
<b>Mammary neoplasia</b>	0	0	0	0	0	0	1	0	1	0
<b>Hardarian Gland neoplasia</b>	0	0	1	0	0	0	0	0	0	0
<b>Adrenal neoplasia</b>	0	1	0	0	0	0	0	0	0	0

The only finding which appears to be treatment/dose-related is the rate of deaths attributed to respiratory disease during the first 52 weeks of the study.

Neoplastic: Tumor incidence was unaffected by treatment and were of the types normally encountered CD-1 mice at

Summary of neoplastic findings for all animals distributed by organ system (70 mice/group):

	Males					Females				
	0	10	30	100	200	0	10	30	100	200
<b>Adrenals:</b>										
Cortical Adenoma	-	-	-	1	1	-	-	-	-	-
Phaeochromocytoma	7	6	3	8	2	1	-	-	-	1
Malignant Phaeochromocytoma	0	1	-	-	-	-	-	-	-	-
<b>Bone:</b>										
Osteoma	-	1	-	1	-	-	1	1	-	-
Osteosarcoma	-	-	-	-	-	-	1	-	-	-
Chondroma	-	-	-	-	-	-	1	-	-	1
<b>Brain:</b>										
Meningioma	-	-	-	-	-	-	1	-	-	-
<b>Harderian Glands:</b>										
Adenoma	7	5	7	2	2	7	2	-	1	2
Adenocarcinoma	-	-	-	-	-	-	2	-	1	2
<b>Hemopoietic:</b>										
Malignant Lymphoma	13	12	7	9	5	13	12	13	6	9
Histoicytic sarcoma	2	6	5	-	3	3	4	6	3	4
Myeloid Leukemia	1	-	-	2	-	-	-	-	-	-
<b>GI Tract:</b>										
Cecum - Adenocarcinoma	1	-	-	-	-	-	-	-	-	-
Colon - Adenoma	-	-	1	1	-	-	-	-	-	-
Adenocarcinoma	-	-	-	1	1	-	-	1	-	-
Duodenum - Adenoma	-	-	1	-	-	-	-	-	-	-
Adenocarcinoma	3	3	4	-	-	-	-	-	-	-
Jejunum - Adenocarcinoma	-	-	-	-	-	-	-	-	-	1
Stomach - Squamous cell papilloma	-	1	-	-	-	-	-	-	-	-
Adenoma	-	-	1	-	-	-	1	-	-	-
Tongue - Adenoma	1	-	-	-	-	-	-	-	-	-
Rectum - Squamous cell papilloma	-	-	-	-	-	-	1	-	-	-
	Males					Females				
	0	10	30	100	200	0	10	30	100	200

<b>Kidneys:</b>										
Adenoma	1	-	-	1	-	-	-	-	-	-
<b>Liver:</b>										
Hepatocellular adenoma	6	9	10	4	7	2	3	2	-	-
Hepatocellular carcinoma	4	7	4	1	1	-	-	-	-	-
Hemangioma	1	-	-	-	-	-	-	-	-	-
Hemangiosarcoma	1	4	2	-	-	-	-	2	-	-
Cholangioma (M) / Ito cell tumor (F)	-	1	-	-	-	-	-	1	-	-
<b>Lungs:</b>										
Bronchioloalveolar Adenoma	22	16	17	20	11	15	15	11	7	5
Bronchioloalveolar Adneocarcinoma	6	5	8	2	1	4	4	-	-	1
<b>Mammary:</b>										
Adenocarcinoma	-	-	-	-	-	1	1	2	1	-
<b>Ovaries:</b>										
Schwannoma	-	-	-	-	-	-	1	-	-	-
Granulosa Cell Tumor	-	-	-	-	-	1	2	1	1	0
Luteoma	-	-	-	-	-	1	3	3	3	1
Cystadenoma	-	-	-	-	-	-	-	1	-	-
Leiomyoma	-	-	-	-	-	-	-	-	-	1
Sertoli cell adenoma	-	-	-	-	-	1	-	-	-	-
Tubulostromal adenoma	-	-	-	-	-	-	-	1	1	0
Undifferentiated stromal tumor	-	-	-	-	-	-	-	0	1	0
<b>Pancreas:</b>										
Islet cell adenoma	1	1	-	2	-	-	1	-	-	-
<b>Pituitary:</b>										
Adenoma	-	-	-	1	-	3	3	-	-	-
<b>Salivary Gland:</b>										
Squamous cell carcinoma	-	-	-	1	-	-	-	-	-	-
<b>Spleen:</b>										
Hemangioma	-	-	-	-	-	-	1	-	-	-
Hemangiosarcoma	-	-	-	-	-	1	-	-	-	-
<b>Testes:</b>										
Interstitial (Leydig) cell adenoma	5	1	4	3	-	-	-	-	-	-
Adenoma	-	-	-	-	1	-	-	-	-	-
<b>Thymus:</b>										
Thyoma (Lymphoid)	-	1	1	-	-	6	4	9	4	2
Thyoma (Epithelial)	-	-	-	-	-	-	-	1	-	-
<b>Thyroids:</b>										
Follicular cell adenoma	1	-	-	1	-	-	-	-	-	-

	Males					Females				
	0	10	30	100	200	0	10	30	100	200
<b>Uterine / Cervix:</b>										
Leiomyoma						3	1	-	2	-
Leiomyosarcoma						1	1	1	1	1
Malignant schwannoma						-	-	-	1	-
Endometrial Polyp						4	9	6	3	2
Endometrial adenoma						-	2	-	-	-
Endometrial stromal cell sarcoma						-	-	2	-	2
Histiocytic sarcoma						-	1	1	-	-
<b>Vagina:</b>										
Histiocytic sarcoma						2	-	-	-	-
<b>Hemangiomas:</b>										
Mesenteric lymph node	1	-	-	-	-	-	-	-	-	-
Prostate	-	1	-	-	-	-	-	-	-	-
Spleen	2	-	-	1	-	-	-	-	-	-
Uterus						1	1	2	-	1
<b>Hemangiosarcoma:</b>										
Uterus						-	2	-	-	-

**Summary of individual study findings:**

Adequacy of the carcinogenicity study and appropriateness of the test model: Due to the significantly lower survival and greater than 10% decrease in body weight gain in the high-dose group, the ExecCAC recommended that the sponsor look at histopathology in the mid- and low-dose animals. The requested raw mouse data has been submitted and reviewed. Final study results including the mid- and low-dose tissues will be resubmitted to the ExecCAC following statistical review. Labeling will be revised if necessary.

Evaluation of tumor findings: Preliminary data did not demonstrate a dose-related or significant increase in the tumor incidence rate when compared with either concurrent controls or in-house historical controls.

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**Study title: YM905 - 104-Week Carcinogenicity Gavage Study with YM905 in Rats**

**Key study findings:** preliminary findings were negative

**Study number:** R905-TX-024 (20020924)

**Volume #, and page #:** EDR

**Conducting laboratory and location:**

**Date of study initiation:** August 31, 1999

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, and % purity:** YM905 Batch No. K9059804. —

**CAC concurrence:** No [See Appendix 1]

**Study Type:** 2 year bioassay (104 weeks)

**Species/strain:** CDF® (F-344) — .BR rats —

**Number/sex/group; age at start of study:** 60/sex/group, 6 weeks of age

**Animal housing:** one animal/cage randomly distributed in study room with 12 hour light cycles; mice were identified by tail tattoo and cages were color coded by treatment group

**Formulation/vehicle:** 0.5% aqueous methylcellulose

**Drug stability/homogeneity:** Dosing solutions were prepared weekly. Stability studies verified that the concentrations ranging between 0.01 to 200 mg/ml were stable for 8 days at room temperature, protected from light. Routine analyses indicated that all formulations were within  $\pm 10\%$  of target with the exception of 15 mg/kg/day formulation prepared for week 14 which contained 112% YM905.

**Methods:**

Doses: 3, 10 or 20 mg/kg/day in males; 3, 7.5 and 15 mg/kg/day in females

Basis of dose selection: Doses were selected based on mortalities and adverse clinical behavior in the 26-week chronic toxicology study where rats were administered doses of 3, 10, 30, 60 or 100 mg/kg/day ( — Report No. VVH 018/993353). For the female rats, the Exec CAC recommended doses of 3, 7.5 and 15 mg/kg/day based on mortalities at 30 and 60 mg/kg/day. For male rats, 20 mg/kg/day was selected as the high dose based on one death at 100 mg/kg and decreases in final body weight relative to control of 21% at 100/75 mg/kg and 11% at 30 mg/kg. 3 mg/kg/day was considered the NOAEL in both sexes.

Restriction paradigm for dietary restriction studies: none

Route of administration: oral gavage, 5 ml/kg

Frequency of drug administration: once daily

Dual controls employed: no

Interim sacrifices: none

Satellite PK or special study group(s): 12/sex/YM905 group (treated for 104 weeks)

Deviations from original study protocol: There were no significant deviations from protocol that would adversely impact the validity of the assay.

Statistical methods: Data was independently reviewed by CDER biostatistician. (See Appendix 2)

**Observations and times:**

Clinical signs: Animals and cages were inspected at least twice daily for morbidity and mortality. More detailed examinations including palpitations were performed weekly.

Animals judged to be *in extremis* were euthanized.

Body weights: Individual body weights were recorded prior to treatment (at randomization) and weekly for the duration of the study.

Food consumption: Food consumption was measured and recorded weekly beginning week 39.

Clinical pathology: Blood samples were collected from the jugular vein from moribund rats prior to sacrifice when possible and following an overnight fast for regularly scheduled terminations. Blood smears were prepared retained pending requested examination.

Organ weights: na

Gross pathology: Animals were euthanized by sodium methohexital and exsanguinated. Full necropsies performed on all animals including the satellite animals at the time of scheduled or unscheduled termination. The necropsy procedure included an examination of the external features and orifices, cranial, thoracic and abdominal cavities and organs/tissues.

Histopathology: The following tissues were collected from all main study rats:

adrenal (2)	lesions	sciatic nerve
aorta	liver	seminal vesicle (2)
brain	lung w/bronchi	skeletal muscle (thigh)
cecum	lymph nodes	skin
cervix	- mandibular	spinal cord (3)
colon	- mesenteric	spleen
duodenum	mammary gland	sternum w/marrow
epididymis (2)	masses w/lymph nodes	stomach
eye w/optic nerve (2)	ovary (2)	testes (2)
femur w/marrow	pancreas	thymus
Harderian gland (2)	pituitary	thyroid w/parathyroid (2)
heart	prostate	tongue
ileum	rectum	trachea
jejunum	salivary glands	urinary bladder
kidney (2)	- mandibular (2)	uterus
lacrimal gland	- parotid	vagina

All tissues from each main study rat in the control and high-dose groups and from each main study rat that died or was sacrificed at an unscheduled interval from the low- and mid-dose groups were examined microscopically. Macroscopic lesions and/or masses were examined microscopically from all animals. Suspected target organs noted necropsy or at the high dose were examined microscopically from each rat. Histopathological peer review was performed by \_\_\_\_\_

Toxicokinetics: During week 52, 3 rats/sex/group per timepoint were bled at 0.5, 2, 4, 8 and 24 hours postdose. By ascending animal number, the toxicokinetic rats were divided into three subsets: Subset I animals were bled at 0.5, Subset II animals were bled at 2 and 8 hours, and Subset III animals were bled at 4 and 24 hours. The first Group 4 female in Subset I was also bled at the 4 hour timepoint, and the second Group 4 female in Subset I was bled at the 24 hour timepoint. Samples were collected from the jugular vein. Frozen plasma samples were sent to \_\_\_\_\_ for analyzed.

Other: Serology Testing: During week 38, blood samples were collected from the first two surviving control rats/sex that exhibited clinical signs consistent with sialodacryoadenitis

(SDA) virus infection. Serology testing was performed for the rat corona virus/SDAV. Samples were sent to \_\_\_\_\_ for analysis.

**Results:**

Mortality (Figures R1 and R2): There was a particularly high mortality rate in females dosed at 7.5 and 15 mg/kg between weeks 23 and 26, the same time SDA (sialodacryoadenitis) viral infections were showing up. This decrease in survival was partly attributed to the SDA infections occurring during this interval. It is hypothesized that the increased mortality of the 15 mg/kg females, and to a lesser extent, the 7.5 mg/kg females, reflects an interaction between the compromised health status of the rats and the effects of YM905. Histologically the cause of death was not identified in many of the rats that died or were sacrificed in extremis.

Dose (mg/kg/day)	Males				Females			
	0	3	10	20	0	3	7.5	15
No. animals/sex/group	60	60	60	60	60	60	60	60
Unscheduled Deaths:								
Weeks 1 - 13	0	1	0	0	0	0	0	0
14 - 26	0	1	5	7	1	3	7	17
27 - 52	0	2	0	1	3	3	4	6
53 - 78	4	3	3	2	2	3	4	4
79 - 91	7	5	8	6	4	5	0	5
92 - 104	14	17	8	9	8	3	12	5
Total Mortality	25	29	24	25	18	17	27	36
% Mortality	42	48	40	42	30	28	45	60
Total Survival	35	31	36	35	42	43	33	24
% Survival	58	52	60	58	70	72	55	40

Clinical signs: A large percentage of the rats from all groups were affected by SDA virus, as noted by body weight decreases and clinical signs (swollen ventral-cervical area, clear or red discharge from eyes, crusting around eyes). SDA virus infections were confirmed serologically and histologically.

Body weights (Figures R3 and R4): Mean body weights were significantly lower for both the high-dose males and females over the course of the entire study.

Dose (mg/kg/day)	Males			Females		
	3	10	20	3	7.5	15
Mean Body Weights relative to Controls (%):						
Week 53	101	98	93	99	100	97
Week 105	98	97	89	100	98	92

A transient loss in mean body weight in all groups was also noted during the period of SDA infection (weeks 23-25).

Figure R1: Percentage survival versus period of treatment - Male Rats

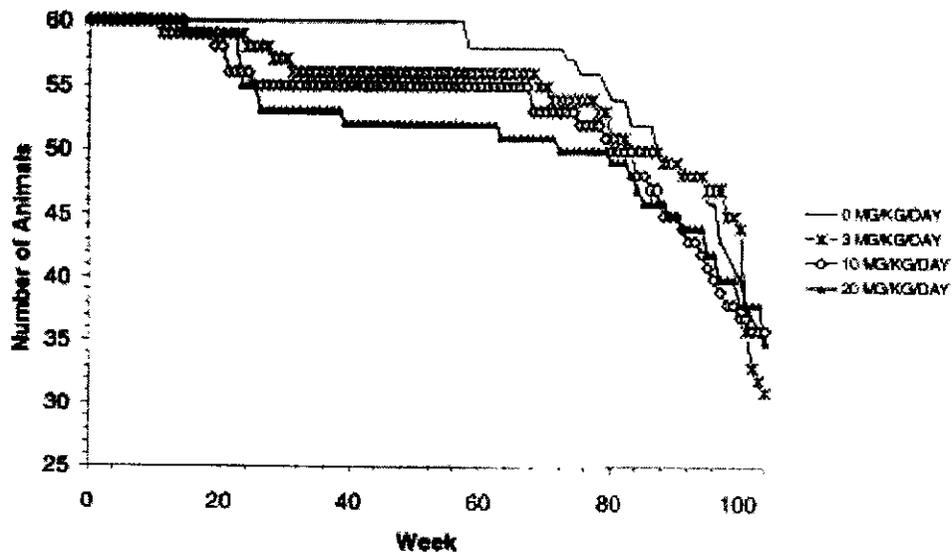


Figure R2: Percentage survival versus period of treatment - Female Rats

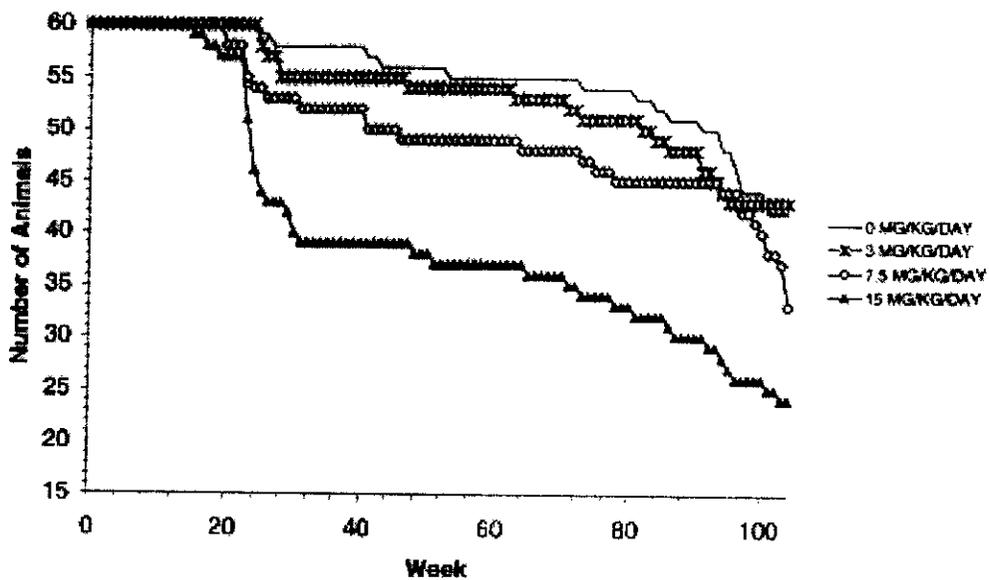


Figure R3: Group mean body weight versus period of treatment - Male Rats

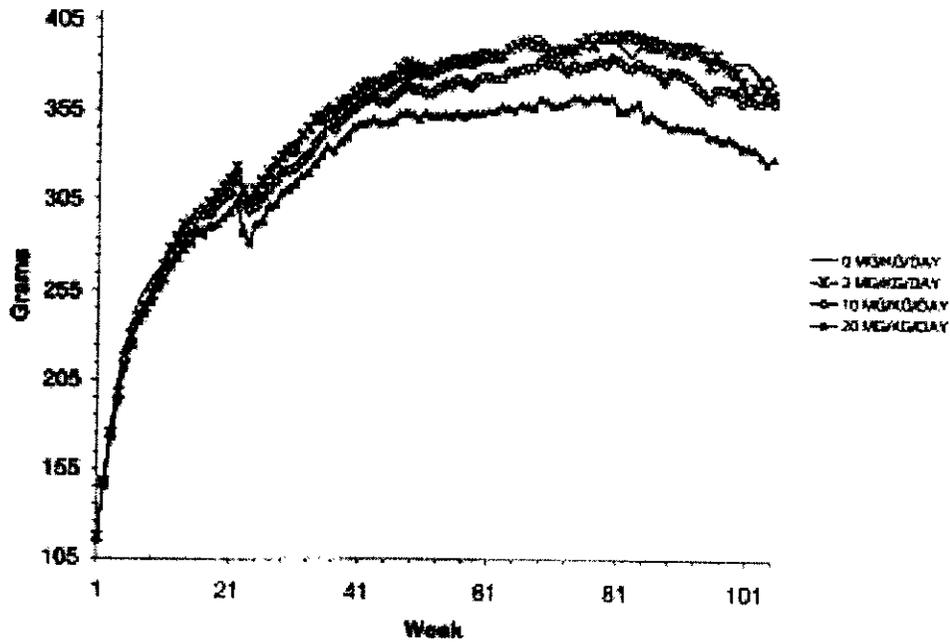
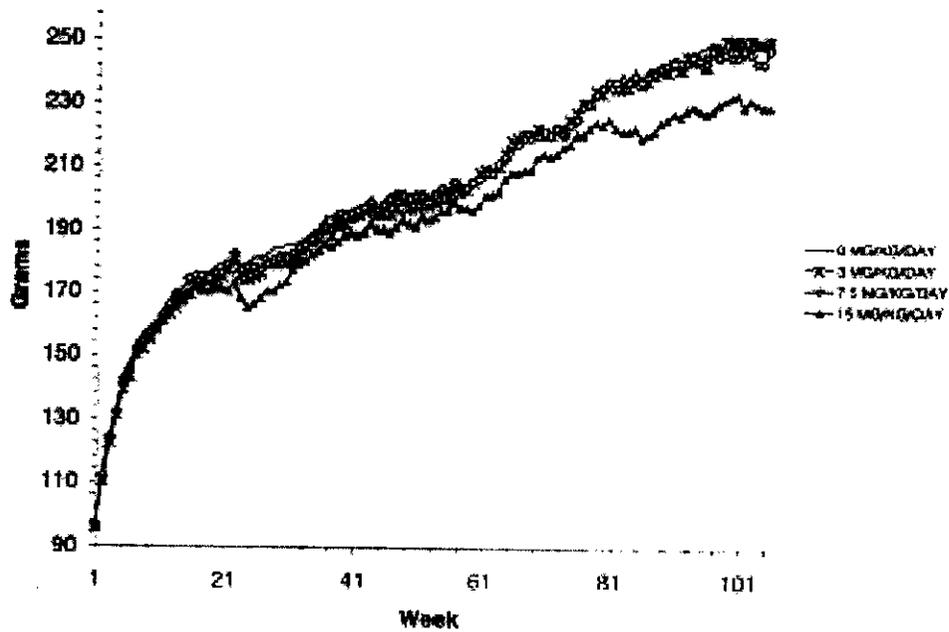


Figure R4: Group mean body weight versus period of treatment - Female Rats



Food consumption: There was only a slight (non-significant) reduction in mean food consumption in the high-dose groups.

Dose (mg/kg/day)	Males			Females		
	3	10	20	3	7.5	15
Mean food consumption relative to Controls (%):						
Week 53	99	96	94	99	102	103
Week 104	106	100	96	101	94	95

Clinical pathology: no data submitted

Toxicokinetics: Mean C<sub>max</sub> and AUC values were measured for YM905 and its major metabolite BY348C during Week 52. Plasma concentrations are expressed in terms of YM905 free base.

Analyte	PK Parameter	Males			Females		
		3	10	20	3	7.5	15
YM905	C <sub>max</sub> (ng/ml)	1.8	7.8	21.3	4.0	8.1	26.7
	AUC <sub>24</sub> (ng.h/ml)	-	64.5	115.7	17.4	67.1	246.1
	T <sub>max</sub> (hrs)	0.5	2	2	2	2	2
BY348C	C <sub>max</sub> (ng/ml)	37.5	138.5	300.7	76.5	167.3	297.9
	AUC <sub>24</sub> (ng.h/ml)	338	1310	2533	760	2229	4427
	T <sub>max</sub> (hrs)	2	2	2	4	4	2
BY-348C:YM905 AUC ratios		-	19.45	20.96	41.83	31.81	17.23

At 24 hours post-dose, plasma concentrations of YM905 were not quantifiable in any dose group, while quantifiable levels of BY348C were quantifiable in males dosed at 20 mg/kg and females dosed at 7.5 and 15 mg/kg. The data suggests saturation of metabolic pathways and the possibility of a capacity limited process for the elimination of YM905 in female rats.

Gross pathology: There were no treatment-related macroscopic findings reported. Gross abnormalities were of the type and incidence frequently observed in F-344 rats and included pituitary enlargement (usually a manifestation of pituitary adenoma), mottled lungs (mostly a manifestation of agonal congestion and hemorrhage), splenic enlargement (observable manifestation of large granular lymphocyte leukemia) and testicular changes characteristic for this strain of rat.

Histopathology: SDA occurred at about 6 months into the study. Histologically, this infection presented differently according to where in the course of the disease a rat was at the time of death. The most affected organ was the Harderian gland, in which inflammation, glandular necrosis, and regenerative hyperplasia were observed. Squamous metaplasia was frequently seen, subtle in most cases, but in some, it was very florid in nature. The more lasting chronic manifestations of the disease were chronic inflammatory infiltrates, dilated atrophic acini, and fibrosis. The lesions frequently were unilateral and often were restricted to discrete lobules within the affected glands. In relatively few instances, there was overt involvement of mandibular salivary glands and parotid salivary glands. When it was present, there was edema, inflammation, glandular necrosis, sometimes accompanied by squamous metaplasia of ductal epithelium. After recovery from SDA, atrophy was

frequently observed within the parotid gland, characterized by smaller-than-normal glandular acini and increase stroma. In contrast, there seemed to be little residual effect in the mandibular salivary gland after recovery from the disease based on the microscopic evaluation of this tissue from all male rats.

**Non-neoplastic:** There were no non-neoplastic findings that were either dose-related or significantly different from either concurrent controls or in-house historical controls.

**Neoplastic:** The most common findings were pituitary adenomas in both sexes, there was also a high incidence of islet cell carcinoma in the pancreas of controls males, but not in any of the treatment groups, benign interstitial cell tumor of the testes in all males, and large granular lymphocytic leukemia primarily in males but also in females across groups.

	Males				Females			
	0	30	10	20	0	30	10	20
<b>Hemopoietic:</b>								
Myeloid Leukemia	21	21	21	16	9	13	8	8
<b>Liver:</b>								
Hepatocellular adenoma	4	1	2	5	0	0	0	0
<b>Pancreas:</b>								
Adenoma - Islet cell	3	1	0	1	1	1	0	0
Carcinoma - Islet cell	1	0	0	0	0	0	0	0
<b>Pituitary:</b>								
Adenoma	24	22	15	14	22	16	18	17
Carcinoma	0	0	0	0	1	0	0	0
<b>Testes:</b>								
Interstitial (Leydig) cell adenoma	49	39	40	43				
Mesothelioma	4	0	0	1				
<b>Thyroids:</b>								
Follicular cell adenoma	1	0	0	0	0	0	0	0
Follicular cell carcinoma	1	0	1	0	2	0	0	1
C-cell adenoma	1	1	1	1	2	0	0	2
C-cell carcinoma	2	1	0	3	3	0	0	0

#### Summary of individual study findings:

**Adequacy of the carcinogenicity study and appropriateness of the test model:** Due to the significantly lower survival and greater than 10% decrease in body weight gain, the ExecCAC recommended that the sponsor look at histopathology in the mid- and low-dose animals. The requested raw rat data has been submitted and reviewed. Final study results including the mid- and low-dose tissues will be resubmitted to the ExecCAC when submitted electronically for statistical review. Labeling will be revised if necessary.

**Evaluation of tumor findings:** Preliminary data did not demonstrate a dose-related or significant increase in the tumor incidence rate when compared with either concurrent controls or in-house historical controls.

**Carcinogenicity summary:** Analysis of tissues from controls and high-dose animals, and 80% of mouse tissues and 60% of rat tissues from the low- and mid-dose groups, did not demonstrate a significant increase in tumor incidence rates following chronic administration of YM905.

**Carcinogenicity conclusions:**

**Recommendations for further analysis:** The Sponsor has submitted the raw data following the requested complete analysis of all tissues from low- and mid-animals. A final electronic report will be submitted as soon as possible for final review and statistical analysis. Labeling will be subject to revision following review of the completed mouse and rat carcinogenicity studies by the Division and the ExecCAC.

**Labeling Recommendations:** At this time, the Sponsor's proposed labeling is acceptable.

No increase in tumors was found following the administration of solifenacin succinate to male and female mice for 104 weeks at doses up to 200 mg/kg/day (5 and 9 times human exposure at the maximum recommended human dose (MRHD), respectively), and male and female rats for 104 weeks at doses up to 20 and 15 mg/kg/day, respectively (<1 times exposure at the MRHD).'

**Addendum/appendix listing:**

Appendix 1: ExecCAC report

Appendix 2: Statistical review no. 1

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**VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:****Study Title: Effects of YM905 on Fertility and Early Embryonic Development to Implantation in Mice by Oral Administration**

**Key study findings:** The only potential reproductive effect observed in this study was an increase in testicular weights in YM905 treated males. However, this did not result in any observable effect on male fertility. The NOAEL for fertility and early embryonic development in mice was 250 mg/kg/day, the apparent maximum tolerated dose in males and females.

**Study no.:** R905-TX-027

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** —

**Date of study initiation:** October 6, 1999 (F0 Dosage period: 10/19 to 12/12/99)

**GLP compliance:** yes

**QA reports:** yes

**Drug, lot #, radiolabel, and % purity:** K9059804 —

**Formulation/vehicle:** aqueous 0.5% methylcellulose

**Methods:**

Species/strain: — :CD-1® (ICR) BR mouse —

Doses employed: 0, 30, 100 and 250 mg/kg/day

Route of administration: oral gavage, 10 ml/kg

Study design: Male mice were administered the test article or vehicle once daily beginning 28 days before the cohabitation period (maximum 14 days) and continuing through the day before sacrifice. Female mice were administered the test article or vehicle once daily beginning 15 days before cohabitation and continuing through presumed gestation day 6. C-sections were performed on the females on presumed gestation day 13. After completion of the female necropsies, males were sacrificed and necropsied.

Number/sex/group: 24/sex/group

Parameters and endpoints evaluated:

- fertility: Estrous cycling in females was evaluated by examination of vaginal cytology for the 14 days prior to cohabitation and until sperm was observed in the vaginal contents and/or a copulatory plug was observed *in situ*. After necropsy, the following male reproductive organs were individually weighed and retained for potential histopathology examinations: testes (right and left), epididymides (right and left), seminal vesicles (with and without fluid) and prostate. In females, the number of corpora lutea in each ovary were recorded and the uterus examined for pregnancy, number and distribution of implantation sites, and viable and nonviable embryos. Ovaries from all female mice and the vagina and uterus from any nonpregnant mice were retained for potential histopathology examination.
- paternal/maternal toxicity: Mice were observed at least twice daily for clinical signs of toxicity. Body weights were recorded twice weekly in males, and in females twice weekly prior to cohabitation, daily between gestation days 0-6 and on gestation days 7, 10 and 13. Food consumption was recorded weekly in males, and in females twice weekly prior to and during cohabitation and on gestation days 0, 3, 7, 10 and 13.
- fetal: Conceptuses were examined to the extent possible.

**Results:**

**Mortality:** Three (3/24) male mice and four (4/24) female mice dosed at 250 mg/kg/day, and five (5/24) female mice dosed at 100 mg/kg/day were found dead on study.

Four of the deaths in the 100 mg/kg females were considered gavage accidents based on the timing of the deaths (occurring within 1 hour of dosing), the absence of any clinical observations before death, and necropsy observations (perforation of lungs). The fifth female dosed at 100 mg/kg/day was sacrificed on dosing day 9. This female presented with labored breathing, dehydration, cold to touch, ungroomed coat and urine stained abdominal fur beginning on day 7. At necropsy, the small intestines and cecum were distended with gas.

All deaths in mice dosed at 250 mg/kg/day were considered dose-related as no other cause of death could be found. All deaths occurred within 1 hour of dosing. The three deaths in males occurred on dosing days 7, 8 and 55, and the four deaths in females on dosing days 6 (2), 9 and 10. Only one male and one female presented with any adverse clinical signs prior to death: dehydration, gasping and rales in male found dead on day 8, and perioral substance in female found dead on day 9. At necropsy, the male that died on day 55 had intestines distended with gas. No adverse gross necropsy findings were found in the remaining males or female animals.

**Clinical signs:** The number of male mice with rales and urine-stained fur were significantly increased ( $p < 0.01$ ) in the 250 mg/kg/day group compared with controls.

**Body weight:** unremarkable

**Food consumption:**

**Males:** Mean food consumption was significantly reduced (10-20%) in the 100 and 250 mg/kg groups over the entire dosing duration.

**Females:** unremarkable

**In-life observations:** All mating and fertility parameters (numbers of days to insemination, mice that mated and number of pregnancies) were similar across groups. Estrous cycling parameters in females were unaffected by treatment.

**Terminal and necroscopic evaluations:**

**Males:** Intestines were distended with gas in one of the 250 mg/kg males found dead. The absolute and relative weights of the left testis (~10%) were increased in the YM905 dosing groups (statistically significant only in the 250 mg/kg/day group).

**Females:** The litter averages for corpora lutea, implantations, litter sizes, viable and nonviable embryos per litter were comparable between groups.

**Summary of individual study findings:** The NOAEL for males and females was considered to be 30 mg/kg/day based on unexplained mortalities at 100 (females only) and 250 mg/kg/day (males and females), and increased testicular weights in YM905 treated males. There were no effects on fertility or early embryo-fetal development observed at the highest dose tested, 250 mg/kg/day.

**Study Title:** Effects of YM905 on Embryo-Fetal Development in Mice by Oral Administration

**Key study findings:** The NOAEL for developmental effects was 30 mg/kg/day based on reduced fetal weights observed at 100 and 250 mg/kg/day and the increased incidence of cleft palate at 250 mg/kg/day.

**Study no.:** R905-TX-029

**Location:** EDR - Pharm/Tox Datasets

**Conducting laboratory and location:** —

**Date of study initiation:** October 12, 1999 (Dosage period: 10/27 to 11/9/99)

**GLP compliance:** yes

**QA reports:** yes

**Drug, lot #, radiolabel, and % purity:** K9059804. —

**Formulation/vehicle:** aqueous 0.5% methylcellulose

**Methods:**

Species/strain: ~ CD-1® (ICR) BR female mice —

Doses employed: 0, 30, 100 and 250 mg/kg/day

Route of administration: oral gavage, 10 ml/kg

Study design: Female mice (aged ~85 days) were cohoused with male mice in-house. Dosing of presumed pregnant females occurred on gestation days 6 through 15.

Number/sex/group: 24 females/group

Parameters and endpoints evaluated: During treatment, all mice were monitored for mortality, clinical signs, body weight gain and food consumption. At scheduled termination on day 18, all mice were necropsied and the pregnancy status determined, placentas were removed and weighed and the number of corpora lutea counted. Litters were evaluated for number and position of implantations, number of live fetuses, number of early/late resorptions and or deaths. Fetuses were weighed, sexed and examined for external malformations and deviations.

**Results:**

**Mortality:** Six mice were found dead in the 250 mg/kg/day group. Only one of these deaths was attributed to gavage error.

- Female 485 was found dead on gestation day (GD) 9. This mouse lost 7 g after GD 7. This mouse was pregnant with 15 live fetuses. There were no remarkable clinical observations prior to death other than the weight loss and all maternal and fetal tissues appeared normal at necropsy.
- Female 492 was found dead on GD 9. This mouse lost 7 g after GD 7. The only adverse clinical observation was rales beginning on GD 8. Distention of the intestines was noted at necropsy. This mouse was pregnant with 11 fetuses that appeared normal at necropsy.
- Female 487 was found dead on GD 12. This mouse lost 9 g after GD 8. Adverse clinical observations included decreased food consumption, excessive salivation, tremors, bradypnea, hypothermia and urine-stained abdominal fur. This mouse was pregnant with 13 fetuses. All maternal and fetal tissues appeared normal at necropsy.
- Female 476 was found dead on GD13, approximately 4.5 hours after the 8<sup>th</sup> dose. A perforated esophagus was evident at necropsy and the death was attributed to intubation error. Clinical appearance, body weight gains and feed consumption were normal. The mouse was not pregnant.
- Female 495 was found dead on GD 14. Adverse clinical observations included decreased food consumption (days 10-13), tremors, bradypnea, hypothermia and a red perivaginal substance on

GD 14. This mouse was pregnant with 16 fetuses. Maternal and fetal tissues appeared normal at necropsy.

- Female 478 was found dead on GD 15. This mouse lost 5 g after GD 13. Death was preceded by tremors, bradypnea and hypothermia. This mouse was pregnant with 14 live fetuses. All maternal and fetal tissues appeared normal at necropsy.

**Clinical signs:** Adverse clinical signs were observed as noted above only in the 250 mg/kg/day animals which died.

**Body weight:** Mean maternal body weight gains were significantly reduced during the dosing period at 100 mg/kg/day (~11%) and in the surviving dams dosed at 250 mg/kg/day (~8%).

**Food consumption:** Food consumption was significantly reduced on gestation days 10 to 13 in the 30 mg/kg/day group, days 10 to 16 in the 100 mg/kg/day group, and days 13 to 16 in the 250 mg/kg/day group.

**Terminal and necroscopic evaluations:**

**Dams:** There were no signs of adverse maternal physiological effects at necropsy. The litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, early and late resorptions, placental weights, percentage of dead or resorbed conceptuses per litter and percent live male fetuses were comparable across dosing groups.

Reproductive Parameters	Dosage Groups (mg/kg/day)			
	0	30	100	250
Mated	24/24	24/24	24/24	24/24
Pregnancies	23/24	24/24	24/24	21/24
Surviving dams w/viable fetuses	23 (100%)	24 (100%)	24 (100%)	16 (100%)
Mean # of corpora lutea	14.3	14.1	14.6	14.6
Mean # of implantations	12.9	13.0	13.7	13.1
Dams with any resorptions	10/23	11/24	11/24	9/21
Mean # of resorptions	0.5	0.7	1.1	0.8
Mean litter size: live fetuses	12.4	12.2	12.5	12.2
dead fetuses	0.0	0.1	0.1	0.0
% Dead or resorbed conceptuses/litter	3.9	6.3	7.9	6.8
Mean placental wt. (g/litter)	0.09	0.09	0.08	0.08
Mean fetal wts. (g/litter)	1.34	1.34	1.26*	1.26*
- male fetuses	1.36	1.35	1.29	1.29
- female fetuses	1.32	1.33	1.22*	1.23*
% Live male fetuses/litter	49.6	47.6	49.2	48.4

\* p<0.05

**Offspring:** Reductions in mean fetal body weights were significantly reduced at maternal doses of 100 and 250 mg/kg/day, with female fetal weights showing a greater reduction than male weights.

There were no significant differences in the incidence rates or types of soft tissue and skeletal alterations and/or variations between dosing groups. The fetal incidence rate of cleft palate was increased in all three dosing groups (significant only in the 250 mg/kg/day group) compared to the

concurrent control. The litter incidence of cleft palate was increased over the ranges observed historically at the testing facility in both the 100 and 250 mg/kg/day group.

Dose (mg/kg/day)	0	30	100	250
<b>No. Litters Evaluated</b>	<b>23</b>	<b>23</b>	<b>24</b>	<b>16**</b>
<b>No. Fetuses Evaluated</b>	<b>295</b>	<b>296</b>	<b>303</b>	<b>196</b>
<b>Alterations:</b>				
- Litters with alterations	21/23 (91.3%)	16/24 (66.7%)	21/24 (87.5%)	12/16 (75%)
- Fetuses with alterations	54 (18.9%)	32 (10.9%)	54 (17.9%)	29 (14.8%)
<b>Variations:</b>				
- Litters with variations	20/23 (87.0%)	15/24 (62.5%)	18/24 (75.0%)	7/16* (43.8%)
- Fetuses with variations	52 (18.2%)	28 (9.5%)	50 (16.6%)	19 (9.7%)
<b>Malformations:</b>				
- Litters with malformations	1/23 (4.3%)	1/24 (4.2%)	3/24 (12.5%)	<b>5/16 (31.2%)</b>
- Fetuses with malformations	2 (0.7%)	4 (1.4%)	4 (1.3%)	<b>10 * (5.1%)</b>
<b>Fetuses w/cleft palate</b>				
# of litters	1 (4.3%)	1 (4.3%)	3 (12.5%)	4 (25.0%)
# of fetuses	2 (0.7%)	4 (1.4%)	4 (1.4%)	8 (4.1%) *
Range/litter	0-2	0-4	0- 2	0-3
<b>Fetuses w/ eye lids open</b>	0	0	0	1

\*p<0.05

\*\* litters from the dead females were not included in the evaluations

	Historical Controls (15 studies in CD-1 mice)	
	Litters	Fetuses
Number examined	319	1933
Total no. of Cleft Palates	4 (1.25%)	4 (0.21%)
Range/Study	0 - 2 (0 - 7.7%)	0 - 2 (0 - 1.2%)

Other malformations observed in fetuses with and without cleft palate included the following:

- 30 mg/kg/day: cervical ribs and fused sternbrae (n=1)
- 100 mg/kg/day: variation in interfrontal skull ossification (n=1); multiple malformations of cervical, thoracic, lumbar and sacral vertebrae and ribs (n=1)
- 250 mg/kg/day: variation in interfrontal skull ossification (n=2)

The Sponsor has discounted the increased incidence of cleft palate as a secondary effect to maternal toxicity. However, since the only observable effect YM905 had were reductions in weight gain and feed consumption in the surviving dams, also seen at a similar magnitude in the 100 mg/kg/day group, a direct effect resulting from fetal exposure to YM905 cannot be ruled out. Based on the results of this study, YM905 should be considered a potential teratogen in mice.

**Toxicokinetics (Study R905-TX-034):** Plasma concentrations of YM905 in samples taken on GDs 6 and 15 were measured by HPLC. The values of plasma concentrations from mice that were not pregnant were excluded from the mean calculations. The Cmax and AUC0-24 values are summarized below:

Dose (mg/kg/day)	Cmax (ng/ml)		AUC <sub>24</sub> (ng.h/ml)	
	GD 6	GD 15	GD 6	GD 15
30	867.2	304.85	2564	1469
100	1121.16	748.83	6061	4439
250	1719.19	1122.40	12379	9760

Tmax occurred 1-2 hours post-dosing. The mean plasma concentrations of YM905 were not quantifiable at 30 and 100 mg/kg/day. The rate of systemic exposure of female mice to YM905, characterized by Cmax, increased with increasing dose, but were less than the proportionate dosing increment. AUC levels were lower on GD 15 when compared to GD 6.

**Summary of individual study findings:** The NOAEL for maternal toxicity was not determined due to decreased weight gain and feed consumption at the lowest dose tested, 30 mg/kg/day. The NOAEL for developmental effects was 30 mg/kg/day based on reduced fetal weights observed at 100 and 250 mg/kg/day and the increased incidence of cleft palate at 250 mg/kg/day. Based on the increased incidence of cleft palate observed in mice from litters exposed to 250 mg/kg/day, YM905 should be considered a potential teratogen in mice.

**Study title: Investigational Study for Fetal Cleft Palate in Oral Administration of YM905 to Pregnant Mice**

**Key study findings:** There were two incidences of cleft palate in fetuses exposed in utero to 250 mg/kg/day YM905: one fetus in the GD 6-9 exposure group; one in the GD 10-15 exposure group and none in the GD6-15 exposure group. The total number of YM905 exposed litters and fetuses affected were 2/67 (3%) litters and 2/835 (0.24%) fetuses.

**Study no.:** R905-TX-033

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** Yamanouchi

**Date of study initiation:** January 25, 2000

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, radiolabel, and % purity:** Lot K9059804, —

**Formulation/vehicle:** 0.5% methylcellulose solution

**Methods:**

**Species/strain:** ~ CD-1 (ICR) mice —

**Doses employed:** 0 and 250 mg/kg/day

**Route of administration:** oral gavage, 10 ml/kg/day

**Study design:** To determine the effect of YM905 on fetal palate formation the treatment period was divided into two portions: critical stage of palate formation (GD 10-15) and early stage gestation (GD 6-9). An additional YM905 group and the vehicle control groups were treated between GD 6-15. Dams were C-sectioned on GD 18.

**Number/sex/group:** 24 females/group

**Parameters and endpoints evaluated:** Mortality and clinical behavior were assessed 3 times daily. At termination, contents of the thoracic and abdominal cavities were examined macroscopically and the ovaries and uterus were removed to assess corpora lutea and fetuses. Fetuses were observed for viability and pre- and post-implantation loss. Live fetuses were sexed and examined for external malformations, and fetal body weight and placental weight were determined.

**Results:**

**Mortality:** One dam in each of the GD 6-15 and GD 10-15 treatment groups died immediately after dosing due to intubation errors on GD 14 and 11, respectively. Three more animals had to be sacrificed in extremis due to intubation errors: two in the GD 6-15 treatment group and one in the GD 6-9 treatment group.

**Clinical signs:** Two other dams in the GD 6-15 treatment group also showed signs consistent with intubation error, e.g., irregular respiration, decreased locomotor activity, soiled fur and dosing solution backflow during administration, but were able to recover and complete the dosing period.

**Terminal and necroscopic evaluations:**

**Dams:** The contents of thoracic and abdominal cavities showed no macroscopic lesion in any dam, not was any significant difference found in the number of corpora lutea between the control and treatment groups.

**Offspring:** No significant difference was found between the control and treatment groups with respect to the number of implantations, post-implantation loss, the number of live fetuses, sex ratio, fetal body weight or placental weight.

Cleft palate was noted in 1 fetus in the GD 6-9 (1/309 = 0.32%) treatment group and one in the GD 10-15 (1/278 = 0.36%) treatment group. There were no cleft palates in the vehicle control (0/312) or GD 6-15 (0/248) treatment groups.

**Summary of individual study findings:** There were two incidences of cleft palate in this study. One each in the GD 6-9 and GD 10-15 treatment groups resulting in an incidence rate of 0.32% and 0.36%, respectively, for these two groups. When all YM905 exposed groups are combined, the

incidence rate is 0.24%. The Sponsor has argued that these are incidental and not related to treatment based on the following:

- 1) The sensitive exposure period for palate formation is between gestations days 10 and 15, therefore the cleft palate in the GD 6-9 treatment group cannot be drug related.
- 2) The incidence rate in this study is within the incidence range of other facilities background data — 0-1.2%; other literature reports 0-2.32%.

The cleft palate in the GD 6-9 treatment group cannot be ruled out as being dose related due to the likely presence of residual drug exposures extending into the critical period GD 10-15. The incidence rate compared to other studies is low, but in light of no occurrences in the control groups and the previous finding of 12.5% and 25% of litters in the 100 mg/kg and 250 mg/kg groups, respectively, with cleft palate in study 905-TX-029, the relationship between drug exposure and cleft palate cannot be ruled out.

**Study title: Effects of YM905 on Prenatal/Postnatal Development in Mice by Oral Administration, Including Maternal Function**

**Key study findings:** Thirteen F0 females died during treatment: 3 (2 pregnant, 1 non-pregnant) 100 mg/kg females and 8 (6 pregnant, 2 non-pregnant) 250 mg/kg females died during gestation, and 2 - 250 mg/kg females died during lactation. There was an increase in pup mortality peripartum in litters from females dosed at 100 and 250 mg/kg and an increase in pup mortality postpartum in the 250 mg/kg group. Other preweaning findings included decreased pup weight and weight gain, and delayed development. Five pups died postweaning: 1 from the 100 mg/kg/group and 4 from the 250 mg/kg/day group. Vaginal patency was delayed in F1 female offspring from both the 100 and 250 mg/kg/groups. There were no effects on fertility or reproductive parameters in the F1 offspring, and with the exception of an increase in the percent of male fetuses/litter, there were no developmental effects in the F2 offspring. There were no incidences of cleft palate reported in this study. The reproductive/developmental NOAEL of YM905 is 30 mg/kg/day.

**Study no.:** 905-TX-032

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** —

**Date of study initiation:** July 25, 2000

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, radiolabel, and % purity:** Lot K9059804. —

**Formulation/vehicle:** 0.5% methylcellulose solution

**Methods:**

Species/strain: ~ CD-1@(ICR)BR mice —

Doses employed: 0 (vehicle), 30, 100 and 250 mg/kg/day

Route of administration: oral gavage, 10 ml/kg

Study design: F0 females were treated between presumed gestation day 6 and through the lactation period.

Number/sex/group:

F0: 24 females/group at 0, 30 and 100 mg/kg; 30 females at 250 mg/kg

F1: At weaning on day 21 postpartum, one male and one female pup from each available litter per group were randomly selected for continued evaluation.

Parameters and endpoints evaluated:

- F0 generation mice were observed for viability, clinical signs, abortions and premature deliveries. Body weights and food consumption values for the F0 generation mice were recorded. Mice were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes and pup viability at birth. Pup viability, litter size, pup weights, clinical observations, surface righting, pinna unfolding, eye opening and acoustic startle response and maternal behavior were observed and recorded during the preweaning period.

Mice that did not deliver a litter were sacrificed on DG 23 and examined for gross lesions. Uteri were examined to confirm the absence of implantation sites. On post delivery day 21, dams and pups not selected for continued evaluation were sacrificed and necropsied. The dams were examined for the number and distribution of implantation sites, The uterus, ovaries, vagina and mammary glands of any dam that delivered any stillborn pups were retained. All F1 generation pups were examined for apparent hydrocephaly and alterations in the hard palate.

- F1 generation male and female mice were observed for viability, clinical observations and general appearance during the postweaning period. Body weights were recorded weekly during the postweaning period and at sacrifice. The age of vaginal patency or preputial separation was evaluated. Male and female mice were evaluated in a passive avoidance test for learning and for motor activity. An open field observation was conducted to assess the number of rears in open field, autonomic functions, reactivity and sensitivity, excitability, gait pattern in the open field, severity of gait abnormalities, air righting reaction and visual placing response and abnormal clinical signs.

At approximately 90 days of age, the F1 generation mice were assigned to cohabitation. Female mice were sacrificed on DG 18, C-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Each mouse was examined for uterine contents. Upon completion of C-sectioning of the F1 generation female mice, male mice were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed.

#### Results:

**F0 Generation Mice and Litters:** Pregnancy occurred in 20, 22, 19 and 25 of the mice assigned to dosage groups of 0, 30, 100 and 250 mg/kg/day, respectively. Three and seven mice (including one and two non-pregnant mice) in the 100 and 250 mg/kg/day dosage groups, respectively, were found dead during the gestation period. These deaths occurred between GD10 and 17. Two 250 mg/kg mice were found dead on day 19 of lactation. One F0 - 250 mg/kg female mouse was missing and presumed dead on GD 18. All other mice survived to scheduled sacrifice.

Body weights and body weight gains during the gestation and lactation periods were unaffected by dose. Food consumption values were unremarkable during gestational, but were significantly reduced in the 100 and 250 mg/kg/day F0 females on lactation days 10-14.

At 100 and 250 mg/kg/day, peripartum pup mortality was increased. There were two litters in each of these groups that had no liveborn pups, resulting in a significantly reduced delivery index (number of liveborn pups/number of implantation sites). The gestation index (number of mice with live offspring/number of pregnant mice) was also reduced in the 100 and 250 mg/kg/day groups.

	F0 Generation Mice: Delivery Observations			
	0	5	15	50
No. of mated F0 females	24	24	24	24
No. of delivered litters w/live fetuses	20	21	17	20
Fertility Index	83.3	91.7	81.0	90.9
Duration of gestation (d)	19.7 ± 0.9	20.0 ± 0.5	19.9 ± 0.6	19.9 ± 0.7
Implantation sites/delivered litter	12.6 ± 1.8	12.0 ± 2.6	13.0 ± 1.9	12.5 ± 1.9
Mice w/all stillborn pups	0	1	2	2
Delivery Index (%)	90.1	90.4	76.9 **	86.0
Gestation Index (%)	100	90.9	88.2	90.0
No. of F1 pups delivered/litter	11.4 ± 2.4	11.4 ± 2.2	11.3 ± 3.4	11.9 ± 2.2
- liveborn	11.4 ± 2.4	11.4 ± 2.3	11.3 ± 3.4	11.9 ± 2.2
- stillborn	0	1 (0.1 ± 0.5)	0	0

At 250 mg/kg, postpartum pup mortality was also increased, resulting in a significant reduction in the viability index (number of live pups on DL 21/number of live pups on DL 4).

	F0/F1 Generations: Postpartum to Weaning Observations			
	0	5	15	50
Mice w/all pups dying DL days 1-4	1	1	2	1
Mice w/all pups dying DL days 5-21	0	0	0	2
Pups found dead or presumed cannibalized (%):				
- day 1	1.3	0.0	1.2	0.9
- days 2-4	1.8	7.0	6.0	15.0 **
- days 5-7	2.3	6.2 *	2.5	8.3 **
- days 8-14	0.5	1.0	1.3	3.6
- days 15-21	0.9	0.0	1.3	0.7
Viability Index (%)	96.9	93.0	92.9	84.2 **
Weaning Index (%)	96.4	92.9	94.9	87.7 **

\* p < 0.05; \*\* p < 0.01

Average pup body weights per litter and average body weight change per litter were significantly reduced at 100 and 250 mg/kg on lactation days 14 and 21, and in the 250 mg/kg group on lactation day 4.

	F1 Generation Pups: Postpartum to Weaning Observations			
	0	5	15	50
Pup weight/litter (g):				
- day 1	1.6 ± 0.1	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2

- day 4	2.5 ± 0.2	2.5 ± 0.4	2.4 ± 0.2	2.2 ± 0.4 *
- day 7	3.8 ± 0.4	3.8 ± 0.6	3.4 ± 0.5	3.4 ± 0.5
- day 14	6.3 ± 0.7	6.6 ± 1.2	5.4 ± 1.0 *	5.5 ± 0.9 *
- day 21	9.5 ± 1.4	9.5 ± 2.0	7.3 ± 2.0 **	8.0 ± 1.6 *

\* p• 0.05; \*\* p• 0.01

In the 250 mg/kg group, there was a significant reduction in the percentage of pups per litter that could surface right or had unfolded pinna(e) on DL 5. The percentage of pups per litter with open eyes on DLs 14, 15 and 16 was significantly reduced and the average day that 50% of the pups had open eyes was significantly delayed in both the 100 and 250 mg/kg/day groups. The acoustic startle reflex was not affected by administration of the test article.

Day 100% of pups achieved the following developmental landmarks:	F1 Generation Pups: Developmental Landmarks			
	0	5	15	50
- reflex & physical development	10	11	12	11
- pinna unfolding	7	8	6	7
- eye opening	17	18	18	18
- acoustic startle	19	20	20	20

***F1 Generation Mice - Postweaning:*** One male mouse in the 100 mg/kg maternal dose group and four mice (two males and two females) in the 250 mg/kg maternal dosage group were found dead during the first week postweaning. Based on the generally low body weights at weaning, the death of each of these mice was attributed to a failure to thrive postweaning.

The average day of preputial separation in male mice did not differ significantly between groups. However, the average day of vaginal patency in female mice was significantly delayed in females from the 100 and 250 mg/kg groups.

	F1 Generation Mice: Sexual Saturation Indices			
	0	5	15	50
Number of males examined	19	19	13	13
- preputial separation *	31.1 ± 2.0	30.5 ± 1.5	33.6 ± 3.7	31.0 ± 1.5
Number of females examined	18	19	13	13
- vaginal patency *	32.4 ± 3.0	31.7 ± 2.9	36.8 ± 4.8 **	5.4

\* average day postpartum

\*\* p<0.05

There were no significant differences between groups in learning or memory, average number of movements or the time spent in movement, or parameters of the functional observational battery.

Significant reductions in body weights occurred on postweaning days 1, 8 and 15 for male and/or female mice from the 100 and 250 mg/kg groups. For the remainder of the postweaning period (including gestation), body weights were comparable between groups and body weight gains for the F1 generation mice were generally comparable for the entire postweaning period.

#### F1 Generation Pups: Postweaning Observations

	0	5	15	50
<b>Male Pup weight postweaning (g):</b>				
- day 1	9.8 ± 2.5	11.0 ± 1.7	7.6 ± 2.1 *	8.8 ± 2.8
- day 8	18.7 ± 3.8	21.2 ± 2.5	14.2 ± 5.4 **	17.5 ± 3.7
- day 15	25.5 ± 3.5	27.4 ± 2.4	21.2 ± 4.9 **	24.5 ± 3.9
- day 22	28.5 ± 2.8	29.9 ± 2.7	26.6 ± 2.4	28.3 ± 3.2
- day 72 (precohabitation)	36.9 ± 3.8	38.9 ± 3.6	35.8 ± 2.4	36.9 ± 2.9
<b>Female Pup weight postweaning (g):</b>				
- day 1	9.5 ± 2.2	10.2 ± 1.7	6.8 ± 1.7 **	8.2 ± 2.6
- day 8	16.6 ± 2.7	17.4 ± 2.0	13.0 ± 3.5 **	13.3 ± 3.1 **
- day 15	21.2 ± 2.6	21.4 ± 2.3	18.0 ± 3/5 **	18.9 ± 3.0 *
- day 22	22.9 ± 2.6	22.6 ± 2.2	21.2 ± 1.8	21.7 ± 2.2
- day 72 (precohabitation)	28.9 ± 2.6	28.8 ± 3.3	27.6 ± 2.4	27.6 ± 2.6
<b>Maternal weight gain (g):</b>	31.6 ± 5.4	32.2 ± 4.1	33.6 ± 3.4	30.7 ± 5.5

\* p &lt; 0.05; \*\* p &lt; 0.01

There were no significant differences in mating or fertility in male or female offspring, e.g., days of cohabitation, mice that mated, copulation or fertility indices, or the number of pregnant mice. The percentage of live male fetuses per litter was significantly (p < 0.01) increased in the 250 mg/kg maternal dosage group compared to the control group value. There were no other affects on any other C-sectioning or litter parameters and no fetal gross external alterations occurred.

	<b>F1 Generation Pups: Reproductive Parameters</b>			
	0	5	15	50
<b>F1 Males:</b>				
- no. in cohabitation	19	18	11	11
- days in cohabitation	2.2 ± 1.6	2.8 ± 2.1	2.1 ± 1.2	3.2 ± 1.5
- copulation Index (%)	94.7	88.9	100.0	100.0
- fertility Index (%)	100.0	100.0	100.0	100.0
<b>F1 Females:</b>				
- no. in cohabitation	18	19	13	11
- days in cohabitation	2.3 ± 2.2	3.0 ± 2.9	2.2 ± 1.2	3.2 ± 1.5
- copulation Index (%)	100.0	100.0	100.0	100.0
- fertility Index (%)	100.0	100.0	100.0	100.0
- corpora lutea	15.0 ± 2.5	15.4 ± 2.2	15.9 ± 2.5	15.4 ± 2.6
- implantations	13.2 ± 3.2	13.4 ± 2.2	13.8 ± 1.5	13.5 ± 3.3
- litter size	11.9 ± 3.1	12.4 ± 2.4	13.3 ± 1.7	12.8 ± 3.1
- resorptions	1.3 ± 1.3	1.0 ± 1.0	0.5 ± 0.7	0.7 ± 0.8
- fetal weight (g)	1.36 ± 0.08	1.35 ± 0.12	1.39 ± 0.11	1.30 ± 0.10
- male fetuses/litter	44.3 ± 12.4	49.6 ± 17.7	46.6 ± 13.2	63.1 ± 11.6 *

**Summary of individual study findings:** The reproductive/developmental NOAEL of YM905 is 30 mg/kg/day. Findings in F1 offspring included peripartum mortalities, reduced pup weights and delays in the onset of reflex and physical development occurred at the 100 and 250 mg/kg/day dosages and postpartum pup mortality occurred at 250 mg/kg. Deaths continued during the first week postweaning in 100 mg/kg F1 males and 250 mg/kg/day F1 males and females. Body weights were reduced for the first 15 days of the postweaning period in males and female F1 pups from the 100

and 250 mg/kg groups. Sexual maturation (delayed vaginal patency) was delayed in the F1 female pups from the 100 and 250 mg/kg groups.

Despite the delay in sexual maturation in F1 females, there were no effects on fertility and reproductive parameters in F1 males or females, or in F2 fetal development with the exception of an increase in the percentage of live male fetuses/litter from the 250 mg/kg/ml group.

There were no incidences of cleft palate in the F1 or F2 offspring of females exposed to 250 mg/kg YM905, 0/215 F1 pups and 141 live F2 fetuses.

**Study title: Effects of YM905 on Fertility and Early Embryonic Development to Implantation in Male Rats by Oral Administration**

**Key study findings:** NOAEL = 50 mg/kg/day, the highest dose tested

**Study no.:** R905-TX-011

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** —

**Date of study initiation:** November 5, 1996

**GLP compliance:** yes

**QA reports:** Yes

**Drug, lot #, radiolabel, and % purity:** Lot no. K01. —

**Formulation/vehicle:** 0.5% methylcellulose

**Methods:**

Species/strain: — CD (SD) rats

Doses employed: 0, 5, 15 or 50 mg/kg/day

Route of administration: oral gavage, 5 ml/kg/day

Study design: Males were treated for a total of 6 weeks: 4 weeks prior to mating to untreated females and 2 weeks during the mating period.

Number/sex/group: 20 males/group

Parameters and endpoints evaluated:

males: successful mating, impregnation

females: numbers of corpora lutea, implantations, live embryos and pre- and post-implantation losses

**Results:**

Mortality: none

Clinical signs: mydriasis (0/20 in control, low- and mid-dose groups, 5/20 in high-dose group)

Body weight: unremarkable

Food consumption: unremarkable

In-life observations: All males successfully mated and impregnated untreated females. There was no significant difference in the pre-copulation intervals between treated and control males.

Parameter	control	5 mg/kg	15 mg/kg	50 mg/kg
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successful mating	19/20	20/20	20/20	20/20
impregnation	18/20	20/20	20/20	20/20
Pre-copulation interval	3.0 ± 1.3	2.2 ± 1.4	2.6 ± 1.4	2.6 ± 1.3

Toxicokinetics (Study R905-TX-044): YM905 C<sub>max</sub> occurred at approximately 1 hour post-dosing. C<sub>max</sub> for the metabolite BY-348C occurred between 1 and 2 hours post-dosing.

Analyte	C <sub>max</sub> (ng/ml)		
	5 mg/kg	15 mg/kg	50 mg/kg
YM905	0.4 ± 0.9	14.9 ± 21.8	11.6 ± 4.7
BY-348C	22.2 ± 30.5	32.9 ± 26.2	57.8 ± 38.3

Terminal and necroscopic evaluations: Reproductive findings at caesarian section in females showed no differences between females who had been mated with control or dosed males in terms of number of corpora lutea, implantations and live embryos or pre- and post-implantation losses.

	Male Rats: YM905 Dose (mg/kg)			
	0	5	15	50
Number of dams observed	18	20	20	20
corpora lutea	19.2 ± 2.3	19.2 ± 2.1	20.0 ± 3.5	19.4 ± 2.6
implantations	17.1 ± 1.4	17.5 ± 1.5	17.2 ± 2.1	17.1 ± 2.0
pre-implantation loss (%)	11.0	8.9	13.8	12.1
post-implantation loss (%)	5.5	8.3	8.1	6.7
-early	5.5	7.7	8.1	6.5
-late	0.0	0.6	0.0	0.3
live embryos	16.0 ± 1.6	16.1 ± 2.1	15.8 ± 2.2	15.9 ± 1.6

**Summary of individual study findings:** Administration of up to 50 mg/kg/day YM905 to male mice for 4 weeks prior to mating and throughout the mating period with untreated females did not result in any adverse effects on fertility, mating performance or early embryonic development in resulting litters.

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**Study title: Effects of YM905 on Fertility and Early Embryonic Development to Implantation in Female Rats by Oral Administration**

**Key study findings:** Maternal NOAEL = 50 mg/kg/day based on suppressed body weight gain; fertility and early embryonic NOAEL = 100 mg/kg/day, the highest dose tested

**Study no.:** R905-TX-012

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** —

**Date of study initiation:** September 29, 1998

**GLP compliance:** yes

**QA reports:** Yes

**Drug, lot #, radiolabel, and % purity:** Lot no. K01, —

**Formulation/vehicle:** 0.5% methylcellulose

**Methods:**

Species/strain: — CD (SD) female rats

Doses employed: 0, 15, 50 or 100 mg/kg/day

Route of administration: oral gavage, 5 ml/kg/day

Study design: Females were treated for 14 days prior to mating to untreated males, throughout the mating period until day 7 of presumed gestation.

Number/sex/group: 20 females/group

Parameters and endpoints evaluated:

females: copulation and fertility indices

litters: numbers of corpora lutea, implantations, live embryos and pre- and post-implantation losses

**Results:**

**Mortality:** none

**Clinical signs:** mydriasis was observed in females receiving • 50 mg/kg/day.

**Body weight:** suppressed body weight gain at 100 mg/kg/day

**Food consumption:** decreased food intake at 100 mg/kg/day

**In-life observations:** There were no treatment-related effects observed on the copulation and fertility indices of females, pre- and post-implantation losses or number of implantations or live embryos.

	Female Rats: YM905 Dose (mg/kg)			
	0	15	50	100
Number of paired females	20	20	20	20
Successful matings (%)				
1 <sup>st</sup> mating period	20 (100)	18 (90.0)	20 (100)	18 (90.0)
1 <sup>st</sup> and 2 <sup>nd</sup> mating periods	20 (100)	20 (100)	20 (100)	19 (95.0)
No. of pregnancies (%)				
1 <sup>st</sup> mating period	16 (80.0)	17 (94.4)	19 (95.0)	16 (88.9)
1 <sup>st</sup> and 2 <sup>nd</sup> mating periods	16 (80.0)	19 (95.0)	19 (95.0)	17 (89.5)
Pre-copulation Interval	2.8 ± 1.4	3.7 ± 3.3	2.9 ± 1.2	2.7 ± 1.7

Terminal and necropsic evaluations: Reproductive findings at caesarian section in females showed no differences in terms of number of corpora lutea, implantations and live embryos or pre- and post-implantation losses.

	Female Rats: YM905 Dose (mg/kg)			
	0	15	50	100
Number of dams observed	16	19	19	17
corpora lutea	17.7 ± 3.5	19.2 ± 2.3	18.8 ± 2.8	18.2 ± 3.4
implantations	14.8 ± 5.6	16.2 ± 3.5	16.6 ± 1.7	16.0 ± 2.3
pre-implantation loss (%)	16.3	15.6	11.7	12.0
post-implantation loss (%)	7.6	6.8	9.2	7.0
-early	7.2	6.5	8.9	7.0
-late	0.4	0.3	0.3	0.0
live embryos	13.7 ± 5.1	15.1 ± 3.5	15.1 ± 2.8	14.9 ± 2.3

Toxicokinetics (Study R905-TX-046): C<sub>max</sub> and AUC values of YM905 (unchanged) increased greater than dose-proportionally. T<sub>max</sub> was prolonged with increasing dose levels and occurred at 8 hours in high-dose animals. Conversely, C<sub>max</sub> and AUC values of the metabolite (BY-348C) were approximately 3-5 and 3-13 times greater than those of the unchanged YM905.

Dose (mg/kg/day)	YM905			BY-348C		
	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (h)	AUC <sub>24</sub> (ng.h/ml)	C <sub>max</sub> (ng/ml)	T <sub>max</sub> (h)	AUC <sub>24</sub> (ng.h/ml)
15	9.6 ± 7.2	0.5	25.6	52.3 ± 96.1	2.0	327.2
50	44.7 ± 31.4	2.0	233.5	175.9 ± 147.3	2.0	1347.6
100	155.6 ± 113.1	8.0	2117.8	492.6 ± 710.6	8.0	5944.8

**Summary of individual study findings:** Oral administration of YM905 at doses of 5, 50 and 100 mg/kg/day for 14 days prior to mating to untreated males, through mating and presumed gestation day 7 resulted in no observable adverse effects on fertility, reproduction parameters or early fetal development. Maternal toxicity reflected by decreased weight gain was observed at 100 mg/kg/day.

**Study title: Effects of YM905 on Embryo-Fetal Development in Rats by Oral Administration**

**Key study findings:** NOAEL = 50 mg/kg/day, the highest dose tested

**Study no.:** R905-TX-014

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** —

**Date of study initiation:** November 28, 1996

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, radiolabel, and % purity:** Lot no. K01 —

**Formulation/vehicle:** 0.5% methylcellulose

**Methods:**

Species/strain: -.:CD (SD) rats (presumed pregnant)

Doses employed: 0, 5, 15 or 50 mg/kg/day

Route of administration: oral gavage, 5 ml/kg/day

Study design: presumed pregnant females were treated on gestation days 7 through 17

Number/sex/group: 20 females/group

Parameters and endpoints evaluated: numbers of corpora lutea, implantations, litter size, live embryos, pre- and post-implantation losses, sex ratio, fetal and placental weights, and visceral and skeletal anomalies/malformations

**Results:**

Mortality: none

Clinical signs: mydriasis: 0/20 at 0 and 5 mg/kg, 2/20 at 15 mg/kg and 12/20 at 50 mg/kg

Body weight: unremarkable

Food consumption: unremarkable

In-life observations: unremarkable

Terminal and necroscopic evaluations:

**Dams:** One female in the 50 mg/kg group had both light and brown discoloration of the spleen and kidneys as well as an enlarged kidney. There were no significant differences in the number of corpora lutea, implantations, live embryos, pre- and post-implantation losses, or placental weights.

	Female Rats: YM905 Dose (mg/kg)			
	0	5	15	50
Number of dams observed	20	20	20	20
No. corpora lutea	19.6 ± 2.2	19.7 ± 2.8	19.5 ± 2.7	19.3 ± 2.2
No. implantations	17.7 ± 1.3	17.5 ± 1.5	17.2 ± 2.0	17.6 ± 1.7
Implantation Index	90.8 ± 6.8	89.7 ± 10.7	88.9 ± 9.1	91.9 ± 7.1
Placental Weight (mg)	549 ± 38	597 ± 104	538 ± 48	564 ± 52
No. live fetuses	16.6 ± 1.4	16.1 ± 2.0	16.3 ± 1.9	16.3 ± 1.5

**Offspring:** There were no significant differences in fetal weights or sex ratios. There were no significant visceral or skeletal abnormalities or variations noted in fetuses.

	Female Rats: YM905 Dose (mg/kg)			
	0	5	15	50
Number of litters	20	20	20	20
No. live fetuses	16.6 ± 1.4	16.1 ± 2.0	16.3 ± 1.9	16.3 ± 1.5
% Males	48.5	47.2	51.5	45.5
Wt. of male fetuses (g)	3.73 ± 0.22	3.77 ± 0.31	3.74 ± 0.20	3.90 ± 0.23
Wt. of female fetuses (g)	3.56 ± 0.22	3.56 ± 0.27	3.60 ± 0.22	3.70 ± 0.20
Number of dead fetuses (%)	5.9	7.7	5.2	7.7
- early deaths	5.7	7.7	5.2	7.7

- late deaths	0.3	0.0	0.0	0.0
fetuses w/external anomalies	0	0	1 (0.3%)	2 (0.6)

**Summary of individual study findings:** Oral administration of YM905 at doses of 5, 15 and 50 mg/kg/day from gestation day 7 through 17 in female rats did not result in any observable adverse effects on reproduction or early fetal development. Mydriasis was observed in 10% of the females at 15 mg/kg and 60% of the females at 50 mg/kg.

**Study title: Effects of YM905 on Embryo-Fetal Development in Rabbits by Oral Administration**

**Key study findings:** NOAEL = 50 mg/kg/day, the highest dose tested

**Study no.:** R905-TX-016

**Location:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:**

**Date of study initiation:** January 26, 1997

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, radiolabel, and % purity:** Lot no. K01, purity not identified

**Formulation/vehicle:** 0.5% methylcellulose

**Methods:**

Species/strain: New Zealand White rabbits (presumed pregnant)

Doses employed: 0, 5, 10, 25 or 50 mg/kg/day

Route of administration: oral gavage, 5 ml/kg/day

Study design: presumed pregnant females were treated on gestation days 6 through 18

Number/sex/group: 20 females/group

Parameters and endpoints evaluated: numbers of corpora lutea, implantations, live embryos, pre- and post-implantation losses, sex ratio, fetal and placental weights, and visceral and skeletal anomalies/malformations

**Results:**

**Mortality:** there were no deaths, abortions or premature deliveries

**Clinical signs:** mydriasis: unremarkable

**Body weight:** decreased maternal weight in high-dose does

**Food consumption:** dose-related decrease in average weekly food consumption from day 6 to 29

	Control	10 mg/kg	25 mg/kg	50 mg/kg
Food Consumption	159 ± 24	155 ± 24	147 ± 26	139 ± 25

In-life observations: unremarkable

**Terminal and necroscopic evaluations:**

**Dams:** There were no significant differences in the number of corpora lutea, implantations, litter size, live embryos, pre- and post-implantation losses, or placental weights.

	Female Rabbits: YM905 Dose (mg/kg)			
	0	10	25	50
Number of does observed	20	20	20	20
No. of pregnancies	19	20	20	20
No. corpora lutea	9.6 ± 1.9	10.1 ± 1.3	9.6 ± 1.7	9.7 ± 1.1
No. implantations	9.2 ± 1.9	9.6 ± 1.5	9.2 ± 2.0	9.4 ± 1.2
Litter size	8.9 ± 2.3	9.3 ± 1.5	9.0 ± 2.0	9.1 ± 1.2
live fetuses	8.9 ± 2.3	9.3 ± 1.5	9.0 ± 2.0	9.1 ± 1.2
dead fetuses	0	0	0	0
Resorptions	0.3 ± 0.6	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.4
early	4 (0.2 ± 0.6)	3 (0.2 ± 0.4)	1 (0.0 ± 0.2)	1 (0.0 ± 0.2)
late	1 (0.0 ± 0.2)	2 (0.1 ± 0.3)	1 (0.0 ± 0.2)	4 (0.2 ± 0.4)
Does with any resorptions	3 (15.8%)	5 (25%)	2 (10%)	5 (25%)
Does with viable fetuses	19 (100%)	20 (100%)	20 (100%)	20 (100%)

**Offspring:** There were no significant differences in fetal weights or sex ratios. There were no significant visceral or skeletal abnormalities or variations noted in fetuses.

	Female Rabbits: YM905 Dose (mg/kg)			
	0	10	25	50
Litters w/live fetuses	19	20	20	20
Litter size	8.9 ± 2.3	9.3 ± 1.5	9.0 ± 2.0	9.1 ± 1.2
% male fetuses	49.1	49.5	52.2	56.0
Live fetal weight (g)/litter	43.14 ± 5.75	44.48 ± 4.41	43.60 ± 4.08	42.74 ± 4.50
- males	43.66 ± 6.00	45.46 ± 4.10	43.78 ± 5.07	42.61 ± 4.99
- females	43.22 ± 6.22	44.13 ± 4.67	43.48 ± 3.94	43.00 ± 4.35
Placental weight (g)/litter	5.28 ± 0.85	5.38 ± 0.86	5.32 ± 0.56	5.23 ± 0.77
Post-implantation mortality	5 (2.9%)	5 (2.6%)	3 (1.6%)	5 (2.7%)
% dead or resorbed				
conceptuses/litter	3.7 ± 9.5	2.5 ± 4.6	1.4 ± 3.4	2.6 ± 4.6

**Summary of individual study findings:** Oral administration of YM905 at doses of 10, 25 and 50 mg/kg/day from gestation day 6 through 18 in female rabbits did not result in any observable adverse effects on reproduction or early fetal development. Decreased maternal weight gain was observed at 50 mg/kg/day and there was a dose-related decrease in food consumption during treatment (GD6-18) to termination (GD 29).

**Study title: Toxicokinetic Study in Pregnant New Zealand White Rabbits**

**Key study findings:** Study demonstrated sufficient systemic exposures to validate developmental studies in rabbits.

**Study no.:** R905-TX-030

**Submission, Volume, and page:** EDR NDA 51218, Item 5: Pharm/Tox Datasets

**Conducting laboratory and location:** —

(Study no. NOE004/002568)

**Date of study initiation:** February 23, 2000

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, radiolabel, and % purity:** K9059804 —

**Formulation/vehicle:** 0.5% methylcellulose

**Methods:**

Species/strain: New Zealand White female rabbits —

Doses employed: 10, 25 and 50 mg/kg/day

Route of administration: oral gavage

Study design: Virgin females were mated with New Zealand White males of established fertility. Following mating, each female was injected i.v. with 25 i.u. of LH to ensure successful ovulation. The day of mating was designated Day 0 of gestation. Animals were dosed from GD 6 to 18 inclusive at a volume of 5 mg/kg based on the animals bodyweight on the day of mating.

Number/sex/group: 5 females/group

Parameters and endpoints evaluated: Females were monitored daily for mortality, clinical signs of toxicity and food consumption. Body weights were recorded on days 0, 3, 6, 10, 14 and 18 after mating. Blood samples for TK analysis were collected on days 6 and 18 at predose (day 18 only), 1, 2, 4, 8 and 24 hours postdose. Terminal evaluations included determination of pregnancy status, numbers of corpora lutea and implantation sites. Fetuses were not examined.

**Results:**

**Mortality:** Two females dosed at 50 mg/kg/day died on study. The first was found dead after dosing on GD 13. This female had dilated pupils at 1, 2, 4 and 8 hours post dosing on day 6 and during days 7 to 13 had shown signs of decreased fecal pellets. Necropsy revealed a few strands of fine connective tissue attached to the gastro-intestinal tract and uterus, and a small amount of thick yellow precipitate in the urinary bladder. The left uterine horn contained 5 implantations and one early resorption; the right uterine horn showed no signs of any implantations, but contained a large amount of pale purulent material. Death was considered related to a uterine infection. The other female was found dead on GD 18 between the scheduled 1 and 2 hour blood draws. Necropsy of this female was unremarkable and cause of death was not determined.

**Clinical signs:** Decreased fecal pellet production was recorded in 2/5 females at 25 mg/kg/day and 3/5 females at 50 mg/kg/day. Pupil dilation was observed at 50 mg/kg/day in 2/5 females on day 6 and 18.

**Body weight:** All animals showed some weight loss following dosing and blood sampling on day 6. Group mean gains at 50 mg/kg/day were slightly lower than the other groups between days 6 and 10, but were similar on day 18. A full assessment is not possible without a concurrent control group.

**Food consumption:** Excluding the female that died on day 13, there were no significant dose-related differences in food consumption.

**Gross Pathology:** Necropsy on day 19 revealed no obvious treatment related effects of YM905. Again, excluding the female that died on day 13, there were no dose-related effects on the number of pregnancies, corpora lutea, implantation sites or resorptions.

**Toxicokinetics:** Inter-individual variation in plasma YM905 and BY-348C concentrations was high, with coefficients of variation generally being greater than 60% and in the range of 14-224%. Mean C<sub>max</sub> and AUC values for YM905 and BY-348C are summarized in the following tables:

YM905	Day	10 mg/kg	25 mg/kg	50 mg/kg
C <sub>max</sub> (ng/ml)	6	26.49 ± 52.99	22.89 ± 41.81	76.93 ± 50.74
	18	28.19 ± 39.96	67.77 ± 58.24	305.50 *
AUC <sub>0-24</sub> (ng.h/ml)	6	379 ± 758	327 ± 711	1027 ± 951
	18	396 ± 754	595 ± 929	2174 *

\*n=2

BY-348C	Day	10 mg/kg	25 mg/kg	50 mg/kg
C <sub>max</sub> (ng/ml)	6	14.19 ± 9.87	82.84 ± 59.02	527.39 ± 305.47
	18	35.23 ± 25.14	134.42 ± 52.16	335.57 *
AUC <sub>0-24</sub> (ng.h/ml)	6	78 ± 97	591 ± 619	3044 ± 863
	18	220 ± 213	1118 ± 463	4260 *

\*n=2

The half-lives of YM905 and BY-348C were in the range of 2.2 to 8.0 hours. The data appear to indicate that there is a time-dependent increase in the systemic exposure of YM905 and BY-348C and this increase is greater for YM905 than its metabolite, suggesting a decrease in the clearance of YM905 after repeated doses.

#### **Reproductive and developmental toxicology summary:**

**Segment I studies:** Male and female mice were treated with YM905 for 4 weeks and 2 weeks, respectively, prior to mating, during cohabitation and through presumed gestation day 6 in the females. Females were C-sectioned on presumed gestation day 13. The NOAEL for males and females was considered to be 30 mg/kg/day based on unexplained mortalities at 100 (females only) and 250 mg/kg/day (males and females), and increased testicular weights in YM905 treated males. There were no observable effects in mice on fertility, reproduction or early embryo-fetal development observed at the highest dose tested, 250 mg/kg/day.

Male rats were treated with YM905 for 4 weeks at doses up to 50 mg/kg/day and females for 2 weeks at doses up to 100 mg/kg/day prior to mating, during cohabitation and through presumed

gestation day 7 in the females. Mydriasis observed in males at • 15 mg/kg and in females at • 50 mg/kg/day and reduced body weight gain and food consumption in females at 100 mg/kg/day were the only adverse clinical effects observed in adult animals. There were no observable effects on fertility, reproduction or early embryonic development observed at the highest doses tested, 50 mg/kg/day in males and 100 mg/kg/day in females.

Segment II studies: Female mice were treated from gestation days 6 through 15 at doses between 30 and 250 mg/kg/day. There were dose-related mortalities (5/20) at 250 mg/kg/day and maternal body weight gains and food consumption were significantly reduced at 100 and 250 mg/kg/day. There were no adverse effects on maternal reproductive parameters. However, fetal body weights were significantly reduced in the 100 and 250 mg/kg litters. There was also an increase in the incidence of cleft palate in the absence of any other significant increase in soft tissue or skeletal malformations or variations.

In a follow-up investigational study in mice, two more fetuses with cleft palate were seen from litters exposed *in utero* to 250 mg/kg/day between gestation days 6-9, 10-15 or 6-15. In this study, cleft palates were seen in one fetus in each of the GD 6-9 and GD 10-15 groups but not in the GD 6-15 group. The critical window for cleft palate in mice occurs after GD 10.

Female rats were treated from gestation days 7 through 17 at doses between 5 and 50 mg/kg/day. The only maternal effect was mydriasis at • 15 mg/kg/day. There were no effects seen on any of the reproductive or fetal development parameters evaluated at the highest dose tested, 50 mg/kg/day, in rats.

Female rabbits were treated from gestation days 6 through 18 at doses between 5 and 50 mg/kg/day. The only maternal effect was mydriasis. There were no effects seen on any of the reproductive or fetal development parameters evaluated in rabbits.

Segment III study: F0 female mice were treated from gestation days 6 through 15 at doses between 30 and 250 mg/kg/day. Thirteen F0 females died during treatment: 3 (2 pregnant, 1 non-pregnant) 100 mg/kg females and 8 (6 pregnant, 2 non-pregnant) 250 mg/kg females died during gestation, and 2 - 250 mg/kg females died during lactation. There was an increase in pup mortality peripartum in litters from females dosed at 100 and 250 mg/kg and an increase in pup mortality postpartum in the 250 mg/kg group. Other preweaning findings included decreased pup weight and weight change and delayed development. Five pups died postweaning: 1 from the 100 mg/kg/group and 4 from the 250 mg/kg/day group. Vaginal patency was delayed in F1 female offspring from both the 100 and 250 mg/kg/groups. There were no effects on fertility or reproductive parameters in the F1 offspring, and with the exception of an increase in the percent of male fetuses/litter, there were no developmental effects in the F2 offspring. There were no incidences of cleft palate reported in this study.

Cleft Palate: The incidence rate for cleft palate was significantly above background levels in the Segment II study conducted in mice dosed between gestation days 6 and 15 with 250 mg/kg/day solifenacin. The sponsor has argued that this effect was the result of maternal toxicity. Although there were deaths in the female mice at this dose, the only significant findings in those that survived and had litters containing fetuses with cleft palate were decreases in maternal body weight and feed consumption. These parameters were also decreased to a similar extent at 100 mg/kg/day, a dose that did not produce a significant increase (over background) for cleft palates. In my opinion, a direct

link between administration of solifenacin at 250 mg/kg/day and cleft palate cannot be ruled out based on maternal toxicity.

### Cleft Palate in Mice

	Historical Controls (15 studies in CD-1 mice)			
	Litters		Fetuses	
Number examined	319		1933	
Total no. of Cleft Palates	4 (1.25%)		4 (0.21%)	
Range/Study	0 - 2 (0 - 7.7%)		0 - 2 (0 - 1.2%)	
<b>YM905 Segment II Study:</b>	<b>0</b>	<b>30</b>	<b>100</b>	<b>250</b>
Number of Litters	23	23	24	16
Number of Litters w/CP	1	1	3	4
% of Litters w/CP	4.3%	4.3%	12.5%	25%
Number of Fetuses	295	296	303	196
Number of Fetuses w/CP	2	4	4	8
% of Fetuses w/CP	0.68%	1.35%	0.99%	4.08%
<b>YM905 Cleft Palate Study:</b>	<b>0</b>	<b>250 mg/kg</b>		
		<b>GD 6-9</b>	<b>GD 10-15</b>	<b>GD 6-15</b>
Number of Litters	24	23	23	21
Number of Litters w/CP	0	1	1	0
% of Litters w/CP	0	4.3%	4.3%	0
Number of Fetuses	312	309	278	248
Number of Fetuses w/CP	0	1	1	0
% of Fetuses w/CP	0	0.32%	0.36%	0
<b>YM905 Segment III Study:</b>	<b>0</b>	<b>30</b>	<b>100</b>	<b>250</b>
Number of Litters	20	21	17	20
Number of Litters w/CP	0	0	0	0
Number of Pups	228	227	170	215
Number of Pups w/CP	0	0	0	0
<b>Combined YM905 Exposures:</b>	<b>0</b>	<b>30</b>	<b>100</b>	<b>250</b>
Number of Litters	67	44	41	36
Number of Litters w/CP	1	1	3	4
% of Litters w/CP	1.5%	2.3%	7.3%	11.1%
Number of Fetuses	835	523	473	411
Number of Fetuses w/CP	2	4	4	8
% of Fetuses w/CP	0.24%	0.76%	0.85%	1.95%

Summary NOAELs and LOAELs for reproductive and developmental effects of YM905 in mice, rats and rabbits:

Reproductive Parameter / Developmental Effect:	Maternal/Paternal YM905 Dose: mg/kg (mean AUC value. ng.h/ml)					
	Mice		Rats		Rabbits	
	NOAEL	LOAEL	NOAEL	LOAEL	NOAEL	LOAEL
NOAEL in adult males	30	100 (2708)	50 (1904)	na	na	na
NOAEL in pregnant females	30 (1469-2564)	100 (4439-6061)	50 (1348)	100	25 (327-595)	50 (1027-2174)
Male fertility, reproduction and early embryonic development	250	*	50	*	ND	ND
Female fertility, reproduction and early embryonic development	250 (9760-12379)	*	100 (5945)	*	ND	ND
Embryo-fetal development	30 (1469-2564)	100 (4439-6061)	50 (1348)	*	50 (1027-2174)	*
Cleft Palate	100 (4439-6061)	250 (9760-12379)	50 (1348)	*	50 (1027-2174)	*
F1 Postpartum development	30 (1469-2564)	100 (4439-6061)				
F1 fertility and reproduction parameters	250 (9760-12379)	*				
F2 embryo-fetal development	30 (1469-2564)	100 (4439-6061)				

\* No effects were seen at the highest dose test

C<sub>max</sub> and AUC<sub>24</sub> values at steady state in males and females are as follows (clinical study nos. 905-CL-004 and 905-CL-029):

	Males			Females		
	5 mg	10 mg	20 mg	5 mg	10 mg	20 mg
C <sub>max</sub> (ng/ml)	32 ± 13	63 ± 26	88 ± 35	33 ± 9	63 ± 21	72 ± 25
AUC (ng.h/ml)	646 ± 273	1248 ± 525	1801 ± 748	626 ± 205	1223 ± 391	1428 ± 530

The proposed clinical dose for VESICARE® is 5 or 10 mg daily, with 5 mg being the recommended start dose.

**Reproductive and developmental toxicology conclusions:**

**Fertility:** YM905 had no effect on fertility, reproductive functions or early embryonic development when administered to mice and rats at systemic exposure levels 5 and 10 fold greater in male and female mice, respectively, and <1 and 5 fold greater in male and female rats when compared to the highest mean AUC values achieved in humans. These values represent the highest doses tested in mouse and rat reproductive and developmental studies.

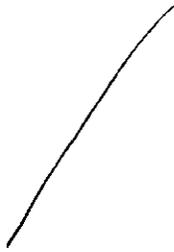
**Embryo-fetal toxicity:** Mouse fetal body weights were significantly reduced following *in utero* exposures to YM905 at • 100 mg/kg/day. There were no effects on embryo-fetal development in mice, rats or rabbits. Exposures (based on comparison of mean AUC<sub>24</sub> values) were approximately 2 fold greater in mice, approximately 1/5<sup>th</sup> in rats and 1.8 fold greater in rabbits than expected exposure in humans.

Cleft palate was observed only in mice at the highest dose tested. This dose was maternally toxic and approximately 10 fold higher than expected exposures in female patients. Exposures which did not result in any fetuses with cleft palate resulted in systemic exposures 5 fold higher than expected in female patients.

**Developmental effects:** Effects observed in F1 mouse offspring exposed from gestation day 6 through lactation included increase in pup peripartum mortality, decreased pup weight and weight gain, and delayed development at maternal doses • 100 mg/kg, and an increase in pup mortality postpartum at a maternal dose of 250 mg/kg. Vaginal patency was also delayed in F1 female offspring at • 100 mg/kg. There were no effects on fertility or reproductive parameters in the F1 offspring, and with the exception of a potential increase in the percent of male fetuses, there were no developmental effects in the F2 offspring. Doses which did not result in any observable developmental effects 1<sup>st</sup> generation offspring mice resulted in systemic exposures approximately 2 fold greater than expected in female patients.

**Labeling recommendations:**

1 Draft Labeling Page(s) Withheld



### VIII. SPECIAL TOXICOLOGY STUDIES:

- 1) Acute skin and eye irritation studies in rabbits were submitted in the original IND submission (4/2/99).
  - Minor skin irritation (erythema) was noted.
  - Severe and seemingly irreversible ocular mucosal damage, especially opacity and edema to the cornea and falling of the nictating membrane was observed with 10 and 100 mg/eye (unrinsed). Rinsing appeared to ameliorate the effect. Effects were reversible over time at 1 mg (unrinsed) and following rinsing 10-30 seconds after instillation at higher concentrations.
- 2) Solifenacin was not antigenic in the delayed type skin reaction assay in guinea pigs and did not induce hemolysis in human peripheral blood.

**Conclusions:** Care should be exercised in keeping the product away from the eyes and directions to rinse eyes immediately if exposed to solifenacin.

**APPEARS THIS WAY  
ON ORIGINAL**

## IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

**Conclusions:** From a Pharm/Tox perspective, we recommend approval of this NDA.

### General Toxicology Issues:

**Cardiac Effects in Dogs:** ECG effects were observed in the 4-week and 13-week toxicology studies. In the 4-week study at a dose of 30 mg/kg/day there was a prolongation of the P-wave, the PR interval and the QRS duration and QT interval in some dogs. However, QT interval values in all animals were within the normal range and C<sub>max</sub> was 54 to 87-fold higher than at the therapeutic dose of 10 mg/day in humans. In the 13-week study the only changes observed were prolongation of the P-wave and PR interval at 25/18 mg/kg/day which produced C<sub>max</sub> of 24-32-fold higher than the therapeutic dose. There were no cardiovascular or ECG effects observed in the 52-week toxicity study at doses up to 20 mg/kg/day and C<sub>max</sub> values of 45 to 54-fold higher than the proposed therapeutic dose.

Following observations of ventricular repolarization effects in dogs, Yamanouchi performed *in vitro* studies to assess the effect of YM905 on action potential parameters in isolated dog cardiac Purkinje fibers and the effect of YM905 on HERG-mediated potassium current. In Purkinje fibers, YM905 showed no increase but rather a slight decrease in the duration of the action potential at concentrations up to 0.3  $\mu$ M with stimulation of 0.5 and 1.0 Hz. The Sponsor attributed this decrease to potential effects on sodium channels. CHO cells with stable expression of the HERG-K channel showed evidence of inhibition of the potassium current with an approximate IC<sub>50</sub> of 0.3  $\mu$ M. This concentration is ~80-fold higher than the mean steady state maximum unbound plasma concentration of YM905 in humans at the therapeutic dose of 10 mg per day.

In pentobarbital anesthetized dogs intravenously administered YM905 produced a dose-dependent prolongation of the PR interval at 1 and 3 mg/kg and complete AV blockage at 10 mg/kg. However, there was no effect on QTc intervals at doses up to 10 mg/kg.

**ADME:** *In vivo* distribution studies in albino and pigmented mice with <sup>14</sup>C-solifenacin demonstrated similar distribution patterns except for the eyes. In the eyes of pigmented mice C<sub>max</sub> values occurred at 4 hours with levels decreasing slowly (t<sub>1/2</sub> = 134 hours) compared to other tissues. The C<sub>max</sub> values of radioactivity in the eyes were 7 times higher than those in the plasma. *In vitro* studies confirmed that solifenacin was highly, but reversibly, bound to melanin (bound fraction = 37.5 to 73.2%). Solifenacin binding to pigmented ocular tissues was not associated with development of ophthalmologic or histopathologic changes in the eyes of mice, rats or dogs evaluated following chronic administration. Furthermore, solifenacin does not absorb UVA, UVA or visible light.

In rats, clearance from the testes was slower than for other tissues, with 85% of the radioactivity present 24 hours post-dosing. The prolonged presence of solifenacin in the testes was not associated with development of microscopic lesions and had no effect upon the mating performance or fertility of male rats or mice.

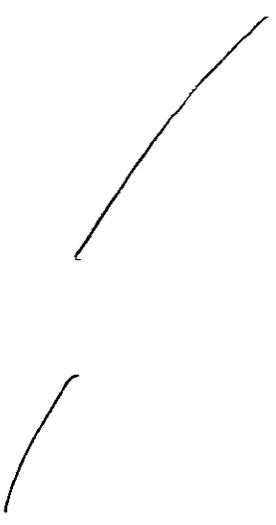
**Developmental effects:** Delayed growth of the fetuses and live born pups was observed in mice at maternally toxic doses. In addition, there was an increased incidence of cleft palate in litters exposed *in utero* to 100 mg/kg (12.5%) and 250 mg/kg (25%). Under conditions of stress or toxicity, mice

are particularly sensitive to this effect with a normal background rate of approximately 8%. Maternal toxicity and mortalities did occur at these dosing levels. In follow up and subsequent studies, including the multigenerational study in mice, the incidence rate of cleft palate was within historical ranges. Never the less, the magnitude of findings in the original developmental study cannot be ignored or a connection to drug exposures ruled out. The combination of reduced fetal and pup weights, delayed maturation, and potentially drug induced cleft palate warrant a pregnancy category of C.

**Labeling with basis for findings:**

- 1) **Clinical Pharmacology:** Nonclinical data should not be used to make efficacy claims when human data is available.

**Clinical Pharmacology**



- 2) Solifenacin was not mutagenic in the standard genotox battery. Preliminary data suggest that chronic administration of solifenacin does not increase the risk for carcinogenicity. Finally, there were no effects on fertility in male or female mice or rats at the highest doses tested. The following table compares nonclinical AUC values with the mean AUC values in male (1248 ng.h/ml) and female (1223 ng.h/ml) subjects at the recommended maximum dose of 10 mg/day.

Study	Species	Sex	High-dose (mg/kg)	AUC (ng.h/ml)	Multiple of human AUC
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Carcinogenicity	Mice	Males	200	6220	5
		Females	200	11,133	9
	Rats	Males	20	116	<1
		Females	15	246	<1
Fertility & early fetal development	Mice	Males	250	• 6220	• 5*
		Females	250	12,379	10
	Rats	Males	50	~1904	1.5
		Females	100	2118	1.7

\* estimated on AUC value following multiple doses at 200 mg/kg/day

**Carcinogenesis, Mutagenesis, Impairment of Fertility**

Solifenacin succinate was not mutagenic in the *in vitro* *Salmonella typhimurium* or *Escherichia coli* microbial mutagenicity test or in the *in vitro* chromosomal aberration test in human peripheral blood lymphocytes with or without metabolic activation or in the *in vivo* micronucleus test in rats.

No increase in tumors was found following the administration of solifenacin succinate to male and female mice for 104 weeks at doses up to 200 mg/kg/day (5 and 9 times human exposure at the maximum recommended human dose (MRHD), respectively), and male and female rats for 104 weeks at doses up to 20 and 15 mg/kg/day, respectively (<1 times exposure at the MRHD).

Solifenacin succinate had no effect on reproductive function, fertility or early embryonic development of the fetus in male and female mice treated with 250 mg/kg/day (13 times exposure at the MRHD) of solifenacin succinate, and in male rats treated with 50 mg/kg/day (<1 times exposure at the MRHD) and female rats treated with 100mg/kg/day (1.7 times exposure at the MRHD) of solifenacin succinate.

- 3) In mice, administration of solifenacin during organogenesis resulted in reduced fetal weights at 100 and 250 mg/kg, and an increased incidence of cleft palate at 250 mg/kg/day. Solifenacin did not produce any adverse effects in rats or rabbits at the highest doses tested. Following administration of solifenacin from gestation day 6 through lactation, reduced pup weights and delays in physical development (e.g., eye opening and vaginal patency) occurred at the 100 and 250 mg/kg/day dosages and postpartum pup mortality occurred at 250 mg/kg. The only effect

observed in F2 fetuses was an increase in males in the 250 mg/kg/day group. The NOAEL for peri/postnatal development was 30 mg/kg/kg. The following table compares nonclinical AUC values with the mean AUC values in female (1223 ng.h/ml) subjects at the recommended maximum dose of 10 mg/day.

Study	Species	Sex	Dose (mg/kg)	AUC (ng.h/ml)	Multiple of human AUC
Embryo-Fetal Development	Mice	Females	30	2564	2
			100	6061	5
			250	9760	8
	Rats	Females	50	234	<1
	Rabbits	Females	50	2174	1.8
Prenatal/Postnatal Development	Mice	Females	250	9760	8

#### Pregnancy, Teratogenic Effects, Pregnancy Category

Pregnancy Category ~C

Reproduction studies have been performed in mice, rats and rabbits. After oral administration of <sup>14</sup>C- solifenacin succinate to pregnant mice, drug-related material has shown to cross the placental barrier. No embryotoxicity or teratogenicity was observed in mice treated with 30 mg/kg/day (1.2 times exposure at the MRHD)

Administration of solifenacin succinate to pregnant mice at doses of 100 mg/kg and greater times exposure at the MRHD) during the major period of organ development resulted in reduced fetal body weights. Administration of 250 mg/kg ~ times exposure at the MRHD) to pregnant mice resulted in an increased incidence of cleft palate. In utero and lactational exposures to maternal doses of solifenacin succinate of 100 mg/kg/day and greater ~ times exposure at the MRHD) resulted in reduced peripartum and postnatal survival, reductions in body weight gain, and delayed physical development (eye opening and vaginal patency). An increase in the percentage of male offspring was also observed in litters from offspring exposed to maternal doses of 250 mg/kg/day. -

ε No embryotoxic effects were observed in rats at up to 50 mg/kg/day (<1 times exposure at the MRHD) or in rabbits at up

to 50 mg/kg/day (1.8 times exposure at the MRHD). There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, VESICARE<sup>®</sup> should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

### **Labor and Delivery**

The effect of VESICARE<sup>®</sup> on labor and delivery in humans has not been studied. There were no effects on natural delivery in mice treated with 30 mg/kg/day (1.2 - times exposure at the MRHD). Administration of solifenacin succinate at 100 mg/kg/day (3.6 - times exposure at the MRHD) or greater increased in peripartum pup mortality.

### **Nursing Mothers**

After oral administration of <sup>14</sup>C-solifenacin succinate to lactating mice, radioactivity was detected in maternal milk. There were no adverse observations in mice treated with 30 mg/kg/day (1.2 - times exposure at the MRHD). Pups of female mice treated with 100 mg/kg/day (3.6 - times exposure at the MRHD) or greater revealed reduced body weights, postpartum pup mortality or delays in the onset of reflex and physical development during the lactation period.

It is not know whether solifenacin succinate is excreted in human milk. Because many drugs are excreted in human milk, VESICARE<sup>®</sup> should not be administered during nursing. A decision should be made whether to discontinue nursing or to discontinue VESICARE<sup>®</sup> in nursing mothers.

## **X. APPENDIX/ATTACHMENTS:**

**Addendum to review:** none

**Other relevant materials (Studies not reviewed, appended consults, etc.):** none

**Appendix/Attachments:**

Appendix 1: ExecCAC minutes from May 27, 2003

Appendix 2: Statistical review of mouse and rat carcinogenicity studies

**Any compliance issues:** none

## APPENDIX 1

### Executive CAC

**Date of Meeting: May 27, 2003**

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair  
Abby Jacobs, Ph.D., HFD-540, Alternate Member  
David Jacobson-Kram, Ph.D., HFD-024, Alternate Member  
John Leighton, Ph.D., HFD-150, Alternate Member  
Alex Jordan, Ph.D., HFD-580, Team Leader  
Lynnda Reid, Ph.D., HFD-580, Presenting Reviewer

Author of Minutes: Lynnda Reid

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

NDA # 21,518

Drug Name: VESICARE™ (solifenacin succinate)

Sponsor: Yamanouchi Pharmaceutical Co., Ltd.

Background: Solifenacin succinate (YM905) is a new molecular entity shown to have antagonistic action on muscarinic M3 receptors. The proposed indication is for the —

Two-year bioassays were conducted in rats and mice to assess carcinogenicity potential. The protocols for both studies were approved by the executive CAC on July 27, 1999.

Rat Carcinogenicity Study: YM905 was administered orally by gavage at 3, 10 or 20 mg/kg/day in males, and 3, 7.5 or 15 mg/kg/day in females. SDA (sialodacryoadenitis) viral infections occurred at about 6 months into the study. There was a particularly high mortality rate in females dosed at 7.5 and 15 mg/kg between weeks 23 and 26 at the beginning of the infection.

Mean body weights were significantly lower for both the high-dose males and females over the course of the entire study.

Histological examinations were routinely performed only on tissues from high-dose and control animals. There was no statistically significant increase in tumor incidence in the treated groups when compared to controls.

Mouse Carcinogenicity Study: YM905 was administered orally by gavage at 10, 30, 100 or 200 mg/kg/day. There was a treatment/dose-related increase in deaths attributed to respiratory disease during the first 52 weeks of the study. Mortality was significantly increased in both males and females receiving 100 mg/kg/day and in males receiving 200 mg/kg/day apparent during the first year of the study. After week 53, mortality in each dose group was similar to the lower doses.

Overall, bodyweight gain reductions in males receiving 100 or 200 mg/kg were 19 and 20%, respectively. Those in females receiving 30, 100 or 200 mg/kg/day were 17, 23 and 36%,

respectively. Statistically significant decreases in high-dose animals were observed beginning between weeks 4-5 in males and weeks 10-12 in females.

Beginning week 20, overall food consumption was reduced by approximately 5% in males and females dosed at 200 mg/kg/day. Overall food intake in animals receiving 10, 30 or 100 mg/kg/day was unaffected by treatment.

Histological examinations were routinely performed only on tissues from high-dose and control animals. There was no statistically significant increase in tumor incidence in the treated groups when compared to controls.

#### Executive CAC Recommendations and Conclusions:

##### Rat Study:

- The Committee agreed that the study doses were adequate since they had received concurrence from the Exec CAC based on MTD (lethality at 60 mg/kg/day).
- The Committee considered whether the viral infection should invalidate the study, and decided that by itself it probably doesn't disqualify the study. However, they would like more details, particularly in regards to the relationship of the infection and effects on study animals (e.g., loss of weight gain, mortality, etc.), and would like to know if any treatment was used for the infection.
- The Committee noted that with significantly lower survival in the high-dose group and greater than 10% body weight loss (both of which occurred), the sponsor needs to look at histopathology in the mid- and low-dose animals.

##### Mouse Study:

- The Committee agreed that the study doses were adequate since they had received concurrence from the Exec CAC based on MTD (lethality at 60).
- As with the rats, the committee would like the sponsor to more fully characterize the respiratory infections and indicate if any treatment was given.
- As with the rats, the committee recommends that the sponsor look at histopathology in the remaining treatment groups because of the low survival and greater than 10% body weight loss at the higher dose levels.

CAC concurrence on the absence of drug-related neoplasms in rats and mice cannot be given until the diagnoses for all tissues and groups have been reevaluated.

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

## APPENDIX 2

Statistical Review and Evaluation  
Carcinogenicity

NDA No:	21-518
Trade Name:	YM905
Applicant:	Yamanouchi Pharmaceutical Co., Ltd.
Research Laboratory:	—
Pharmacologist:	Lynnda Reid, Ph.D. (HFD-870)
Statistical Reviewer:	Moh-Jee Ng, M.S. (HFD-715)

## SUMMARY

- In the 2-year mouse study, there were statistically significant positive trends in survival rate and statistically significant difference in survival distributions among treatment groups for both males and females. There was no statistically significant positive linear trend in tumor incidence rate detected for both males and females.
- In the 2-year rat study, there were statistically significant positive trends in survival rate and statistically significant difference in survival distributions among treatment groups for females but not in males. There was no statistically significant positive linear trend in tumor incidence rate detected for both males and females.

## 1. Introduction

This reviewer evaluated the carcinogenic potential of YM905 that was administered daily by oral gavage for a 104 weeks. This report includes the results of the analyses of the survival and tumor data.

## 2. Studies Designs

The study designs of mice and rats are summarized in the following Table.

Table 1

Species	Mice	RatS
Study Number	R905-TX-023	R905-TX-024
Strain	— CD-1(CR) BR	CDF@(F-344) — 3R
Route of Administration	Oral	Oral
Frequency of Drug Administration	Daily	Daily
Dose Unit	mg/kg/day	mg/kg/day
Dose Level	0, 10, 30, 100, 200 for male 0, 10, 30, 100, 200 for female	0, 3, 10, 20 for male 0, 3, 7.5, 15 for female
Control Group Dose Level	0.5% methylcellulose aqueous solution at a dose volume of 10 mL/kg/day	0.5% methylcellulose aqueous solution at a dose volume of 5 mL/kg/day
Number of Animals/sex/per treatment group	70 males/group 70 females/group	60 males/group 60 females/group
Length of Study	104 weeks	104 weeks

For mice, there were one control group and four treated groups known as low, medium, medium-high, and high dose. The dose levels were 0, 10, 30, 100 and 200 mg/kg/day, and 70 animals of each sex/group were used. For rats, there were one control group and three treated groups known as low, medium, and high dose. The dose levels were 0, 3, 10, 20 for males; and 0, 3, 7.5 and 12 for females; and 60 animals of each sex/group were used. All surviving males and females were necropsied following a minimum of 104 weeks of dosing. The terminal sacrifice started at and after week 104.

### 3. Sponsor's Tumor Analyses and Findings

The sponsor used the Log-rank method to analyze the number of animals with tumors across treatment groups (Mantel 1966, Peto 1974, Peto et al. 1980). The sponsor used a one-tailed Fisher's exact test for the pairwise comparison of each treatment group against the control group when there were fewer than ten observed tumors.

The sponsor listed the following findings in its reports.

#### In survival analysis:

For Mice:

- There was a statistical significantly difference in mortality in females receiving 100 mg/kg/day ( $p=0.004$ ) when compared with the control group.
- There were statistical significantly differences in mortality in males receiving 100 mg/kg/day ( $p=0.019$ ) and 200 mg/kg/day ( $p=0.003$ ) when compared with the control group.
- The sponsor concluded that the increased mortalities in higher dose groups were apparent during the first year of the study. After week 53, the mortalities for all groups were almost comparable for both females and males.

For Rats:

- There was a statistical significantly difference in the mortality in females receiving 15 mg/kg/day when compared with the control group.

#### In tumor analysis:

- No significantly positive linear trend in incidence rate in tumor data was detected for both male and females.

The sponsor concluded that there was no carcinogenic potential in treated with YM905 for both mice and rats in both males and females for 104 weeks.

### 4. Reviewer's Evaluation

This reviewer performed independent analyses on the survival and tumor data submitted by the sponsor, using the programs written by Dr. Ted Guo of Division of Biostatistics II. The primary statistical methods used were described by Peto *et al.* (1980), and Lin and Ali (1994). These methods adjust differences in animal mortality and take the fatal or prevalence context of observation of the tumor into consideration. The intervals used for the adjustment of mortality were 0-52, 53-78, 79-91 and 92-103 weeks and terminal sacrifice for animals. The actual doses were used as weights in the analyses.

The statistical analyses of carcinogenicity study data consisted of two parts, namely, the survival data analysis and the tumor data analysis. The survival data analysis was: 1) to examine the differences in survival distributions among the treatment groups (homogeneity test); and 2) to determine if there is a positive trend in the proportion of deaths with respect to the dose levels (Trend test). Two statistical tests were used in the survival data analysis: the Cox test and the generalized Kruskal-Wallis test. The theoretical background of these tests was described by Lin and Ali (1994) and Thomas *et al* (1977).

The tumor data analysis was to determine if there is a positive trend in the proportions of a selected tumor type in a selected organ/tissue with respect to the dose levels. The tumors were classified as either fatal (lethal) or non-fatal (non-lethal), according to Peto *et al* (1980). The reviewer applied the death-rate method to data of fatal tumors and the prevalence method to data of non-fatal tumors. For tumors that caused death for some, but not for all, animals, a combined test was performed.

A rule for adjusting the effect of multiple testings proposed by Haseman (1983) can be used to adjust for the effect of multiple testings in pairwise comparisons. Haseman's rule says that rare tumors should be tested at 0.05 level of significance and common tumors should be tested at 0.01 level of significance. A similar rule proposed by the Office of Biostatistics, CDER/FDA for trend tests was used in this review for tests for positive trend. The rule states that in order to keep the overall false-positive rate at the nominal level of approximately 0.1, tumor types with spontaneous tumor rates of 1% or less (rare tumors) should be tested at 0.025 significance level, otherwise (common tumors) at 0.005 significance level (Lin and Rahman, 1998).

#### **4.1 Evaluation of Carcinogenicity Study on Mice**

This reviewer's evaluation comprises the following components:

- Survival data analysis
- Tumor data analysis

##### **4.1.1 Survival Data Analysis of Mice**

The survival data analysis determines whether the dose-mortality trend is statistically significant. A positive result indicates that the mortality increases as the dose level increases.

- Tables 2 and 3 present the cumulate percentages of death by dose group for female and male, respectively. The time interval "Final Kill 104-106" presents the terminal-sacrifice interval. Figure 1 and 3 present bar charts of the cumulate percentages fo death for female and male, respectively.
- Figures 2 and 4 present plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for female and male, respectively.
- Tables 4 and 5 present results of test for dose-mortality trend for female and male using the methods described in the paper "Trend and Homogeneity Analysis of Proportions and Life Table Data" version 2.1, by Donald G. Thomas, National Cancer Institute.

Table 2  
Cumulative Percentage of Death in Female Mice

Dose Group	Weeks	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CONTROL	0-52	70	3	67	95.7	4.3
	53-78	67	13	54	77.1	22.9
	79-91	54	7	47	67.1	32.9
	92-103	47	16	31	44.3	55.7
	FINALKILL104-106	31	31	0		
LOW (10 mg/kg/day)	0-52	70	5	65	92.9	7.1
	53-78	65	6	59	84.3	15.7
	79-91	59	13	46	65.7	34.3
	92-103	46	8	38	54.3	45.7
	FINALKILL104-106	38	38	0		
MEDIUM (30 mg/kg/day)	0-52	70	10	60	85.7	14.3
	53-78	60	19	41	58.6	41.4
	79-91	41	4	37	52.9	47.1
	92-103	37	13	24	34.3	65.7
	FINALKILL104-106	24	24	0		
MEDIUM-HIGH (100 mg/kg/day)	0-52	70	31	39	55.7	44.3
	53-78	39	6	33	47.1	52.9
	79-91	33	5	28	40.0	60.0
	92-103	28	7	21	30.0	70.0
	FINALKILL104-106	21	21	0		
HIGH (200 mg/kg/day)	0-52	70	18	52	74.3	25.7
	53-78	52	10	42	60.0	40.0
	79-91	42	3	39	55.7	44.3
	92-103	39	10	29	41.4	58.6
	FINALKILL104-106	29	29	0		

Figure 1  
Bar Chart of Cumulative Percentage of Death in Female Mice

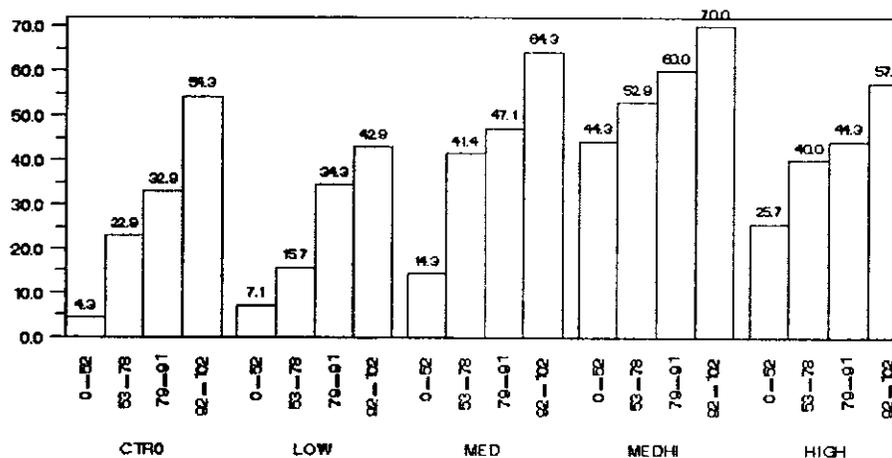


Figure 2  
Kaplan-Meier Survival Functions for Female Mice

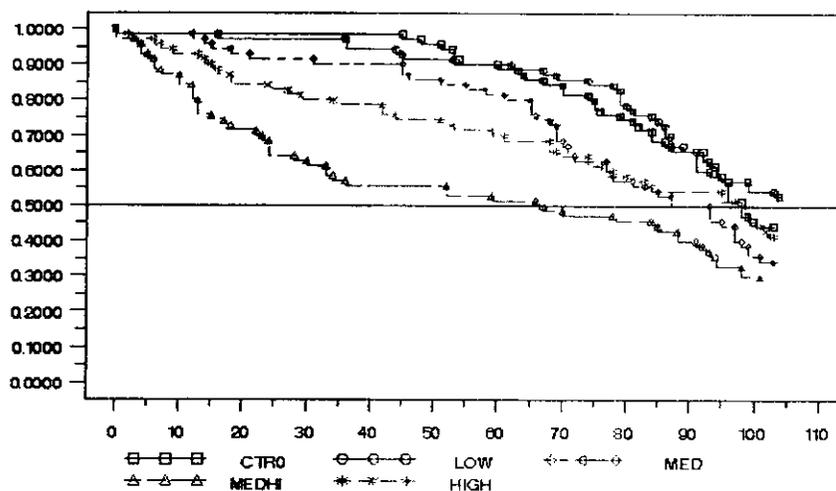


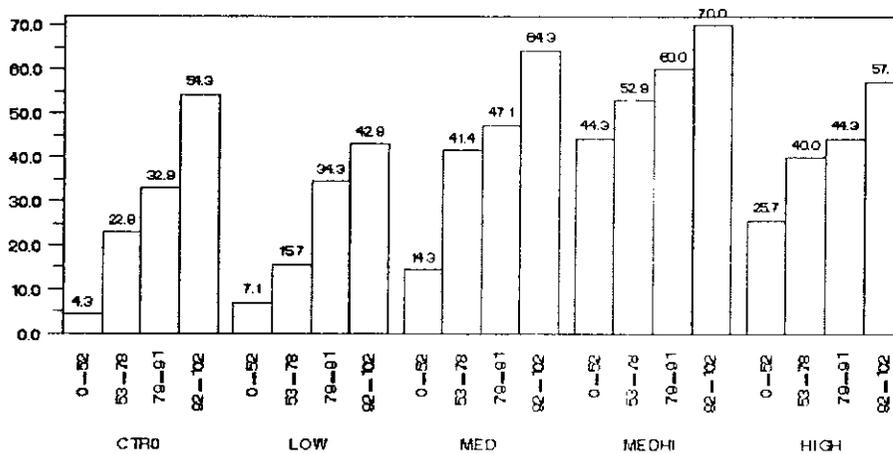
Table 3

Cumulative Percentages of Death in Male Mice

Dose Group	Weeks	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CONTROL	0-52	70	2	68	97.1	2.9
	53-78	68	13	55	78.6	21.4
	79-91	55	5	50	71.4	28.6
	92-103	50	12	38	54.3	45.7

	FINALKILL104-106	38	38	0		
LOW (10 mg/kg/day)	0-52	70	4	66	94.3	5.7
	53-78	66	11	55	78.6	21.4
	79-91	55	11	44	62.9	37.1
	92-103	44	4	40	57.1	42.9
	FINALKILL104-106	40	40	0		
MEDIUM (30 mg/kg/day)	0-52	70	9	61	87.1	12.9
	53-78	61	12	49	70.0	30.0
	79-91	49	10	39	55.7	44.3
	92-103	39	9	30	42.9	57.1
	FINALKILL104-106	30	30	0		
MEDIUM-HIGH (100 mg/kg/day)	0-52	70	13	57	81.4	18.6
	53-78	57	12	45	64.3	35.7
	79-91	45	7	38	54.3	45.7
	92-103	38	12	26	37.1	62.9
	FINALKILL104-106	26	26	0		
HIGH (200 mg/kg/day)	0-52	70	26	44	62.9	37.1
	53-78	44	10	34	48.6	51.4
	79-91	34	6	28	40.0	60.0
	92-103	28	2	26	37.1	62.9
	FINALKILL104-106	26	26	0		

Figure 3  
Bar Chart of Cumulative Percentage of Death in Male Mice



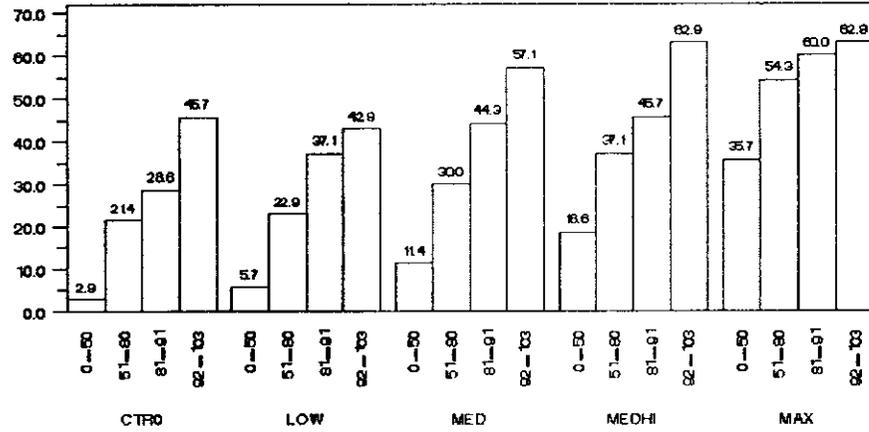
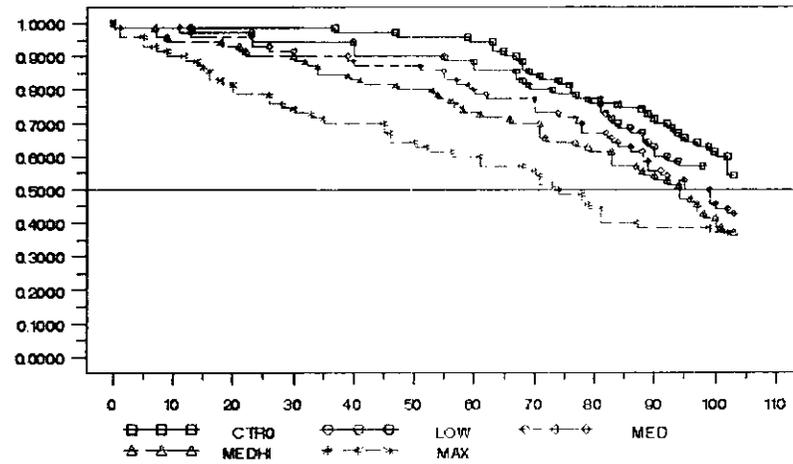


Figure 4  
Kaplan-Meier Survival Functions for Male Mice



The dose-mortality trend tests and homogeneity tests for female mice (presented in Table 4) and male mice (presented in Table 5) are statistically significant using the Cox test and the Kruskal-Wallis test.

Table 4  
Results of Tests for Dose-Mortality trend for Female Mice

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	12.2508	0.0066	16.8656	0.0008
Dose-Mortality Trend	6.7609	0.0093	12.5838	0.0004
Homogeneity	19.0117	0.0008	29.4494	0.0000

Table 5  
Results of Tests for Dose-Mortality trend for Male Mice

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	1.0654	0.7854	1.5660	0.6671
Dose-Mortality Trend	15.2996	0.0001	22.1656	0.0000
Homogeneity	16.3650	0.0026	23.7316	0.0001

At the end of the 1<sup>st</sup> year, the group of female mice receiving 100 mg/kg/day had a very high mortality rate of 44.3% compared with 4.3%, 7.1%, 14.3% and 25.7% for the groups of female mice receiving control, 10, 30, and 200 mg/kg/day.

**4.1.2 Tumor Data Analysis for Mice**

The tumor data analysis determines whether the positive linear trend in tumor incidence is statistically significant. This reviewer tested the trend for every organ and tumor combination with the data provided by the sponsor. This reviewer tested the dose-tumor trend across the control group and the three treated groups. The daily doeses of 0, 10, 30, 100 and 200 mg/kg/day were used as weights for those tests. The time intervals used for the adjustment of mortality were 0-52, 53-78, 79-91, 91-104 weeks, and terminal sacrifice. The resulting p-values are compared against the p-value cutoff points set by the FDA procedures.

The results of tumor analysis show that there is no significantly positive linear trend in tumor incidence rate in both males and females.

**4.1.3 Conclusion of Mouse Study**

In the 2-year mouse study, there were significantly positive trends in survival rate and statistically significant difference in survival distributions among the treatment groups in both females and males. There was no significantly positive linear trend in incidence rate in the tumor data of both males and females.

**4.1.4 Evaluation of Validity of the Study Designs**

This reviewer’s analysis did not find any tumor type with a significantly positive trend in the

mouse study. However, before drawing the conclusion that the drug is not carcinogenic in mice, it is important to look into the following two issues pointed out in the paper by Haseman (1984). The two issues are:

- 1) Were enough animals exposed to a drug for a sustained amount of time to the risk of late developing tumors?
- 2) Were dose levels high enough to pose a reasonable tumor challenge to the tested animals?

This is no consensus among experts in the field regarding the number of animals and the length of time at risk, although most carcinogenicity studies are designed to run for two years with fifty animals per treatment group. The following are some rules of thumb regarding these two issues suggested by experts in this field: Haseman (1984) investigated the first issue. He gathered data from 21 studies using Fischer 344 rats and B6C3F1 mice conducted at the National Toxicology Program (NTP). It was found that, on an average, approximately 50% of the animals in the high dose group survived the two-year study period. Haseman suggested that, as a rule of thumb, a 50% survival of 50 initial animals in the high dose group, between weeks 80-90, would be considered as a sufficient number of animals under an adequate exposure. However, the percentage can be lower or higher if the number of animals used in each treatment/sex group is larger or smaller than 50 so that there will be between 20-30 animals still alive during these weeks. In addition, Chu, Cueto, and Ward (1981) suggested that "To be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50% survival at one-year." It appears, from the above sources, that the proportions of survival at 52 weeks, 80-90 weeks, and two years are of interest in determining the adequacy of exposure and number of animals at risk. For the adequacy of the chosen dose levels, it is generally accepted that the high dose should be close to the MTD (maximum tolerated dose). Chu, Cueto, and Ward (1981) suggested the following rules on this issue:

- I) "A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dose group relative to the controls." or
- II) "The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical." Or
- III) "In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls."

Bart, Chu, and Tarone (1979) stated that the mean body weight curves over the entire study period should be taken into consideration with the survival curves, when adequacy of dose levels is to be examined. In particular, "usually, the comparison should be limited to the early weeks of a study when no or little mortality has yet occurred in any of the groups. Here a depression of the mean weight in the treated groups is an indication that the treatment has been tested on levels at or approaching the MTD."

Based on the above suggestions and recommendations, this reviewer examined the validity of the experimental design of the mouse study.

#### ANALYSIS OF MICE SURVIVAL AND BODY WEIGHT DATA

The following are the summary survival data of mice for the medium-high dose and high dose groups at weeks 52, 91, and end of the study.

Table 6

**I. Survival data for Medium-High and High Doses of Mice**

I. Dose	II. Sex	End of 52 Weeks	End of 91 Weeks	End of Study
Medium-High Dose	Male	81%	54%	37%
	Female	56%	40%	30%
High dose	Male	63%	40%	37%
	Female	74%	56%	43%

- The survival rate for females in the medium-high dose group at week 91 was 40% (Table 6) which is less than 50% survival of 70 initial animals. However, the high dose group had a 56% survival at week 91.
- The survival rates for males at week 91 (40%) is less than 50% survival of 70 initial animals in the high dose group.
- From the percentages in the table above and the survival criteria mentioned, it may be reasonable to conclude that there are enough mice exposed for a sustained amount of time to the drug at the medium-high dose and high dose groups for both males and females.

The following table summarizes the percentages of weight gain of treated groups as compared to control groups for mice.

**Table 7  
Mean Body Weight Gain for Mice**

Sex	Groups	Mean Body Weight (grams)		Mean Body Weight Gain	% Differences in MBWG
		Beginning Study	End of Study		
Male	Control	32	44	12	
	Low	33	44	11	-8.3
	Medium	31	43	12	0
	Medium-High	32	41	9	-25
	High	31	41	10	-16.7
Female	Control	25	37	12	
	Low	26	37	11	-8.3
	Medium	26	36	10	-16.7
	Medium-High	25	36	11	-8.3
	High	24	33	9	-25

- The body weight gain data result in Table 7 suggested that the medium-high and high doses for males and the medium and high doses for females might be over the MTD according to the criterion proposed by Chu, Cueto, and Ward (1981). The mortality rates for the medium high and high dose groups were also significantly higher than that of the control groups for both males and females. This is another indication that the medium and high doses are over MTD. The above evaluation of validity of the study designs was based on the information contained in the data of body weight gain and mortality of the mouse study. The information about clinical signs and histopathologic effects attributed to the drug should also be included in the final evaluation.

## 4.2 Evaluation of Carcinogenicity Study on Rats

This reviewer's evaluation comprises the following components:

- Survival data analysis
- Tumor data analysis

### 4.2.1 Survival Data Analysis of Rats

The survival data analysis determines whether the dose-mortality trend is statistically significant. A positive result indicates that the mortality increases as the dose level increases.

- *Tables 8 and 9 present the cumulative percentages of death by dose group for female and male, respectively. The time interval "Final Kill 104-106" presents the terminal-sacrifice interval. Figures 5 and 7 present bar charts for the cumulative percentages of death for female and male, respectively.*
- Figures 6 and 8 present plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for female and male, respectively.
- Tables 10 and 11 present results of test for dose-mortality trend for female and male using the methods described in the paper "Trend and Homogeneity Analysis of Proportions and Life Table Data" version 2.1, by Donald G. Thomas, National Cancer Institute.

Table 8  
Cumulative Percentages of Death in Female Rats

Dose Group	Weeks	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
Control	0-52	60	4	56	93.3	6.7
	53-78	56	2	54	90.0	10.0
	79-91	54	4	50	83.3	16.7
	92-104	50	8	42	70.0	30.0
	FINALKILL105-105	42	42	0		
Low (3 mg/kg/day)	0-52	60	6	54	90.0	10.0
	53-78	54	3	51	85.0	15.0
	79-91	51	5	46	76.7	23.3
	92-104	46	3	43	71.7	28.3
	FINALKILL105-105	43	43	0		
Medium (10 mg/kg/d)	0-52	60	11	49	81.7	18.3
	53-78	49	4	45	75.0	25.0
	92-104	45	12	33	55.0	45.0
	FINALKILL105-105	33	33	0		
High (25 mg/kg/d)	0-52	60	23	37	61.7	38.3
	53-78	37	4	33	55.0	45.0
	79-91	33	3	30	50.0	50.0
	92-104	30	6	24	40.0	60.0
	FINALKILL105-105	24	24	0		

Figure 5  
Bar Chart of Cumulative Percent Death of Female Rats

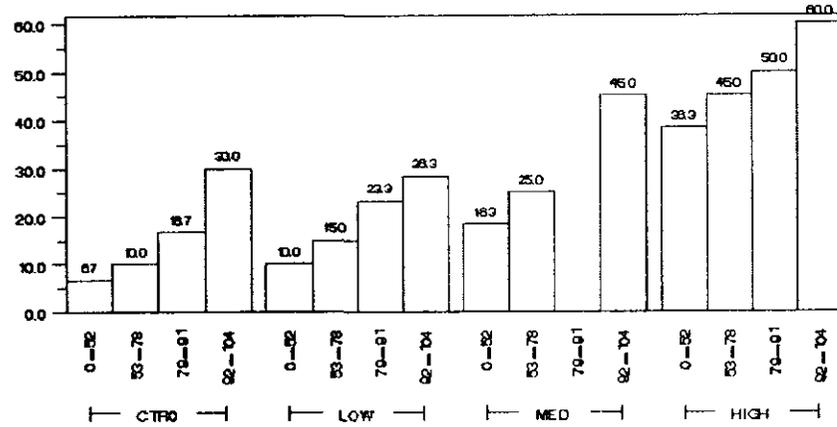
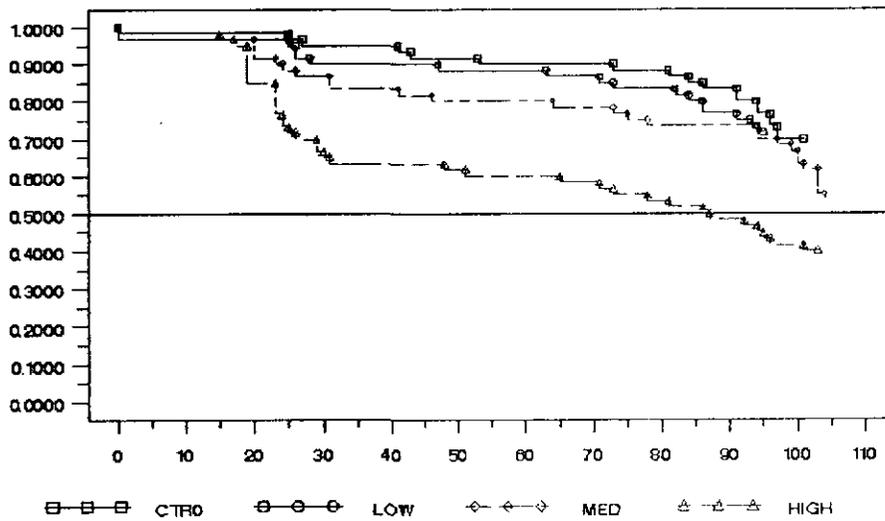


Figure 6  
Kaplan-Meier Survival Functions for Female Rats



**Table 9**  
Cumulative Percentages of Death in Male Rats

Dose Group	Weeks	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
Control	53-78	60	4	56	93.3	6.7
	79-91	56	7	49	81.7	18.3
	92-104	49	14	35	58.3	41.7
	FINALKILL105-105	35	35	0		
Low (3 mg/kg/day)	0-52	60	4	56	93.3	6.7
	53-78	56	3	53	88.3	11.7
	79-91	53	5	48	80.0	20.0
	92-104	48	17	31	51.7	48.3
	FINALKILL105-105	31	31	0		
Medium (10 mg/kg/d)	0-52	60	5	55	91.7	8.3
	53-78	55	3	52	86.7	13.3
	79-91	52	8	44	73.3	26.7
	92-104	44	8	36	60.0	40.0
	FINALKILL105-105	36	36	0		
High (25 mg/kg/d)	0-52	60	8	52	86.7	13.3
	53-78	52	2	50	83.3	16.7
	79-91	50	6	44	73.3	26.7
	92-104	44	9	35	58.3	41.7
	FINALKILL105-105	35	35	0		

Figure 7  
Bar Chart of Cumulative Percent Death of Male Rats

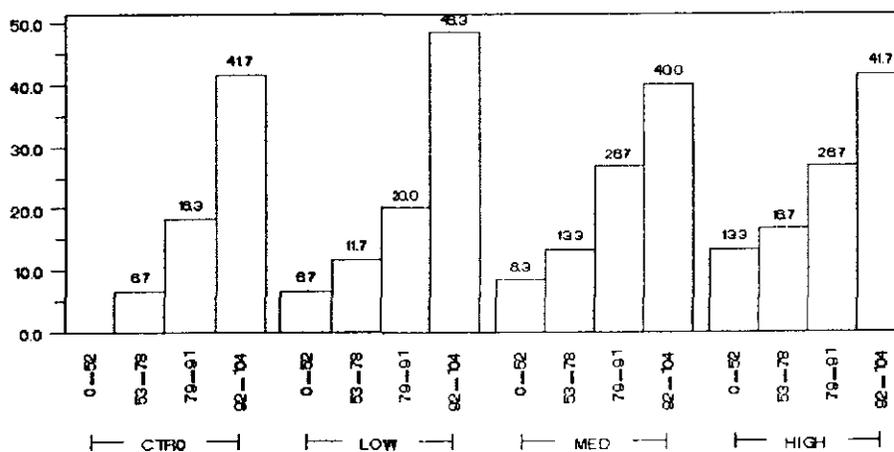
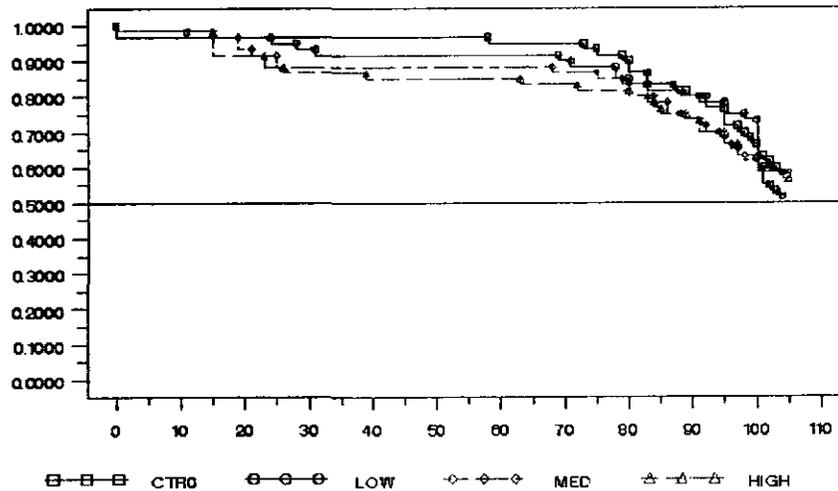


Figure 8  
Kaplan-Meier Survival Function for Male Rats



The dose-mortality trend test and homogeneity test for female rats (presented in Table 10) is statistically significant using the Cox test and the Kruskal-Wallis test but not in males rats (presented in Table 11).

Table 10  
Results of Tests for Dose-Mortality trend for Female rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	4.2299	0.1206	4.7885	0.0912
Dose-Mortality Trend	18.2384	0.0000	21.1945	0.0000
Homogeneity	22.4683	0.0001	25.9830	0.0000

Table 11  
Results of Tests for Dose-Mortality trend for Male rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	0.3743	0.8293	0.1487	0.9284
Dose-Mortality Trend	0.0073	0.9319	0.1961	0.6579
Homogeneity	0.3816	0.9440	0.3447	0.9514

At the end of the 1<sup>st</sup> year, the group of female rats receiving 15 mg/kg/day had a very high mortality rate of 38.3% compared with 6.7%, 10%, and 18.3% for the groups receiving 0, 3, and 7.5 mg/kg/day.

#### 4.2.2 Tumor Data Analysis for Rats

The tumor data analysis determines whether the positive linear trend in tumor incidence is statistically significant. This reviewer tested the trend for every organ and tumor combination with the data provided by the sponsor. This reviewer tested the dose-tumor trend across the control group and the three treated groups. The daily doses 0, 3, 7.5, and 15 mg/kg/day for females and 0, 3, 10, and 20 mg/kg/day for males were used as weights for those tests. The time intervals used for the adjustment of mortality were 0-52, 53-78, 79-91, 91-103 weeks, and terminal sacrifice. The resulting p-values are compared against the p-value cutoff points set by the FDA procedures.

The results of tumor analysis show that there is no significantly positive linear trend in tumor incidence rate in both males and females.

#### 4.2.3 Conclusion of Rat Study

In the 2-year rat study, there was no significantly positive trend in survival rate and no statistically significant difference in survival distributions among treatment groups in males. However, The dose-mortality trend for female rats was statistically significant using the Cox test and the Kruskal-Wallis test. There was no significantly positive linear trend in incidence rate in tumor data of both males and females. Since this reviewer's analysis did not detect any tumor type with a significantly positive trend in the rat study, it is necessary to evaluate the validity of the study design using the criteria described in section 4.1.4 in the following analysis of survival and body weight data.

#### ANALYSIS OF RAT SURVIVAL AND BODY WEIGHT DATA

The following are the summary survival data of rats for the high dose group at weeks 52, 91, and end of the study.

Table 12  
II. Survival data for High Dose of Rats

III. Sex	End of 52 Weeks	End of 91 Weeks	End of Study
Male	87%	73%	58%
Female	62%	50%	40%

The survival rates for the high dose group were 40% and 58% for females and males, respectively. With 60 initial animals used in the treatment groups, it may be reasonable to conclude that there were enough rats exposed for a sustained amount of time to the drug for both males and females.

The following table summarizes the percentages of weight gain as compared to control groups for rats.

Table 13  
Mean Body Weight Gain for Rats

Sex	Groups	Mean Body Weight (grams)		Mean Body Weight Gain	% Differences in MBWG
		Beginning Study	End of Study		
Male	Control	118	371	253	
	Low	117	365	248	-2
	Medium	115	360	245	-3.2
	High	116	329	213	-15.8
Female	Control	98	251	153	
	Low	96	250	154	+0.7
	Medium	96	247	151	-1.3
	High	97	230	133	-13.1

The high dose for male and female rats had 15.8% and 13.1% weight gain decrement, respectively. The body weight gain data suggested for high doses used for male and female rats might be over the MTD according to the criterion proposed by Chu, Cueto, and Ward (1981). The significantly higher mortality rates of the high dose group of female rats also indicated that the high dose might be over MTD for female rats. The above evaluation of validity of the study designs was based on the information contained in the data of body weight gain and mortality of the mouse study. The information about clinical signs and histopathologic effects attributed to the drug should also be included in the final evaluation.

## 5. References

- 1) Bart, Chi, and Tarone (1979). "Statistical Issues in Interpretation of Chronic Bioassay Tests for Carcinogenicity." Journal of the National Cancer Institute. Vol. 62, pp.957-974.
- 2) Chu, Cueto and Ward (1981). "Factors in the evaluation of 200 national cancer institute carcinogen bioassay." Journal of Toxicology and environmental Health. Vol. 8, pp.251-80.
- 3) Haseman, J. K. (1983). "A re-examination of false-positive rates for carcinogenesis studies." Fundamental and Applied Toxicology, 3, pp.334-9
- 4) Haseman, J. K. (1985). "Issues in carcinogenicity testing: Dose selection." Fundamental and Applied Toxicology. Vol. 5. Pp. 66-78.
- 5) Lang, P. L. (1992). "Spontaneous neoplastic lesions and selected non-neoplastic lesions in the CrI:CD BR rat." Charles River Laboratories. Table 5b. neoplasms 24 Month Studies Female CD®Rats. pp.23
- 6) Lin, K. K. and M. Ali (1994), "Statistical Review and Evaluation of Animal Tumorigenicity Studies" Statistical in the Pharmaceutical Industry, Second Edition, Revised and Expanded, edited by C.R.Buncher and J.Y. Tsay, Marcel Dekker, Inc., New York. pp. 19-57.
- 7) Lin, K. K. and M. A. Rahman (1998). "Overall False Positive Rates in Tests for Linear Trend in Tumor Incidence in Animal Carcinogenicity Studies of New Drug."

- Journal of Biopharmaceutical Statistics, 8(1), 1-15 (1998)
- 8) Peto *et al* (1980). "Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments," In Long-Term and Short-Term Screening Assays for Carcinogens: An Critical Appraisal, International Agency for Research on Cancer, Lyon, France. IARC monographs supplement, 2, pp.311-426
  - 9) Thomas *et al* (1977). "Trend and Homogeneity Analyses of Proportions and Life Table Data," Computer and Biomedical Research, 10, pp.373-381.

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