CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-595

STATISTICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
OFFICE OF BIOSTATISTICS

Statistical Review and Evaluation

CLINICAL STUDIES

NDA:	21-595
Name of drug:	——— (trospium chloride) 20 mg tablets
Applicant:	Indevus Pharmaceuticals Inc.
Indication:	Treatment of the symptoms associated with overactive bladder
Documents reviewed:	Vol. 1.1; electronic submission
	\\CDSESUB1\N21595\N_000\2003-04-28\clinstat
	\\CDSESUB1\N21595\N_000\2004-02-23\clinstat
Project manager:	Dale Cutright
Clinical reviewer:	Suresh Kaul, M.D.
Dates:	Received 4/27/03; user fee (10 months) 2/27/04;
	Extended PDUFA date to 5/28/04 for addl. study submitted
Statistical reviewer:	Kate Meaker, M.S.
Statistics team leader:	Mike Welch, Ph.D.
Biometrics division director:	Ed Nevius, Ph.D.

Keywords: NDA review, clinical studies

1 EXECUTIVE SUMMARY OF STATISTICAL FINDINGS

1.1 CONCLUSIONS AND RECOMMENDATIONS

This application seeks approval for the indication of the treatment of symptoms of overactive bladder for a single dose regimen of Trospium. It includes two prospectively planned placebo-controlled Phase 3 studies in the U.S., along with supportive evidence from a placebo-controlled European study. The results from the U.S. studies support the efficacy of Trospium on two co-primary endpoints and two additional secondary endpoints of interest (all p-values ≤ 0.012). The collective evidence for the efficacy assessment shows that two principle trials, IP631-003 and IP631-005, provide consistent evidence of efficacy for the four endpoints of interest. The results of these two studies give sufficient statistical evidence to support the efficacy of Trospium for the desired indication.

1.2 OVERVIEW OF CLINICAL PROGRAM AND STUDIES REVIEWED

This application seeks approval for the indication of the treatment of symptoms of overactive bladder for a single dose regimen of Trospium. It includes two prospectively planned placebo-controlled Phase 3 studies in the U.S., along with supportive evidence from a placebo-controlled European study. The results from the U.S. studies, IP631-003 and IP631-005, support the efficacy of Trospium on two co-primary endpoints and two additional secondary endpoints of interest (all p-values ≤ 0.012).

The strength of the supportive evidence from the European study is not straightforward, primarily because the planned endpoints in that study, MP94D2.14, were not the clinical endpoints assessed in the U.S. studies. The planned endpoints were two urodynamic variables, and Trospium was statistically significantly different from placebo on both. If the Medical Officer feels that the urodynamic endpoints provide direct evidence of efficacy, then that study does provide supportive evidence.

1.3 PRINCIPAL FINDINGS

The U.S. studies were prospectively planned with advice from the division. The two co-primary endpoints are Change in toilet voids per 24 hours and Change in average number of urge incontinence episodes per 24 hours. Two secondary variables of interest to the Medical officers are Change in volume voided per toilet void and Change in average urgency severity associated with toilet voids. None of these four endpoints was prospectively planned in the European study, MP94D2.14. In that study, the planned co-primary efficacy variables are Maximum cystometric bladder capacity and Bladder volume at first unstable detrusor contraction. These are both urodynamic variable. A summary of the endpoints and results by study is shown in Table 1.

Table 1: Summary of Results for 3 Studies

Endpoint	Study IP631-003	Study IP631-005	Study MP94D2.14
Change in toilet voids per 24 hrs	p < .001 ◊	p < .001 ◊	NA
Change in average number of urge incontinence episodes per 24 hrs	p = 0.012 ◊	p < .001	p = 0.68 (t-test)
Change in volume voided per toilet void	p < .001	p < .001	p < 0.01 (t-test)
Change in average urgency severity associated with toilet voids	p < .001	p < .001	NA
Maximum cystometric bladder capacity (ml)	NA	NA	p < 0.01 ◊ (t-test)
Bladder volume at first unstable detrusor contraction (ml)	NA	NA	p = 0.02 ◊ (t-test)

◊ Primary endpoints
NA = not primary or secondary endpoints in a study
Source: SAS datasets

2 STATISTICAL REVIEW AND EVALUATION OF EVIDENCE

2.1 INTRODUCTION AND BACKGROUND

There are three clinical studies which address the efficacy of Trospium for the treatment of symptoms of overactive bladder. These include two U.S. studies, IP631-003 and IP631-005, which are both prospectively planned placebo-controlled studies. Both were planned to address the clinical endpoints desired by the Division for this indication. These are the principle studies to support efficacy. The third study is a European study, MP94D2.14, which was planned to address different, urodynamic, endpoints. This provides supportive evidence of efficacy.

2.2 DATA ANALYZED AND SOURCES

The sponsor provided SAS datasets of all the efficacy variables. These were adequate to carry out my own analyses and to confirm analyses performed by the sponsor in the submission.

The efficacy endpoints of interest to the Medical Officers were collected using daily diaries. Patients recorded information on all micturitions (toilet voids or incontinence episodes) during 24 hours. In the two US studies (003 and 005) the prospectively planned primary and

secondary endpoints were based on the diary data. For these studies, complete diary data was provided.

In the European study, the primary endpoints were urodynamic measures, not based on the diary data. The sponsor reported that "due to various reasons (diary was not brought back or not filled in, patients were unable to do so, etc.)" not all patients in that study have diary data for analysis. The analyses of the endpoints drawn from the diary are post hoc for this study, and only offer supportive evidence. Since the application included two principle studies with complete data for these endpoints, this was not a review issue.

2.3 STATISTICAL EVALUATION OF EVIDENCE ON EFFICACY / SAFETY

2.3.1 SPONSOR'S RESULTS AND CONCLUSIONS

The sponsor originally submitted only one U.S. study (003) along with the European study. The sponsor concluded that the single prospectively planned study, with support of the urodynamic endpoints results from the European trial, were sufficient to support Trospium for the indication of the treatment of symptoms of overactive bladder. However, based on that evidence, the relevance of the urodynamic endpoints from the European toward assessing the desired diary endpoints was a review issue.

The NDA review was extended when the sponsor submitted a major amendment for the clinical pharmacology reviewer. When the extension was made, the sponsor also submitted the study report and data for the second U.S. study (005). The results of the two U.S. studies together are sufficient to assess the efficacy without having to rely on the European study for confirmation.

2.3.2 STATISTICAL METHODOLOGIES

The sponsor planned to assess the efficacy endpoints using ANOVA models. The model would include terms for treatment, center, and a treatment-by-center interaction term. The homogeneity and normality assumptions would be tested using relevant methods. These analyses are appropriate for this type of data.

2.3.3 DETAILED REVIEW OF INDIVIDUAL STUDIES

2.3.3.1 Study IP631-003

Study IP631-003 is a multicenter, double-blind, placebo-controlled, parallel arm study conducted in the U.S. Subjects were male or female, 18 years and older, with symptoms of overactive bladder for at least 6 months prior to enrollment. After completing screening, subjects were randomized on a 1:1 basis to one of the two blinded treatment groups. They received either Trospium 20 mg or placebo for a 12-week treatment period.

This study was prospectively planned to address two co-primary endpoints desired by the Division. These are Change in toilet voids per 24 hrs and Change in average number of urge incontinence episodes per 24 hrs. Two additional endpoints were also of interest to the Medical Officer. These are Change in volume voided per toilet void and Change in average urgency severity associated with toilet voids. Both were planned in the protocol as secondary endpoints. All four of these endpoints were collected in daily diaries. The primary timepoint was the change from baseline to Week 12.

In the protocol the sponsor planned to analyze all these endpoints using an ANOVA model with terms for treatment, center, and a treatment-by-center interaction. If the interaction term was not significant (α=0.10) it would be dropped. The homogeneity or variance assumption would be checked using Levene's test and the normality assumption would be checked using Q-Q plots and the Shapiro-Wilks test. If the data were not normally distributed, a rank transformation was planned.

The results for the four endpoints of interest are shown in Table 2. Trospium was statistically significantly better than placebo for all four endpoints. This study provides strong support for the efficacy of Trospium.

Table 2: Study IP631-003 Efficacy Results

	Trospium	Placebo	Comparison
Change in toilet voids per 24 hrs	n=253	n=256	
Baseline: Mean (SE)	12.74 (0.16)	12.93 (0.16)	
Change from Baseline: Mean (SE)	-2.37 (0.17)	-1.29 (0.17)	-1.08 p < .001 ◊
Change in average number of urge incontinence episodes per 24 hrs	n=253	n=256	
Baseline: Mean (SE)	3.89 (0.17)	4.33 (0.21)	
Change from Baseline: Mean (SE)	-2.20 (0.16)	-1.98 (0.18)	-0.22 p = 0.012 ◊
Change in volume voided per toilet void (mL)	n=248	n=253	
Baseline: Mean (SE)	155.09 (3.10)	156.62 (3.08)	
Change from Baseline: Mean (SE)	32.14 (3.08)	7.72 (3.05)	24.42 p < .001
Change in average urgency severity associated with toilet voids	n=253	n=256	
Baseline: Mean (SE)	1.77 (0.03)	1.77 (0.03)	
Change from Baseline: Mean (SE)	-0.22 (0.03)	-0.04 (0.03)	-0.18 p < .001

◊ Primary endpoints
Source: SAS datasets

2.3.3.2 Study IP631-005

Study IP631-005 is nearly identical to study 003. The study design and patient population were the same. It is a multicenter, double-blind, placebo-controlled, parallel arm study conducted in the U.S. Subjects were male or female, 18 years and older, with symptoms of overactive bladder for at least 6 months prior to enrollment. After completing screening, subjects were randomized on a 1:1 basis to one of the two blinded treatment groups. They received either Trospium 20 mg or placebo for a 12-week treatment period.

The only difference between study 005 and 003 is that study 005 was prospectively planned to address only one of the two co-primary endpoints desired by the Division. The single primary endpoint in this study was the Change in toilet voids per 24 hrs. The Change in average number of urge incontinence episodes per 24 hrs was planned as a secondary endpoint, along with the Change in volume voided per toilet void and Change in average urgency severity associated with toilet voids. All four of these endpoints were collected in daily diaries. The primary timepoint was the change from baseline to Week 12.

In the protocol the sponsor planned to analyze all these endpoints using an ANOVA model with terms for treatment, center, and a treatment-by-center interaction. If the interaction term was not significant (α=0.10) it would be dropped. The homogeneity or variance assumption would be checked using Levene's test and the normality assumption would be checked using Q-Q plots and the Shapiro-Wilks test. If the data were not normally distributed, a rank transformation was planned.

The results for the four endpoints of interest are shown in Table 3. Trospium was statistically significantly better than placebo for all four endpoints. This study provides strong support for the efficacy of Trospium.

Table 3: Study IP631-005 Efficacy Results

	Trospium	Placebo	Comparison
Change in toilet voids per 24 hrs	n=323	n=325	
Baseline: Mean (SE)	12.94 (0.17)	13.17 (0.17)	
Change from Baseline: Mean (SE)	-2.67 (0.17)	-1.76 (0.15)	-0.91 $p < .001$ ◊
Change in average number of urge incontinence episodes per 24 hrs	n=323	n=325	
Baseline: Mean (SE)	3.83 (0.16)	3.91 (0.16)	
Change from Baseline: Mean (SE)	-2.31 (0.14)	-1.73 (0.14)	-0.58 $p < .001$
Change in volume voided per toilet void (mL)	n=319	n=320	
Baseline: Mean (SE)	154.12 (2.60)	154.13 (2.58)	
Change from Baseline: Mean (SE)	35.62 (3.26)	9.65 (2.33)	25.97 $p < .001$
Change in average urgency severity associated with toilet voids	n=323	n=325	
Baseline: Mean (SE)	1.79 (0.03)	1.75 (0.03)	
Change from Baseline: Mean (SE)	-0.21 (0.03)	-0.02 (0.03)	-0.19 $p < .001$

◊ Primary endpoint
Source: SAS datasets

2.3.3.3 Study MP94D2.14

Study MP94D2.14 is a multicenter, double-blind, placebo-controlled, parallel arm study conducted in Europe. Subjects were male or female, 18 years and older, with urge syndrome (detrusor instability) verified by urodynamic measures up to 3 months prior to enrollment. After completing screening, subjects were randomized on a 2:1 basis to the Trospium or placebo blinded treatment groups. They received either Trospium 20 mg or placebo for a 28-day treatment period.

The planned co-primary endpoints for study D2.14 were the change from baseline in the maximum cystometric bladder capacity and the change from baseline in the volume at first unstable contraction. These urodynamic variables are measured during the office visits at baseline and Week 4. These measures are not the endpoints desired by the Division to assess this indication.

Daily diary data was planned in this study, but was not collected from all subjects. According to the sponsor, "due to various reasons (diary was not brought back or not filled in, patients were unable to do so, etc.)" not all patients in that study have diary data for analysis. The analyses of the endpoints drawn from the diary are post hoc for this study, and only offer supportive evidence. The diary data which was provided did not include two of the 4 endpoints of interest.

This study was not designed with input from the Division. There are two specific differences between this study and the principle ones. The first is that the treatment period was only 28 days (4 weeks) in this study, versus 12 weeks in the principle studies. The second difference is that the endpoints in this study were not the primary endpoints of interest. Therefore, the results from this study offer only supportive evidence. In that capacity, the results of the planned urodynamic endpoints do show a statistically significant difference versus placebo, but only one of the two available diary endpoints shows a significant difference.

Table 4: Study MP94D2.14 Efficacy Results

	Trospium	Placebo	Comparison
Change in toilet voids per 24 hrs	Not reported	Not reported	NA
Change in average number of urge incontinence episodes per 24 hrs	n=33	n=24	(post hoc; t-test)
Baseline: Mean (SE)	2.88 (0.41)	2.31 (0.33)	
Change from Baseline: Mean (SE)	-1.59 (0.54)	-1.27 (0.50)	-0.32 p = 0.68
Change in volume voided per toilet void (mL)	n=82	n=36	(post hoc; t-test)
Baseline: Mean (SE)	156.55 (7.94)	139.14 (9.58)	
Change from Baseline: Mean (SE)	49.03 (8.60)	11.06 (8.89)	37.97 p < 0.01
Change in average urgency severity associated with toilet voids	Not reported	Not reported	NA
Maximum cystometric bladder capacity (ml)	n=177	n=83	(t-test)
Baseline: Mean (SE)	222.01 (6.60)	231.95 (9.70)	
Change from Baseline: Mean (SE)	70.7 (8.09)	5.4 (12.82)	65.3 p < 0.01 ◊
Bladder volume at first unstable detrusor contraction (ml)	n=173	n=83	(t-test)
Baseline: Mean (SE)	117.92 (5.35)	114.72 (8.04)	
Change from Baseline: Mean (SE)	97.4 (9.46)	41.4 (9.75)	56.0 p = 0.02 ◊

◊ Primary endpoints
NA = not primary or secondary endpoints in a study
Source: SAS datasets

2.3.4 STATISTICAL REVIEWER'S FINDINGS

In the two principle studies, 003 and 005, Trospium was statistically significantly better than placebo for the primary endpoint(s) as well as with the key secondary endpoints of interest (all p-values ≤0.012). The treatment effects observed were similar across the two studies. These consistent results support the efficacy of Trospium for this indication.

2.4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

For each of the two principle studies, I performed subgroup analyses by gender. In both studies, the majority of the patients were female (75% in study 003 and 81% in study 005). The results are shown in Tables 5 and 6. These analyses are for descriptive purposes only and were not planned for statistical conclusions.

In both studies the treatment effect versus placebo for urgency severity was higher in females. In study 003 the treatment effect for the other three endpoints was similar across gender. In study 005 the treatment effect for toilet voids was higher in women but the treatment effect on urge incontinence episodes was higher in men. I discussed these results with the Medical Officer, and he felt they were not a review issue.

Table 5: Study IP631-003: Efficacy Results by Gender

Descriptive Statistics: Mean (Std. Dev.)	Males			Females		
	Trospium n=56	Plac. n=73	Diff.	Trospium n=197	Plac. n=183	Diff.
Change in toilet voids per 24 hrs	-2.01 (2.84)	-0.87 (2.13)	-1.14	-2.47 (2.66)	-1.47 (2.76)	-1.00
Change in avg. number of urge incontinence episodes per 24 hrs	-1.69 (1.72)	-1.48 (2.71)	-0.21	-2.35 (2.73)	-2.18 (2.84)	-0.17
Change in volume voided per toilet void	19.99 (52.32)	-3.56 (29.93)	23.55	35.43 (51.96)	12.23 (48.52)	23.20
Change in avg. urgency severity associated with toilet voids	-0.16 (0.55)	-0.07 (0.62)	-0.09	-0.24 (0.53)	-0.03 (0.45)	-0.21

Source: SAS datasets

Table 6: Study IP631-005: Efficacy Results by Gender

Descriptive Statistics: Mean (Std. Dev.)	Males			Females		
	Trospium n=61	Plac. n=59	Diff.	Trospium n=261	Plac. n=260	Diff.
Change in toilet voids per 24 hrs	- 1.47 (3.46)	- 1.16 (2.08)	- 0.31	- 2.92 (2.65)	- 1.86 (2.59)	- 1.06
Change in avg. number of urge incontinence episodes per 24 hrs	- 2.08 (1.93)	- 1.14 (2.22)	- 0.94	- 2.36 (2.64)	- 1.87 (2.57)	- 0.49
Change in volume voided per toilet void	20.70 (45.86)	- 3.33 (32.69)	24.03	39.15 (60.32)	12.64 (43.05)	26.51
Change in avg. urgency severity associated with toilet voids	- 0.17 (0.44)	- 0.14 (0.55)	- 0.03	- 0.22 (0.51)	0.01 (0.47)	- 0.23

Source: SAS datasets

2.5 STATISTICAL AND TECHNICAL ISSUES

The sponsor originally submitted only one U.S. study (003) along with the European study. The NDA review was extended when the sponsor submitted a major amendment for the clinical pharmacology reviewer. When the extension was made, the sponsor also submitted the study report and data for the second U.S. study (005). The results of the two U.S. studies together are sufficient to assess the efficacy without having to rely on the European study for confirmation. Without the addition of study 005, the relevance of the endpoints used in the European study to support the desired efficacy endpoints would have been a review issue.

2.6 STATISTICAL EVALUATION OF COLLECTIVE EVIDENCE

This application includes two prospectively planned, placebo-controlled studies. They both measured the desired endpoints and had sufficient sample size to detect treatment differences versus placebo. In both studies Trospium was statistically significantly better than placebo for the primary and secondary endpoints of interest to assess the efficacy of Trospium for the indication of the treatment of symptoms of overactive bladder. This is sufficient statistical evidence to support the indication.

2.7 CONCLUSIONS AND RECOMMENDATIONS

This application seeks approval for the indication of the treatment of symptoms of overactive bladder for a single dose regimen of Trospium. It includes two prospectively planned placebo-controlled Phase 3 studies in the U.S., along with supportive evidence from

a placebo-controlled European study. The results from the U.S. studies support the efficacy of Trospium on two co-primary endpoints and two additional secondary endpoints of interest (all p-values ≤ 0.012). The collective evidence for the efficacy assessment shows that two principle trials, IP631-003 and IP631-005, provide consistent evidence of efficacy for the four endpoints of interest. The results of these two studies give sufficient statistical evidence to support the efficacy of Trospium for the desired indication.

APPEARS THIS WAY
ON ORIGINAL

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

/s/

Katherine Meaker
5/27/04 11:10:48 AM
BIOMETRICS

S. Edward Nevius
5/27/04 11:13:21 AM
BIOMETRICS
Concur with review.

Screening of New NDA for Statistical Filing
Division of Biometrics II

NDA #: 21-595

Applicant: Indevus Pharmaceuticals, Inc.

Trade/Generic Name: trospium chloride 20 mg tablets

Indication: treatment of symptoms associated with overactive bladder

Date of Submission: Apr 28, 2003

Filing Date: Jun 12, 2003

User Fee Goal Date: Feb 28, 2004

Project Manager: Ms. Cutright (HFD-580)

Medical Reviewer: Dr. Kaul

Comments: This is a completely electronic submission (\\Cdsesub1\n21595\N_000\2003-04-28) and it can be filed. Twelve controlled clinical studies and eight uncontrolled studies were conducted in patients with OAB/urge syndrome or neurogenic bladder. Efficacy are comprised of patient urinary diary data, urodynamic measurement data, and global assessments. Review emphasis will be on the single, principle trial IP631-003, a large, multicenter, double-blind, randomized, placebo-controlled U.S. study. The reviewer should also examine three other controlled studies (MP94D2.04, MP94D2.14, and MP94D2.15) for supportive evidence of efficacy. The sponsor used both parametric and nonparametric methods to evaluate the co-primary endpoints of change in average daily number of micturations (toilet voids) and change in average daily number of incontinence episodes. Efficacy results for the latter endpoint appear to be sensitive to the analysis approach, so it will be an important review issue to validate the sponsor's reliance on the rank-based method. Sensitivity of all results to the LOCF assumptions should also be a review concern.

Checklist for Fileability	Remarks (NA if not applicable)
Indexes sufficient to locate study reports, analyses, protocols, ISE, ISS, etc.	Easily accessible in EDR
Original protocols & subsequent amendments submitted	OK
Study designs utilized appropriate for the indications requested	OK
Endpoints and methods of analysis spelled out in the protocols	Review concern
Interim analyses (if present) planned in the protocol and appropriate adjustments in significance level made	NA
Appropriate references included for novel statistical methodology (if present)	NA
Data and reports from primary studies submitted to EDR according to Guidances	Access to EDR data files and documentation OK
Safety and efficacy for gender, racial, geriatric, and/or other necessary subgroups investigated	OK

Reviewer: M. Welch

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

/s/

Mike Welch
6/13/03 10:13:18 AM
BIOMETRICS

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
OFFICE OF BIOSTATISTICS

STATISTICAL REVIEW AND EVALUATION OF RAT AND MOUSE CARCINOGENICITY STUDIES

NDA:.	21-595
Name of drug:	Trospium Chloride 20 mg tablets
Sponsor:	Indevus Pharmaceuticals, Inc.
Indication:	Overactive bladder
Documents reviewed:	MP 194 (43-1811)
	1. 78-Week Dietary Carcinogenicity study in mice.
	\\Cdsesub1\n21595\N_000\2003-04-28\pharmtox\tox\71everett.pdf
	2. Histological evaluation extension from the 78-week carcinogenicity study in mice
	\\Cdsesub1\n21595\N_000\2003-04-28\pharmtox\tox\72perry.pdf
	MP 194 (43-1827): 104-Week Dietary Carcinogenicity study in rats
	\\Cdsesub1\n21595\N_000\2003-04-28\pharmtox\tox\75mulhern.pdf
Project manager:	Dale Cutright
Pharmacology reviewer:	Laurie McCleod-Flynn, Ph.D.
Dates:	Electronic Submission, Dated April 28, 2003
Statistical reviewer:	Joan Buenconsejo, M.S.
Secondary reviewer:	Karl Lin, Ph.D.
Biometrics division director:	S. Edward Nevius, Ph.D.

Keywords: NDA review, carcinogenicity studies, survival

Background

In this NDA submission, an 18-month carcinogenicity study on CD-1 mice and a 24-month carcinogenicity study on Sprague-Dawley rats were conducted. These studies were intended to assess the carcinogenic potential of trospium chloride, a compound with anticholinergic activity being developed for the treatment of patients with an overactive bladder, following oral administration by the dietary route for 104 weeks in rats and for 78 weeks in mice.

Study Design

The designs of the carcinogenicity studies were similar with primary differences arising in the rodent species. The current review evaluates and presents results separately for each species.

1. Study in Mice (Reference No. 431811)

Two separate experiments, one in male and one in female were conducted. In each of these two experiments, there were two control groups and three treatment groups. Two hundred fifty males and 250 females were assigned to control and treated groups of equal size. Groups of 50 males and 50 females were given trospium chloride (MP 194) in the diet for 78 weeks at concentrations formulated to achieve dose levels of 2, 20 and 200 mg/kg/day in diet. (Table 1) Two groups of 50 male and 50 female mice received untreated diet and served as the control groups.

Viability was checked once each morning and once as late as practicable on each day. Body weight and food consumption were recorded weekly from one week prior to the start of treatment and for the first 13 weeks of treatment and then every 4 weeks thereafter until the end of the study. Water consumption was monitored by visual inspection throughout the study. Clinical signs were monitored daily and a detailed clinical examination and palpation was carried out once each week.

Following completion of 78 weeks of treatment, all surviving animals in the first control group and all treated groups were killed and subjected to a full histopathological examination. All animals were sacrificed by exposure to nitrogen asphyxiation (up to 29 June 1987) or to carbon dioxide asphyxiation (30 June 1987 onwards), followed by exsanguination. Because the Sponsor noticed an apparent intergroup difference in incidence of a lesion in the lung, it was decided that a histopathological examination be extended to include the lungs from all animals in Control II. A blinded histological re-evaluation of lung tumors was conducted by the Sponsor upon the request of the Agency.

2. Study in Rats (Reference No. 431827)

Two separate experiments, one in male and one in female were conducted. In each of these two experiments, there were two control groups and three treatment groups. Two hundred fifty males and 250 females were assigned to control and treated groups of equal size. Groups of 50 males and 50 females were given trospium chloride (MP 194) in the diet for 104 weeks at concentrations formulated to achieve dose levels of 2, 20, and 200 mg/kg/day. (Table 8) Meanwhile, two groups of 50 male and 50 female rats received untreated diet to act as controls.

Viability was checked once each morning and once as late as practicable on each day. Body weight and food consumption were recorded weekly from one week prior to the start of treatment and for the first 13 weeks of treatment and then every 4 weeks thereafter until the end of the study. Water consumption was monitored by visual inspection throughout the study. Clinical signs were monitored daily and a detailed clinical examination and palpation was carried out once each week.

Following completion of 104 weeks of treatment, all surviving animals were killed and subjected to necropsy where a full list of organs and tissues were weighed/collected and examined microscopically. Method of killing was by exposure to carbon dioxide, followed by exsanguination. It should be noted that of the 2 control groups, only the control group 1 was examined histopathologically.

Sponsor's Analysis and Results

Survival Analysis:

Mortality data has been presented graphically using Kaplan-Meier survival curves and pairwise comparisons using Wilcoxon rank sum test modified censored survival data. *P*-values for the multiple comparisons are reported. Their results showed that in the male rat study, the mortality in the intermediate group (28%) and high dose (30%) groups was significantly lower than that of the two control groups (44% and 36% for control group 1 and control group 2, respectively). The death pattern of the male dose groups did not achieve statistical significance. The mortality patterns were similar to those observed in the female rats. Meanwhile, the result in the male mice study indicated that the mortality in the intermediate group (38%) and high dose (86%) groups was significantly higher than that of the two control groups (20% and 12% for control group 1 and control group 2, respectively) suggesting evidence of toxicity primarily related to reduced gut motility resulting in intestinal impaction. A statistical significant effect was not present in the female dose groups, although the mortality in the high dose groups (30%) appears to be higher than the two control groups (22% and 24% for control group 1 and control group 2, respectively).

Body Weight Analysis:

Body weight data were analyzed using either parametric ANOVA when group variances appeared homogenous or non-parametric test such as Kruskal-Wallis ANOVA when group variances were heterogeneous. Pairwise comparisons are performed via Student's t-test using Fisher's F-protected LSD for homogeneous variances, while Dunn Z test are performed for non-homogenous group variances. In the rat study, there were marked reductions in body weight gain in the male high dose group (23%) and intermediate group (9%), which in terms of absolute weight, attained statistical significance ($p<0.01$-$p<0.001$) from Week 20 onwards and ($p<0.05$-$p<0.001$) from Week 28 onwards, respectively. Meanwhile, dose related reduction in body weight gain is also shown in female high dose group (37%) and intermediate group (28%), which in terms of absolute weight, attained statistical significance from week 20 onwards in the intermediate group and week 3 onwards in the high dose group. The Sponsor suggested that the

female rats at 20 mg/kg/day and both the males and females at 200 mg/kg/day exceeded the maximum tolerated dose level (MTD). However, exceeding the MTD does not imply any evidence of carcinogenic potential at any dose level, therefore, the Sponsor established a no effect level at 2 mg/kg/day.

Meanwhile in the mouse study, the high dose male group showed reduced body weight gain (28%) compared to the control group that may be attributable to weight loss prior to death. There was also an equivocal reduction in body weight gain in the female low (21%), intermediate (12%) and high dose (16%) groups compared to the controls without a clear dose-related pattern, which in terms of absolute weight, attained statistical significance (at the $p<0.05$, $p<0.01$-$p<0.001$ level) from Week 28 onwards in the low and high dose groups. However, there was no conclusive evidence of any carcinogenic action of MP194 after 78 weeks of treatment to mice in either sex.

Tumor Trend Analysis:

Tumors thought to be of pathological interest were classified by the pathologists into one of the following categories:

1. definitely fatal
2. probably fatal
3. probably non-fatal
4. definitely non-fatal
5. observable during life

For statistical purposes, the Sponsor classified categories (1) and (2) as fatal tumors, and the remainders as non-fatal tumors. Observable tumor incidences were analyzed for dose-response and pair-wise intergroup differences using the log-rank test with time of observation as the event time. Non-fatal tumor prevalences were analyzed for dose-response and intergroup differences using the Hoel-Walburg test.

In the rat study, their results have shown that administration of MP 194 or trospium chloride in both sexes did not increase the overall tumor incidence (and in some groups there was a reduction. The results indicated no consistent increase in the proportion of tumors, which were malignant, and it also did not induce tumors of types not normally seen in Sprague-Dawley rats. Based on their tumor trend analysis, they suggested that there were no statistically significant increase in incidence of any one type of tumor. The Sponsor concluded that there was no evidence of carcinogenic potential at any dose level in male or female rats.

In the mouse study, the Sponsor also concluded that there was no evidence of carcinogenic potential at any dose level. However, their findings suggested an increased incidence of pulmonary adenomas in male mice of the intermediate and low dose groups when compared with control group I. They also found an increased incidence of pulmonary adenomas in the low dose group females when compared with Control group I. Although the data did not fall out with the historical incidences recorded at [redacted] in recent comparative carcinogenicity studies, bearing in mind the differences in mortality between the groups, the Sponsor considered it appropriate to subject the data to time adjusted statistical analysis. Their analysis indicated a statistically significant trend of increasing incidence of lung adenomas in

male mice when the number at risk was taken into account. When non-fatal carcinomas were also included, the trend remained. However, there was little difference between intermediate and high dose groups. Similarly in the female group, the sponsor found a statistically significant difference between Control I and low dose groups when lung adenomas and adenomas plus non-fatal carcinomas were considered. Although there were number of other relatively low tumor incidence present in the High dose males and females groups (such as hepatocytic carcinoma in male mice, mammary carcinoma, haemangiosarcoma, and mutilobular osteoma in female mice), the Sponsor suggested that none of these tumors were of a type not previously diagnosed in Control mice. Therefore, the low tumor incidence may not have resulted from the treatment.

The sponsor, upon the request of the Food and Drug Administration (FDA), re-evaluated the lung sections from the original carcinogenicity study in mice in a blinded fashion to ensure no bias in reading of the original slides occurred. Their results are as follow:

1. In the original and re-evaluated data, the sponsor found that the incidence of male animals in the 20mg/kg/day (intermediate dose) group bearing either benign or malignant tumors is statistically significantly increased when compared with the 1st control group, but not when compared with Control groups 1 and 2 combined.
2. The number of male animals carrying adenomas in the re-evaluated data is statistically significantly increased when compared with the 1st control group but not when compared with Control groups 1 and 2 combined.
3. In the original and re-evaluated data, the number of female animals bearing bronchiolo-alveolar adenomas or carcinomas in the 2 mg/kg/day (low dose) group is statistically significantly increased when compared with Control group 1 or with Control groups 1 and 2 combined, but not when compared with Control group 2 alone. This increase does not occur in the male group, nor is there any increasing trend with a tenfold increase in dose in female group.
4. When the number of female animals bearing either benign or malignant tumor was analyzed, the incidence in the 2 mg/kg/day animals only achieved statistical significance when compared with the 1st control group or control groups 1 and 2 combined. No comparison with the 2nd control group, whose incidence values lie within the historical control range of incidences achieved statistical significance.
5. In both data sets, the number of female animals bearing any proliferative lesions in the 2 mg/kg/day group is statistically significantly increased when compared with control group 1 or with groups 1 and 2 combined, but not when compared with the 2nd control group.
6. No evidence of any effect in females in the 20 mg/kg/day (intermediate dose) group.

Statistical Analysis Methods

This reviewer conducted an independent analysis on the carcinogenicity data submitted by the Sponsor. The analysis conformed to the Food and Drug Administration's Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May, 2001). In addition, this reviewer's analysis was primarily conducted using *e*Review of Animal Carcinogenicity, a review tool developed for and utilized by CDER reviewers.

Mortality Analysis

Tests for homogeneity and dose mortality trends were conducted using survival analysis methods described by Cox (1972) and the Kruskal-Wallis Test (Gehan, 1965; Breslow, 1970; Thomas, Breslow, and Gart, 1977) where the latter test weights early failures more heavily.

Tumor Data Analysis (Trend Test)

This reviewer conducted the trend tests on tumor incidence rates using the method described by Peto et. al. (1980) and the method of exact permutation trend test developed by the Division of Biometrics II. The sponsor classified tumors as fatal, possibly fatal, incidental, or possibly incidental, in which case, this reviewer combined fatal and possibly fatal as one group called fatal, and combined incidental and possibly incidental in another group called incidental. Data of incidental and fatal tumors were analyzed via the prevalence and death-rates methods, respectively. A combined test was used to analyze tumors classified as both fatal and incidental. The method of exact permutation trend test was used to counter underestimation of p-values when tumor incidence across the treatment group was small. All tests are performed separately for males and females for both species.

Multiple Testing Adjustment

A rule proposed by Haseman (1983) could be used to adjust the effect of multiple testing. A similar rule proposed by the Division of Biometrics, CDER/FDA was used in this review. The rule states that in order to keep the overall false-positive rate at the nominal level of approximately ten percent, tumor types with a spontaneous tumor rate of no more than one percent should be tested at 0.025 level, otherwise the level should be set at 0.005. (Lin, 1995, 1997; Lin and Rahman, 1998a, 1998b) The ten percent overall false positive rate is seen by CDER statisticians as appropriate in a new drug regulatory setting.

Evaluation of Validity of the Design of the Study

An evaluation of validity of the study design will be conducted in a negative study (that is, an analysis did not indicate any tumor type with a significant positive linear trend) before drawing the conclusion that the drug was not carcinogenic in rodents.. Readers are referred to papers by Haseman (1984) and Chu, Cueto and Ward (1981) for further information about evaluating the validity of the study design for negative studies.

Results and Discussion

1. Study in Mice (Reference No. 431811)

Survival Analysis: The intercurrent mortality data are given in Tables 2 and 4 for males and females, respectively. The Kaplan-Meier curves for death rate are given in Figures 1A and 1B for males and females, respectively. The homogeneity of survival was tested separately for males and females using the Cox test (Cox, 1972) and the Generalized Wilcoxon test (Gehan, 1965). Results of the tests are presented in Tables 3A-3C and 5A-5C for males and females, respectively. In male mice, the dose-mortality trend is statistically significant and the survival

distributions of the treatment groups are not homogeneous. None were found to be significant in the female mice.

Tumor data analysis: Following Peto et al., (1980) this reviewer applied the 'death rate method' and the 'prevalence method' on these two new categories of tumors, respectively. A combined test of 'death rate method' and the 'prevalence method' was performed for tumor types occurring in both categories. For the calculation of p values, the exact permutation method and the approximation method based on normal distribution were used. In all tissues except for lungs, scores used for the tumor trend analyses were 0, 2, 20, and 200 (equivalent to the dose levels) for the control 1, low, intermediate, and high dose groups, respectively in male and female mice. Meanwhile, scores used for the tumor trend analyses in the lungs were 0, 0, 2, 20, and 200 (equivalent to the dose levels) for the control 1, control 2, low, intermediate, and high dose groups, respectively in male and female mice. The time intervals used were 0 – 52, 53 – 78 weeks and terminal sacrifice. One set of analyses were performed in all tissues except for lung (i.e. control 1 vs. treatment groups). Meanwhile, three sets of analyses were initially performed in the lung tissue, and they are:

(1) Control 1 versus treatment
(2) Control 2 versus treatment
(3) Pooled control versus treatment

The tumor incidence rates and the tumor types with asymptotic p-values less than 0.05 for dose-response relationships are listed in Tables 6 and 7 for male and female mice, respectively. Any tumor incidence rates with asymptotic p-values greater than 0.05 are not presented. As per request by the pharmacology reviewer, additional pairwise analyses between different control groups and treatment groups were performed when there is at least one positive significant trend observed. In addition, tumor trend analyses were performed again for combination tumors identified to be relevant by the pharmacology reviewer.

From Tables 6 and 7, it is shown that on the basis of the Division's p-value adjustment rule, no significant positive dose-related trend is found either in male or female mice. In addition, no significant positive trend was found when tumors were combined (i.e. hepatocellular adenoma and hepatocellular carcinoma of the liver; pulmonary adenoma and pulmonary carcinoma of the lung).

2. Study in Rats (Reference No. 431827)

Survival Analysis: The intercurrent mortality data are given in Tables 9 and 11 for males and females, respectively. The Kaplan-Meier curves for death rate are given in Figures 2A and 2B for males and females, respectively. The homogeneity of survival was tested separately for males and females using the Cox test (Cox, 1972) and the Generalized Wilcoxon test (Gehan, 1965). Results of the tests are presented in Tables 10A-10C and 12A-12C for males and females, respectively. In female rats, the dose-mortality trend is statistically significant and the survival distributions of the treatment groups are not homogeneous. None were found to be significant in the male rats.

Tumor data analysis: This reviewer applied the same tests performed in the mouse data to the rat data. However, for the calculation of p values, Monte Carlo sampling technique is applied, because of slow computing of the exact permutation method and the approximation method in the StatXact software.

The tumor incidence rates and the tumor types with asymptotic p-values less than 0.05 for dose-response relationships are listed in Tables 13 and/or 14 for male and female rats, respectively. Any tumor incidence rates with asymptotic p-values greater than 0.05 are not presented. As per request by the pharmacology reviewer, additional pairwise analysis between control group 1 and treatment groups were performed when there is at least one positive significant trend observed. Scores used were 0, 2, 20, and 200 (equivalent to the dose levels) for the control 1, low, intermediate, and high dose groups, respectively in male and female rats. The time intervals used were 0 – 52, 53 – 78, 79 – 91, 92 – 104 weeks and terminal sacrifice.

From Table 13, the result shows that on the basis of the Division's p-value adjustment rule, no significant positive dose-related trend is found in male rats. There is also no significant positive dose-related trend found in female rats as no asymptotic p-values are less than 0.05 for dose-response relationships in any tumor sites.

3. Historical Control Analysis

Based on the results presented in subsections 1 and 2 (under results and discussion), this reviewer found no significant positive trend in either mouse or rat study. As an additional examination, historical control analysis was conducted. Historical control data on hepatocellular adenoma and carcinoma, as well as pulmonary adenoma and carcinoma for mice are available, while no historical control data are available on rats.

This reviewer conducted the historical control analysis using method proposed by Tarone (1982) in testing for trend in proportions. The historical control tumor incidence of the lung and liver from 6 studies in CD-1 Mice is presented in Table 14A. The pooled historical control rates based on 6 studies of the pulmonary adenoma of the lungs are 81/400 = 0.20 and 43/400=0.11, for male and female mice, respectively. Meanwhile, the pooled historical control rates on the hepatocellular carcinoma in the liver are 21/250 =0.08 and 1/250 = 0.004, for male and female mice, respectively.

The method of moment estimates and the maximum likelihood estimates for the beta-binomial parameters $(\hat{\alpha}, \hat{\beta})$ are almost identical. Table 15 presents the beta-parameter estimates, the estimated pooled historical control rates $(\hat{p})$, the adjusted total number of animals $(\hat{n})$ from the historical control and the score test (χ^2) for adenoma in the lung, for males and females, respectively and hepatocellular carcinoma in the liver for male mice. Incorporation of historical control information provided evidence of no significant tumor increase association with the administration of trospium chloride (MP194).

Additional analyses were performed that includes adding carcinoma in the lung in the pooled lung control rates, and adding hepatocellular adenoma in the pooled liver control rates. The

results are provided in Table 16, and it shows that no significant tumor increase is associated with the administration of trospium chloride when historical control is incorporated into the analysis.

4. Evaluation of the validity of the Mouse and Rat study design

In light of the criteria presented in the paper by Haseman (1984) and Chu, Cueto and Ward (1981), this reviewer investigated the validity of the experimental design of the mouse and rat carcinogencity studies.

As shown in Tables 2 and 4, the mortality rate at the high dose group was higher than that of the controls, for male and female mice, respectively. This is particularly disconcerting in the male mice since most of the animals (86%) did not survive before week 80. Meanwhile, 30% of the female mice did not survive before week 80. Based on the survival criterion Haseman proposed, it could be concluded that not enough male mice were exposed to the drug for a sufficient amount of time as less than 50% of the animals in male mice were alive in the high dose group before week 80. The same conclusion can be made to the female mice since the study was terminated before week 80.

Because body weight data was not provided by the Sponsor, this reviewer will use the results the Sponsor has provided in its report. Tables 17 and 18 present sponsor's summary on body weight reduction in the mice study comparing the treatment and control groups. The results showed that the high dose group had 28% decrement in body weight gain in males, while females in the high dose group had 16% decrement in body weight gain. The body weight gain data shows that for male and female mice, the high dose level (200 mg/kg/day) exceeded the maximum tolerated dose.

In conclusion, the CD-1 mouse study is not valid in terms of the length of drug exposure and tumor challenge to the tested animals described above.

Tables 9 and 11 present the summary of the survival data on rats. Based on the survival criterion Haseman proposed, it could be concluded that enough rats in both sexes were exposed to the drug for a sufficient amount of time as more than 50% of the animals were alive in the high dose group at the time points evaluated.

Similar to the mouse study, body weight data on rats were not provided by the Sponsor. Instead, this reviewer will use the results summarized by the Sponsor. Tables 19 and 20 present Sponsor's summary on body weight reduction in the rat study comparing the treatment and the control groups. The results showed that the intermediate dose group had 28% decrement in body weight gain in female rats and at least 37% decrement in body weight gain in high dose group. Meanwhile, males in the high dose group had 23% decrement in body weight gain. The body weight gain data of the rat studies suggested that the female rats at 20 mg/kg/day and both the males and females at 200 mg/kg/day exceeded the maximum tolerated dose level (MTD).

Summary

In this submission report, animal carcinogenicity studies in rats and in mice were included. These studies were intended to assess the carcinogenic potential of trospium chloride (MP194) in rats and mice with appropriate drug levels for about 104 weeks for rats and 78 weeks for mice.

Mouse Study: This study had two control groups and 3 treatment groups (dose levels: 0, 0, 2, 20, 200 mg/kg/day in males and females). Test results showed the dose-mortality trend is statistically significant and the survival distributions of the treatment groups are not homogeneous in male mice but none was found in female mice. Tests on tumor data showed no significant positive trend either in male or female mice.

The only concern this reviewer has on the mouse study is the high rates of mortality in the high dose group (particularly in the male mice) before week 80. This led to the conclusion that there were not enough animals living long enough to be exposed to the drug. Based on the reduction in body weight gain in both sexes (particularly in the male mice), it can be concluded that the high dose (200 mg/kg/day) was over the maximum tolerated dose

Rat Study: This study had two control groups and 3 treatment groups (dose levels: 0, 0, 2, 20, 200 mg/kg/day in males and in females). Test results showed the dose-mortality trend is statistically significant and the survival distributions of the treatment groups are not homogeneous in the female rats but none found were found in male rats. Out of the 2 control groups, only control group 1 is examined histopathologically. Tests on tumor data showed no significant positive trend in either male or female rats. Historical control data for any tumors types are not available such that additional analyses can not be conducted.

There were sufficient numbers of animals living long enough for the risk of late-developing tumors. However, the body weight gain data demonstrated that the high dose (200 mg/kg/day) exceeded the maximum tolerated dose for both male and female rats.

The sponsor indicated in their letter dated January 20, 2003 that the original read and the re-read of the slides for the lung neoplasms was in agreement such that additional datasets requested were not reproduced. Therefore, re-evaluation data on the mouse study was not conducted by this reviewer.

Reference

Food and Drug Administration (FDA), (2001), Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals

D.R. Cox (1972), "Regression Models and Life Tables (with discussion)," Journal of Royal Statistical Society, Series B, 34, 187-220

E.A. Gehan (1965), "A Generalized Wilcoxon Test for Comparing K Samples Subject to Unequal Patterns of Censorship," Biometrika, 52, 203-223

N.E. Breslow (1970), "A generalized Kruskal-Wallis test for comparing K samples subject to unequal patterns of censorship," Biometrika, 57, 579-594

D.G. Thomas, N.E. Breslow, and J.J. Gart (1977), "Trend and Homogeneity Analyses of Proportions and Life Table Data," Computer and Biomedical Research, 10, 373-381.

R. Peto, M.C. Pike, N.E. Day, 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: A Critical Appraisal, World Health Organization.

J.K. Haseman (1983), "A Reexamination of False-Positive Rates for Carcinogenesis Studies," Fundamental and Applied Toxicology, 3, 334-339.
K.K. Lin (1995), "A Regulatory Perspective on Statistical Methods for Analyzing New Drug Carcinogenicity Study Data," Bio/Pharm Quarterly, 1(2), 18-20

K.K. Lin (1997), "Control of Overall False Positive Rates in Animal Carcinogenicity Studies of Pharmaceuticals," presented at 1997 FDA Forum on Regulatory Sciences, December 8-9, 1997, Bethesda, MD.

K.K. Lin and M.A. Rahman (1998a), "Overall False Positive Rates in Tests for Linear Trend in Tumor Incidence in Animal Carcinogenicity Studies of New Drugs," Journal of Pharmaceutical Statistics, with discussions, 8(1), 1-22

K.K. Lin and M.A. Rahman (1998b), "False Positive Rates in Tests for Trend and Differences in Tumor Incidence in Animal Carcinogenicity Studies of Pharmaceuticals under ICH Guidance S1B," unpublished report, Division of Biometrics 2, Center for Drug Evaluation and Research, Food and Drug Administration

J.K. Haseman (1984), "Statistical Issues in the Design, Analysis and Interpretation of Animal Carcinogenecity Studies," Environmental Health Perspective, 58, 385-392.

J.K. Haseman (1984), "Issues in Carcinogenecity Testing: Dose Selection," Fundamental and Applied Toxicology, 5, 66-78.

J.K. Haseman (1999), personal communication to K.K. Lin

K.C. Chu, C. Cueto, and J.M. Ward (1981), "Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays," Journal of Toxicology and Environmental Health, 8, 251-280

R.E. Tarone (1982), "The Use of Historical Control Information in Testing for a Trend in Proportions", Biometrics, 38, 215-220

Tables

1. MICE STUDY: *(Reference No. 431811)*

Table 1: Dose levels (mg/kg/day)

	Control 1	Control 2	Low (mg/kg/day)	Medium (mg/kg/day)	High (mg/kg/day)
Mice (M/F)	0	0	2	20	200

Mortality Analysis:

Table 2: Intercurrent Mortality Rate Male Mice

Week	Control 1		Control 2		2 mg/kg/day		20 mg/kg/day		200 mg/kg/day	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 – 52	4	8	1	2	4	8	2	4	19	38
53 – 78	6	20	5	12	5	18	17	38	24	86
Terminal Sacrifice	40	80	44	88	41	82	31	62	7	14

Table 3A: Intercurrent Mortality Comparison Male Mice (Control 1 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	0.5551	0.7576	0.3165	0.8536
Dose-Mortality Trend	88.6461	0.0000	80.6842	0.0000
Homogeneity	89.2011	0.0000	81.0007	0.0000

Table 3B: Intercurrent Mortality Comparison Male Mice (Control 2 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	1.3120	0.5189	1.0649	0.5872
Dose-Mortality Trend	104.6622	0.0000	98.5839	0.0000
Homogeneity	105.9742	0.0000	99.6488	0.0000

Table 3C: Intercurrent Mortality Comparison Male Mice (Pooled Control vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	1.6046	0.6583	1.4183	0.7012
Dose-Mortality Trend	119.2005	0.0000	111.1226	0.0000
Homogeneity	120.8051	0.0000	112.5409	0.0000

Table 4: Intercurrent Mortality Rate Female Mice

Week	Control 1		Control 2		2 mg/kg/day		20 mg/kg/day		200 mg/kg/day	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 – 52	3	6	1	2	1	2	5	10	5	10
53 – 78	8	22	11	24	7	16	7	24	10	30
Terminal Sacrifice	39	78	38	76	42	84	38	76	35	70

Table 5A: Intercurrent Mortality Comparison Female Mice (Control 1 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	0.9048	0.6361	1.0512	0.5912
Dose-Mortality Trend	2.0017	0.1571	1.9364	0.1641
Homogeneity	2.9066	0.4063	2.9876	0.3935

Table 5B: Intercurrent Mortality Comparison Female Mice (Control 2 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	1.0018	0.6060	1.0220	0.5999
Dose-Mortality Trend	1.9485	0.1628	2.1501	0.1426
Homogeneity	2.9503	0.3994	3.1721	0.3658

Table 5C: Intercurrent Mortality Comparison Female Mice (Pooled Control vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	1.0720	0.7838	1.1450	0.7662
Dose-Mortality Trend	1.9023	0.1678	2.0019	0.1571
Homogeneity	2.9743	0.5621	3.1469	0.5336

Tumor Trend Analysis:

Table 6: Tumor Incidence Rates (Male Mice) with *P*-values (Asymptotic Method) Less Than 0.05

i. Control 1 vs. Treatment

Organ Name	Tumor Name	Overall tumor type	Tumor rate as PCT. in control group	CTR1	LOW	MED	HIGH	P-Value (Exact Method)
Liver	Hepatocellular Carcinoma[M]	Both fatal and Incidental	0.00	0	0	3	1	0.0366 > 0.025
Lung	Brochiolo-Alveolar Adenoma[B]	Both Fatal and Incidental	8.00	4	7	11	4	0.0162 > 0.005

ii. Control 2 vs. Treatment

Organ Name	Tumor Name	Overall tumor type	Tumor rate as PCT. in control group	CTR2	LOW	MED	HIGH	P-Value (Exact Method)
Lung	Brochiolo-Alveolar Adenoma[B]	Both Fatal and Incidental	14.00	10	7	11	4	0.0762 > 0.005

iii. Pooled Control vs. Treatment

Organ Name	Tumor Name	Overall tumor type	Tumor rate as PCT. in control group	CTR1	CTR2	LOW	MED	HIGH	P-Value (Exact Method)
Lung	Brochiolo-Alveolar Adenoma[B]	Both Fatal and Incidental	14.00	4	10	7	11	4	0.0365 > 0.005

Table 7: Tumor Incidence Rates (Female Mice) with *P*-values (Asymptotic Method) Less Than 0.05

i. Control 1 vs. Treatment

Organ Name	Tumor Name	Overall tumor type	Tumor rate as PCT. in control group	CTR1	LOW	MED	HIGH	P-Value (Exact Method)
Skin and Subcutis	Haemangiosarcoma[M]	Fatal	0.00	0	0	0	1	0.2564 > 0.025

2. RATS STUDY: *(Reference No. 431827)*

Table 8: Dose levels (mg/kg/day)

	Control 1	Control 2	Low (mg/kg/day)	Medium (mg/kg/day)	High (mg/kg/day)
RATS (M/F)	0	0	2	20	200

Mortality Analysis:

Table 9: Intercurrent Mortality Rate Male RATS

Week	Control 1		Control 2		2 mg/kg/day		20 mg/kg/day		200 mg/kg/day	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 – 52	2	4	0	0	2	4	2	4	0	0
53 – 78	6	16	6	12	4	12	4	12	6	12
79 – 91	8	32	10	32	5	22	1	14	5	22
92 – 103	6	44	2	36	10	42	7	28	4	30
Terminal Sacrifice	28	56	32	64	29	58	36	72	35	70

Table 10A: Intercurrent Mortality Comparison Male RATS (Control 1 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	2.8430	0.2413	2.6391	0.2673
Dose-Mortality Trend	1.3764	0.2407	1.3602	0.2435
Homogeneity	4.2195	0.2387	3.9993	0.2615

Table 10B: Intercurrent Mortality Comparison Male RATS (Control 2 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	1.8227	0.4020	1.6643	0.4351
Dose-Mortality Trend	0.7041	0.4014	0.7515	0.3860
Homogeneity	2.5268	0.4705	2.4157	0.4907

Table 10C: Intercurrent Mortality Comparison Male RATS (Pooled Control vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	2.8057	0.4226	2.5505	0.4662
Dose-Mortality Trend	1.3398	0.2471	1.3834	0.2395
Homogeneity	4.1456	0.3867	3.9339	0.4150

Table 11: Intercurrent Mortality Rate Female RATS

Week	Control 1		Control 2		2 mg/kg/day		20 mg/kg/day		200 mg/kg/day	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 – 52	1	2	0	0	0	0	0	0	1	2
53 – 78	5	12	4	8	6	12	2	4	4	10
79 – 91	7	26	9	26	13	38	5	14	3	16
92 – 103	15	56	8	42	10	58	11	36	5	26
Terminal Sacrifice	22	44	29	58	21	42	32	64	37	74

Table 12A: Intercurrent Mortality Comparison Female RATS (Control 1 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	6.5642	0.0375	7.2578	0.0265
Dose-Mortality Trend	8.5984	0.0034	7.4330	0.0064
Homogeneity	15.1626	0.0017	14.6908	0.0021

Table 12B: Intercurrent Mortality Comparison Female RATS (Control 2 vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	6.9938	0.0303	7.8002	0.0202
Dose-Mortality Trend	5.7119	0.0169	5.0913	0.0240
Homogeneity	12.7057	0.0053	12.8914	0.0049

Table 12C: Intercurrent Mortality Comparison Female RATS (Pooled Control vs. Treatment)

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	7.6599	0.0536	8.1688	0.0426
Dose-Mortality Trend	7.5390	0.0060	6.5742	0.0103
Homogeneity	15.1989	0.0043	14.7430	0.0053

Tumor Trend Analysis:

Table 13: Tumor Incidence Rates (Male RATS) with *P*-values (Asymptotic Method) Less Than 0.05

Organ Name	Tumor Name	Overall tumor type	Tumor rate as PCT. in control group	CTR1	LOW	MED	HIGH	P-Value (Exact Method)
Ileum	Leionyosarcoma (TA)	Fatal	0.00	0	0	0	1	0.2498 > 0.025
Brain	Meningiomata (TA)	Incidental	0.00	0	0	0	1	0.2613 > 0.025
Skin/Subcutis	Sarcoma (TA)	Incidental	0.00	0	2	1	4	0.0306 > 0.025

Historical Control Data Analysis:

Table 14A: Historical Control Data for Mouse Carcinogenicity Studies

Study	Bronchiolo-Alveolar Adenoma (LUNG)		Hepatocellular Carcinoma (LIVER)	
Number	Male N=400	Female N=400	Male N=250	Female N=250
A	17	10	3	0
B	21	8	8	0
C	11	5	2	1
D	15	6	2	0
E	8	6	6	0
F	9	8		
Total (%)	81 (20%)	43 (11%)	21 (8%)	1 (0.4%)

Table 14B: Tumor Incidence in Current Study

Group	Bronchiolo-Alveolar Adenoma (LUNG)		Hepatocellular Carcinoma (LIVER)
	Male N=250	Female N=250	Male N=250
Control 1	4	1	0
Control 2	10	4	0
Pooled	14	5	0
Low	7	10	0
Medium	11	4	2
Dose	4	3	1

Table 15: Trend test in proportions using historical control information

Group	Bronchiolo-Alveolar Adenoma (LUNG)		Hepatocellular Carcinoma (LIVER)
	Male	Female	Male
$\hat{\alpha}$	5.57	5.26	2.72
$\hat{\beta}$	20.1	38.0	29.7
$\tilde{p}$	0.15	0.09	0.02
$\tilde{n}$	275.7	293.27	282.4
χ^2	2.03	0.86	0.016
$p-value$	0.15	0.36	0.90

Table 16: Trend test in proportions using historical control information

Group	Bronchiolo-Alveolar Adenoma + Carcinoma (LUNG)		Hepatocellular Carcinoma + Adenoma (LIVER)
	Male	Female	Male
$\hat{\alpha}$	8.69	4.2	3.33
$\hat{\beta}$	23.9	24.8	23.5
$\tilde{p}$	0.16	0.09	0.02
$\tilde{n}$	282.6	279.0	276.8
χ^2	2.47	0.90	0.07
$p-value$	0.12	0.34	0.79

Body Weight Analysis:

Table 17: Mean Body Weight (gms) for Male and Female Mice

	Male			Female		
Group	Day 0 of Study	End of Study	Weight Gain	Day 0 of Study	End of Study	Weight Gain
Control 1	27.5	45.7	18.2	22.1	39.0	16.9
Control 2	28.4	45.7	17.3	22.4	37.2	14.8
Control (Average)	28.0	45.7	17.8	22.3	38.1	15.9
Low	28.2	44.4	16.2	22.6	35.1	12.5
Medium	28.6	44.7	16.1	22.7	36.7	14.0
High	28.5	41.3	12.8	22.0	35.3	13.3

Table 18: Percent Reduction in Mean Body Weight Gain from Concurrent Controls

Group	% Male	% Female
Low	9	21
Medium	9	12
High	28	16

Table 19: Mean Body Weight (gms) for Male and Female Rats

	Male			Female		
Group	Day 0 of Study	End of Study	Weight Gain	Day 0 of Study	End of Study	Weight Gain
Control 1	208	654	446	130	465	335
Control 2	204	687	483	131	464	333
Control (Average)	206	670.5	464.5	130.5	464.5	334
Low	206	671	465	131	437	306
Medium	208	631	423	129	368	239
High	209	567	358	132	342	210

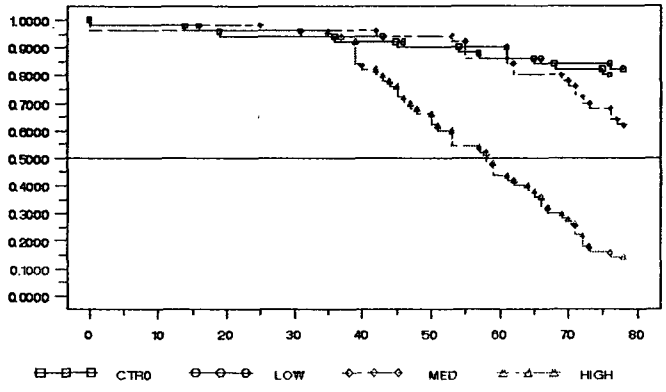
Table 20: Percent Reduction in Mean Body Weight Gain from Concurrent Controls

Group	% Male	% Female
Low	0	8
Medium	9	28
High	23	37

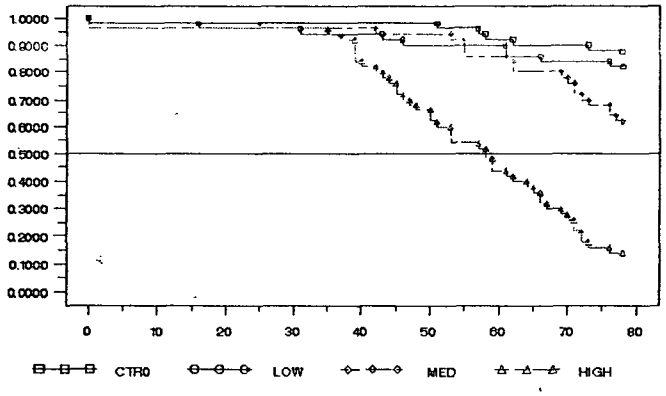
Figures

Figure 1A: Kaplan-Meier Curve for MOUSE (MALE)

Control 1 vs. Treatment



Control 2 vs. Treatment



Pooled Control vs. Treatment

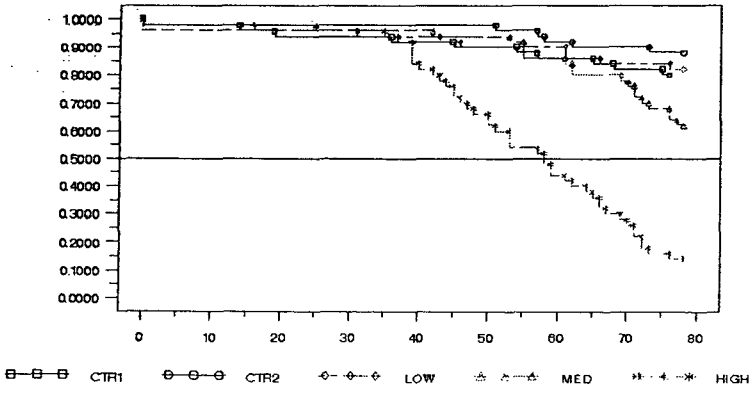
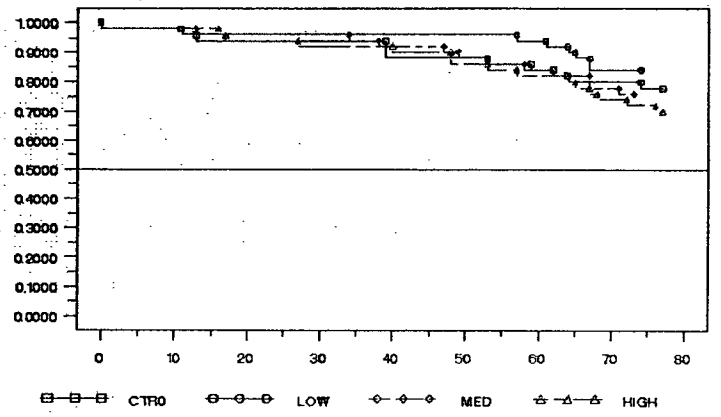
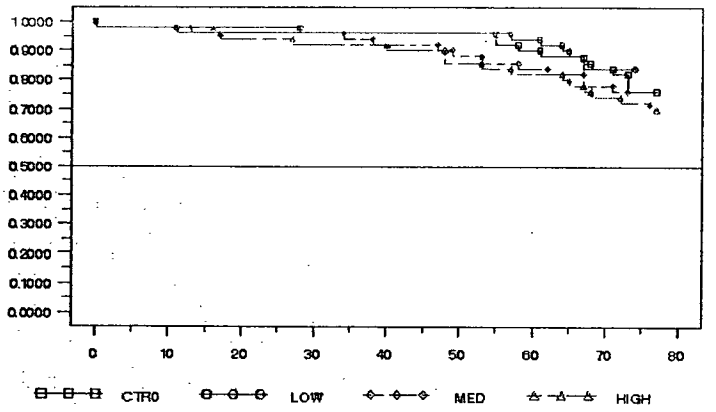


Figure 1B: Kaplan-Meier Curve for MOUSE (FEMALE)
Control 1 vs. Treatment



Control 2 vs. Treatment



Pooled Control vs. Treatment

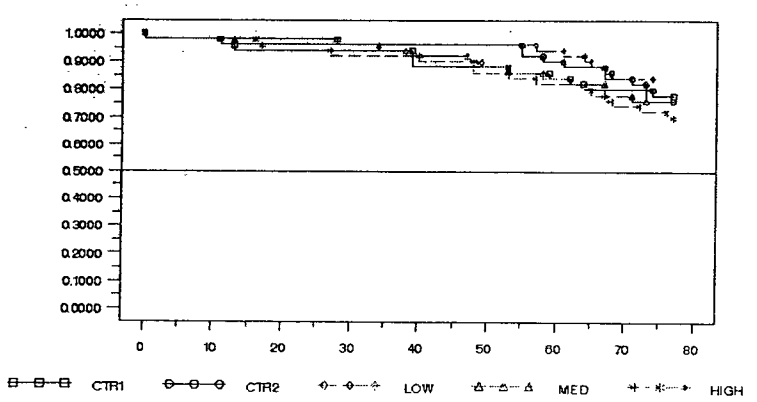
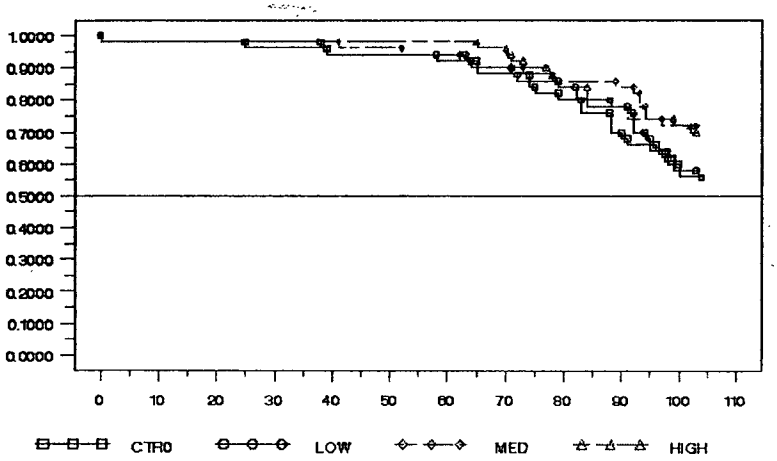
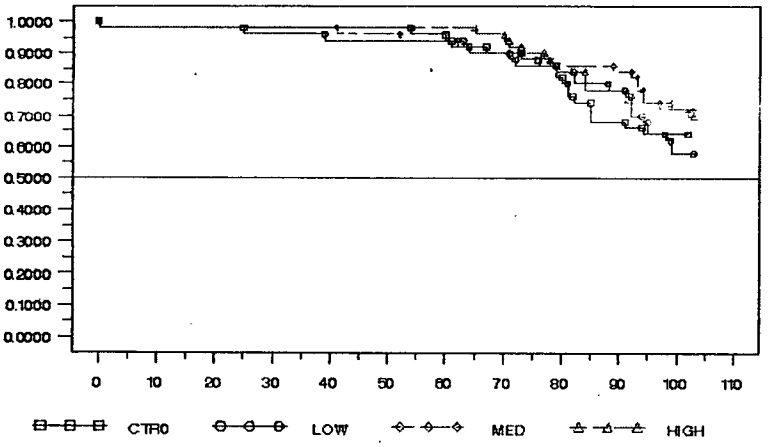


Figure 2A: Kaplan-Meier Curve for RAT (MALE):
Control 1 vs. Treatment



Control 2 vs. Treatment



Pooled Control vs. Treatment

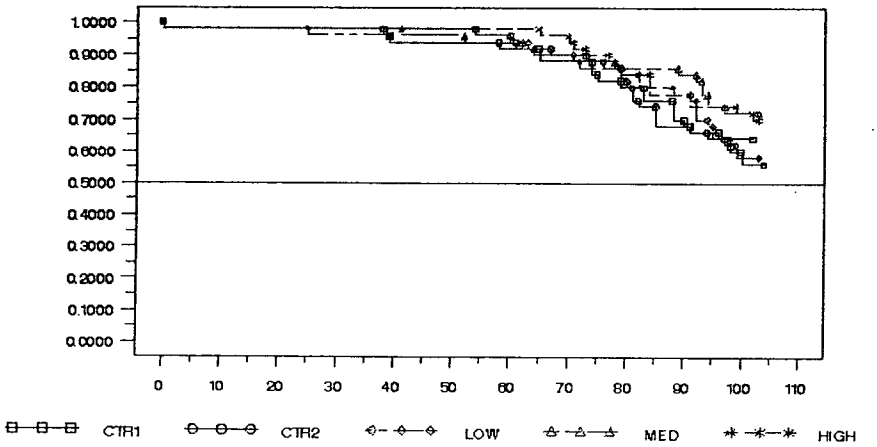
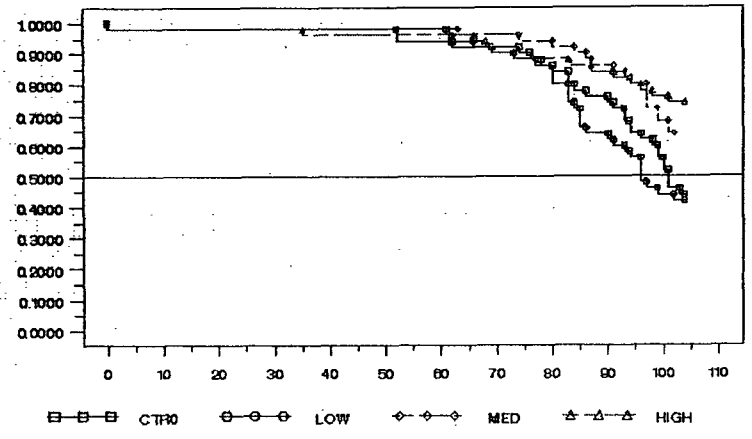
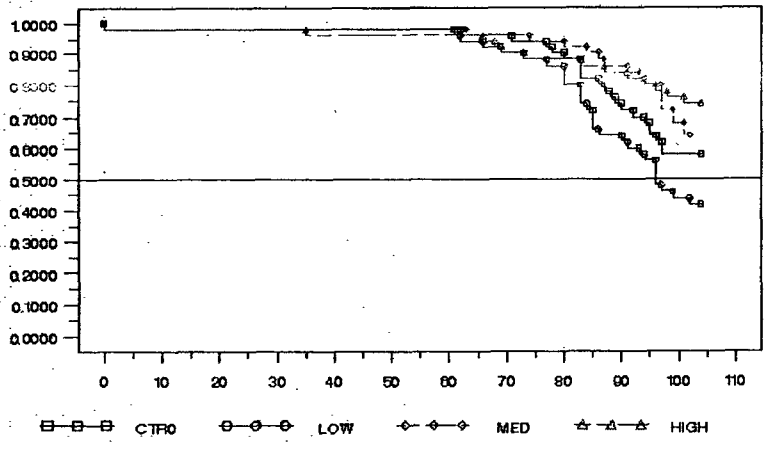


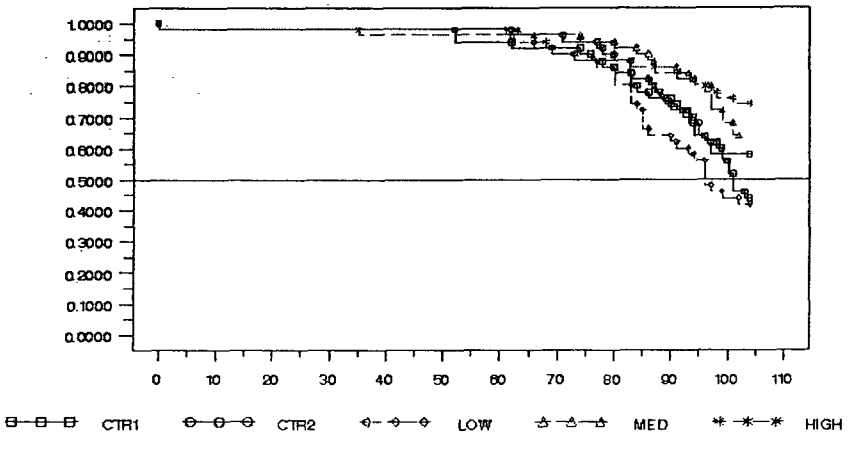
Figure 2B: Kaplan-Meier Curve for RAT (FEMALE):
Control 1 vs. treatment



Control 2 vs. Treatment



Pooled Control vs. Treatment



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

/s/

Joan Buenconsejo
2/12/04 01:31:38 PM
BIOMETRICS

Karl Lin
2/12/04 01:57:04 PM
BIOMETRICS
Concur with review

NDA 21-595
Sanctura

CAC/ECAC Report

Not applicable for this application.