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RESEARCH**

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STATISTICAL REVIEW(S)



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STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

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Supplement #:
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Indication(s): Improve symptoms in PAH and CTEPH
Applicant: Bayer
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EXECUTIVE SUMMARY

This submission contains two phase 3 trials to support two separate but related indications. Trial 12934 (PATENT-1) was a randomized, double-blind, placebo-controlled, multi-center, multinational study to evaluate the efficacy and safety of oral riociguat (1 mg, 1.5 mg, 2 mg, or 2.5 mg tid) in patients with symptomatic pulmonary arterial hypertension (PAH). Trial 11348 (CHEST-1) was a randomized, double-blind, placebo-controlled, multicenter, multinational study to evaluate the efficacy and safety of oral riociguat (1 mg, 1.5 mg, 2 mg, or 2.5 mg tid) in patients with chronic thromboembolic pulmonary hypertension (CTEPH).

Both studies met their primary objective of showing an improvement compared to placebo in 6-minute walk distance, a symptomatic benefit. No statistical issues were identified with these trials.

INTRODUCTION

1.1 Overview

Table: List of all studies included in analysis

	Phase and Design	Treatment Period	Follow-up Period	# of Subjects per Arm	Study Population
<i>Study 12934</i>	<i>Phase 3</i>	<i>12 weeks</i>	<i>12 weeks</i>	<i>Riociguat 1.0-2.5 mg group: 254 Placebo group: 126 Riociguat 1.0-1.5 mg group: 63</i>	<i>Symptomatic PAH (Group 1)</i>
<i>Study 11348</i>	<i>Phase 3</i>	<i>16 weeks</i>	<i>16 weeks</i>	<i>Riociguat 1.0-2.5 mg group: 173 Placebo group: 88</i>	<i>CTEPH</i>

Both studies used a primary endpoint of change from baseline in 6-minute walk distance.

1.2 Data Sources

Electronic datasets and Study Reports:

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<\\cdsesub1\evsprod\NDA204819\0000\m5\datasets>

STATISTICAL EVALUATION

1.3 Data and Analysis Quality

The data quality and analysis quality were both excellent.

PAH trials are in general, prone to have substantial missing data. Handling those missing observations is difficult and the best way to deal with it is to try to avoid it. These two trials had

relatively few subjects with missing data. In addition, the sponsor conducted many sensitivity analyses to examine the effect of different assumptions on the missing data. These sensitivity analyses consistently showed a moderately large effect on the primary endpoint.

1.4 Evaluation of Efficacy

1.4.1 Study Design and Endpoints

Trial 12934 (PATENT-1) was a randomized, double-blind, placebo-controlled, multi-center, multinational study to evaluate the efficacy and safety of oral riociguat (1 mg, 1.5 mg, 2 mg, or 2.5 mg tid) in patients with symptomatic pulmonary arterial hypertension (PAH). The primary endpoint was change in 6 minute walk distance (6MWD)

Trial 11348 (CHEST-1) was a randomized, double-blind, placebo-controlled, multicenter, multinational study to evaluate the efficacy and safety of oral riociguat (1 mg, 1.5 mg, 2 mg, or 2.5 mg tid) in patients with chronic thromboembolic pulmonary hypertension (CTEPH). The primary endpoint was change in 6MWD.

1.4.2 Statistical Methodologies

In trial 12934, the primary efficacy analysis was the analysis of the change in 6MWD from baseline to week 12 (last observation until week 12) in subjects valid for ITT, with imputation of missing values for subjects who withdrew or died before 12 weeks. The riociguat 1.0-2.5 mg and placebo groups were compared using analysis of covariance (ANCOVA), with baseline 6MWD as a covariate and treatment group, stratification group (therapy-naïve / add-on), and region as main effects. The primary statistical method would be the stratified Wilcoxon test if the Shapiro-Wilk test for normality of residuals was statistically significant. Least squares (LS) mean and 95% confidence intervals (CIs) of the treatment difference were calculated based on the ANCOVA. Superiority of the riociguat 1.0-2.5 mg group over the placebo group was to be declared if the two-sided p-value was less than or equal to 0.05.

In trial 11348, The primary efficacy analysis was the analysis of the change in 6MWD from baseline to week 16 (last observation until week 16) in subjects valid for ITT, with imputation of missing values for subjects who withdrew or died before 16 weeks. The riociguat 1.0-2.5 mg and placebo groups were compared using analysis of covariance (ANCOVA), with baseline 6MWD as a covariate and treatment group and region as main effects. The primary statistical method would be the stratified Wilcoxon test if the Shapiro-Wilk test for normality of residuals was statistically significant. Least squares (LS) mean and 95% confidence intervals (CIs) of the treatment difference were calculated based on the ANCOVA. Superiority of the

riociguat 1.0-2.5 mg group over the placebo group was to be declared if the two-sided p-value was less than 0.05.

1.4.3 Patient Disposition, Demographic and Baseline Characteristics

1.4.3.1 Trial 12934

The patient disposition is shown in Table 1. Close to 90% of the subjects in all 3 groups completed treatment.

	Riociguat 1.0–2.5 mg N=254 (100%)		Placebo N=127 (100%)		Riociguat 1.0–1.5 mg N=64 (100%)	
Completed treatment	237	(93.3%)	111	(87.4%)	57	(89.1%)
Prematurely discontinued	17	(6.7%)	16	(12.6%)	7	(10.9%)
Adverse event	8	(3.1%)	7	(5.5%)	1	(1.6%)
Death	0	–	2	(1.6%)	1	(1.6%)
Lack of efficacy	0	–	1	(0.8%)	0	–
Lost to follow-up	1	(0.4%)	0	–	0	–
Non-compliance with study drug	1	(0.4%)	0	–	0	–
Protocol violation	1	(0.4%)	3 ^a	(2.4%)	3 ^a	(4.7%)
Withdrawal by subject	6	(2.4%)	3	(2.4%)	2	(3.1%)

Table 1 Patient disposition for trial 12934 (Table 8-2 of Study Report)

The patient demographic characteristics are shown in Table 2 and Table 3. The demographics were comparable between the three groups. The riociguat 1.0-2.5 arm had more subjects with baseline 6MWD less than 380 m compared to the placebo group. However, the mean PVR in the riociguat group was lower than the mean in the placebo group. The latter is not a statistically significant difference, but low PVR is associated with less severe disease; i.e. it suggests that the subjects randomized to riociguat 1.0-2.5 may not have had much more severe symptoms at baseline than the placebo group.

Characteristic	Riociguat 1.0–2.5 mg N=254 (100%)		Placebo N=126 (100%)		Riociguat 1.0–1.5 mg N=63 (100%)	
Sex						
Male	51	(20.1%)	28	(22.2%)	14	(22.2%)
Female	203	(79.9%)	98	(77.8%)	49	(77.8%)
Race / Ethnicity						
White	161	(63.4%)	78	(61.9%)	33	(52.4%)
Black or African American	4	(1.6%)	1	(0.8%)	1	(1.6%)
Asian	79	(31.1%)	38	(30.2%)	22	(34.9%)
Multiple races	1	(0.4%)	1	(0.8%)	0	–
Hispanic or latino	9	(3.5%)	8	(6.3%)	7	(11.1%)
Age (years)						
N	254		126		63	
Mean (SD)	51.1 (16.6)		50.7 (16.5)		48.8 (16.1)	
Median (Min-Max)	52.5 (18-80)		51.0 (18-79)		49.0 (18-77)	
Age group						
Age <65 years	188	(74.0%)	94	(74.6%)	49	(77.8%)
Age ≥65 years	66	(26.0%)	32	(25.4%)	14	(22.2%)

Table 2 Patient demographic characteristics for trial 12934 (Table 8-5 of Study Report)

Characteristic	Riociguat 1.0–2.5 mg N=254 (100%)		Placebo N=126 (100%)		Riociguat 1.0–1.5 mg N=63 (100%)	
WHO functional class						
I	5	(2.0%)	4	(3.2%)	5	(7.9%)
II	108	(42.5%)	60	(47.6%)	19	(30.2%)
III	140	(55.1%)	58	(46.0%)	39	(61.9%)
IV	1	(0.4%)	3	(2.4%)	0	–
Missing	0	–	1	(0.8%)	0	–
6MWD category						
<320 m	67	(26.4%)	27	(21.4%)	14	(22.2%)
≥320 m	187	(73.6%)	99	(78.6%)	49	(77.8%)
6MWD category						
<380 m	139	(54.7%)	53	(42.1%)	30	(47.6%)
≥380 m	115	(45.3%)	73	(57.9%)	33	(52.4%)
PVR (dyn*s*cm ⁻⁵)						
N	232		107		58	
Mean (SD)	791.0 (452.6)		834.1 (476.7)		847.8 (548.2)	
Median (Min-Max)	685.2 (241.5-2613.3)		740.0 (286.1-2545.5)		729.7 (258.1-3617.4)	
PAPmean (mmHg)						
N	235		109		58	
Mean (SD)	47.1 (14.8)		48.9 (15.2)		52.1 (16.2)	
Median (Min-Max)	45.7 (23.0-96.0)		48.0 (24.3-94.0)		51.2 (26.7-107.0)	

Table 3 Patient demographic characteristics for trial 12934 (Table and 8-8 of Study Report)

1.4.3.2 Trial 11348

The patient disposition is shown in Table 4. More than 90% of the subjects in both groups completed treatment.

	Riociguat 1.0–2.5 mg N=174 (100%)		Placebo N=88 (100%)	
Completed treatment	160	(92.0%)	83	(94.3%)
Prematurely discontinued	14	(8.0%)	5	(5.7%)
Adverse event	4	(2.3%)	2	(2.3%)
Death	2	(1.1%)	2	(2.3%)
Lack of efficacy	2	(1.1%)	1	(1.1%)
Non-compliance with study drug	1	(0.6%)	0	–
Protocol violation	3 ^a	(1.7%)	0	–
Withdrawal by subject	2	(1.1%)	0	–

Table 4 Patient disposition for trial 11348 (Table 8-2 of Study Report)

The patient demographic characteristics are shown in Table 5 and Table 6 . The demographics were comparable between the two groups.

Characteristic	Riociguat 1.0–2.5 mg N=173 (100%)		Placebo N=88 (100%)	
Sex				
Male	55	(31.8%)	34	(38.6%)
Female	118	(68.2%)	54	(61.4%)
Race / Ethnicity				
White	120	(69.4%)	65	(73.9%)
Black or African American	7	(4.0%)	1	(1.1%)
Asian	37	(21.4%)	20	(22.7%)
Multiple races	1	(0.6%)	0	–
Hispanic or Latino	8	(4.6%)	2	(2.3%)
Age (years)				
N	173		88	
Mean (SD)	59.3 (13.9)		59.2 (12.7)	
Median (Min-Max)	62.0 (19-80)		61.0 (26-77)	
Age group				
Age <65 years	99	(57.2%)	52	(59.1%)
Age ≥65 years	74	(42.8%)	36	(40.9%)

Table 5 Patient demographic characteristics for trial 11348 (Table 8-5 of Study Report)

Characteristic	Riociguat 1.0–2.5 mg N=173 (100%)		Placebo N=88 (100%)	
WHO functional class				
I	3	(1.7%)	0	–
II	55	(31.8%)	25	(28.4%)
III	107	(61.8%)	60	(68.2%)
IV	8	(4.6%)	2	(2.3%)
Missing	0	–	1	(1.1%)
6MWD category				
<320 m	60	(34.7%)	25	(28.4%)
≥320 m	113	(65.3%)	63	(71.6%)
6MWD category				
<380 m	109	(63.0%)	50	(56.8%)
≥380 m	64	(37.0%)	38	(43.2%)
PVR (dyn*s*cm ⁻⁵)				
N	151		82	
Mean (SD)	790.7 (431.6)		779.3 (400.9)	
Median (Min-Max)	711.1 (195.2-3942.0)		691.4 (258.1-2046.8)	

Table 6 Patient demographic characteristics for trial 11348 (Table and 8-8 of Study Report)

1.4.4 Results and Conclusions

1.4.4.1 Trial 12934

The results for the primary endpoint are shown in Table 7. Note that the Shapiro-Wilk test for normality of the ANCOVA residuals was significant ($p=0.0001$). Therefore, the stratified Wilcoxon p-value was formally used for determining statistical significance, as pre-specified in the statistical analysis plan. The cumulative distribution functions for change in 6MWD are shown in Figure 1 (those subjects with worst case imputations were not included in estimating the cumulative distribution functions in this figure). The LS mean difference shown in the table is not a good estimate of the treatment effect for the same reason that the ANCOVA is not a good test (errors not normally distributed). A better estimate would be the Hodges-Lehmann estimate associated with the stratified Wilcoxon test[†], which I calculated as 29 m with a 95% CI of (18 m, 40 m).

[†] Randles, Ronald H., and Douglas A. Wolfe. *Introduction to the theory of nonparametric statistics*, by Randles, Ronald H.; Wolfe, Douglas A. New York: Wiley, c1979. Wiley series in probability and mathematical statistics 1 (1979).

A Bayesian estimate of the treatment effect for a uniform prior would be the mean of the posterior distribution, which is normal with mean 35.78 and standard deviation 8.02. This Bayesian estimate also uses the assumption that the estimator is normally distributed.

Statistic	Riociguat 1.0–2.5 mg N=254	Placebo N=126	Riociguat 1.0–1.5 mg N=63
Baseline			
Mean (SD)	361.4 (67.7)	367.8 (74.6)	363.2 (66.6)
Median (Min-Max)	374.5 (160-468)	391.0 (150-450)	385.0 (158-448)
Change from baseline to last visit			
Mean (SD)	29.6 (65.8)	–5.6 (85.5)	31.1 (79.3)
Median (Min-Max)	30.0 (–430-279)	8.5 (–400-204)	32.0 (–415-190)
Treatment comparison	Riociguat 1.0-2.5 mg – placebo		
LS mean difference	35.78		
95% CI	20.06 to 51.51		
p-value (ANCOVA)	<0.0001		
p-value (stratified Wilcoxon test)	<0.0001		

ANCOVA model with baseline value, treatment group, region, and stratification group as fixed effects, stratified Wilcoxon test by region and stratification group

Last visit = Last observed value (not including follow-up) for subjects who completed the study or withdrew, except imputed worst value in case of death or clinical worsening without a termination visit or a measurement at that termination visit. Worst value imputation for 6MWD at last visit was performed for 2 subjects in the riociguat 1.0-2.5 mg group and 6 subjects in the placebo group.

Table 7 Results for primary endpoint in Trial 12934 (Table and 9-2 of Study Report and confirmed by FDA)

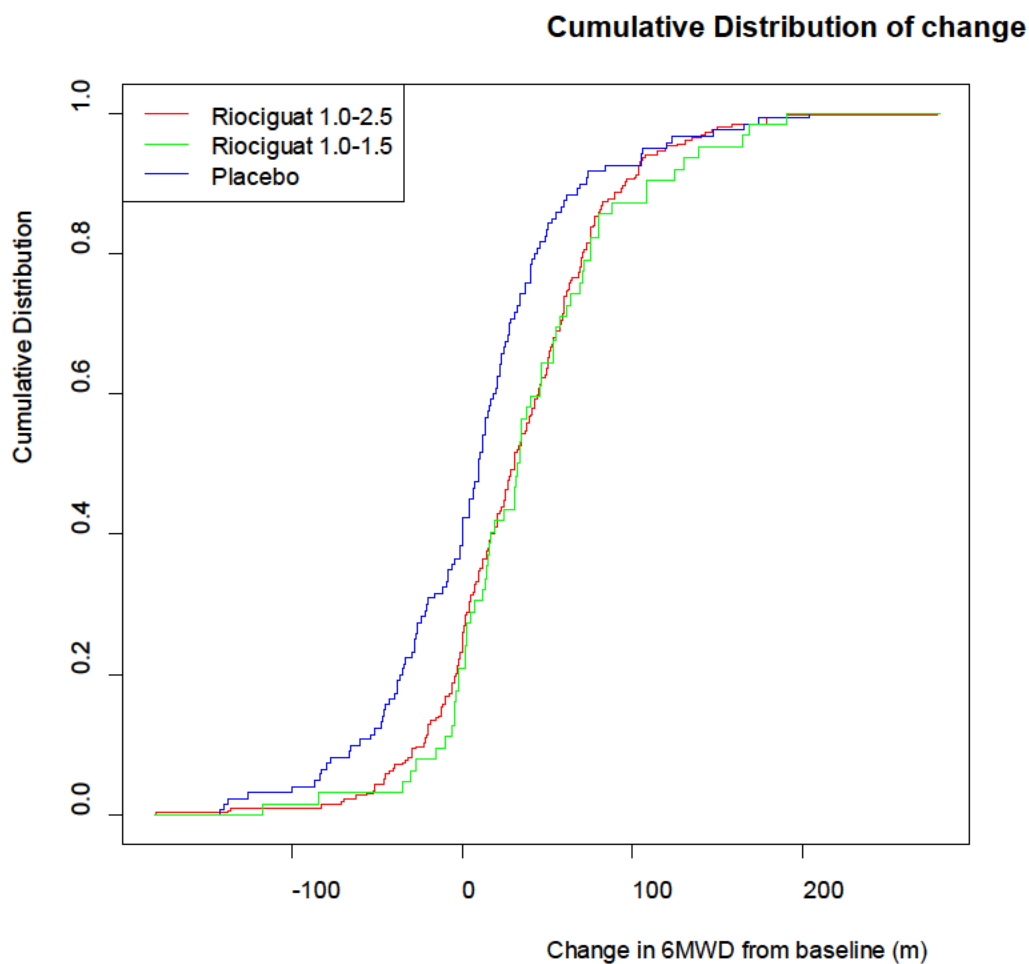


Figure 1 Cumulative distribution of change in 6MWD for three treatment groups in trial 12934 (FDA).

The secondary endpoints are listed in Table 8. These were tested in sequential order in the order listed in the table. The scatterplot of PVR and 6MWD measurements for those subjects with observed values of both variables is shown in Figure 2. There is a weak relationship between the two variables.

Variable	Treatment effect ANCOVA p-value	Shapiro-Wilk test p-value	Stratified Wilcoxon test p-value	Statistically significant	Statistically significant in hierarchical testing
6MWD (primary)	<0.0001	0.0001	<0.0001	Yes	Yes
PVR	<0.0001	0.0001	<0.0001	Yes	Yes
NT-proBNP	0.0157	0.0001	<0.0001	Yes	Yes
WHO functional class	—	—	0.0033	Yes	Yes
Time to clinical worsening	0.0285 ^a	—	0.0046 ^b	Yes	Yes
Borg CR 10 scale ^c	—	—	0.0022	Yes	Yes
EQ-5D questionnaire	0.0197	0.0001	0.0663	No	No
LPH questionnaire	0.0009	0.0001	0.0019	Yes	No

P-values used to determine statistical significance are given in bold.

^a Mantel-Haenszel estimate p-value for incidence of clinical worsening

^b Stratified log-rank test p-value for time to clinical worsening.

Table 8 Secondary endpoints for study 12934 (Table 9-6 of Study Report)

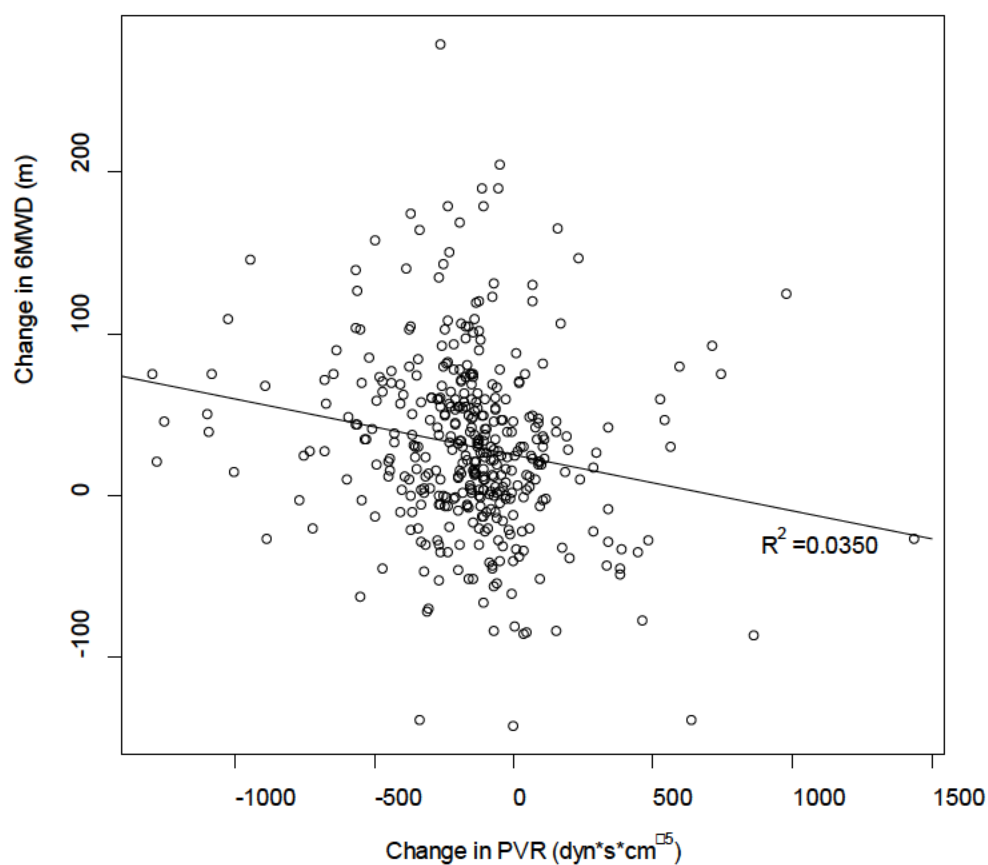


Figure 2 Scatterplot of change in PVR and change in 6MWD for subjects in study 12934 (FDA).

1.4.4.2 Trial 11348

The results for the primary endpoint are shown in Table 9. Note that the Shapiro-Wilk test for normality of the ANCOVA residuals was significant ($p=0.0001$). Therefore, the stratified Wilcoxon p-value was formally used for determining statistical significance, as pre-specified in the statistical analysis plan. The cumulative distribution functions for change in 6MWD are shown in Figure 3 (those subjects with worst case imputations were not included in estimating the cumulative distribution functions in this figure). The LS mean difference shown in the table is not a good estimate of the treatment effect for the same reason that the ANCOVA is not a good test (errors not normally distributed). As in the other trial, a better estimate would be the Hodges-Lehmann estimate associated with the stratified Wilcoxon test, which I calculated as 39 m with a 95% CI of (25 m, 54 m).

A Bayesian estimate of the treatment effect for a uniform prior would be the mean of the posterior distribution, which is normal with mean 45.69 and standard deviation 10.69. This Bayesian estimate also uses the assumption that the estimator is normally distributed.

Statistic	Riociguat 1.0–2.5 mg N=173	Placebo N=88
Baseline		
Mean (SD)	342.3 (81.9)	356.0 (74.7)
Median (Min-Max)	360.0 (150-557)	372.0 (152-474)
Change from baseline to last visit		
Mean (SD)	38.9 (79.3)	–5.5 (84.3)
Median (Min-Max)	42.0 (–376-335)	5.0 (–389-226)
Treatment comparison	Riociguat 1.0-2.5 mg – placebo	
LS mean difference	45.69	
95% CI	24.74 to 66.63	
p-value (ANCOVA)	<0.0001	
p-value (stratified Wilcoxon test)	<0.0001	

ANCOVA model with baseline value, treatment group, and region as fixed effects, stratified Wilcoxon test by region

Last visit = Last observed value (not including follow-up) for subjects who completed the study or withdrew, except imputed worst value in case of death or clinical worsening without a termination visit or a measurement at that termination visit. Worst value imputation for 6MWD at last visit was performed for 4 subjects in the riociguat 1.0-2.5 mg group and 4 subjects in the placebo group.

Table 9 Results for primary endpoint in Trial 11348 (Table and 9-2 of Study Report and confirmed by FDA)

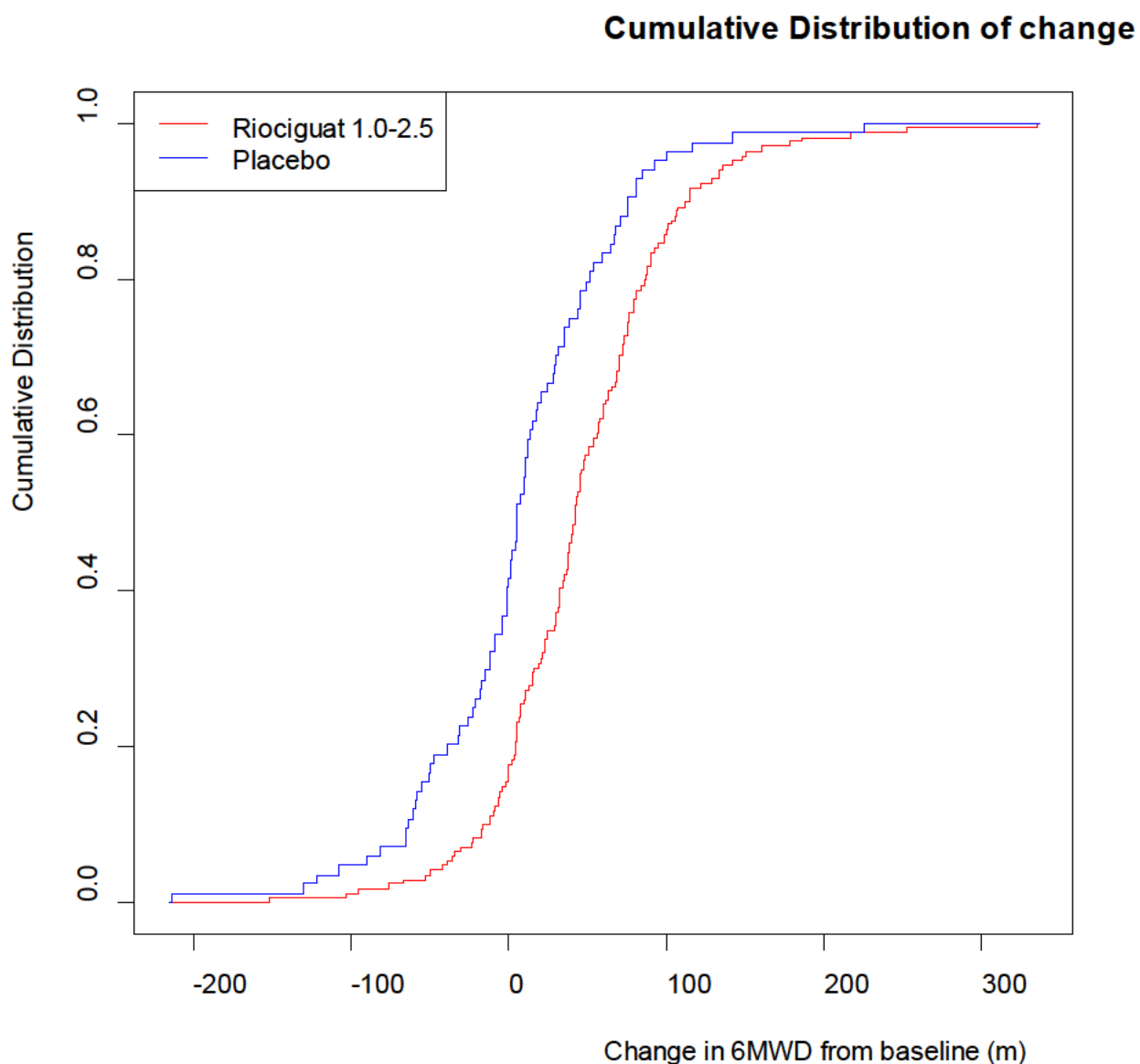


Figure 3 Cumulative distribution of change in 6MWD for three treatment groups in trial 11348 (FDA).

The secondary endpoints are listed in Table 10. These were tested in sequential order in the order listed in the table. The scatterplot of PVR and 6MWD measurements for those subjects with observed values of both variables is shown in Figure 4. There is a weak relationship between the two variables ($R^2=0.073$).

Variable	Treatment effect ANCOVA p-value	Shapiro-Wilk test p-value	Stratified Wilcoxon test p-value	Statistically significant	Statistically significant in hierarchical testing
6MWD (primary)	<0.0001	0.0001	<0.0001	Yes	Yes
PVR	<0.0001	0.0001	<0.0001	Yes	Yes
NT-proBNP	0.0293	0.0001	<0.0001	Yes	Yes
WHO functional class	–	–	0.0026	Yes	Yes
Time to clinical worsening	0.2180 ^a	–	0.1724 ^b	No	No
Borg CR 10 scale ^c	–	–	0.0035	Yes	No
EQ-5D questionnaire	0.0002	0.0001	<0.0001	Yes	No
LPH questionnaire	0.0165	0.0001	0.1220	No	No

P-values used to determine statistical significance are given in bold.

^a Mantel-Haenszel estimate p-value for incidence of clinical worsening

^b Stratified log-rank test p-value for time to clinical worsening.

^c Subjects enrolled before amendment 3 used the Modified Borg Dyspnoea Scale.

Table 10 Secondary endpoints for study 11348 (Table 9-6 of Study Report)

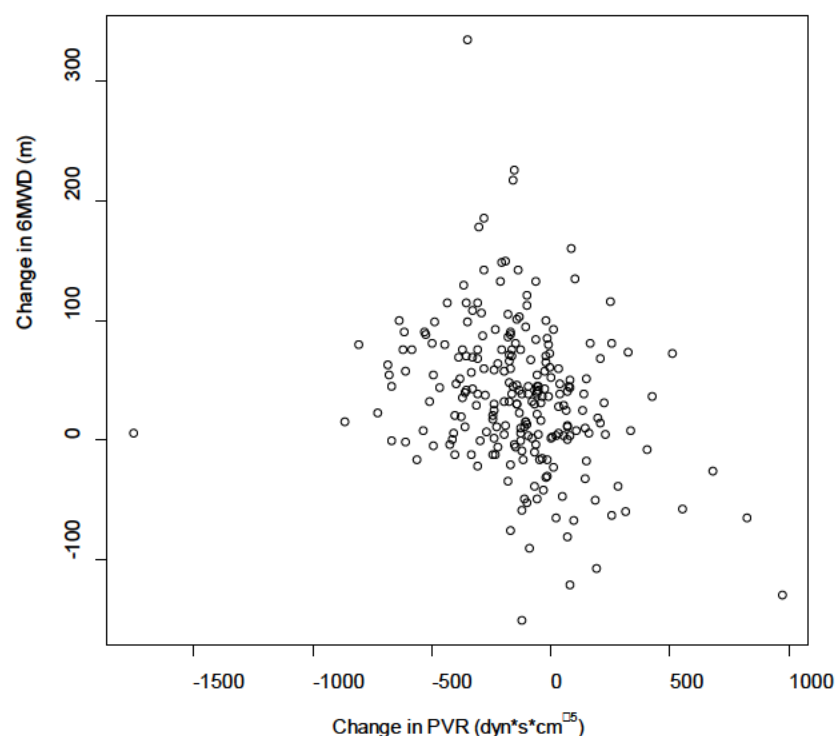


Figure 4 Scatterplot of change in PVR and change in 6MWD for subjects in study 11348 (FDA).

1.5 Evaluation of Safety

See clinical review.

1.6 Benefit-Risk Assessment (Optional)

See clinical review.

FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

1.7 Gender, Race, Age, and Geographic Region

The results for the primary endpoint (6MWD) for subgroups are shown in Table 11 and Table 12. In trial 12934, the treatment effect appeared to be larger in the older age subgroup compared to the younger age. However, in trial 11348, the treatment effect appeared to be about the same in both age subgroups. The biggest treatment effect across regions was observed in Europe and China in both trials.

PAIRWISE COMPARISON: BAY 63-2521 Individual Titration - Placebo				
SUBGROUP	DIFF. OF MEANS	95% LOWER CIL FOR DIFF.	95% UPPER CIL FOR DIFF.	
Female	36.90	20.20	53.60	
Male	31.38	-9.34	72.10	
Age < 65	27.27	8.14	46.41	
Age ≥ 65	54.77	30.18	79.37	
White	37.99	17.88	58.10	
Black or African American	32.89	-70.29	136.07	
Asian	38.78	10.93	66.63	
Race not reported	-4.73	-58.13	48.67	
North America	4.07	-49.32	57.46	
South America	-4.04	-39.36	31.28	
Europe	46.10	21.47	70.73	
China	46.13	5.48	86.79	
Asia/Pacific	40.67	8.29	73.05	

Table 11 Results for 6MWD in trial 12934 (Table 14.2.5/1 of Study Report)

PAIRWISE COMPARISON: BAY 63-2521 Individual Titration - Placebo			
SUBGROUP	DIFF. OF MEANS	95% LOWER CIL FOR DIFF.	95% UPPER CIL FOR DIFF.
Female	54.21	27.73	80.70
Male	39.80	8.60	71.00
Age < 65	43.93	14.52	73.34
Age ≥ 65	48.63	18.85	78.41
White	44.34	20.41	68.27
Black or African American	63.83	-162.89	290.56
Asian	55.42	3.61	107.24
Race not reported	5.79	-64.87	76.44
North America	18.63	-29.83	67.10
South America	18.54	-37.89	74.96
Europe	46.63	19.59	73.67
China	101.85	38.94	164.76
Asia/Pacific	14.80	-77.48	107.08

Table 12 Results for 6MWD in trial 11348 (Table 14.2.5/1 of Study Report)

1.8 Other Special/Subgroup Populations

NA.

SUMMARY AND CONCLUSIONS

1.9 Statistical Issues

No significant statistical issues were identified that would affect approval.

1.10 Collective Evidence

It might not make sense to pool the evidence together since the two trials are for separate indications.

1.11 Conclusions and Recommendations

The two studies showed a symptomatic benefit in improving 6MWD. There are no approved drugs for CTEPH, but the magnitude of the effect in the PAH trial was similar to the magnitude of the treatment effect for other approved drugs (approximately 30 m improvement compared to placebo).

1.12 Labeling Recommendations (as applicable)

Since the assumptions for ANCOVA were not met, the statistical analysis plan stated that formal testing would be done using the stratified Wilcoxon test. For the same reason that the test based on ANCOVA is not valid, the treatment effect should also not be estimated by ANCOVA. I recommend the label does not show the LS mean estimated by ANCOVA; instead, it should show the median change from baseline in both groups or the Hodges-Lehmann estimate of the difference in medians.

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/s/

JOHN P LAWRENCE
07/01/2013

HSIEN MING J HUNG
07/01/2013



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDIES

IND/NDA Number:	NDA 204-819
Drug Name:	Riocignat
Indication(s):	105 Week Rat and Mouse Carcinogenicity Studies
Applicant:	Sponsor: Bayer Pharma AG 42096 Wuppertal, Germany
Documents Reviewed:	Electronic submission submitted on February 12, 2013 Electronic data submitted on February 12, 2013
Review Priority:	Standard
Biometrics Division:	Division of Biometrics -6
Statistical Reviewer:	Mohammad Atiar Rahman, Ph.D.
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Keywords:	Carcinogenicity, Dose response

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1. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of riocignat when administered orally daily through dietary admixture at appropriate drug levels for 105 weeks. Results of this review have been discussed with the reviewing pharmacologist Dr. Hausner.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as dose increases.

2. Rat Study

Two separate experiments were conducted, one in male and one in female rats. In each of these two experiments there were three treated groups and one control group. Two hundred Hsd Cpb:WU Wister rats of each sex were assigned randomly to the treated and control groups in equal size of 50 rats per group. The dose levels for treated groups were 5, 10, or 20 mg/kg/day. In this review these dose groups would be referred to as the low, medium, and high dose groups, respectively. The rats in the control group remained untreated.

During the administration period all rats were observed twice daily morbidity and mortality (once on weekends and public holidays). A detailed clinical examination was performed once before the start of treatment and once weekly thereafter. The rats were palpated regularly for the appearance of masses during the clinical observations.

Body weights of all rats were measured once before the beginning of the study and weekly thereafter up to the scheduled necropsy.

2.1. Sponsor's analyses

2.1.1. Survival analysis

The sponsor performed some descriptive analysis by calculating the number, percentage, and cumulative percentage of number of deaths at quarterly intervals from the start of the study until week 105.

Reviewer's comment: *The sponsor mentioned that they analyzed the mortality data. However, this reviewer could not find out the actual statistical procedures they used in the submitted report.*

Sponsor's findings: Sponsor's analysis showed that during the first year of treatment intercurrent mortality was low. The numbers of intercurrent deaths at the end of Week 105 were 19, 21, 31, 31 for male rats, and 22, 15, 15, 14 for female rats in the control, low, medium and high dose groups, respectively. Sponsor's analysis showed that the survival rates of males receiving 5 mg/kg and females up to 20 mg/kg were similar to those in the control groups throughout the period of the study. At 10 and 20 mg/kg the number of males surviving till the end of study was reduced, however, dose-dependency was missing. The sponsor concluded that the survival was not affected by the treatment with the test substance up to 5 mg/kg in males and up to 20 mg/kg in females.

2.1.2. Tumor data analysis

The sponsor analyzed the tumor incidence data using the methods outlined in the paper of Peto et al. (1980) for positive dose response relationships and the Fisher exact test for pairwise comparisons of the treated groups with the control. For Peto analysis the sponsor first classified the tumor types as fatal and incidental, and analyzed them using the death rate and prevalence methods, respectively. For the evaluation of incidental tumors, the experimental period was divided into partitions using the ad hoc run procedure described in Peto et al.

The sponsor carried out the statistical evaluation of all neoplastic findings in a first step using the asymptotic test. However, tumors displaying p-values < 0.05 in asymptotic tests or tumors of special interest were re-evaluated using the survival adjusted exact stratified test

Adjustment for multiple testing: In order to control the false positive error, the sponsor tested the common and the rare tumors at 0.005 and 0.025 significance levels, respectively (Haseman, 1983) for positive dose response relationship, and 0.01–0.05 for pairwise comparisons. Tumors are considered as common with a background rate of $> 1\%$ and as rare with a background incidence of $< 1\%$.

Reviewer's comment: *The use of 0.005 and 0.025 significance levels for common and rare tumor types, respectively for the test of positive dose response relationships was actually suggested by Lin and Rahman (1998), while the use of 0.01 and 0.05 significance levels for common and rare tumor types, respectively for pairwise comparisons of the treated groups with the control was suggested by Haseman (1983).*

Sponsor's findings: The sponsor's analyses did not show statistically significant dose response relationship among the treatment groups in any of the observed tumor type. Pairwise comparisons also did not show increased incidence in any of the observed tumors.

2.2. Reviewer's analyses

To verify the sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically.

2.2.1. Survival analysis

The survival distributions of rats in all five treatment groups were estimated using the Kaplan-Meier product limit method. For control, low, medium, and high dose groups, the dose response relationship was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 1A and 1B in the appendix for male and female rats, respectively. The intercurrent mortality data are given in Tables 1A and 1B in the appendix for male and female rats, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 2A and 2B in the appendix for male and female rats, respectively.

Reviewer's findings: This reviewer's analysis showed 19 (38%), 22 (44%), 31 (62%), and 31 (62%) number (percent) of deaths in male rats and 24 (48%), 15 (30%), 15 (30%), and 14 (28%) number (percent) of deaths in female rats in control, low, medium, and high dose groups, respectively. The tests showed statistically significant dose response relationship in mortality across control and treated groups in male rats. The pairwise comparisons showed statistically significant increased mortality in the male rat high dose group and decreased

mortality in the female rat high dose group compared to their respective control.

2.2.2. Tumor data analysis

The tumor data were analyzed for dose response relationships and pairwise comparisons of control group with each of the treated groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period (w_{\max}) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of $s_h = 1$. An animal that dies at week w_h without a tumor before the end of

the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}} \right)^k < 1$. The adjusted group size is defined as $\sum s_h$. As an interpretation, an

animal with score $s_h = 1$ can be considered as a whole animal while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values of the tested tumor types are listed in Tables 3A and 3B in the appendix for male and female rats, respectively.

Multiple testing adjustment: For the adjustment of multiple testing of dose response relationship, the FDA guidance for the carcinogenicity study design and data analysis suggests the use of test levels $\alpha=0.005$ for common tumors and $\alpha=0.025$ for rare tumors for a submission with two species, and a significance level $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors for a submission with one species in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. For multiple pairwise comparisons of treated group with control the FDA guidance suggested the use of test levels $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors, in order to keep the false-positive rate at the nominal level of approximately 10% for both submissions with two or one species.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Lin and Rahman (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer's findings: Following tumor types showed p-values less than or equal to 0.05 either for dose response relationship or pairwise comparisons of treated groups and control.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Control in Rats

Sex	Organ Name	Tumor Name	Cont	Low	Med	High	Dose Resp	P_Value		
								C vs. L	C vs. M	C vs. H
Male	ADRENAL GLANDS	Tumor medullary benign,	15	22	17	22	0.0540	0.1116	0.4112	0.0387

Based on the criteria of adjustment for multiple testing discussed above, none of the observed tumors was considered to have statistically significant dose response relationship in either sex. The pairwise comparison also did not show statistically significant increased incidence in any observed tumor type in any treated group in either sex compared to their respective control.

3. Mouse Study

Two separate experiments were conducted, one in male and one in female mice. In each of these two experiments there were three treated groups and one control group. Two hundred SPF-bred CD-1(ICR)BR mice of each sex were assigned randomly to the treated and control groups in equal size of 50 mice per group. The dose levels for treated groups were 50, 100 or 200 ppm, which are equivalent to 6.21, 12.38 and 24.91 mg/kg/day in male mice, and 7.64, 15.66 and 32.47 mg/kg/day in female mice. In this review these dose groups were referred to as the low, medium, and high dose groups, respectively. Animals in the control group remained untreated.

During the administration period all mice were inspected twice a day for morbidity and mortality (once on weekends and public holidays). Detailed clinical examinations were performed once before the start of treatment and once weekly thereafter. The mice were palpated regularly for the appearance of masses during the clinical observations.

The individual body weights for mice were determined just prior to the first administration, once a week during the first 13 weeks and once every 4 weeks thereafter.

3.1. Sponsor's analyses

3.1.1. Survival analysis

The sponsor used similar methodologies to analyze the mouse survival data as they used to analyze the rat survival data.

Sponsor's findings: The sponsor analysis showed 22, 24, 23 and 36 deaths of male mice, and 34, 27, 31 and 32 deaths of female mice at the end of Week 105 in the control, low, medium and high dose groups, respectively. The male mice high dose group showed treatment related increased mortality. In female mice the mortality in the high dose group was comparable to that observed in the control group.

3.1.2. Tumor data analysis

The sponsor used similar methodologies to analyze the mouse tumor data as they used to analyze the rat tumor data.

Adjustment for multiple testing: The sponsor used similar procedure to adjust the multiple testing in the mouse tumor data analysis as they used to adjust the multiple testing in the rat data analysis.

Sponsor's findings: The sponsor's analyses showed a carcinogenic effect on the large bowel (cecum/colon) in male and female mice beginning at 100 ppm. The histopathological evaluation of the gastrointestinal tract revealed in the large bowel an adenocarcinoma in two females at 100 ppm and one male at 200 ppm. The sponsor commented that the tumors were localized in the cecum (females) or in the colon infiltrating the cecum (male). Adenoma of the colon was diagnosed in one male at 200 ppm. No statistically significant result of dose response relationship or pairwise comparisons was mentioned in the sponsor's report.

3.2. Reviewer's analyses

Similar to the rat study, to verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses of mouse data. Data used in this reviewer's analyses were provided by the sponsor electronically.

For the analysis of both the survival data and the tumor data this reviewer used similar methodologies as he used for the analyses of the rat survival and tumor data.

3.2.1. Survival analysis

The Kaplan-Meier curves for survival rates of all treatment groups are given in Figures 2A and 2B in the appendix for male and female mice, respectively. The intercurrent mortality data of all treatment groups are given in Tables 4A and 4B in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals for control, low, medium, and high dose groups are given in Tables 5A and 5B in the appendix for male and female mice, respectively.

Reviewer's findings: This reviewer's analysis showed 22 (44%), 24 (48%), 23 (46%), and 36 (72%) number (percent) of deaths in male mice, and 34 (68%), 27 (54%), 31 (62%), and 32 (64%) number (percent) of deaths in female mice in control, low, medium, and high dose groups, respectively. The tests showed statistically significant dose response relationship in mortality across the treatment groups in male mice. The pairwise comparison show statistically significant increased mortality in male mice high dose group compared to their control.

3.2.2. Tumor data analysis

The tumor rates and the p-values of the tested tumor types are given in Tables 6A and Table 6B in the appendix, for male and female mice respectively.

Reviewer's findings: Following tumor type showed p-values less than or equal to 0.05 either for dose response relationship or pairwise comparisons of treated groups and control.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Control in Mice

			Cont	Low	Med	High	P_Val ue			
Sex	Organ Name	Tumor Name	N=50	N=50	N=50	N=50	Dose Resp	C vs L	C vs M	C vs H
~~~~~										
Male	LUNGS	Carci noma bronchi ol o-al veol ar	4	6	11	4	0.4210	0.4014	0.0466	0.4244
Femal e	ADRENAL GLANDS	Tumor medul lary beni gn	0	0	1	3	0.0145*	.	0.5385	0.1186

Based on the criteria of adjustment for multiple testing discussed in the rat data analysis section, the incidence of adrenal glands benign medullary tumor was considered to have statistically significant dose response relationship in female mice. The pairwise comparisons did not show statistically significant increased incidence in any observed tumor type in any treated group compared to their respective control in either sex.

#### 4. Evaluation of the validity of design of rat and mouse studies

As has been noted, except for the incidence of adrenal glands benign medullary tumor in female mice, no other tumor types showed statistically significant dose response relationship or increased incidence compared to their respective control. However, before drawing any conclusion regarding the carcinogenic or non-carcinogenic potential of the study drug in rats and mice, it is important to look into the following two issues, as have been pointed out in the paper by Haseman (1984).

- (i) Were enough animals exposed, for a sustained amount of time, to the risk of late developing tumors?
- (ii) Were dose levels high enough to pose a reasonable tumor challenge to the animals?

There is no consensus among experts regarding the number of animals and length of time at risk, although most carcinogenicity studies are designed to run for two years with about fifty to sixty animals per treatment group. The following are some rules of thumb regarding these two issues as suggested by experts in this field.

Haseman (1985) has done an investigation on 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 the average, approximately 50% of the animals in the high dose group survived the two-year study period. Also, in a personal communication with Dr. Karl Lin of Division of Biometrics-6, Haseman suggested that, as a rule of thumb, a 50% survival of 50 initial animals or 20 to 30 animals still alive in the high dose group, between weeks 80-90, would be considered as a sufficient number and adequate exposure. 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 these three 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.

Regarding the question of adequate dose levels, it is generally accepted that the high dose should be close to the maximum tolerated dose (MTD). In the paper of Chu, Cueto and Ward (1981), the following criteria are mentioned for dose adequacy. A high dose is considered as close to MTD if any of the criteria is met.

- (i) "A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dosed group relative to the controls."

(ii) “The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical.”

(iii) “In addition, doses are considered adequate if the dosed animals show a slight increased mortality compared to the controls.”

We will now investigate the validity of the riocignat rat and mouse carcinogenicity study, in the light of the above guidelines.

#### 4.1. Rat Study

The following is the summary of survival data of rats in the high dose groups:

##### Percentage of Survival in the High Dose Group at the End of Weeks 52, 78, and 91 in Rats

	Percentage of survival		
	End of 52 weeks	End of 78 weeks	End of 91 weeks
Male	96%	76%	62%
Female	98%	94%	88%

Based on the survival criterion Haseman proposed, it may be concluded that enough rats were exposed to the high dose for a sufficient amount of time in both sexes.

The following table shows the percent difference in mean body weight gain in rats from the concurrent control, defined as

$$\text{Percent difference} = \frac{(\text{Final BW} - \text{Baseline BW})_{\text{Treated}} - (\text{Final BW} - \text{Baseline BW})_{\text{Control}}}{(\text{Final BW} - \text{Baseline BW})_{\text{Control}}} \times 100$$

##### Percent Difference in Mean body Weight Gain from Controls in Rats

Male			Female		
Low	Medium	High	Low	Medium	High
-4.62	-9.85	-4.31	-4.62	-6.15	-8.21

Source: “Body weights – summary main study animals” of Sponsor’s report (Part of Table 8)

Therefore, relative to the control the male rats in high dose group had about 5% and the female rats had about 8% decrements in their body weight gains.

The mortality rates at the end of the experiment were as follows:

##### Mortality Rates at the End of the Experiment in Rats

	<b>Control</b>	<b>Low</b>	<b>Medium</b>	<b>High</b>
Male	38%	44%	62%	62%
Female	48%	30%	30%	28%

This shows that the mortality rates in the male rats high dose group is 24% higher than their control, while that in female rats is 20% lower than their control.

Thus, from the mortality and the body weight gain data it can be concluded that the used high dose level might have reached the MTD in both sexes. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

#### 4.2. Mouse Study

The following is the summary of survival data of mice in the high dose groups:

##### Percentage of Survival in the High Dose Group at the End of Weeks 52, 78, and 91 in Mice

	____Percentage of survival____		
	End of 52 weeks	End of 78 weeks	End of 91 weeks
Male	74%	58%	42%
Female	88%	60%	50%

Based on the survival criterion Haseman proposed, it may be concluded that not enough mice were exposed to the high dose for a sufficient amount of time in either sex.

The following table shows the percent difference in mean body weight gain in mice from the concurrent control,

##### Percent Difference in Mean body Weight Gain from Controls

Male			Female		
Low	Medium	High	Low	Medium	High
6.72	6.72	15.13	16.36	17.27	30.91

Source: Source: "Body weights – summary main study animals" of Sponsor's report (Part of Table 8)

Therefore, relative to control the high dose male mice had less than 15% and the female mice had less than 30% increment in their body weight gain.

The mortality rates at the end of the experiment were as follows:

##### Mortality Rates End of the Experiment

	<b>Control</b>	<b>Low</b>	<b>Medium</b>	<b>High</b>
Male	44%	48%	48%	72%
Female	68%	54%	62%	64%

This shows that the mortality rate was 28% higher than the control in the male mice high dose group, while 4%



lower in the female mice high dose group compared to their respective control.

Thus, from the body weight gain and mortality data it can be concluded that the used high dose level might have reached or exceeded the MTD in males, while it might not have reached the MDT in female mice. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

## 5. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of riocignat when administered orally daily through dietary admixture at appropriate drug levels for 105 weeks.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as dose increases.

**Rat study:** Two separate experiments were conducted, one in male and one in female rats. In each of these two experiments there were three treated groups and one control group. Two hundred Hsd Cpb:WU Wister rats of each sex were assigned randomly to the treated and control groups in equal size of 50 rats per group. The dose levels for treated groups were 5, 10, or 20 mg/kg/day. The rats in the control group remained untreated.

During the administration period all rats were observed twice daily for mortality and morbidity. A detailed clinical examination was performed once before the start of treatment and once weekly thereafter. The rats were palpated regularly for the appearance of masses during the clinical observations.

Body weights of all rats were measured once before the beginning of the study and weekly thereafter up to the scheduled necropsy.

The tests showed statistically significant dose response relationship in mortality across control and treated groups in male rats. The pairwise comparisons showed statistically significant increased mortality in the male rat high dose group and decreased mortality in the female rat high dose group compared to their respective control.

The tests did not show statistically significant dose response relationship in any observed tumor in either sex. The pairwise comparison also did not show statistically significant increased incidence in any observed tumor type in any treated group compared to their respective control.

The mortality and body weight gain data indicate that the used high dose level might have reached the MTD in both sexes of rats. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

**Mouse Study:** Two separate experiments were conducted, one in male and one in female mice. In each of these two experiments there were three treated groups and one control group. Two hundred SPF-bred CD-1(ICR)BR mice of each sex were assigned randomly to the treated and control groups in equal size of 50 mice per group. The dose levels for treated groups were 50, 100 or 200 ppm, which is equivalent to 6.21, 12.38 and 24.91 mg/kg/day in male mice, and 7.64, 15.66 and 32.47 mg/kg/day in female mice. The mice in the control group remained untreated.

During the administration period all mice were inspected twice a day for morbidity and mortality. Detailed clinical examinations were performed once before the start of treatment and once weekly thereafter. The mice were palpated regularly for the appearance of masses during the clinical observations.

The individual body weights for mice were determined just prior to the first administration, once a week during the first 13 weeks and once every 4 weeks thereafter.

The tests showed statistically significant dose response relationship in mortality across the treatment groups in male mice. The pairwise comparison show statistically significant increased mortality in male mice high dose group compared to their control.

The tests showed statistically significant dose response relationship in the incidence of adrenal glands benign medullary tumor in female mice. The pairwise comparisons did not show statistically significant increased incidence in any observed tumor type in any treated group compared to their respective control in either sex.

The mortality and body weight gain data indicate that the used high dose level might have reached or exceeded the MTD in males, while it might not have reached the MDT in female mice. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

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## 6. Appendix

**Table 1A: Intercurrent Mortality Rate  
Male Rats**

Week	0 mg/kg/day		5 mg/kg/day		10 mg/kg/day		20 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
~~~~~								
0 - 52	2	4.00	3	6.00
53 - 78	8	16.00	7	14.00	4	12.00	9	24.00
79 - 91	5	26.00	9	32.00	11	34.00	7	38.00
92 - 104	6	38.00	5	42.00	14	62.00	12	62.00
Ter. Sac.	31	62.00	29	58.00	19	38.00	19	38.00

Total	N=50		N=50		N=50		N=50	

* Cum. %: Cumulative percentage except for Ter. Sac.

**Table 1B: Intercurrent Mortality Rate
Female Rats**

Week	0 mg/kg/day		5 mg/kg/day		10 mg/kg/day		20 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
~~~~~								
0 - 52	5	10.00	.	.	4	8.00	1	2.00
53 - 78	1	12.00	4	8.00	4	16.00	2	6.00
79 - 91	8	28.00	3	14.00	1	18.00	3	12.00
92 - 104	8	44.00	8	30.00	6	30.00	8	28.00
Ter. Sac.	28	56.00	35	70.00	35	70.00	36	72.00
-----								
Total	N=50		N=50		N=50		N=50	

* Cum. %: Cumulative percentage except for Ter. Sac.

**Table 2A: Intercurrent Mortality Comparison  
Male Rats**

Test	Statistic	P_Value#
~~~~~		
Dose-Response	Likelihood Ratio	0.0442
Homogeneity	Log-Rank	0.0557

**Table 2B: Intercurrent Mortality Comparison
Female Rats**

Test	Statistic	P_Value
~~~~~		
Dose-Response	Likelihood Ratio	0.3343
Homogeneity	Log-Rank	0.1057

**Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Male Rats**

		Omg	5mg	10mg	20mg	P_Val ue			
Organ Name	Tumor Name	Cont N=50	Low N=50	Med N=50	Hi gh N=50	Dose Resp	P_Val ue C vs. L	P_Val ue C vs. M	P_Val ue C vs. H
ADRENAL GLANDS	Adenoma cortical	1	0	0	1	0.4707	1.0000	1.0000	0.7198
	Tumor medullary benign,	15	22	17	22	0.0540	0.1116	0.4112	0.0387
	Tumor medullary malignant,	3	4	2	3	0.5254	0.5000	0.8045	0.5838
BODY CAVITIES	Mesothelioma malignant	1	1	0	1	0.5534	0.7531	1.0000	0.7123
BONE	Odontoma, ameloblastic	0	0	1	0	0.4808	.	0.4938	.
	Osteosarcoma	0	0	0	1	0.2308	.	.	0.4675
BRAIN	Tumor granular cell benign,	0	0	3	0	0.4122	.	0.1112	.
DUODENUM	Adenocarcinoma	0	0	0	1	0.2308	.	.	0.4675
FEMUR	Hemangiosarcoma	0	0	0	1	0.2258	.	.	0.4605
HEART	Schwannoma endocardial benign	0	0	1	0	0.4774	.	0.4875	.
	Schwannoma endocardial malignant	0	0	1	0	0.4774	.	0.4875	.
ILEUM	Adenoma, single	1	0	0	0	1.0000	1.0000	1.0000	1.0000
KIDNEYS	Renal lipoma	0	0	0	1	0.2258	.	.	0.4605
	Renal liposarcoma	0	1	0	0	0.7355	0.4938	.	.
	Renal mesenchymal tumor malignant	1	0	0	0	1.0000	1.0000	1.0000	1.0000
LIVER	Adenoma hepatocellular	0	1	0	0	0.7355	0.4938	.	.
	Cholangiocarcinoma,	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Cholangioma benign	0	0	0	1	0.2258	.	.	0.4605
	Hemangioma, single	0	0	0	1	0.2258	.	.	0.4605
LUNGS	Alveolar/bronchiolar adenoma, sin	0	0	0	1	0.2308	.	.	0.4675
LYMPH NODES	Hemangioma, single	0	0	1	0	0.4774	.	0.4875	.
MESENT. LYMPH N	Hemangioma, single	0	3	3	1	0.3978	0.1204	0.1112	0.4605
NOSE	Carcinoma squamous cell	1	0	1	0	0.7939	1.0000	0.7405	1.0000
	Fibroma, myxoid	0	0	1	0	0.4774	.	0.4875	.
PANCREAS	Adenoma exocrine	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Adenoma islet cell	0	0	2	0	0.4638	.	0.2345	.
	Carcinoma islet cell	0	1	1	0	0.5946	0.4938	0.4875	.
PARATHYROID GLA	Adenoma, single	0	2	0	1	0.4072	0.2530	.	0.4605
PITUITARY GLAND	Adenoma pars distalis, multiple	7	7	11	6	0.4503	0.5953	0.1948	0.6145
	Adenoma pars intermedia	1	0	0	0	1.0000	1.0000	1.0000	1.0000
PREPUTIAL GLAND	Adenoma, single, unilateral	0	0	1	1	0.1642	.	0.4875	0.4605
	Carcinoma sebaceous cell, unilate	0	0	1	0	0.4774	.	0.4875	.
	Carcinoma squamous cell, unilater	0	0	0	1	0.2258	.	.	0.4605

**Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Male Rats**

		0mg	5mg	10mg	20mg	P_Val ue			
Organ Name	Tumor Name	Cont	Low	Med	Hi gh	Dose	P_Val ue	P_Val ue	P_Val ue
		N=50	N=50	N=50	N=50	Resp	C vs. L	C vs. M	C vs. H
<div></div>									
PREPUTIAL GLAND	Papilloma squamous cell, unilater	0	1	0	0	0.7355	0.4938	.	.
SALIVARY GLANDS	Adenocarcinoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
SKELETAL MUSCLE	Rhabdomyosarcoma	0	1	0	0	0.7372	0.5000	.	.
	Tumor granular cell benign	0	0	0	1	0.2258	.	.	0.4605
SKIN	Carcinoma squamous cell	1	1	0	0	0.9322	0.7531	1.0000	1.0000
	Fibroma benign	2	0	0	0	1.0000	1.0000	1.0000	1.0000
	Keratoacanthoma, single	0	1	0	0	0.7372	0.5000	.	.
	Lipoma benign	2	0	1	0	0.9260	1.0000	0.8654	1.0000
	Tumor basosquamous benign	0	0	1	0	0.4774	.	0.4875	.
SPLEEN	Hemangioma, single	0	0	0	1	0.2258	.	.	0.4605
	Hemangiosarcoma, single	1	0	0	0	1.0000	1.0000	1.0000	1.0000
SYSTEMIC NEOPLA	Histiocytic Sarcoma	0	0	1	1	0.1642	.	0.4875	0.4605
	Lymphoma malignant, pleomorphic	0	1	0	0	0.7355	0.4938	.	.
	Myeloid Leukemia, emia, not other	1	0	0	0	1.0000	1.0000	1.0000	1.0000
TESTES	Tumor Leydig cell benign	0	2	4	3	0.0716	0.2407	0.0520	0.0976
THYMUS	Thymoma benign	0	1	0	0	0.7355	0.4938	.	.
	Thymoma malignant	0	1	0	1	0.2873	0.4938	.	0.4675
THYROID GLAND	Adenoma C-cell	2	3	2	1	0.7502	0.4881	0.6731	0.8484
	Adenoma follicular cell	2	2	0	0	0.9772	0.6828	1.0000	1.0000
	Carcinoma C-cell	0	1	0	0	0.7355	0.4938	.	.
ZYMBAL GLANDS	Adenoma, cystic with keratinizati	0	0	1	0	0.4774	.	0.4875	.
	Carcinoma sebaceous cell, with ke	0	1	0	0	0.7372	0.5000	.	.
	Carcinoma squamous cell, with foc	0	1	0	0	0.7372	0.5000	.	.

**Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Rats**

		0mg	5mg	10mg	20mg	P_Val ue			
		Cont	Low	Med	Hi gh	Dose	P_Val ue	P_Val ue	P_Val ue
Organ Name	Tumor Name	N=50	N=50	N=50	N=50	Resp	C vs. L	C vs. M	C vs. H
))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))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**Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Rats**

		0mg Cont	5mg Low	10mg Med	20mg High	P_Val ue Dose	P_Val ue C vs. L	P_Val ue C vs. M	P_Val ue C vs. H
Organ Name	Tumor Name	N=50	N=50	N=50	N=50	Resp	C vs. L	C vs. M	C vs. H
SKELETAL MUSCLE	Rhabdomyosarcoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
SKIN	Carcinoma basal cell, near clitor	0	1	0	0	0.7674	0.5294	.	.
	Carci noma squamous cell	0	0	0	1	0.2632	.	.	0.5294
	Fibroma benign	0	0	1	0	0.5116	.	0.5181	.
	Schwannoma malignant	0	0	0	1	0.2632	.	.	0.5294
SPLEEN	Fibrosarcoma	0	0	1	0	0.5088	.	0.5122	.
SYSTEMIC NEOPLA	Lymphoma malignant, lymphocytic	1	0	0	0	1.0000	1.0000	1.0000	1.0000
THYMUS	Osteosarcoma	0	0	1	0	0.5116	.	0.5181	.
	Thymoma benign	0	4	3	0	0.7841	0.0704	0.1343	.
	Thymoma malignant, with fibrosis	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Yolk sac carcinoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
THYROID GLAND	Adenoma C-cell	1	1	2	3	0.1597	0.7762	0.5185	0.3539
TONGUE	Tumor granular cell benign	1	0	0	0	1.0000	1.0000	1.0000	1.0000
UTERUS/HORNS, C	Adenocarcinoma	2	2	2	2	0.5739	0.7353	0.7105	0.7353
	Adenoma, single	0	0	1	2	0.0681	.	0.5122	0.2773
	Contact metastasis, carcinoma, si	0	0	0	1	0.2632	.	.	0.5294
	Endometrial glandular polyp	1	0	2	0	0.7533	1.0000	0.5185	1.0000
	Endometrial stromal polyp, necrot	15	18	9	14	0.8208	0.4565	0.9609	0.7744
	Fibrosarcoma, arising in polyp	1	0	0	2	0.2170	1.0000	1.0000	0.5446
	Leiomyoma benign	0	0	0	1	0.2632	.	.	0.5294
	Leiomyosarcoma	1	2	0	0	0.9460	0.5446	1.0000	1.0000
	Mixed Muellerian tumor malignant	0	0	1	0	0.5088	.	0.5122	.
	Tumor granular cell benign, one h	1	0	0	0	1.0000	1.0000	1.0000	1.0000
VAGINA	Carcinoma squamous cell	0	1	0	1	0.3335	0.5294	.	0.5294
	Fibroma	0	1	0	0	0.7661	0.5238	.	.
	Histiocytoma	0	0	1	0	0.5088	.	0.5122	.
	Tumor granular cell benign	0	0	1	1	0.1981	.	0.5122	0.5294

**Table 4A: Intercurrent Mortality Rate in Male Mice**

Week	0 mg/kg/day		6 mg/kg/day		12 mg/kg/day		50 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
~~~~~								
0 - 52	3	6.00	1	2.00	.	.	13	26.00
53 - 78	10	26.00	3	8.00	12	24.00	8	42.00
79 - 91	1	28.00	10	28.00	6	36.00	8	58.00
92 - 104	8	44.00	10	48.00	5	46.00	7	72.00
Ter. Sac.	28	56.00	26	52.00	27	54.00	14	28.00

Total	N=50		N=50		N=50		N=50	

* Cum. %: Cumulative percentage except for Ter. Sac.

Table 4B: Intercurrent Mortality Rate Female Mice

Week	0 mg/kg/day		6 mg/kg/day		12 mg/kg/day		50 mg/kg/day	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
~~~~~								
0 - 52	3	6.00	2	4.00	2	4.00	6	12.00
53 - 78	18	42.00	9	22.00	11	26.00	14	40.00
79 - 91	8	58.00	10	42.00	5	36.00	5	50.00
92 - 104	5	68.00	6	54.00	13	62.00	7	64.00
Ter. Sac.	16	32.00	23	46.00	19	38.00	18	36.00
-----								
Total	N=50		N=50		N=50		N=50	

* Cum. %: Cumulative percentage except for Ter. Sac.

**Table 5A: Intercurrent Mortality Comparison Male Mice**

Test	Statistic	P_Value
~~~~~		
Dose-Response	Likelihood Ratio	0.0024
Homogeneity	Log-Rank	0.0005

Table 5B: Intercurrent Mortality Comparison Female Mice

Test	Statistic	P_Value
~~~~~		
Dose-Response	Likelihood Ratio	0.6580
Homogeneity	Log-Rank	0.3308



**Table 6A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Male Mice**

		Omg	6mg	12mg	50mg	P_Val ue			
		Cont	Low	Med	Hi gh	Dose	P_Val ue	P_Val ue	P_Val ue
Organ Name	Tumor Name	N=50	N=50	N=50	N=50	Resp	C vs. L	C vs. M	C vs. H
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%									
ADRENAL GLANDS	Adenoma cortical	2	0	1	1	0.5004	1.0000	0.8800	0.7975
	Adenoma subcapsular cell , mixed	0	0	0	1	0.1831	.	.	0.4062
	Tumor medullary benign	0	0	1	0	0.4507	.	0.5000	.
BONE	Osteosarcoma, well -di fferentiated	0	1	0	0	0.7343	0.5190	.	.
BRAIN	Meningi oma, fi brous	0	1	0	0	0.7343	0.5190	.	.
COLON	Adenocarci noma, well -di fferentiat	0	0	0	1	0.1831	.	.	0.4062
	Adenoma, single	0	0	0	1	0.1831	.	.	0.4062
GALLBLADDER	Adenoma, papi llary	0	0	1	0	0.4507	.	0.5000	.
HARDERIAN GLAND	Adenoma, single	3	4	8	3	0.3843	0.5284	0.0959	0.4556
JEJUNUM	Adenocarci noma, well -di fferentiat	1	0	0	0	1.0000	1.0000	1.0000	1.0000
KIDNEYS	Adenoma renal tubule	2	2	0	0	0.9749	0.7207	1.0000	1.0000
	Carci noma renal tubule	1	0	1	0	0.7779	1.0000	0.7533	1.0000
LIVER	Adenoma hepatocell ular	0	2	1	2	0.1151	0.2661	0.5000	0.1687
	Carci noma hepatocell ular	2	2	2	0	0.8839	0.7117	0.6926	1.0000
	Hemangi osarcoma, mul ti ple	1	2	0	2	0.1948	0.5190	1.0000	0.3497
LUNGS	Adenoma bronchi olo-al veol ar	7	6	7	1	0.9644	0.7605	0.6363	0.9890
	Carci noma bronchi olo-al veol ar	4	6	11	4	0.4210	0.4014	0.0466	0.4244
NASAL CAV. /NASO	Adenocarci noma, well -di fferentiat	0	1	0	0	0.7343	0.5190	.	.
PANCREAS	I slet cell adenoma, single	0	1	0	0	0.7324	0.5128	.	.
PITUITARY GLAND	Adenoma pars distalis	0	1	1	0	0.5543	0.5190	0.5000	.
PROSTATE	Adenocarci noma, well -di fferentiat	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Adenoma, single	0	1	0	0	0.7324	0.5128	.	.
SKIN	Fibrosarcoma	1	1	0	0	0.9308	0.7718	1.0000	1.0000
	Malignant neuroendocrine cell tum	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Sebaceous cell carcinoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Squamous cell papilloma, single	0	0	1	0	0.4507	.	0.5000	.
SPLEEN	Hemangi oma, single	0	1	0	0	0.7324	0.5128	.	.
	Hemangi osarcoma, mul ti ple	1	1	0	1	0.4166	0.7718	1.0000	0.6513
STOMACH	Carci noma squamous cell	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Malignant neuroendocrine cell tum	0	1	0	0	0.7324	0.5128	.	.
SYSTEMIC TUMORS	Histiocyti c sarcoma	0	0	2	0	0.4046	.	0.2532	.
	Lymphoma, lymphoblastic	9	11	4	2	0.9758	0.4984	0.9673	0.9825
	Myeloid leukemia, not otherwise s	2	1	1	1	0.5599	0.8888	0.8751	0.7908

**Table 6A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Male Mice**

		0mg	6mg	12mg	50mg	P_Val ue			
		Cont	Low	Med	Hi gh	Dose	P_Val ue	P_Val ue	P_Val ue
Organ Name	Tumor Name	N=50	N=50	N=50	N=50	Resp	C vs. L	M C vs.	H C vs.
TESTES	Tumor Leydi g cel l beni gn	0	4	1	2	0. 2178	0. 0641	0. 5000	0. 1612
THYMUS	Thymoma beni gn	0	0	0	1	0. 1831	.	.	0. 4062
THYROID GLAND	Follicul ar cel l carci noma, sol id	1	0	0	0	1. 0000	1. 0000	1. 0000	1. 0000

**Table 6B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Female Mice**

Organ Name	Tumor Name	0mg	6mg	12mg	50mg	P_Val ue			
		Cont	Low	Med	High	Dose	P_Val ue	P_Val ue	P_Val ue
		N=50	N=50	N=50	N=50	Resp	C vs. L	C vs. M	C vs. H
////////////////////////////////////									
ADRENAL GLANDS	Adenoma cortical	0	1	0	0	0.7710	0.5455	.	.
	Tumor medullary benign	0	0	1	3	0.0145*	.	0.5385	0.1186
	Tumor medullary malignant	0	0	1	0	0.4962	.	0.5385	.
BONE	Osteosarcoma, well-differentiated	1	0	0	0	1.0000	1.0000	1.0000	1.0000
CECUM	Adenocarcinoma, well-differentiated	0	0	2	0	0.4768	.	0.2861	.
HARDERIAN GLAND	Adenoma, single	2	0	3	2	0.3263	1.0000	0.5719	0.6813
HEART	Hemangiosarcoma, single, pericard	1	0	0	0	1.0000	1.0000	1.0000	1.0000
LIVER	Carcinoma hepatocellular	0	0	0	1	0.2290	.	.	0.5000
	Hemangiosarcoma, multiple	0	0	1	2	0.0582	.	0.5455	0.2623
LUNGS	Adenoma bronchiolo-alveolar	4	3	4	3	0.5878	0.8434	0.7127	0.7876
	Carcinoma bronchiolo-alveolar	5	3	3	2	0.8229	0.9050	0.9050	0.9431
MAMMARY GLAND	Adenocarcinoma, poorly-differentiated	1	1	1	0	0.8529	0.7972	0.7909	1.0000
NOSE	Osteosarcoma, well-differentiated	0	0	1	0	0.5000	.	0.5455	.
OVARIES	Cystadenoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Hemangioma, single	0	0	0	1	0.2290	.	.	0.5000
	Luteoma benign	2	2	1	2	0.4550	0.7592	0.9071	0.6940
	Tumor granulosa cell benign	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Tumor sex cord stromal mixed benign	1	0	0	0	1.0000	1.0000	1.0000	1.0000
PITUITARY GLAND	Adenoma pars distalis	1	1	2	0	0.8010	0.7972	0.5585	1.0000
SKIN	Fibrosarcoma	0	0	1	0	0.4962	.	0.5385	.
	Squamous cell carcinoma, well-differentiated	0	0	1	0	0.5000	.	0.5455	.
SPLEEN	Hemangiosarcoma, single	1	1	0	0	0.9489	0.7972	1.0000	1.0000
STERNUM	Fibrosarcoma	0	0	1	0	0.5000	.	0.5455	.
SYSTEMIC TUMORS	Histiocytic sarcoma	1	3	3	3	0.2441	0.3656	0.3769	0.3062
	Lymphoma, NOS	15	19	10	9	0.9091	0.5537	0.9607	0.9358
	Myeloid leukemia, emia, neutrophilic	1	1	1	0	0.8551	0.7972	0.7972	1.0000
THYMUS	Thymoma benign	1	0	0	0	1.0000	1.0000	1.0000	1.0000
UTERUS	Adenocarcinoma, adenosquamous, area	1	0	1	3	0.0622	1.0000	0.7909	0.3187
	Adenoma, single	0	1	0	0	0.7710	0.5455	.	.
	Carcinoma not otherwise specified	0	0	1	0	0.5000	.	0.5455	.
	Fibroma	0	0	1	0	0.4962	.	0.5385	.
	Glandular polyp	3	1	0	2	0.4264	0.9589	1.0000	0.8230
	Leiomyoma, single	2	1	0	3	0.1302	0.9113	1.0000	0.5000
	Leiomyosarcoma	0	0	0	1	0.2290	.	.	0.5000

**Table 6B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Mice**

		0mg	6mg	12mg	50mg	P_Val ue			
		Cont	Low	Med	Hig h	Dose	P_Val ue	P_Val ue	P_Val ue
Organ Name	Tumor Name	N=50	N=50	N=50	N=50	Resp	C vs. L	C vs. M	C vs. H
<i>%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%</i>									
UTERUS	Sarcoma, endometrial stromal	1	1	0	1	0.5192	0.7972	1.0000	0.7623
	Stromal polyp, single	9	7	10	9	0.3602	0.8704	0.6705	0.6092
VAGI NA	L eiomyoma, si ngle	0	0	0	1	0.2290	.	.	0.5000
	Stromal polyp	0	0	2	0	0.4768	.	0.2861	.

Figure 1A: Kaplan-Meier Survival Functions for Male Rats

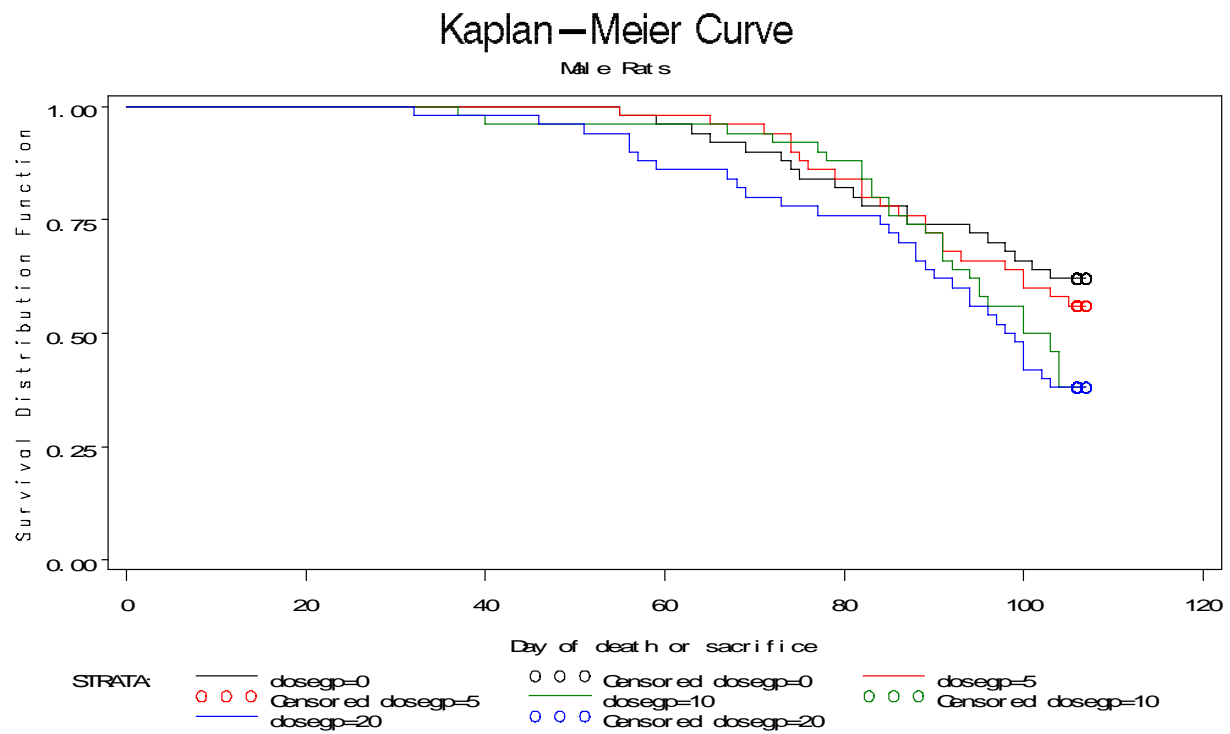


Figure 1B: Kaplan-Meier Survival Functions for Female Rats

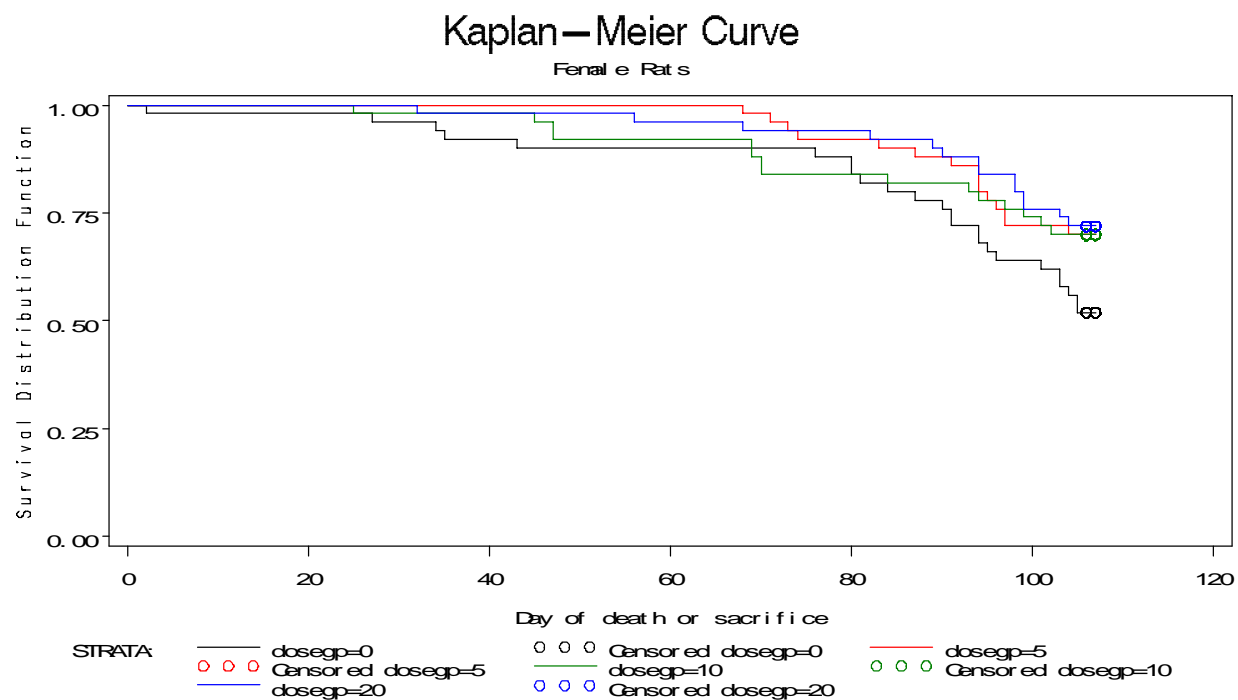


Figure 2A: Kaplan-Meier Survival Functions for Male Mice

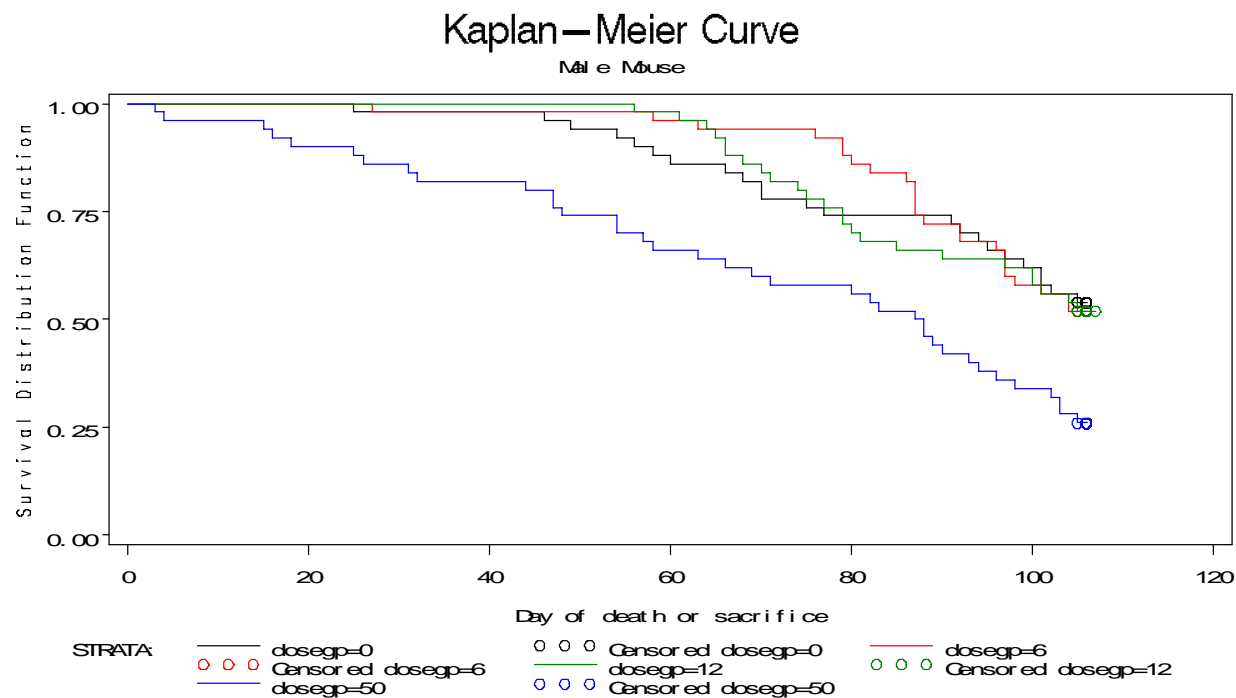
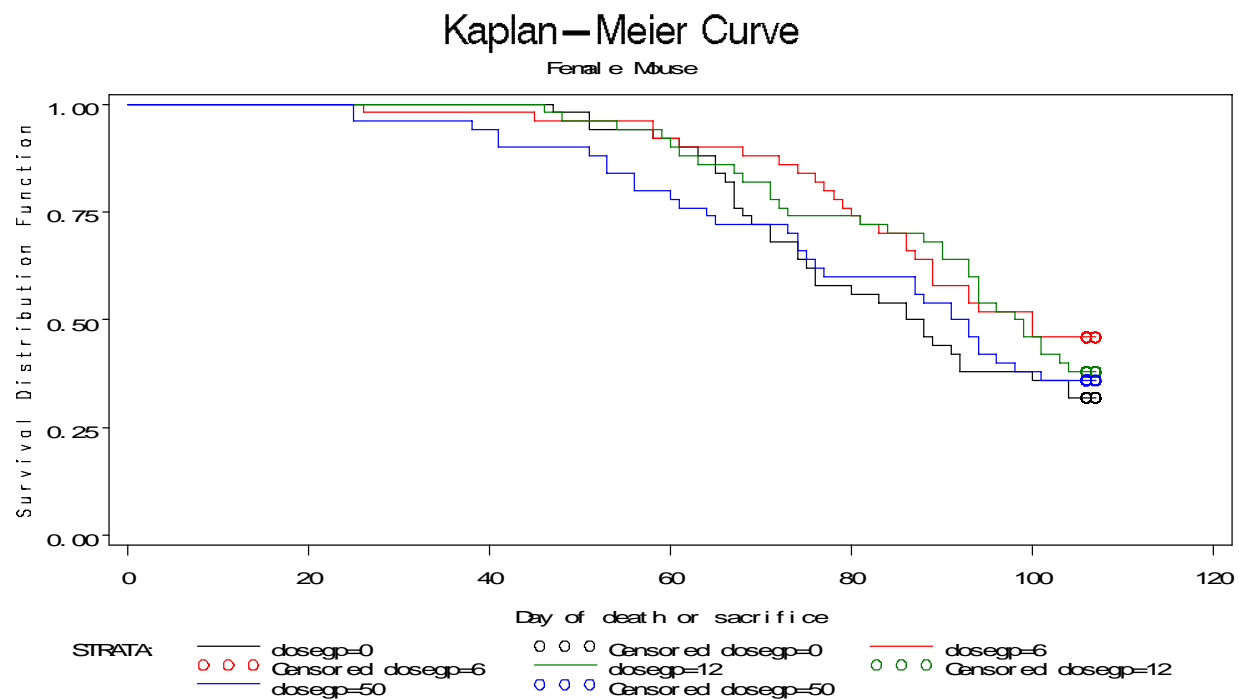


Figure 2B: Kaplan-Meier Survival Functions for Female Mice





## 7. References

1. Peto, R., M.C. Pike, N.E. Day, R.G. Gray, P.N. Lee, S. Parish, J. Peto, Richards, and J. Wahrendorf, "Guidelines for sample sensitive significance test for carcinogenic effects in long-term animal experiments", Long term and short term screening assays for carcinogens: A critical appraisal, International agency for research against cancer monographs, *Annex to supplement, World Health Organization, Geneva*, 311-426, 1980.
2. Bailer AJ, Portier CJ (1988). "Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples." *Biometrics*, 44, 417-431.
3. Bieler, G. S. and Williams, R. L. (1993). "Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity". *Biometrics* 49, 793-801.
4. Tarone RE, "Test for trend in life table analysis", *Biometrika* 1975, 62: 679-82
5. Lin K.K. and Rahman M.A., "Overall false positive rates in tests for linear trend in tumor incidence in animal carcinogenicity studies of new drugs", *Journal of Biopharmaceutical Statistics*, 8(1), 1-15, 1998.
6. Haseman, J, "A re-examination of false-positive rates for carcinogenesis studies", *Fundamental and Applied Toxicology*, 3: 334-339, 1983.
7. Guidance for Industry. Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (Draft Guidance). U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2001.

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/s/  
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04/22/2013

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Concur with review