CENTER FOR DRUG EVALUATION AND RESEARCH

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STATISTICAL REVIEW(S)

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 22-117
Drug Name:	Asenapine
Applicant:	Sponosor: NV Organon PO Box 20 NL-5340BH Oss The Netherlands
	Test Facility: (b) (4)
Documents Reviewed:	Electronic data submitted on September 10, 2008, Also include the sponsor's reports submitted.
Review Priority:	Standard
Biometrics Division:	Division of Biometrics -6
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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 further assess the carcinogenic potential of Asenapine in rats and mice by preparation and examination of all tissues from all animals assigned to the low and medium dose groups with respect to the possible presence of microscopic lymphomas in mice, that were not previously examined on (b) (4) studies No. 0082/074 and No. 0082/075 (104 week subcutaneous administration oncogenicity studies in rats and mice, respectively). The analyses were done for the original study and the present study combined.

After review of the study data from Study Number 0082/074 and 0082/075 by the US FDA, it was concluded that full histopathological examination also had to be performed in the survivors in the low and medium dose groups in rats and in female mice.

Results of this review have been discussed with the reviewing pharmacologist Dr. Chalecka-Franaszek who suggested doing analysis for rat and female mouse studies.

2. Rat Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Three hundred Sprague-Dawley Crl:CD®(SD)IGSBR rats of each sex were randomly allocated to treated and control groups. There were 60 animals per sex in each of groups. At initiation of treatment, treated animals each received dose preparations 0.3, 1.2, or 3.0 mg/kg at a volume-dose of 3 mL/kg. After six weeks of treatment, the dose level of 3.0 mg/kg dose group was increased to 5.0 mg/kg/day, by increasing the volume-dose from 3 to 5 mL/kg. In this review these dose groups would be referred to as the low, medium and high dose group, respectively. Treatment was administrated by subcutaneous injection for about 104 weeks. However, due to excessive mortality, the females were terminated in Weeks 100 to 102. The males were terminated in Weeks 106 to 107. For two control groups, animals of one control group received the control vehicle and animals of the other control group remained untreated for the duration of the study.

The original study (No. 0082/074) design is as the following table:

Group number	Group description	Dose level (mg/kg/day)†	Animals/group Male	Female
1	Placebo Control 1	0	1-60	301-360
2	Low	0.3	61-120	361-420
3	Intermediate	1.2	121-180	421-480
4	High	3.0/5.0#	181-240	481-540
5	Untreated Control 2	0	241-300	541-600

after six weeks the dose was increased to 5 mg/kg/day.
† Dose levels were expressed in terms of the salt (to express in terms of the active entity a factor of 0.709 should be applied).

All animals will be examined at the beginning and the end of the working day to ensure the animals are in good health. Any animal which shows marked signs of ill health may be isolated. Moribund animals will be killed and necropsied. Necropsy will be performed on all survivors after animals have been treated for at least 24 months or if survival of either one of the vehicle, high dose or untreated groups becomes prohibitive

(50%). All animals killed or found dead during the scheduled necropsy period will be considered to have completed the study and any data collected will be treated as such.

2.1. Sponsor's analyses

2.1.1. Survival analysis (from original study No. 0082/074)

Survival function of each treatment group was estimated using the Kaplan-Meier product limit method. The log-rank method will be used for group comparisons. Tests to compare survival incidence will be performed with a one-sided risk for both increasing and decreasing mortality (incidence) with dose. Tests will be performed for dose response and for each treated group against control. The probability of dying before scheduled kill is compared using the Peto et al (1980) method for fatal conditions, which is equivalent to the method of Cox (1972) in that it conditions on the numbers of survivors in each group at each time point. The Peto and Kruskal-Wallis survival analyses include all pair-wise comparisons of all groups, as well as an analysis of dose-related trend. All analyses were carried out using ROELEE84, available from P.N.Lee Statistics and Computing Ltd, 17 Cedar Road Sutton, Surrey, SM2 5DA, United Kingdom.

Sponsor's findings: The Kaplan-Meier product-limit survival curves from the sponsor's report are presented in Figure 1 and Figure 2 for males and females, respectively. Sponsor's analysis showed survival rates of 40.0%, 31.7%, 33.3%, 55.0% and 31.7% in males and 43.3%, 25.0%, 35.0%, 45.0% and 40.0% in females in the untreated control, vehicle control, low, medium, and high dose groups. For both males and females, there was no evidence of increased mortality in low, medium, high dose and untreated groups compared with vehicle group. In males, survival was similar in the two control groups and, compared to the combined controls, was better at 1.2mg/kg/day ($p<0.05$) and similar at 0.3 and 5.0 mg/kg/day. In females, survival was poorer in the vehicle controls than in the untreated controls ($p<0.05$). In females, survival in the asenapine treated groups was similar to that in the untreated controls, but was somewhat better than that in the vehicle controls ($p<0.01$ at 1.2 mg/kg/day, $p<0.05$ at 5.0 mg/kg/day).

Figure 1: Kaplan-Meier plot of Survival in Male Rats

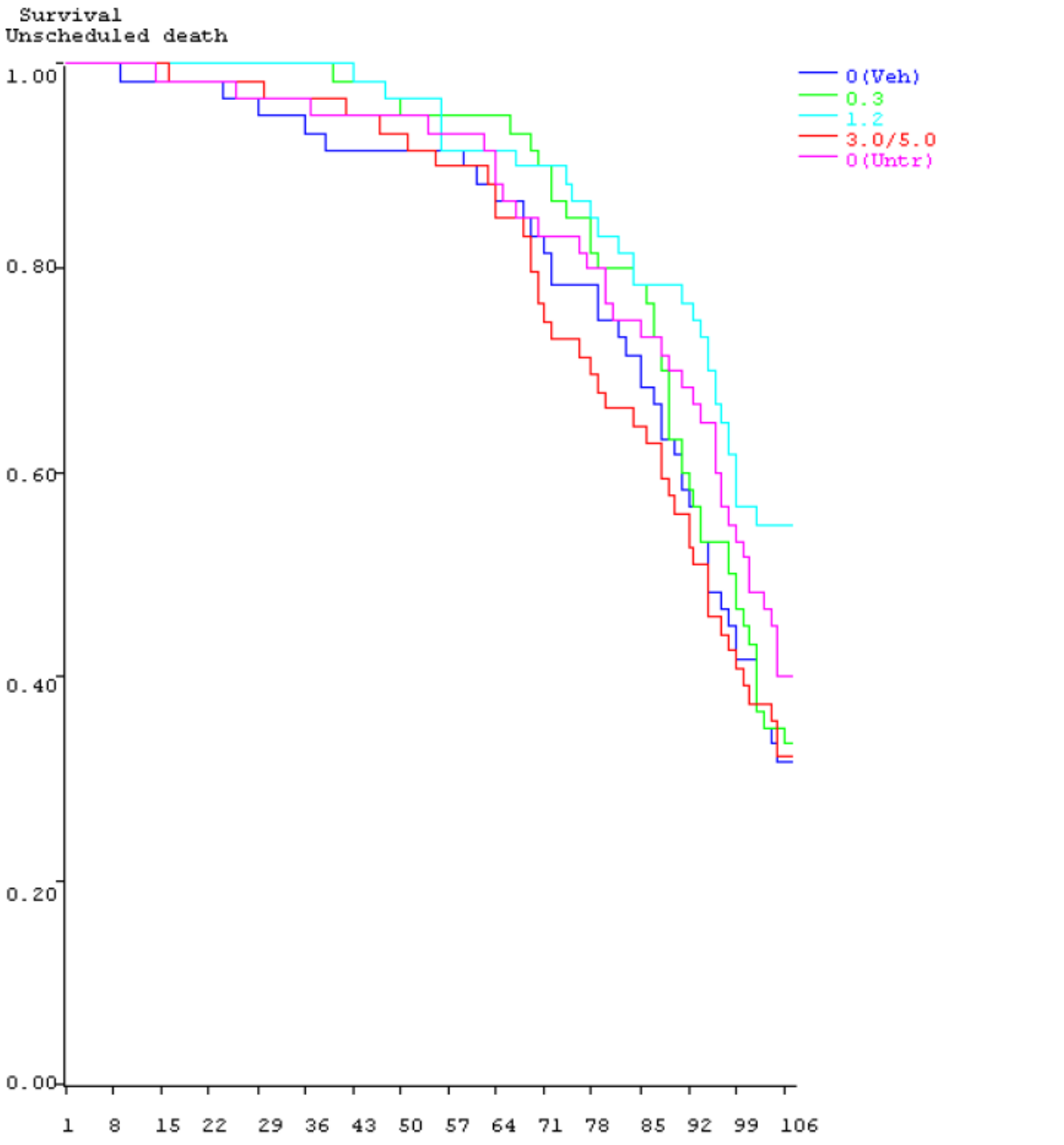
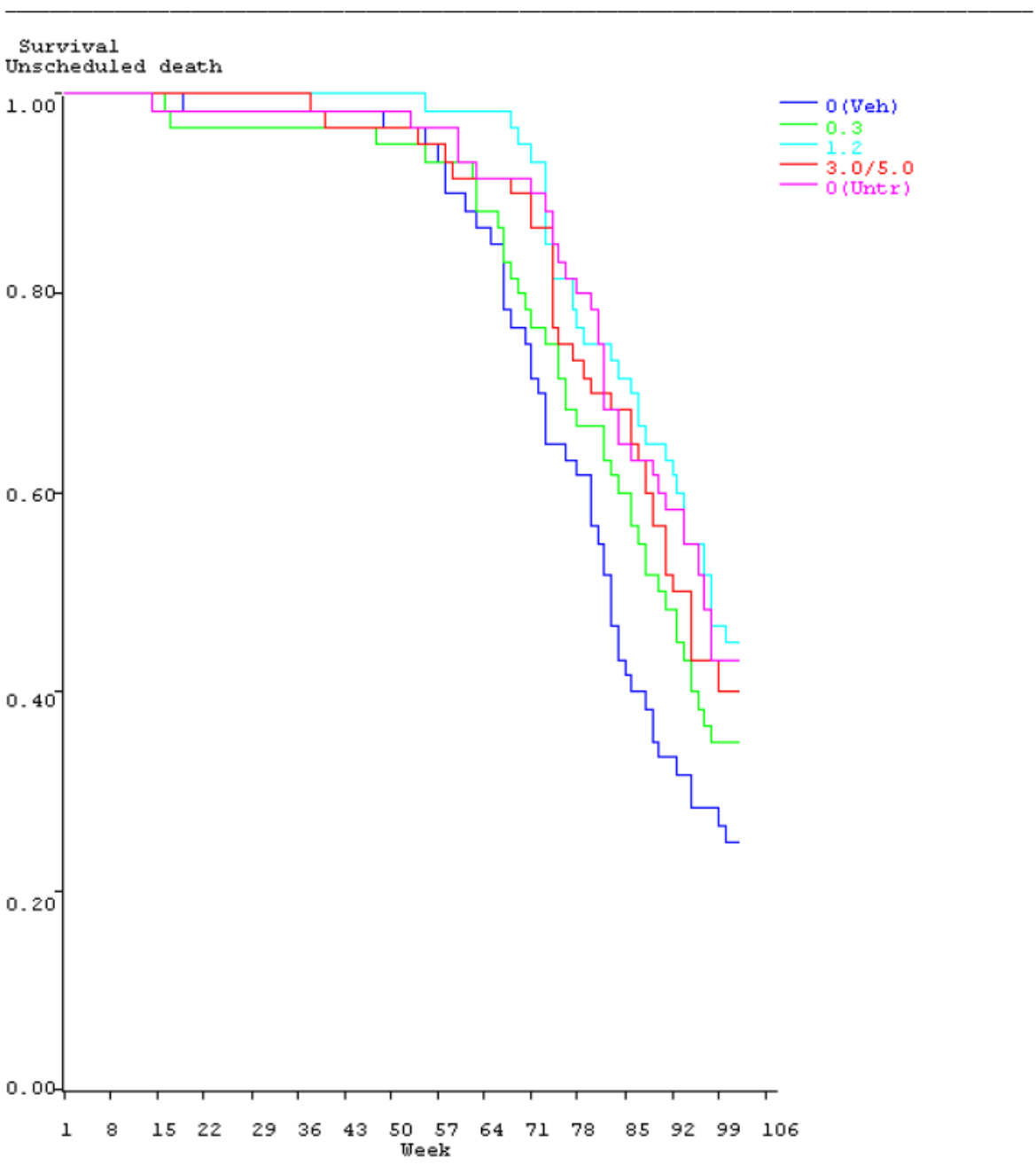


Figure 2: Kaplan-Meier plot of Survival in Female Rats



2.1.2. Tumor data analysis

For histopathology findings, statistical analysis will be performed wherever there are at least 3 tumors, or other lesions, of a given type. The statistical method was based on that described by Peto et al (1980), with extensions to

provide exact tests where incidences were low. The method was used to compare incidences in each specific group with that in the specified control group(s), to test for overall between-group variation and to test for dose-related trend, taking account of between-group differences in survival. For lesions occurring in animals dying spontaneously or sacrificed in extremis during the study, the pathologist will classify the context of observations as one of the three categories: (1) fatal: the lesion was a factor in the demise of the animal; (2) non-fatal: the lesion was not a factor in the demise of the animal; (3) uncertain. Separate analyses will be performed with lesions of uncertain context interpreted as (1) all fatal and (2) all non-fatal. Fixed intervals will be used for the analysis of non-fatal lesions. Fatal and non-fatal lesions will be analysed separately and a combined test will be performed. When the combined analysis is significant ($P<0.05$) the separate analyses will also be reported. Where there are fewer than 10 lesions in any category, the sampling distributions of the test statistics may be obtained through Monte-Carlo simulation. Unadjusted P-values will be reported for tumors. Indication of a possible treatment effect will be assessed on the basis of rare or common tumor type, in line with the FDA guidance "Statistical Aspects of Design, Analysis and Interpretation of Animal Carcinogenicity Studies", August 1997.

Sponsor's findings: Taking into account the survival differences, there was statistically no evidence that Asenapine increased the incidence of any type of tumor significantly.
In contrast, there was strong evidence that Asenapine decreased the incidence of a number of types of tumors, including benign mammary tumors and pituitary pars distalis tumors in females, fatal pituitary pars distalis tumors and injection site fibromas in males, adrenal phaeochromocytomas, squamous cell tumors and histiocytic sarcoma in both sexes. The overall incidence of tumors was significantly reduced in both the 1.2 mg/kg/day and 5 mg/kg/day Asenapine -treated groups. Further, no rare tumors were encountered that could be attributed to Asenapine -treatment. Concluding, Asenapine given subcutaneously to male and female rats at doses of 0.3, 1.2 and 5.0 mg/kg/day did not exhibit any organ specific, systemic or local tumorigenic potential.

5.1.1 Additionally Observed Neoplastic Lesions (extended histopathology intermediate dose groups; Study No. 080114)

The incidences of benign and malignant tumors are summarised per sex, dose-level and per organ/tissue(s) in the following tables.

Benign tumors

Group	1		2		3		4		5	
Dose (mg/kg/day)	0		0.3		1.2		5.0		-	
Sex	M	F	M	F	M	F	M	F	M	F
Lymph node(s)			1							
Ovaries				1						
Pancreas			3		2					
Pituitary gland			6	1	8					
Prostate			1		1					
Uterus				1		2				
Vagina				1						
Subtotal			**11**	**4**	**11**	**2**				

Malignant tumors

Group	1		2		3		4		5	
Dose (mg/kg/day)	0		0.3		1.2		5.0		-	
Sex	M	F	M	F	M	F	M	F	M	F
Hematopoietic system					1					
Pancreas						1				
Subtotal					**1**	**1**				

2.2. Reviewer's analyses

To verify sponsor's analyses and to perform the additional analysis 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 animals in all four treatment groups (three treated groups and one control group) were estimated by the Kaplan-Meier product limit method. Two sets of survival analysis were done in the reviewer's analysis, one set including the untreated control with the three treated groups, and the other set including the vehicle control and the three treated groups. The dose response relationship and homogeneity of survival distributions were tested using the Cox test (Cox, 1972). The intercurrent mortality data are given in Tables 1A and 1B in the appendix for males and females, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1A (1), 1A (2), 1B (1) and 1B (2) in the appendix for males and females, respectively. Results for the tests for dose response relationship and homogeneity of survivals, are given in Tables 2A and 2B in the appendix for males and females, respectively.

Reviewer's findings: The test results showed no statistically significant dose-response mortality and statistically significant difference in mortality in males when compared separately with the untreated and the vehicle control groups. For females, the dose-response in mortality is not statistically significant when compared with the

untreated control and the vehicle control separately. However, the differences in mortality between vehicle control and the medium dose group, and between the vehicle control and the high dose group in females are statistically significant.

2.2.2. Tumor data analysis

The tumor data were analyzed for dose response relationships and pair-wise comparisons of control group with each of the treated groups were performed using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). One critical point for Poly-k test is the choice of the appropriate value of k. 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 (1), 3A (2), 3B (1) and 3B (2) in the appendix for males and females, respectively.

Multiple testing adjustment: Adjustment for the multiple dose response relationship testing was done using the criteria developed by Lin and Rahman (1998). The criteria recommend the use of a significance level α=0.025 for rare tumors and α=0.005 for common tumors for a submission with two species, and a significance level α=0.05 for rare tumors and α=0.01 for common tumors for a submission with only one species study 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 spontaneous tumor rate is less than 1%. The adjustment for multiple pair-wise comparisons was done using the criteria developed by Haseman (1983) that recommends the use of a significance level α=0.05 for rare tumors and α=0.01 for common tumors, in order to keep the false-positive rate at the nominal level of approximately 10%.

It should be noted that the recommended test levels by Lin and Rahman for the adjustment of multiple testing were originally based on the result of a simulation and an empirical study using the Peto method for dose response relationship analysis. However, some later simulation results by Rahman and Lin (2008) indicate that the criteria apply equally well to the analysis using the poly-3 test.

Reviewer's findings: The same type and number of additional tumors were found in this reviewer's tumor analysis as sponsor's in low and medium dose groups. Following tumor types showed p-values less than or equal to 0.05 either tests for dose response relationship and/or pair-wise comparisons between control and each of individual treated groups.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons (Untreated control, low, medium and high dose groups)

	Organ Name	Tumor Name	Untreated_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male	injection site(	histiocytoma, fibrou	1	5	7	2	0.622	0.107	0.041	0.446
Female	mammary glands	adenocarcinoma (M)	11	18	23	25	0.017	0.059	0.020	0.005

Based on the criteria of adjustment for multiple testing of trends proposed by Lin and Rahman, the incidence of none of the above or any other tested tumor types in either sex was considered to have a statistically significant positive dose response relationship. Also based on the criteria by Haseman, the increased tumor

incidences of adenocarcinoma of mammary glands in high dose group in female rats were considered to be statistically significant when compared to the untreated control group because the p-value was less than 0.01.

3. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Two hundred and eighty eight Crl: CD1® (ICR) BR mice of each sex were randomly allocated to high dose group of 60 animals and other groups in equal size of 57 animals. The dose levels for treated groups were 0.5, 1.5, and 5.0 mg/kg/day for males and 0.5, 1.5, 7.5 mg/kg/day for females for 24 months. In this review these dose groups would be referred to as the low, medium, and high dose group, respectively. The high dose level was reduced in Week 25 from 5 to 4.0 mg/kg/day for males and from 7.5 to 5.0 mg/kg/day for females, as a consequence of increased morbidity/mortality. Asenapine was administrated by subcutaneous injection for 104 weeks. However, due to excessive mortality, the males were terminated in Weeks 89/90 and the females in Weeks 98/99.

3.1. Sponsor's analyses

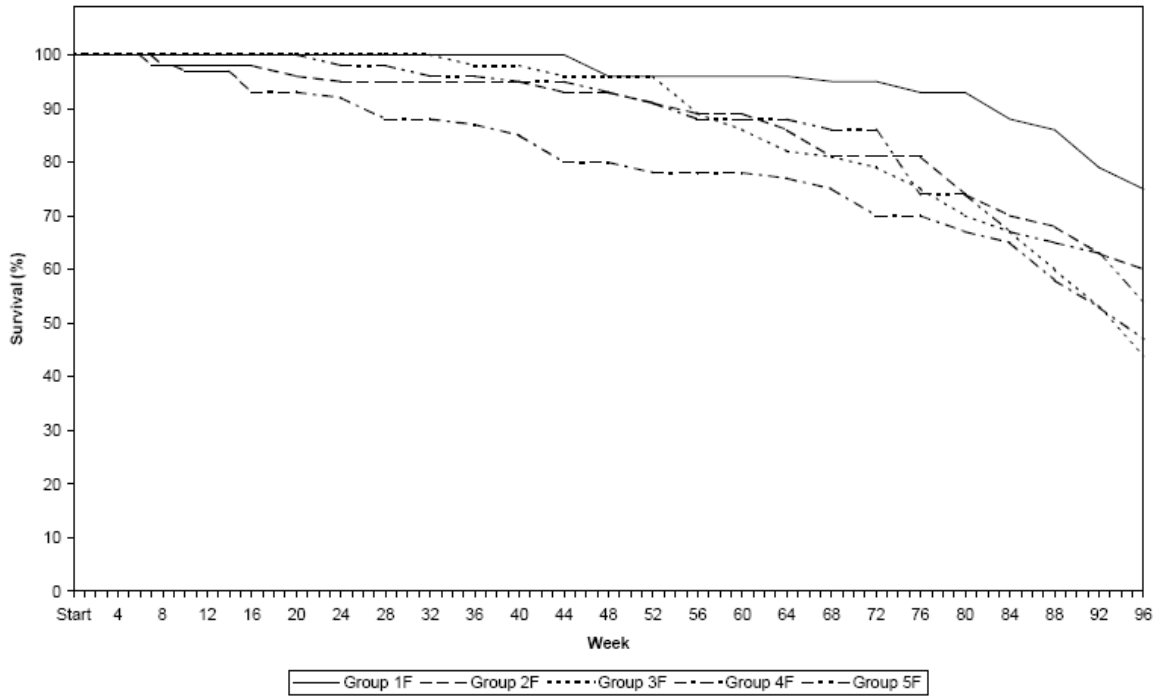
3.1.1. Survival analysis (from original study No. 0082/075)

Survival data from the mouse study were analyzed by the sponsor using the same statistical methodologies that were used to analyze the survival data from the rat study. All statistical analysis was performed for males and females separately.

Sponsor's findings: The Kaplan-Meier product-limit survival curves from the sponsor's report are presented in Figure 4 for females, respectively. Sponsor's analysis showed survival rates of 57.9%, 64.9%, 50.9%, 28.1% and 40.0% in males and 42.1%, 71.9%, 50.9%, 33.3% and 25.0% in females, respectively in the untreated control, vehicle control, low, medium, and high dose groups. A dose-related increase in morbidity and mortality was noted in treated animals but there was no clear evidence of treatment-related responsible for this. A variety of findings including skin/appendage lesions, uro-genital tract lesions, gastrointestinal lesions and haemolymphoreticular tumors were noted as factors contributory to death or morbidity.

Figure 3: Kaplan-Meier plot of Survival in Female Mice

Test article	Vehicle control		Org 5222		Untreated control
Group	1	2	3	4	5
Level (mg/kg/day) Males	0	0.5	1.5	5.0/4.0	0
Level (mg/kg/day) Females	0	0.5	1.5	7.5/5.0	0



3.1.2. Tumor data analysis

Tumor data from the female mouse study were also analyzed by the sponsor using the same statistical methodologies that were used to analyze the tumor data from the rat study.

Sponsor's findings: Examination of the additional tissues from low and medium dose groups females surviving to scheduled termination resulted in the identification of additional tumours in two low dose group animals and in two medium dose group animals. In low dose group animals, these consisted of a single hepatocellular adenoma and a lung bronchio-alveolar carcinoma. In medium dose group animals, the additional tumours identified were an adrenal sub-capsular adenoma and a benign mast cell tumour at one of the three injection sites. Additionally, review of the other tumours identified previously from macroscopic findings resulted in the change of diagnosis of a single ovarian tumour in a low dose group animal from benign sex cord stromal tumour to benign sertoli cell tumour.
There were no tumours of either unusual type or incidence suggestive of systemic oncogenicity. With the exception of haemolymphoreticular tumours, neoplasms in other tissues were generally infrequent, and consistent with the usual pattern in mice of this strain. There was a statistically significant ($P<0.001$) higher

incidence of lymphomas in the high dose females compared with vehicle controls, but the incidence was less than that in untreated control females and lies within the historical control data for this laboratory. This pronounced difference between untreated and vehicle controls suggests that the increasing trend in lymphomas in treated females was probably a chance event rather than an effect of the test article.

3.2. Reviewer's analyses

This reviewer independently performed survival and tumor data analyses from the female mouse study. For the female mouse data analyses this reviewer used similar methodologies that she used to analyze the data from the rat study. Data used in this reviewer's analyses were provided by the sponsor electronically. Two sets of analysis comparing the untreated control and vehicle control separately with the treated groups were done in the reviewer's analysis.

3.2.1. Survival analysis

The intercurrent mortality data are given in Tables 4A in the appendix for females. The Kaplan-Meier curves for death rate are given in Figures 2A (1), 2A (2) in the females, respectively.

Reviewer's findings: The test showed a statistically significant dose-response in survival across either vehicle control group, or untreated control group and treated groups, respectively, and pair-wise differences between medium dose group and vehicle control group, and between high dose group and vehicle control group in survivals in females. There were some differences between reviewer's and sponsor's survival rates and the differences may be caused by the different dates of starting the terminal killing.

3.2.2. Tumor data analysis

The tumor rates and the p-values of the tumor types tested for dose response relationship and pair-wise comparisons of control and treated groups are given in Table 6A (1), 6A (2) in the appendix for males and females, respectively. As suggested by the reviewing pharmacologist Dr. Chalecka-Franaszek, this reviewer also did tumor data analysis for combination of all malignant lymphomas (lymphocytic, plasmocytic, pleomorphic, lymphoblastic, and NOS) in the mouse study.

Reviewer's findings: The same type and number of additional tumors were found in this reviewer's tumor analysis as sponsor's in low and medium dose groups. Following tumor types showed p-values less than or equal to 0.05 either tests for dose response relationship or pair-wise comparisons between either untreated control or vehicle control and each of individual treated groups, respectively.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons (Untreated control, low, medium and high dose groups)

	Organ Name	Tumor Name	Untreated_Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Female	HAEMOLYMPHORETI	MALIGNANT LYMPHOMA								
		-Pleomorphic	14	2	6	14	0.023	0.999	0.941	0.469
		-Lymphocytic	2	1	1	5	0.029	0.509	0.472	0.186
	HAEMOLYMPHORETI	ALL_MALIGNANT_LYMPHO	22	4	8	20	0.014	1.000	0.991	0.480

Based on the criteria of adjustment for multiple testing of trends by Lin and Rahman, the incidence of none of the above or any other tested tumor types in females was considered to have a statistically significant positive dose response relationship. Also based on the criteria of Haseman, the increased tumor incidence of none of treated groups in female mice was considered to be statistically significant when compared to the untreated control group.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons (Vehicle control, low, medium and high dose groups)

			Vehicle Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Female	HAEMOLYMPHORETI	MALIGNANT LYMPHOMA -								
		-Pleomorphic	2	2	6	14	0.000	0.633	0.074	0.000
		-Lymphocytic	3	1	1	5	0.042	0.624	0.584	0.219
	Haemolymphoreti	ALL_MALIGNANT_LYMPHO	7	4	8	20	0.000	0.618	0.306	0.000
	PITUITARY	ADENOMA	1	0	0	3	0.036	0.457	0.438	0.213

Based on the criteria of adjustment for multiple testing of trends by Lin and Rahman, the dose response relationship in the incidence of pleomorphic malignant lymphoma and combination of all types of malignant lymphomas of haemolymphoreti in female mice were considered to be statistically significant since the p-values were less than 0.005. Also based on the criteria of Haseman, the increased tumor incidence of pleomorphic malignant lymphoma and of combination of all types of malignant lymphomas of haemolymphoreti in high dose group in female mice was considered to be statistically significant when compared to the vehicle control group because the p-value is less than 0.01.

4. 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 further assess the carcinogenic potential of Asenapine in rats and mice by preparation and examination of all tissues from all animals assigned to the low and medium dose groups with respect to the possible presence of microscopic lymphomas in mice, that were not previously examined on Covance studies No. 0082/074 and No. 0082/075 (104 week subcutaneous administration oncogenicity studies in rats and mice, respectively).

Rat Study: Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Three hundred Sprague-Dawley Crl:CD®(SD)IGSBR rats of each sex were randomly allocated to treated and control groups. There were 60 animals per sex in each of groups. At initiation of treatment, treated animals each received dose preparations 0.3, 1.2, or 3.0 mg/kg at a volume-dose of 3 mL/kg. The test results showed no statistically significant dose-response mortality and statistically significant difference in mortality in males when compared separately with the untreated and the vehicle control groups. For females, the dose-response in mortality is not statistically significant when compared with the untreated control and the vehicle control separately. However, the differences in

mortality between vehicle control and the medium dose group, and between the vehicle control and the high dose group in females are statistically significant. The tests showed no statistically significant dose response relationship in any of the tested tumor types. Pair-wise comparisons showed a statistically significantly increased incidence of adenocarcinoma of mammary glands in high dose group in female rats compared to the untreated control group.

Mouse Study: Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Two hundred and eighty eight Crl: CD1® (ICR) BR mice of each sex were randomly allocated to high dose group of 60 animals and other groups in equal size of 57 animals. The dose levels for treated groups were 0.5, 1.5, and 5.0 mg/kg/day for males and 0.5, 1.5, 7.5 mg/kg/day for females for 24 months. The test showed statistically significant dose-response across vehicle control group, untreated control group and treated groups, respectively, and pair-wise differences between medium dose group and vehicle control group, and between high dose group and vehicle control group in survivals in females. Tests showed a statistically significant positive dose response relationship across vehicle control and treated groups in the incidence of pleomorphic malignant lymphoma and combination of all types of malignant lymphomas of haemolymphoreti in female mice. Pair-wise comparisons showed a statistically significantly increased incidence of pleomorphic malignant lymphoma and combination of all types of malignant lymphomas of haemolymphoreti in high dose group in females compared to the vehicle control group.

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5. Appendix

Table 1A: Intercurrent Mortality Rate
Male Rats

	Untreated_CONTROL		Vehicle_Control		LOW		MEDIUM		HIGH	
Week	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT
0-52	3	5.0%	5	8.3%	3	5.0%	2	3.3%	5	8.3%
53-78	9	20.0%	8	21.7%	8	18.3%	7	15.0%	14	31.7%
79-91	7	31.7%	12	41.7%	13	40.0%	5	23.3%	8	45.0%
92-105	17	60.0%	16	68.3%	15	65.0%	13	45.0%	14	68.3%
Term. Sac.	24	100.0%	19	100.0%	21	100.0%	33	100.0%	19	100.0%

Table 1B: Intercurrent Mortality Rate
Female Rats

	Untreated_CONTROL		Vehicle_Control		LOW		MEDIUM		HIGH	
Week	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT
0-52	1	1.7%	2	3.3%	3	5.0%	.	.	2	3.3%
53-78	11	20.0%	21	38.3%	17	33.3%	14	23.3%	14	26.7%
79-91	13	41.7%	17	66.7%	11	51.7%	8	36.7%	13	48.3%
92-99	9	56.7%	4	73.3%	8	65.0%	10	53.3%	7	60.0%
Term. Sac.	26	100.0%	16	100.0%	21	100.0%	28	100.0%	24	100.0%

Table 2A: Intercurrent Mortality Comparison
Male Rats

Test	P-Value (across four groups)	P-Value (untreated_control vs low)	P-Value (untreted_control vs medium)	P-Value (untreated_control vs high)
Dose Response	0.1814	0.3035	0.3942	0.1779
Homogeneity	0.0602	0.4845	0.1397	0.2766

Test	P-Value (across four groups)	P-Value (vehicle_control vs low)	P-Value (vehicle_control vs medium)	P-Value (vehicle_control vs high)
Dose Response	0.4595	0.9170	0.0520	0.7595
Homogeneity	0.0379	0.6817	0.0112	0.9733

Table 2B: Intercurrent Mortality Comparison
Female Rats

Test	P-Value (across four groups)	P-Value (untreated_control vs low)	P-Value (untreated_control vs medium)	P-Value (untreated_control vs high)
Dose Response	0.9547	0.2658	0.8542	0.7206
Homogeneity	0.4439	0.2232	0.8019	0.6295

Test	P-Value (across four groups)	P-Value (vehicle_control vs low)	P-Value (vehicle_control vs medium)	P-Value (vehicle_control vs high)
Dose Response	0.1501	0.1998	0.0070	0.0338
Homogeneity	0.0200	0.1633	0.0031	0.0261

Table 3A (1): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Male Rats (untreated control, low, medium and high dose goups)

Organ Name	Tumor Name	Untreated_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
adrenal glands	adenoma, cortical (B	0	1	1	0	0.468	0.494	0.517	.
	pheochromocytoma, be	9	9	7	3	0.954	0.583	0.644	0.888
	pheochromocytoma, ma	2	0	2	1	0.453	0.747	0.334	0.445
auditory sebace	carcinoma, squamo-se	1	1	0	0	0.803	0.747	0.511	0.458
brain	glioma, mixed, malig	0	0	0	1	0.225	.	.	.
	oligodendroglioma, m	1	0	0	0	0.740	0.489	0.511	0.458
haematopoietic	leukemia, granulocyt	0	0	0	1	0.225	.	.	0.470
	lymphoma, malignant	1	2	1	2	0.297	0.492	0.258	0.446
	sarcoma, histiocytic	0	1	0	0	0.494	0.494	.	.
injection site(	carcinoma, squamous	1	0	0	0	0.744	0.494	0.517	0.463
	fibroma (B)	5	2	2	0	0.984	0.783	0.809	0.960
	fibrosarcoma (M)	1	6	5	4	0.320	0.059	0.123	0.152
	histiocytoma, fibrou	1	5	7	2	0.622	0.107	0.041	0.446
	keratoacanthoma (B)	0	1	0	0	0.494	0.494	.	.
	lipoma (B)	0	2	0	0	0.743	0.247	.	.
	liposarcoma (M)	1	0	0	0	0.740	0.489	0.511	0.458
	sarcoma, histiocytic	1	2	0	0	0.886	0.500	0.517	0.463
	schwannoma, malignan	1	1	0	0	0.806	0.753	0.517	0.463
	tumor, hair follicle	0	0	0	1	0.221	.	.	0.463
liver	carcinoma, hepatocel	1	0	0	0	0.740	0.489	0.511	0.458
lymph nodes	haemangioma (B)	2	3	0	1	0.748	0.500	0.769	0.445
mammary glands	fibroadenoma (B)	0	1	0	0	0.494	0.494	.	.
mandibular sali	myoepithelioma, mali	1	0	0	0	0.740	0.489	0.511	0.458
oral cavity & r	papilloma, squamous	1	0	0	0	0.744	0.494	0.517	0.463
pancreas	adenocarcinoma, acin	1	0	0	0	0.744	0.494	0.517	0.463
	adenoma, acinar-isle	1	0	0	0	0.744	0.494	0.517	0.463
	adenoma, islet cell	1	6	3	1	0.822	0.055	0.342	0.715
	carcinoma, islet cel	1	0	1	1	0.359	0.494	0.264	0.715
parathyroid gla	adenoma (B)	4	2	1	3	0.390	0.640	0.833	0.419
pineal gland	pinealoma, malignant	1	0	0	0	0.744	0.494	0.517	0.463
pituitary	adenoma, pars distal	30	30	31	19	0.949	0.581	0.436	0.886
	adenoma, pars interm	0	0	0	1	0.225	.	.	0.470
prostate gland	adenoma, acinar cell	0	1	1	0	0.468	0.494	0.517	.
rectum	sarcoma, histiocytic	1	0	0	0	0.744	0.494	0.517	0.463
skin	carcinoma, NOS (M)	1	0	0	0	0.744	0.494	0.517	0.463
	carcinoma, squamous	0	1	0	0	0.494	0.494	.	.

Table 3A (1)(Continued): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Male Rats (untreated control, low, medium and high dose groups)

Organ Name	Tumor Name	Untreated_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
	keratoacanthoma (B)	1	1	0	1	0.494	0.747	0.517	0.722
	papilloma, squamous	5	0	0	0	0.999	0.969	0.975	0.958
	tumor, basal cell, b	0	0	1	0	0.220	.	0.522	.
	tumor, basal cell, m	1	0	0	0	0.744	0.494	0.517	0.463
	tumor, hair follicle	1	0	0	0	0.744	0.494	0.517	0.463
soft tissues	chondrosarcoma (M)	1	0	0	0	0.740	0.489	0.511	0.458
	fibroma (B)	1	1	3	1	0.432	0.747	0.342	0.715
	fibrosarcoma (M)	0	2	0	0	0.743	0.247	.	.
	histiocytoma, fibrou	0	1	0	2	0.089	0.500	.	0.218
	lipoma (B)	1	0	2	1	0.331	0.494	0.525	0.715
	osteosarcoma (M)	0	1	0	0	0.494	0.494	.	.
	sarcoma, histiocytic	1	0	0	0	0.740	0.489	0.511	0.458
	schwannoma, benign (	1	0	0	0	0.744	0.494	0.517	0.463
	schwannoma, malignan	0	0	1	0	0.220	.	0.522	.
testis	adenoma, Leydig cell	1	0	0	0	0.744	0.494	0.517	0.463
thyroid glands	adenoma, C-cell (B)	3	1	8	3	0.379	0.683	0.120	0.603
	adenoma, follicular	2	3	1	0	0.940	0.500	0.525	0.715
	carcinoma, C-cell (M	2	3	0	1	0.759	0.489	0.769	0.454
	carcinoma, follicula	0	0	1	0	0.221	.	0.517	.

Table 3A (2): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Male Rats (Vehicle control, low, medium and high dose groups)

Organ Name	Tumor Name	Vehicle_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
adrenal glands	adenoma, cortical (B	0	1	1	0	0.479	0.518	0.540	.
	pheochromocytoma, be	7	9	7	3	0.934	0.473	0.514	0.812
	pheochromocytoma, ma	2	0	2	1	0.469	0.765	0.362	0.471
auditory sebace	carcinoma, squamo-se	1	1	0	0	0.817	0.265	0.534	0.481
brain	glioma, mixed, malig	0	0	0	1	0.231	.	.	.
	oligodendroglioma, m	1	0	0	0	0.757	0.512	0.534	0.481
	tumor, granular cell	1	0	0	0	0.757	0.512	0.534	0.481
haematopoietic	leukemia, granulocyt	2	0	0	1	0.545	0.765	0.786	0.481
	lymphoma, malignant	5	2	1	2	0.702	0.783	0.913	0.731
	sarcoma, histiocytic	1	1	0	0	0.818	0.259	0.534	0.481
injection site(	fibroma (B)	4	2	2	0	0.971	0.695	0.725	0.933
	fibrosarcoma (M)	6	6	5	4	0.701	0.429	0.595	0.599
	histiocytoma, fibrou	7	5	7	2	0.924	0.653	0.490	0.890
	keratoacanthoma (B)	1	1	0	0	0.822	0.265	0.540	0.487
	lipoma (B)	0	2	0	0	0.754	0.271	.	.
	sarcoma, histiocytic	2	2	0	0	0.960	0.335	0.786	0.734
	schwannoma, malignan	1	1	0	0	0.817	0.265	0.534	0.481
	tumor, hair follicle	0	0	0	1	0.226	.	.	0.487
kidney	adenoma, renal tubul	1	0	0	0	0.762	0.518	0.540	0.487
lung & bronchi	adenoma, bronchiolo-	1	0	0	0	0.762	0.518	0.540	0.487
lymph nodes	haemangioma (B)	0	3	0	1	0.532	0.139	.	0.487
mammary glands	fibroadenoma (B)	0	1	0	0	0.506	0.518	.	.
oral cavity & r	papilloma, squamous	1	0	0	0	0.757	0.512	0.534	0.481
pancreas	adenoma, islet cell	2	6	3	1	0.891	0.166	0.587	0.481
	carcinoma, islet cel	0	0	1	1	0.177	.	0.540	0.487
parathyroid gla	adenoma (B)	5	2	1	3	0.491	0.793	0.922	0.601
pituitary	adenoma, pars distal	18	30	31	19	0.742	0.050	0.052	0.426
	adenoma, pars interm	0	0	0	1	0.231	.	.	0.494
prostate gland	adenoma, acinar cell	0	1	1	0	0.479	0.518	0.540	.
	schwannoma, malignan	1	0	0	0	0.757	0.512	0.534	0.481
skin	carcinoma, squamous	0	1	0	0	0.506	0.518	.	.
	keratoacanthoma (B)	0	1	0	1	0.300	0.518	.	0.494
	lipoma (B)	1	0	0	0	0.762	0.518	0.540	0.487
	papilloma, squamous	1	0	0	0	0.757	0.512	0.534	0.481
	tumor, basal cell, b	0	0	1	0	0.225	.	0.546	.
soft tissues	fibroma (B)	0	1	3	1	0.347	0.518	0.158	0.487
	fibrosarcoma (M)	0	2	0	0	0.754	0.271	.	.
	histiocytoma, fibrou	0	1	0	2	0.095	0.524	.	0.241

Table 3A (2)(Continued): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Male Rats (Vehicle control, low, medium and high dose groups)

Organ Name	Tumor Name	Vehicle_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
	lipoma (B)	0	0	2	1	0.182	.	0.289	0.487
	osteosarcoma (M)	1	1	0	0	0.822	0.265	0.540	0.487
	sarcoma, histiocytic	1	0	0	0	0.757	0.512	0.534	0.481
	schwannoma, malignan	0	0	1	0	0.225	.	0.546	.
testis	adenoma, Leydig cell	2	0	0	0	0.942	0.765	0.786	0.734
	seminoma, malignant	1	0	0	0	0.757	0.512	0.534	0.481
thyroid glands	adenoma, C-cell (B)	6	1	8	3	0.629	0.949	0.477	0.720
	adenoma, follicular	2	3	1	0	0.946	0.534	0.552	0.734
	carcinoma, C-cell (M	1	3	0	1	0.675	0.336	0.540	0.747
	carcinoma, follicula	1	0	1	0	0.623	0.518	0.289	0.487
	ganglioneuroma (B)	1	0	0	0	0.757	0.512	0.534	0.481

Table 3B (1): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Rats (Untreated control, low, medium and high dose groups)

Organ Name	Tumor Name	Untreated_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
adrenal glands	adenoma, cortical (B	3	0	0	2	0.355	0.852	0.883	0.468
	carcinoma, cortical	0	1	0	1	0.309	0.477	.	0.494
	pheochromocytoma, be	3	0	1	0	0.929	0.856	0.708	0.871
	pheochromocytoma, ma	0	1	1	0	0.500	0.477	0.511	.
brain	astrocytoma, maligna	1	0	0	1	0.439	0.743	0.471	0.511
	tumor, granular cell	2	1	0	1	0.617	0.465	0.764	0.483
eyes	leiomyoma, iris (B)	0	1	0	0	0.511	0.477	.	.
haematopoietic	lymphoma, malignant	1	1	0	0	0.813	0.717	0.505	0.483
	sarcoma, histiocytic	0	1	1	0	0.500	0.477	0.511	.
injection site(	fibroma (B)	1	1	0	1	0.523	0.729	0.511	0.741
	fibrosarcoma (M)	0	1	3	1	0.382	0.477	0.129	0.489
	histiocytoma, fibrou	0	2	0	1	0.441	0.224	.	0.494
	lipoma (B)	0	0	0	1	0.246	.	.	0.489
	sarcoma, histiocytic	0	1	0	0	0.511	0.477	.	.
	tumor, hair follicle	0	0	1	2	0.062	.	0.511	0.236
jejunum	sarcoma, histiocytic	0	1	0	0	0.511	0.477	.	.
kidney	adenoma, renal tubul	0	0	1	0	0.246	.	0.511	.
liver	adenoma, hepatocellu	0	2	1	1	0.415	0.224	0.511	0.489
lymph nodes	haemangioma (B)	0	0	1	1	0.196	.	0.511	0.494
mammary glands	adenocarcinoma (M)	11	18	23	25	0.017	0.059	0.020	0.005
	adenocarcinoma in ad	3	1	1	1	0.741	0.656	0.708	0.674
	adenoma (B)	2	2	4	3	0.334	0.647	0.349	0.479
	fibroadenoma (B)	32	17	21	18	0.950	0.979	0.968	0.989
	fibroma (B)	1	1	1	0	0.732	0.723	0.253	0.483
	myoepithelioma, mali	1	0	0	0	0.739	0.465	0.505	0.483
	tumor, mixed, malign	1	0	0	0	0.739	0.465	0.505	0.483
oral cavity & r	carcinoma, NOS (M)	0	1	0	0	0.511	0.477	.	.
	carcinoma, squamous	1	0	1	0	0.622	0.465	0.253	0.483
ovaries	tumor, sex cord stro	1	1	0	1	0.528	0.717	0.505	0.742
pancreas	adenoma, acinar cell	1	0	0	0	0.743	0.471	0.511	0.489
	adenoma, islet cell	0	0	1	1	0.192	.	0.511	0.489
	carcinoma, islet cel	0	1	1	1	0.293	0.477	0.511	0.489
parathyroid gla	adenoma (B)	0	0	0	1	0.250	.	.	0.494
pituitary	adenoma, pars distal	39	38	30	26	0.980	0.483	0.957	0.954
	adenoma, pars interm	0	0	0	1	0.246	.	.	0.489
	carcinoma, pars dist	1	1	1	0	0.736	0.729	0.258	0.489

Table 3B (1)(Continued): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Rats (Untreated control, low, medium and high dose groups)

Organ Name	Tumor Name	Untreated_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
skin	keratoacanthoma (B)	1	0	0	0	0.743	0.471	0.511	0.489
	tumor, hair follicle	1	0	0	0	0.743	0.471	0.511	0.489
soft tissues	fibroma (B)	0	0	1	0	0.246	.	0.511	.
	fibrosarcoma (M)	0	1	0	0	0.511	0.477	.	.
	lipoma (B)	1	0	2	0	0.641	0.471	0.517	0.489
	schwannoma, benign (	0	1	0	0	0.511	0.477	.	.
soft tissues	tumor, granular cell	1	0	0	0	0.743	0.471	0.511	0.489
thymus	thymoma, benign (B)	0	0	2	2	0.075	.	0.258	0.242
	thymoma, malignant (	0	1	0	0	0.511	0.477	.	.
thyroid glands	adenoma, C-cell (B)	2	7	3	3	0.667	0.055	0.510	0.479
	adenoma, follicular	0	0	1	1	0.196	.	0.511	0.494
	carcinoma, C-cell (M	0	2	1	1	0.418	0.219	0.511	0.489
urinary bladder	papilloma, transitio	0	0	1	0	0.246	.	0.511	.
uterus	adenoma, endometrial	0	0	0	1	0.250	.	.	0.494
	polyp, endometrial s	4	2	6	1	0.881	0.616	0.412	0.805
	polyp, glandular, be	0	1	0	0	0.514	0.471	.	.
vagina	schwannoma, malignan	0	0	1	0	0.246	.	0.511	.
	tumor, granular cell	7	5	3	4	0.741	0.520	0.842	0.699

Table 3B (2): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Rats (Vehicle control, low, medium and high dose groups)

Organ Name	Tumor Name	Vehicle_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
adrenal glands	adenoma, cortical (B	2	0	0	2	0.276	0.779	0.815	0.378
	carcinoma, cortical	2	1	0	1	0.662	0.549	0.815	0.576
	pheochromocytoma, be	2	0	1	0	0.863	0.772	0.590	0.789
	pheochromocytoma, ma	0	1	1	0	0.528	0.533	0.566	.
brain	astrocytoma, maligna	1	0	0	1	.	0.526	0.566	0.299
	tumor, granular cell	0	1	0	1	0.338	0.533	.	0.544
eyes	leiomyoma, iris (B)	0	1	0	0	0.539	0.533	.	.
haematopoietic	lymphoma, malignant	2	1	0	0	0.957	0.540	0.815	0.796
	sarcoma, histiocytic	1	1	1	0	0.770	0.273	0.310	0.538
injection site(	fibroma (B)	0	1	0	1	0.338	0.533	.	0.544
	fibrosarcoma (M)	0	1	3	1	0.419	0.533	0.177	0.544
	histiocytoma, fibrou	0	2	0	1	0.481	0.280	.	0.550
	lipoma (B)	0	0	0	1	0.259	.	.	0.544
	sarcoma, histiocytic	2	1	0	0	0.955	0.539	0.809	0.789
	tumor, basal cell, b	1	0	0	0	0.778	0.520	0.560	0.538
	tumor, hair follicle	0	0	1	2	0.073	.	0.566	0.293
jejunum	sarcoma, histiocytic	0	1	0	0	0.539	0.533	.	.
kidney	adenoma, renal tubul	0	0	1	0	0.259	.	0.566	.
liver	adenoma, hepatocellu	1	2	1	1	0.598	0.549	0.318	0.293
	carcinoma, hepatocel	1	0	0	0	0.783	0.526	0.566	0.544
lymph nodes	haemangioma (B)	0	0	1	1	0.217	.	0.566	0.550
mammary glands	adenocarcinoma (M)	23	18	23	25	0.257	0.712	0.571	0.500
	adenocarcinoma in ad	5	1	1	1	0.908	0.921	0.944	0.930
	adenoma (B)	3	2	4	3	0.482	0.562	0.648	0.438
	fibroadenoma (B)	29	17	21	18	0.947	0.982	0.973	0.990
	fibroma (B)	1	1	1	0	0.770	0.273	0.310	0.538
oral cavity & r	carcinoma, NOS (M)	0	1	0	0	0.539	0.533	.	.
	carcinoma, squamous	0	0	1	0	0.259	.	0.566	.
ovaries	tumor, sex cord stro	0	1	0	1	0.344	0.526	.	0.550
pancreas	adenoma, islet cell	0	0	1	1	0.214	.	0.566	0.544
	carcinoma, islet cel	1	1	1	1	0.463	0.280	0.318	0.293
parathyroid gla	adenoma (B)	1	0	0	1	0.459	0.526	0.566	0.299
pituitary	adenoma, pars distal	42	38	30	26	0.996	0.765	0.998	0.998
	adenoma, pars interm	0	0	0	1	0.259	.	.	0.544
	carcinoma, pars dist	0	1	1	0	0.528	0.533	0.566	.
skin	keratoacanthoma (B)	1	0	0	0	0.783	0.526	0.566	0.544

Table 3B (2)(Continued): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Rats (Vehicle control, low, medium and high dose groups)

Organ Name	Tumor Name	Vehicle_Cont N=60	0.3 mg Low N=60	1.2 mg Med N=60	5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
	tumor, hair follicle	1	0	0	0	0.778	0.520	0.560	0.538
soft tissues	fibroma (B)	0	0	1	0	0.259	.	0.566	.
	fibrosarcoma (M)	0	1	0	0	0.539	0.533	.	.
	lipoma (B)	1	0	2	0	0.675	0.526	0.600	0.544
	schwannoma, benign (	0	1	0	0	0.539	0.533	.	.
thymus	thymoma, benign (B)	1	0	2	2	0.206	0.526	0.600	0.576
	thymoma, malignant (	0	1	0	0	0.539	0.533	.	.
thyroid glands	adenoma, C-cell (B)	4	7	3	3	0.854	0.322	0.633	0.598
	adenoma, follicular	0	0	1	1	0.217	.	0.566	0.550
	carcinoma, C-cell (M	0	2	1	1	0.415	0.274	0.566	0.544
	carcinoma, follicula	1	0	0	0	0.783	0.526	0.566	0.544
urinary bladder	papilloma, transitio	0	0	1	0	0.259	.	0.566	.
uterus	adenocarcinoma, endo	1	0	0	0	0.783	0.526	0.566	0.544
	adenoma, endometrial	0	0	0	1	0.264	.	.	0.550
	polyp, endometrial s	3	2	6	1	0.870	0.562	0.406	0.757
	polyp, glandular, be	0	1	0	0	0.542	0.526	.	.
vagina	schwannoma, malignan	0	0	1	0	0.259	.	0.566	.
	tumor, granular cell	1	5	3	4	0.343	0.133	0.415	0.249

Table 4A: Intercurrent Mortality Rate
Female Mice

	Untreated_CONTROL		Vehicle_Control		LOW		MEDIUM		HIGH	
Week	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT	NO.OF DEATH	PERCENT
0-52	7	12.3%	2	3.5%	6	10.5%	6	10.5%	13	21.7%
53-78	11	31.6%	4	10.5%	10	28.1%	13	33.3%	8	35.0%
79-91	8	45.6%	8	24.6%	6	38.6%	11	52.6%	10	51.7%
92-97	5	54.4%	2	28.1%	5	47.4%	8	66.7%	12	71.7%
Term. Sac.	26	100.0%	41	100.0%	30	100.0%	19	100.0%	17	100.0%

Table 5A: Intercurrent Mortality Comparison
Female Mice

Test	P-Value (across four groups)	P-Value (untreated_control vs low)	P-Value (untreated_control vs medium)	P-Value (untreated_control vs high)
Dose Response	0.0349	0.2609	0.2654	0.1240
Homogeneity	0.0552	0.3753	0.3156	0.0893

Test	P-Value (across four groups)	P-Value (vehicle_control vs low)	P-Value (vehicle_control vs medium)	P-Value (vehicle_control vs high)
Dose Response	0.0002	0.1838	<0.0001	<0.0001
Homogeneity	<0.0001	0.0223	<0.0001	<0.0001

Table 6A (1): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Mice (Untreated control, low, medium and high dose groups)

Organ Name	Tumor Name	Untreated_Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
ADRENAL	BENIGN PHAEOCHROMOCY	0	0	0	1	0.238	.	.	0.481
	MALIGNANT PHAEOCHROM	1	0	0	0	0.739	0.500	0.482	0.475
	SUBCAPSULAR CELL ADE	0	1	1	0	0.475	0.512	0.488	.
BONE	OSTEOMA	1	0	0	0	0.744	0.506	0.488	0.481
EAR	SQUAMOUS CELL PAPILL	1	0	0	0	0.744	0.506	0.488	0.481
FEMUR + MARROW	CHONDROMA	1	0	0	0	0.739	0.500	0.482	0.475
	HAEMANGIOMA	1	0	0	0	0.744	0.506	0.488	0.481
HAEMOLYMPHORETI	GRANULOCYTIC LEUKAEM	0	0	4	1	0.346	.	0.061	0.488
	MALIGNANT LYMPHOMA - Plasmacytic	1	0	0	0	0.744	0.506	0.488	0.481
	MALIGNANT LYMPHOMA - Pleomorphic	14	2	6	14	0.023	0.999	0.941	0.469
	MALIGNANT LYMPHOMA - NOS	2	1	0	0	0.930	0.509	0.734	0.728
	MALIGNANT LYMPHOMA -Lymphocytic	2	1	1	5	0.029	0.509	0.472	0.186
	MALIGNANT LYMPHOMA-Lymphoblastic	3	0	1	1	0.642	0.871	0.655	0.645
HARDERIAN GLAND	ADENOMA	0	0	1	0	0.238	.	0.488	.
Haemolymphoreti	ALL_MALIGNANT_LYMPHO	22	4	8	20	0.014	1.000	0.991	0.480
LIVER	HAEMANGIOMA	1	0	1	0	0.607	0.506	0.741	0.481
	HAEMANGIOSARCOMA	1	1	0	0	0.800	0.253	0.488	0.481
	HEPATOCELLULAR ADENO	0	1	0	0	0.481	0.506	.	.
LUNG	BRONCHIOLO-ALVEOLAR	3	4	1	0	0.977	0.513	0.664	0.860
		7	7	5	5	0.657	0.614	0.550	0.550
MAMMARY GLAND	ADENOCARCINOMA	0	2	3	1	0.442	0.259	0.120	0.481
	ADENOMA	0	1	1	0	0.478	0.506	0.488	.
NECK	MALIGNANT FIBROUS HI	0	0	1	0	0.238	.	0.488	.
OVARY	BENIGN GRANULOSA CEL	0	0	0	1	0.238	.	.	0.481
	BENIGN SERTOLI CELL	0	1	0	0	0.481	0.506	.	.
	BENIGN SEX CORD STRO	0	0	1	0	0.238	.	0.488	.
	CYSTADENOMA	0	1	1	0	0.478	0.506	0.488	.
	LEIOMYOMA	1	1	0	0	0.800	0.259	0.488	0.481
PITUITARY	ADENOMA	2	0	0	3	0.086	0.753	0.734	0.452
RIGHT HIP	BENIGN MAST CELL TUM	0	0	1	0	0.238	.	0.488	.
	MALIGNANT FIBROUS HI	0	0	0	1	0.238	.	.	0.481
SKIN + SUBCUTIS	FIBROSARCOMA	0	0	0	1	0.238	.	.	0.481
	LEIOMYOSARCOMA	0	1	0	0	0.481	0.506	.	.
	MALIGNANT FIBROUS HI	2	1	1	1	0.572	0.509	0.472	0.462
	SEBACEOUS CELL ADENO	0	0	1	0	0.238	.	0.488	.
	SQUAMOUS CELL PAPILL	1	0	1	0	0.607	0.506	0.741	0.481

Table 6A (1) (Continued): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Mice (Untreated control, low, medium and high dose groups)

Organ Name	Tumor Name	Untreated_Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
SPLEEN	HAEMANGIOMA	0	0	0	1	0.242	.	.	0.488
	HAEMANGIOSARCOMA	0	1	1	0	0.478	0.506	0.488	.
STERNUM + MARRO	HAEMANGIOMA	1	0	0	0	0.744	0.506	0.488	0.481
STOMACH	ADENOMA	0	0	0	1	0.238	.	.	0.481
UTERUS	HAEMANGIOSARCOMA	0	0	0	1	0.242	.	.	0.488
	HISTIOCYTIC SARCOMA	3	1	0	0	0.978	0.692	0.865	0.860
	LEIOMYOMA	0	1	0	2	0.101	0.506	.	0.235
	MALIGNANT SCHWANNOMA	0	1	0	0	0.478	0.512	.	.
	STROMAL POLYP	3	5	3	2	0.745	0.370	0.638	0.464
	STROMAL SARCOMA	2	0	0	0	0.936	0.759	0.741	0.734
VAGINA	HISTIOCYTIC SARCOMA	1	0	0	0	0.744	0.506	0.488	0.481

Table 6A (2): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Mice (Vehicle control, low, medium and high dose groups)

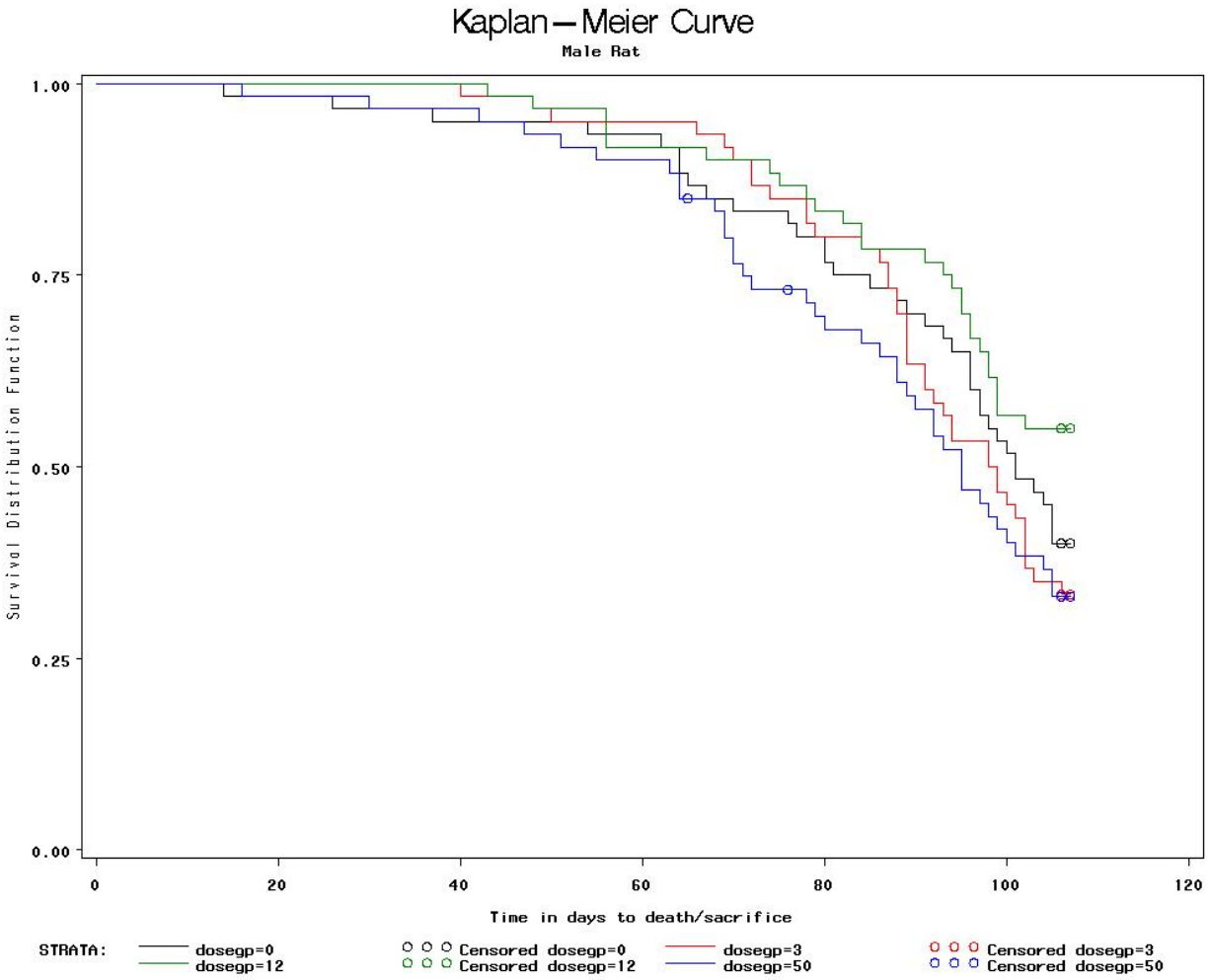
Organ Name	Tumor Name	Vehicle_Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
ADRENAL	BENIGN PHAEOCHROMOCY	2	0	0	1	0.537	0.707	0.687	0.397
	SUBCAPSULAR CELL ADE	0	1	1	0	0.450	0.462	0.438	.
BRAIN	MALIGNANT MENINGIOMA	1	0	0	0	0.704	0.457	0.438	0.432
EAR	NEUROFIBROMA	1	0	0	0	0.704	0.457	0.438	0.432
FEMUR + MARROW	HAEMANGIOMA	1	0	0	0	0.704	0.457	0.438	0.432
	HAEMANGIOSARCOMA	1	0	0	0	0.704	0.457	0.438	0.432
HAEMOLYMPHORETI	GRANULOCYTIC LEUKAEM	2	0	4	1	0.511	0.702	0.251	0.400
	HISTIOCYTIC SARCOMA	1	0	0	0	0.700	0.452	0.433	0.427
	MALIGNANT LYMPHOMA - Nos	0	1	0	0	0.453	0.462	.	.
	MALIGNANT LYMPHOMA - Pleomorphic	2	2	6	14	0.000	0.633	0.074	0.000
	MALIGNANT LYMPHOMA - Lymphocytic	3	1	1	5	0.042	0.624	0.584	0.219
	MALIGNANT LYMPHOMA-Lymphoblastic	2	0	1	1	0.515	0.702	0.409	0.400
HARDERIAN GLAND	ADENOMA	0	0	1	0	0.225	.	0.438	.
Haemolymphoreti	ALL_MALIGNANT_LYMPHO	7	4	8	20	0.000	0.618	0.306	0.000
LIVER	HAEMANGIOMA	0	0	1	0	0.225	.	0.438	.
	HAEMANGIOSARCOMA	0	1	0	0	0.456	0.457	.	.
	HEPATOCELLULAR ADENO	1	1	0	0	0.766	0.707	0.438	0.432
LUNG	BRONCHIOLO-ALVEOLAR	1	4	1	0	0.884	0.137	0.687	0.432
		10	7	5	5	0.752	0.557	0.713	0.713
MAMMARY GLAND	ADENOCARCINOMA	3	2	3	1	0.711	0.419	0.552	0.574
	ADENOMA	0	1	1	0	0.452	0.457	0.438	.
NECK	MALIGNANT FIBROUS HI	0	0	1	0	0.225	.	0.438	.
OVARY	BENIGN GRANULOSA CEL	0	0	0	1	0.225	.	.	0.432
	BENIGN LUTEOMA	3	0	0	0	0.975	0.844	0.827	0.821
	BENIGN SERTOLI CELL	1	1	0	0	0.766	0.707	0.438	0.432
	BENIGN SEX CORD STRO	1	0	1	0	0.568	0.457	0.687	0.432
	CYSTADENOMA	1	1	1	0	0.677	0.707	0.687	0.432
	LEIOMYOMA	0	1	0	0	0.453	0.462	.	.
PITUITARY	ADENOMA	1	0	0	3	0.036	0.457	0.438	0.213
RIGHT HIP	BENIGN MAST CELL TUM	0	0	1	0	0.225	.	0.438	.
	MALIGNANT FIBROUS HI	0	0	0	1	0.225	.	.	0.432
SKIN + SUBCUTIS	FIBROSARCOMA	0	0	0	1	0.225	.	.	0.432
	LEIOMYOSARCOMA	0	1	0	0	0.456	0.457	.	.
	MALIGNANT FIBROUS HI	1	1	1	1	0.439	0.714	0.687	0.680

Table 6A (2)(Continued): Tumor Rates and P-Values for Dose Response Relationship and Pair-wise Comparisons Female Mice (Vehicle control, low, medium and high dose groups)

Organ Name	Tumor Name	Vehicle_Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
	SEBACEOUS CELL ADENO	0	0	1	0	0.225	.	0.438	.
	SQUAMOUS CELL PAPILL	0	0	1	0	0.225	.	0.438	.
SPLEEN	HAEMANGIOMA	1	0	0	1	0.407	0.457	0.438	0.687
	HAEMANGIOSARCOMA	0	1	1	0	0.452	0.457	0.438	.
STOMACH	ADENOMA	0	0	0	1	0.225	.	.	0.432
UTERUS	HAEMANGIOSARCOMA	0	0	0	1	0.229	.	.	0.438
	HISTIOCYTIC SARCOMA	2	1	0	0	0.910	0.434	0.687	0.680
	LEIOMYOMA	2	1	0	2	0.310	0.434	0.687	0.593
	MALIGNANT SCHWANNOMA	0	1	0	0	0.453	0.462	.	.
	STROMAL POLYP	1	5	3	2	0.514	0.067	0.221	0.397

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

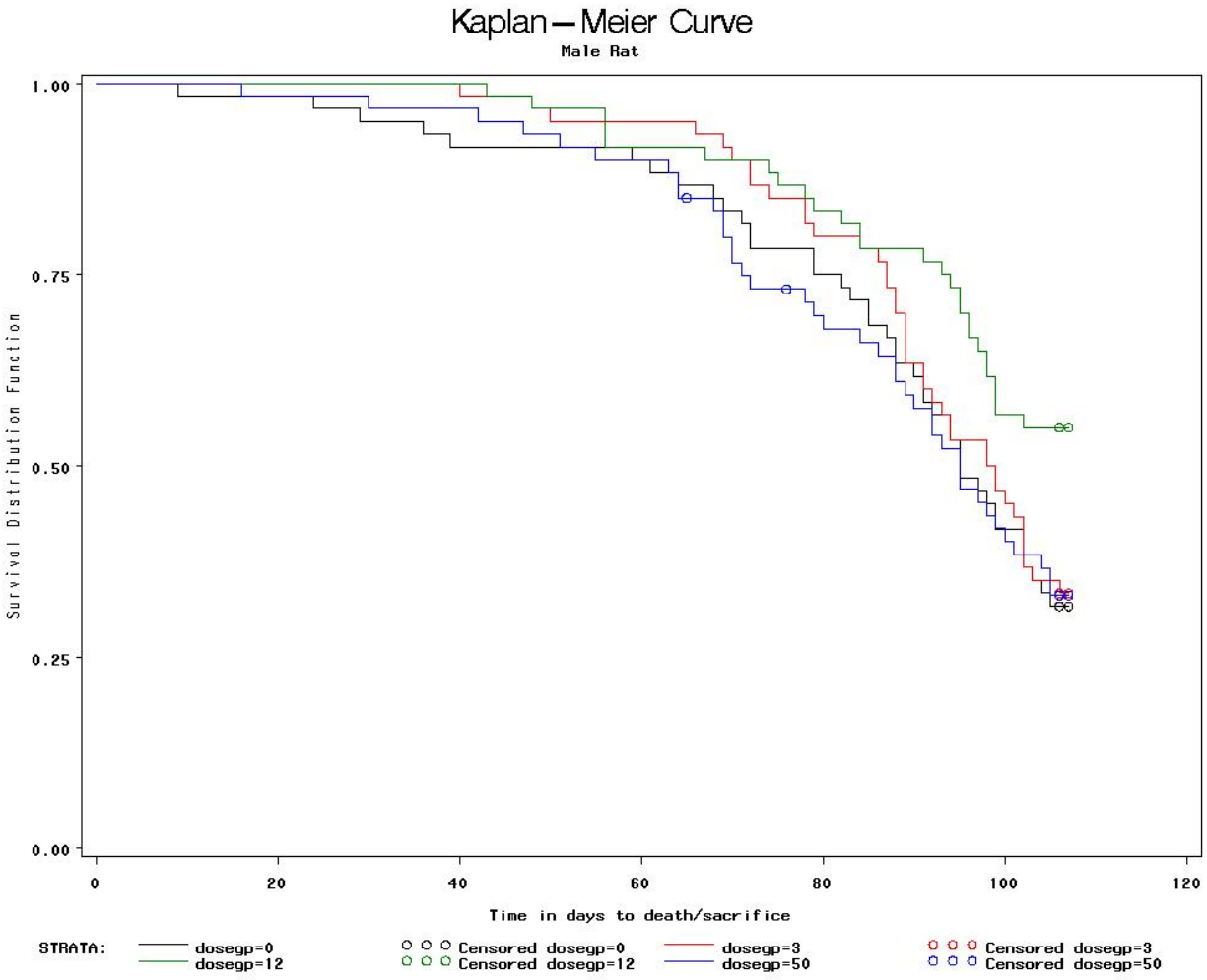
Male Rats (untreated control, low, medium and high dose groups)



X-Axis: Weeks, Y-Axis: Survival rates

Figure 1A (2): Kaplan-Meier Survival Functions for Male Rats

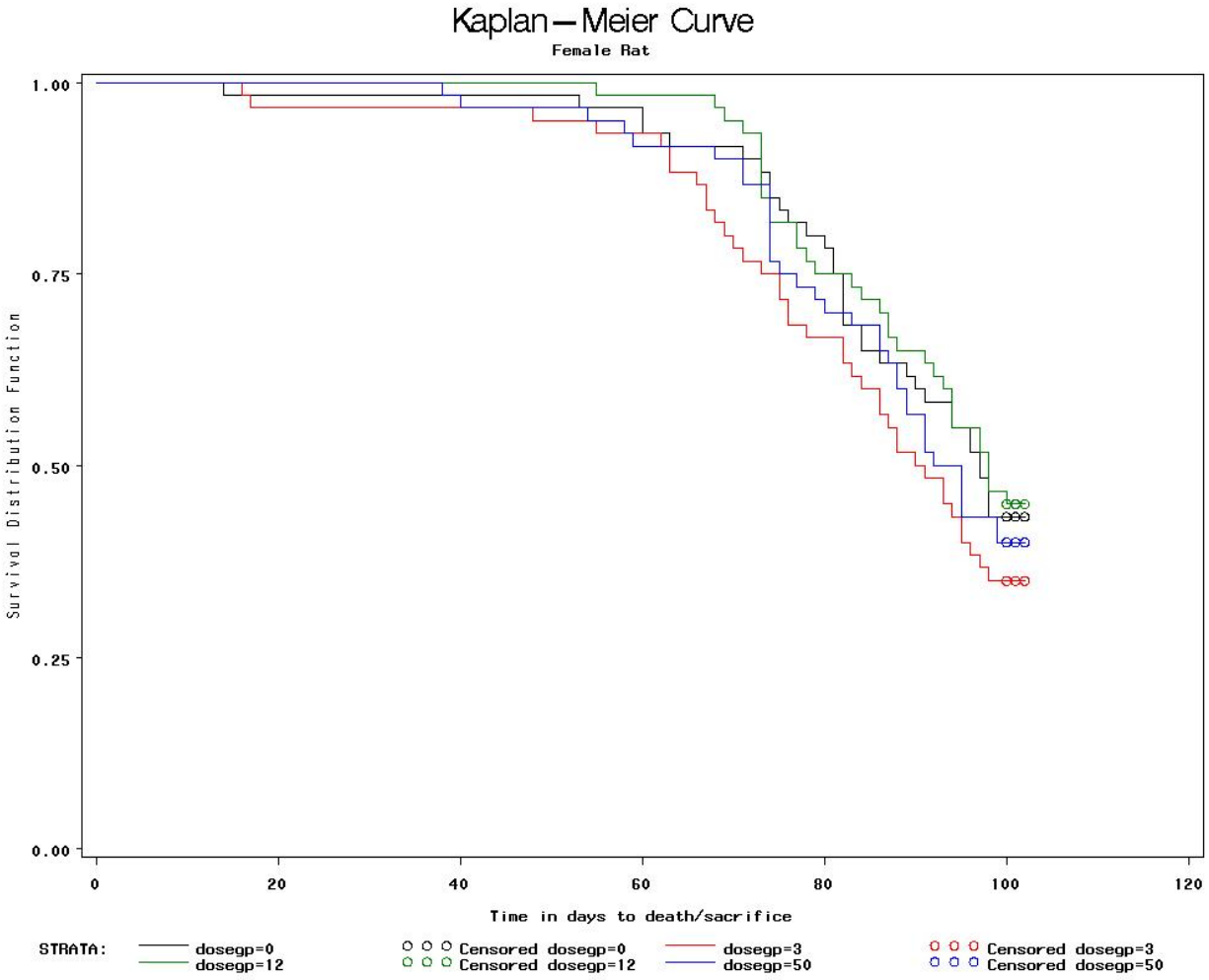
Male Rats (vehicle control, low, medium and high dose groups)



X-Axis: Weeks, Y-Axis: Survival rates

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

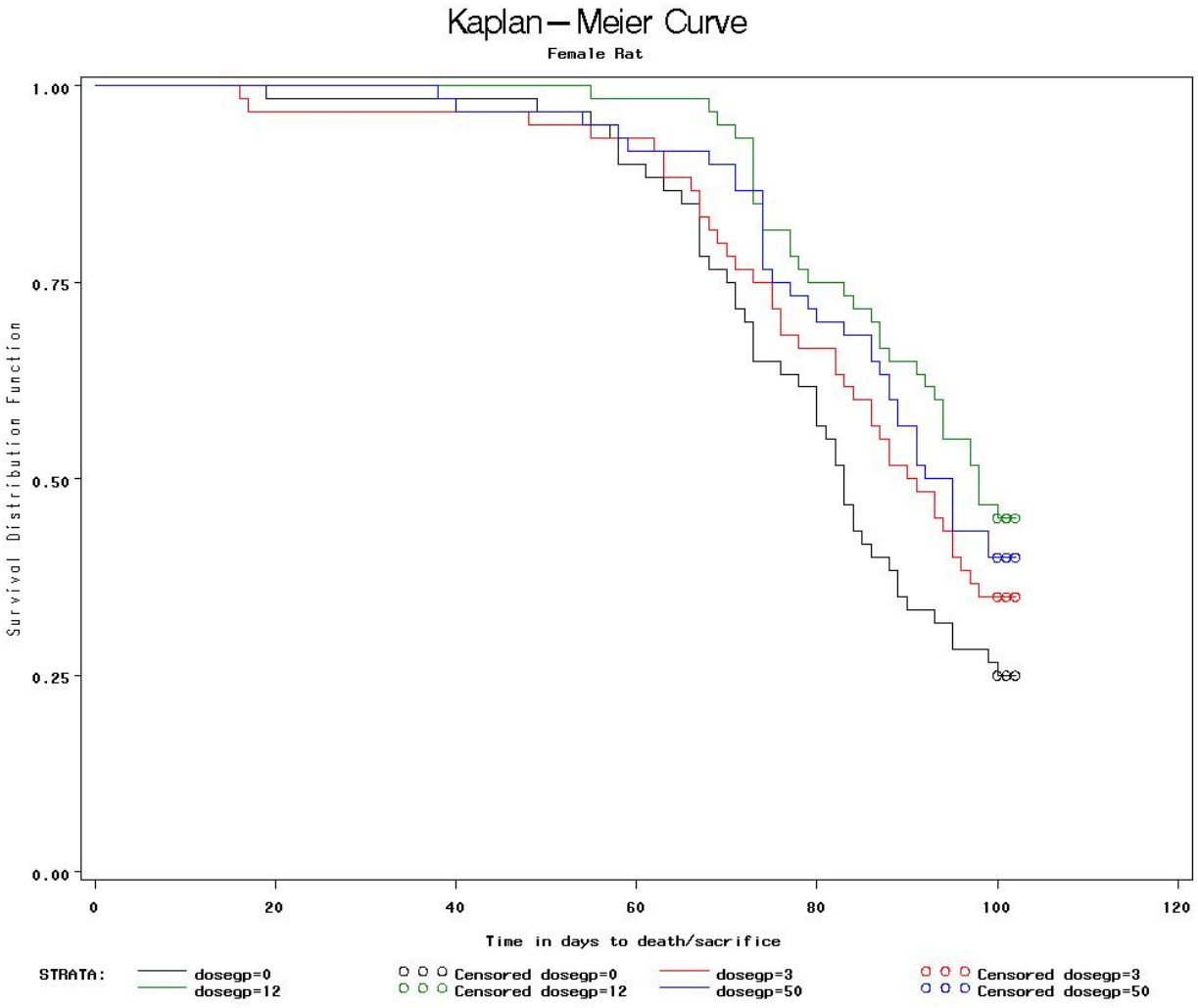
Female Rats (Untreated control, low, medium and high dose groups)



X-Axis: Weeks, Y-Axis: Survival rates

Figure 1B (2): Kaplan-Meier Survival Functions for Female Rats

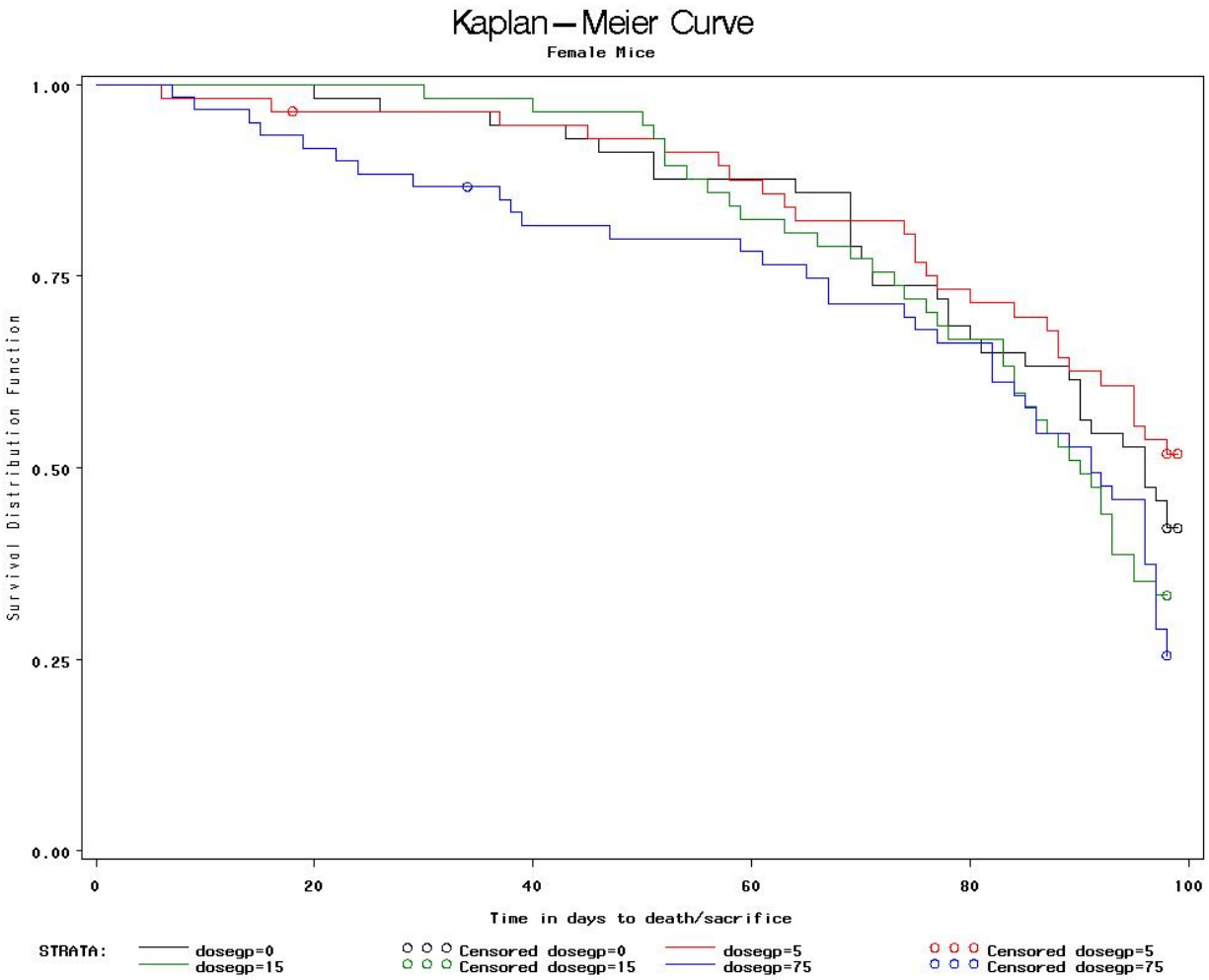
Female Rats (Vehicle control, low, medium and high dose groups)



X-Axis: Weeks, Y-Axis: Survival rates

Figure 2A (1): Kaplan-Meier Survival Functions for Female Mice

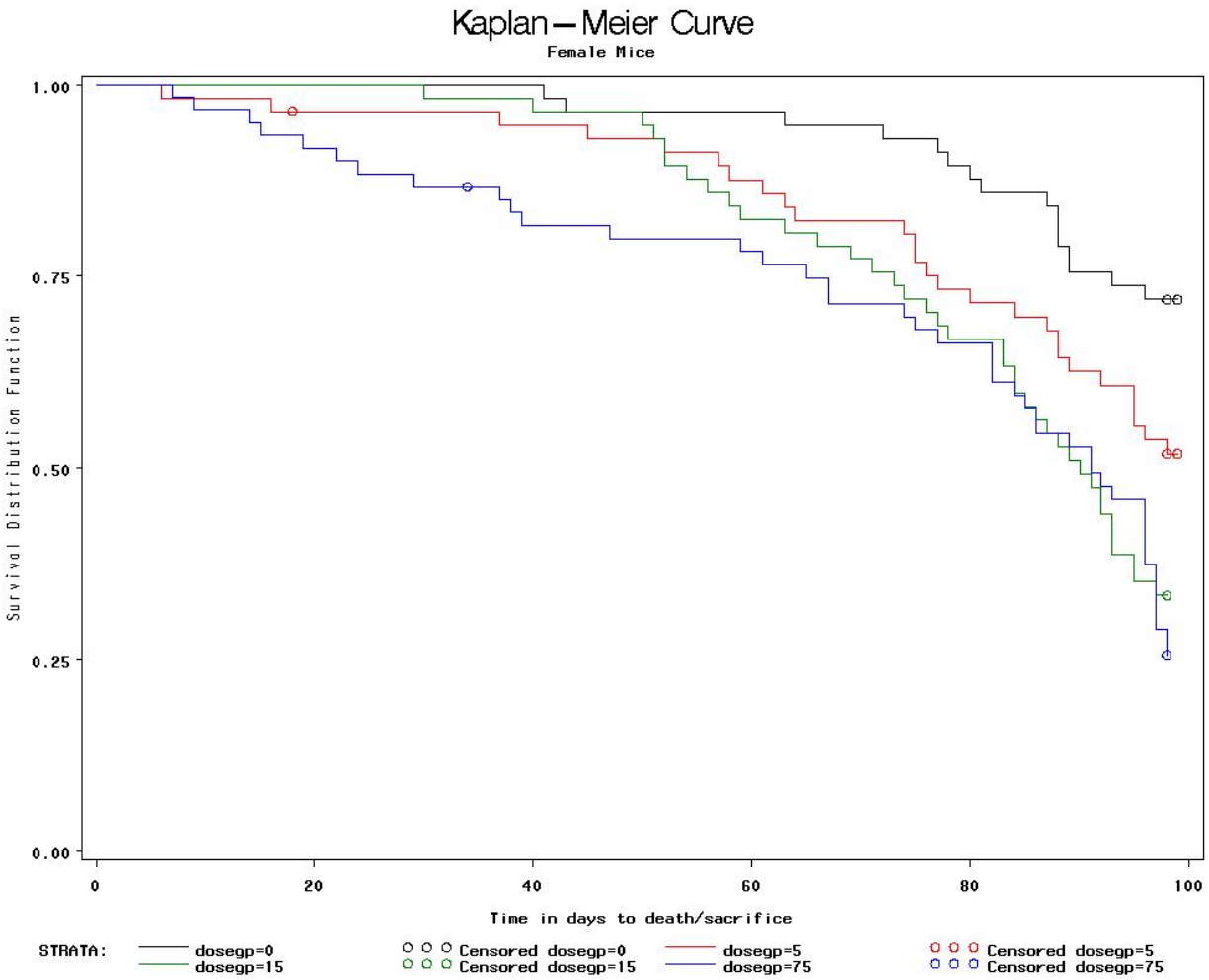
Female Mice (untreated control, low, medium and high dose groups)



X-Axis: Weeks, Y-Axis: Survival rates

Figure 2A (2): Kaplan-Meier Survival Functions for Female Mice

Female Mice (vehicle control, low, medium and high dose groups)



X-Axis: Weeks, Y-Axis: Survival rates

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Min Min
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Karl Lin
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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/Serial Number:	22-117 / N000
Drug Name:	Asenapine
Indication(s):	Bipolar I
Applicant:	Organon
Date(s):	Initial submission date: August 30, 2007
Review Priority:	Standard
Biometrics Division:	Division of Biometrics I
Statistical Reviewer:	George Kordzakhia, Ph.D.
Concurring Reviewers:	Peiling Yang, Ph.D.; H.M. James Hung, Ph.D.
Medical Division:	Division of Psychiatry Products
Clinical Team:	Robert Levin, M.D., Reviewer Gwen Zornberg, M.D., Team Leader
Project Manager:	Mr. Keith Kiedrow
Key Words	ANCOVA, MMRM

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1 EXECUTIVE SUMMARY

1.1 CONCLUSIONS AND RECOMMENDATIONS

At flexible doses of 5 to 10 mg BID (with 10 mg as the starting dose and the option to downtitrate to 5 mg), the asenapine group was statistically significantly superior to placebo in treatment of patients with manic or mixed episodes associated with Bipolar I as measured by the change from baseline in Y-MRS score on Day 21(primary endpoint, Intent-to-treat population) and CGI-BP severity of mania score on Day 21 (key secondary endpoint, Intent-to-treat population).

1.2 BRIEF OVERVIEW OF CLINICAL STUDIES

The development program was designed to investigate in parallel asenapine's efficacy in 2 different indications: treatment of schizophrenia and treatment of acute manic or mixed episodes associated with bipolar I disorder. Two pivotal studies (A75011004 and A7501005) were submitted in support of efficacy of asenapine compared with placebo in treatment of subjects with manic or mixed episodes associated with bipolar I disorder. This reviewer evaluated the bipolar I indication. For studies in support of schizophrenia indication, please refer to a separate statistical review by Dr. Yeh-Fong Chen.

Studies 1004 and 1005 were 3-week randomized, placebo and olanzapine controlled, double-blind, double-dummy, multicenter, international studies with identical design. A total of 611 patients at 61 centers entered Study1004, 488 patients were randomized and 342 patients completed the study. The most common reasons for discontinuing the study were withdrew consent and lack of efficacy. There were 654 enrolled patients at 55 centers in Study1005, 489 patients were randomized and 338 patients completed the study. The most common reasons for discontinuing the study were withdrew consent and lack of efficacy.

1.3 STATISTICAL ISSUES AND FINDINGS

In studies 1004 and 1005, YMRS and CGI-BP total scores were statistically significantly improved (ie, decreased) in the asenapine treatment group compared with the placebo treatment group. Based on the LOCF ANCOVA analysis, the p-values for asenapine vs. placebo with respect to YMRS total score were <0.001 in both studies. The p-values for asenapine vs. placebo with respect to CGI-BP total score were 0.0116 (study 1004) and 0.0017 (study 1005).

In study 1004, the observed asenapine treatment effect compared with placebo appears to be mainly driven by the non US patients subgroup (see Table 14). The observed treatment differences between asenapine and placebo for US and non US subgroups were respectively 0.13 (SE 1.63) and -8.73 (SE 2.32). For study 1005, the observed treatment effects appeared to be consistent across the US and non US subgroups.

One of the inclusion criteria required that to be eligible for the studies a patient had to have YMRS total score ≥20 at screening and at baseline. In both studies there were several patients included in the ITT population with baseline YMRS total score of 18 and 19. However, the primary efficacy results were not affected by the data from these patients.

INTRODUCTION

1.4 OVERVIEW

The development program was designed to investigate in parallel asenapine's efficacy in 2 different indications: treatment of schizophrenia and treatment of acute manic or mixed episodes associated with bipolar I disorder. Two pivotal studies (A75011004 and A7501005) were submitted in support of efficacy of asenapine compared with placebo in treatment of subjects with manic or mixed episodes associated with bipolar I Disorder. This reviewer evaluated the bipolar indication. For studies in support of schizophrenia indication, please refer to a separate statistical review by Dr. Yeh-Fong Chen.

1.5 DATA SOURCES

Data used for review are from the electronic submission received on August 30, 2007. The network path is \\Cdsesub1\evsprod\NDA022117\0000\ in the EDR.

2 STATISTICAL EVALUATION

2.1 EVALUATION OF EFFICACY

2.1.1 OBJECTIVE

The primary objective of trials 1004 and 1005 was to demonstrate the efficacy of asenapine compared with placebo in treatment of subjects with manic or mixed episodes associated with bipolar I disorder.

2.1.2 STUDY DESIGN

Studies 1004 and 1005 were 3-week randomized, placebo and olanzapine controlled, double-blind, double-dummy, parallel-group, multicenter, international studies to investigate efficacy of asenapine in treatment of adult patients with manic or mixed episodes associated with bipolar I disorder. Subjects were randomly assigned to receive asenapine, olanzapine, or placebo treatment in a ratio of 2:2:1.

To be eligible for the studies a patient had to have a primary diagnosis of bipolar I disorder, current episode manic (DSM-IV 296.4x), or mixed (DSM-IV 296.6x) as determined by a structured clinical interview (MINI) at screening; had a YMRS score ≥20 at screening and at baseline; had a current manic or mixed bipolar I episode that must have begun no more than 3 months prior to the screening visit; had a documented history of at least one previous moderate-to-severe mood episode with or without psychotic features (manic or mixed).

The trial included (up to) a 7-day single-blind placebo run in period during which subjects experiencing a manic or mixed episode received single-blind placebo (placebo olanzapine). After placebo run in, the active treatment period was initiated on Day 1 with placebo, asenapine 10 mg BID, or olanzapine 15 mg QD. Thereafter, treatment continued with flexible dosing (asenapine

5- 10 mg BID, olanzapine 5-20 mg QD, or placebo). Subjects remained confined to an inpatient research facility for at least the first 7 days of active treatment (through Day 7), and were subsequently discharged if deemed clinically stable by the investigator. Subjects completing the trial were eligible for enrollment in an extension trial, Protocol A7501006.

Table 1. Chart for Studies 1004 and 1005.

Screening/Placebo Run-In	Treatment Phase	Extension protocol A7501006
Up to 7 days	3 weeks	9 weeks
Placebo	Placebo, 5-10 mg asenapine, 5-20 mg olanzapine	5-10 mg asenapine, 5-20 mg olanzapine

Source: Corresponds to Figure 1 (pg 37), Clinical Study Report A7501004 and Figure 1(pg 35), Clinical Study Report A7501005.

Screening evaluations were conducted between 7 days prior to and the day immediately before the first double-blind dose. After performing all screening procedures, subjects began (up to) 7 days of single-blinded placebo run-in to allow for time to obtain clinical laboratory results and washout of excluded medications, including mood stabilizers. Eligibility determinations were made by the investigator using local or central laboratory results. In case of any unexpected, clinically relevant abnormal values, including the presence of mood stabilizers at levels higher than those outlined in the exclusion criteria, additional samples were to be obtained and analyzed prior to randomization. Lorazepam for the treatment of agitation was allowed at a maximum dose of 4 mg/day during the screening phase and for the first 7 days following the baseline assessment. The use of benzodiazepines after Day 7 was not permitted.

Remark: The Y-MRS, an 11-item, clinician-rated instrument used for assessing the symptoms of mania, was the primary efficacy variable. The Y-MRS was evaluated at screening and Days 1, 2, 4, 7, 14, and 21 (study endpoint).

2.1.3 PATIENT DISPOSITION, DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Study 1004

This trial was carried out from 30 November 2004 until 29 April 2006. The study was conducted at 61 centers, including 32 in the US, 2 in Bulgaria, 6 in India, 2 Korea, 3 Malaysia, 3 Philippines, 2 Romania, 4 Russia, and 7 in the Ukraine.

A total of 488 subjects were randomized to trial medication: 185 subjects to asenapine, 205 subjects to olanzapine, and 98 subjects to placebo (see Table 2). All randomized subjects received at least 1 dose of trial medication. A total of 342 subjects completed the trial. The proportion of patients who withdrew due to an adverse event/SAE related to the disease under study (bipolar disorder) appears to be higher in asenapine group (9.2%) compared with olanzapine group (3.4%) and placebo (4.1%).

Table 2. Study 1004 Summary of subject disposition and discontinuation

	Placebo	Asenapine	Olanzapine	All Subjects
Patients Randomized	98	185	205	488
Intent-to-treat Population	94	183	203	480
Withdrawn during double-blind, n (%)	41 (41.8%)	61 (33.0%)	44 (21.5%)	146 (29.9%)
Adverse Event/SAE	4 (4.1%)	17 (9.2%)	7 (3.4%)	28 (5.7%)
Lack of Efficacy	14 (14.3%)	14 (7.6%)	13 (6.3%)	41 (8.4%)
Withdrew consent	13 (13.3%)	25 (13.5%)	15 (7.3%)	53 (10.9%)
Lost to follow-up	4 (4.1%)	1 (0.5%)	6 (2.9%)	11 (2.3%)
Other	6 (6.1%)	4 (2.2%)	3 (1.5%)	13 (2.7%)
Completed double-blind	57 (58.2%)	124 (67.0%)	161 (78.5%)	342 (70.1%)

Source: Clinical Study Report A7501004, Table 5 (pg. 77)

All treatment groups appear comparable with respect to age, race, weight, and baseline YMRS total score. The proportion of male subjects was higher in the olanzapine group (57.1%) than in the asenapine (49.7%) or placebo (49.0%) groups (see Table 3). There were two patients randomized to asenapine group and included in the ITT population with baseline YMRS total score of 18.

Table 3. Study 1004 Summary of demographics and baseline characteristics (all randomized patients)

Characteristics	Placebo N=98	Asenapine N=185	Olanzapine N=205	All subjects N=488
Gender				
Male	48 (49.0%)	92 (49.7%)	117 (57.1%)	257 (52.7%)
Female	50 (51.0%)	93 (50.3%)	88 (42.9%)	231 (47.3%)
Race				
Caucasian	55 (56.1%)	104 (56.2%)	110 (53.7%)	269 (55.1%)
African	16 (16.3%)	38 (20.5%)	41 (20.0%)	95 (19.5%)
Asian	22 (22.4%)	40 (21.6%)	44 (21.5%)	106 (21.7%)
Other	5 (5.1%)	3 (1.6%)	10 (4.9%)	18 (3.7%)
Age Category				
18-64 years	95 (96.9%)	179 (96.8%)	204 (99.5%)	478 (98.0%)
>=65 years	3 (3.1%)	6 (3.2%)	1 (0.5%)	10 (2.0%)
Age, years				
Mean (SD)	38.1 (12.49%)	39.1 (12.26)	38.4 (10.82)	38.6 (11.71)
Median	38.0	40.0	39.0	39.0
Range	18, 69	18, 76	18, 66	18, 76
Weight, kg				
Mean (SD)	78.1 (19.82)	75.9 (19.20)	77.9 (19.99)	77.2 (19. 65)
Median	77.3	72.6	77.3	75.4
Range	41, 166	38, 144	38, 136	38, 166
YMRS (at baseline)				
Mean (SD)	28.2 (6.27)	29.4 (6.68)	29.7 (6.61)	29.3 (6.58)
Median	26.5	28.0	28.0	28.0
Range	20, 48	18, 54	20, 56	18, 56

Source: Clinical Study Report A7501004, Table 12 (pg 86).

Study 1005

This trial was carried out from 30 November 2004 until 29 April 2006. The study was conducted at 55 centers, 29 in the US, 2 in Bulgaria, 6 in India, 3 in Korea, 1 in Malaysia, 2 in the Philippines, 2 in Romania, 4 in the Russian Federation, 2 in Turkey, and 4 in Ukraine.

A total of 489 subjects were randomized to trial medication: 194 subjects to asenapine, 191 subjects to olanzapine, and 104 subjects to placebo (see Table 4). Of these, 488 subjects received at least 1 dose of trial medication. A total of 338 subjects completed the trial. In the asenapine and olanzapine treatment groups, the most common reason for withdrawal was withdrawal of consent. It appears that the proportion of patients who withdrew due to an adverse event/SAE is higher in the asenapine group: 10.3% asenapine-treated subjects, 4.2% olanzapine-treated subjects, and 6.7% placebo-treated subjects (see Table 4).

Table 4. Study 1005 Summary of subject disposition and discontinuation

	Placebo	**Asenapine**	**Olanzapine**	**All Subjects**
Patients Randomized	104	194	191	489
Intent-to-treat Population	103	189	188	480
Withdrawn during double-blind, n (%)	40 (38.55%)	72 (37.1%)	39 (20.4%)	151 (30.9%)
Adverse Event/SAE	7 (6.7%)	20 (10.3%)	8 (4.2%)	35 (7.2%)
Lack of Efficacy	17 (16.3%)	16 (8.2%)	11 (5.8%)	44 (9.0%)
Withdrew consent	13 (12.5%)	28 (14.4%)	16 (8.4%)	57 (11.7%)
Lost to follow-up	2 (1.9%)	5 (2.6%)	2 (1.0%)	9 (1.8%)
Other	1 (1.0%)	3 (1.5%)	2 (1.0%)	6 (1.2%)
Completed double-blind	64 (61.5%)	122 (62.9%)	152 (79.6%)	338 (69.1%)

Source: Clinical Study Report A7501005, Table 5 (pg. 74)

All treatment groups were comparable with respect to age, race, and weight. The proportion of male subjects was higher in the olanzapine (60.0%) and asenapine groups (58.8%) than in the placebo (50.0%) groups (see Table 5). There was one patient with YMRS baseline score of 3 randomized to asenapine group. The patient was not included in the ITT population. Two patients with YMRS total score of 18 (placebo) and one patient with baseline YMRS total score of 19 (olanzapine group) were included in the ITT population.

Table 5. Study 1005 Summary of Demographics and Baseline characteristics (all patients treated)

Characterisitcs	Placebo N=104	Asenapine N=194	Olanzapine N=190	All subjects N=488
Gender				
Male	52 (50%)	114 (58.8%)	114 (60%)	280 (57.4%)
Female	52 (50%)	80 (41.2%)	76 (40%)	208 (42.6%)
Race				
Caucasian	59 (56.7%)	122 (62.9%)	114 (60%)	295 (60.5%)
African	19 (18.3%)	31 (16.0%)	31 (16.3%)	81 (16.6%)
Asian	19 (18.3%)	35 (18.0%)	34 (17.9%)	88 (18.0%)
Other	7 (6.7%)	6 (3.1%)	11 (5.8%)	24 (4.9%)
Age				
18-64 years	103 (99.0%)	193 (99.5%)	186 (97.9%)	482 (98.8%)
>=65 years	1 (1.0%)	1 (0.5%)	4 (2.1%)	6 (1.2%)
Age, years				
Mean (SD)	39.4 (11.99)	38.7 (11.88)	40.1 (11.30)	39.4 (11.67)
Median	41.5	40.0	40.0	40.0
Range	18, 66	18, 68	19, 67	18, 68
Weight, kg				
Mean (SD)	78.2 (19.17)	77.7 (19.11)	79.7 (19.88)	78.6 (19.41)
Median	77.1	75.5	79.2	77.1
Range	43, 181	41, 146	33, 145	33, 181
YMRS at baseline				
Mean (SD)	29.0 (6.11)	28.1 (5.77)	28.5 (5.89)	28.5 (5.89)
Median	29.0	28.0	28.0	28.0
Range	18, 47	3, 46	19, 51	3, 51

Source: Clinical Study Report A7501005, Table 12 (pg 82).

2.1.4 STATISTICAL METHODOLOGIES

The primary efficacy endpoint, change from baseline to Day 21 on the Y-MRS total score, was analyzed by a fixed-effects analysis of covariance (ANCOVA) using the LOCF method. The primary model used the ITT population with the baseline score as a covariate and allowed for variability due to center and treatment. Small centers were pooled for analysis. The intent-to-treat (ITT) analysis set consisted of all subjects who were randomly assigned to treatment, received at least 1 dose of trial medication, and had at least 1 post-baseline YMRS score.

During the conduct of the trials, it was learned that a small number of subjects, particularly in certain geographic areas within the US with more than one study site in the trial A7501004 or trial A7501005, had enrolled into the trials at more than one study site. That is, these subjects were “repeat” patients and had violated the exclusion criterion 14 (previously participated in an asenapine trial). Prior to blind break, the statistical analysis plan was amended to include efficacy data from these subjects’ initial participation in the trial only in the ITT population. Safety data for these subjects were not excluded from analyses or summary tables.

The robustness of the results against potential bias caused by missing data was checked by a mixed-model analysis using repeated measures. Secondary analyses included analysis of change from baseline in Y-MRS at all assessed time points.

Pooling algorithm for centers: For non-US sites, all investigative sites within a country with fewer than 10 randomized subjects will be combined into a single pooled site for analysis purposes. If a resulting pooled site still has fewer than 10 randomized subjects, then this pooled site will be further combined with the smallest unpooled site within that country. If there is not another unpooled site within that country, then the pooled site will be combined with the smallest pooled site from another country. This pooling process will continue until there are at least 10 randomized subjects in each pooled site. For US sites, all investigative sites within a geographic region with fewer than 10 randomized subjects will be combined into a single pooled site for analysis purposes. If a resulting pooled site still has fewer than 10 randomized subjects, then this pooled site will be further combined with the smallest unpooled site within that region. If there is not another unpooled site within that region, then the pooled site will be combined with the smallest pooled site from another region within the US. This pooling process will continue until there are at least 10 randomized subjects in each pooled site.

2.1.5 RESULTS OF EFFICACY ANALYSES

Primary Analysis

Based on the LOCF ANCOVA analysis, Y-MRS total scores were statistically significantly improved (i.e. decreased) from baseline to Day 21 in the asenapine and olanzapine treatment groups compared with the placebo treatment group. The results are presented in Table 6. For Study 1004, the LS mean change from baseline to Day 21 was -11.5, -7.8, and -14.6 for the asenapine, placebo, and olanzapine treatment groups, respectively ($p=0.0065$ for asenapine vs. placebo and $p<0.0001$ for olanzapine vs. placebo). For Study 1005, the LS mean change from baseline to Day 21 was -10.8, -5.5, and -12.6 for the asenapine, placebo, and olanzapine treatment groups, respectively ($p<0.0001$ for both comparisons with placebo).

Table 6. YMRS Total Score LS mean Change from Baseline to Endpoint (ITT Population)

	Placebo	**Asenapine**	**Olanzapine**
Study 1004			
Number of Patients	94	183	203
Baseline Mean (SD)	28.3 (6.32)	29.4 (6.72)	29.7 (6.64)
Day 21 Mean (SD)	20.4 (12.70)	17.7 (11.91)	14.9 (10.47)
Mean Change from Baseline (SD)	-7.9 (11.46)	-11.7 (11.34)	-14.8 (10.37)
LS Mean Change from Baseline (SE)	-7.8 (1.11)	-11.5 (0.80)	-14.6 (0.76)
P-value vs. Placebo		0.0065	<0.0001
Study 1005			
Number of Patients	103	189	188
Baseline Mean (SD)	29.0 (6.14)	28.3 (5.53)	28.6 (5.88)
Day 21 Mean (SD)	23.5 (12.57)	17.7 (11.29)	16.1 (9.43)
Mean Change from Baseline (SD)	-5.5 (10.63)	-10.5 (11.13)	-12.5 (9.71)
LS Mean Change from Baseline (SE)	-5.5 (1.01)	-10.8 (0.75)	-12.6 (0.76)
P-value vs. Placebo		<0.0001	<0.0001

Source: Clinical Study Report A7501004, Table 18 (pg 96);
Clinical Study Report A7501005, Table 18 (pg 91)
Note: The reported p-values are nominal and are not adjusted for multiplicity.

Supportive analysis

As an exploratory analysis, the same ANCOVA model was applied to analyze change from baseline in Y-MRS at all assessed time points using LOCF method (see Table 7 and Table 8). The results supported the results on the primary endpoint.

Table 7. Study 1004: YMRS Total Score LS Mean Change from Baseline by Day

Visits	Placebo	Asenapine	Olanzapine
Day 2			
Number of Patients	93	175	200
LS mean Change from Baseline (SE)	-1.7 (0.54)	-3.2 (0.40)	-4.4 (0.37)
P-value vs. Placebo		0.0222	<0.0001
Day 4			
Number of Patients	94	183	203
LS mean Change from Baseline (SE)	-3.6 (0.65)	-5.5 (0.46)	-7.4 (0.44)
P-value vs. Placebo		0.0164	<0.0001
Day 7			
Number of Patients	94	183	203
LS mean Change from Baseline (SE)	-5.4 (0.80)	-7.6 (0.58)	-9.7 (0.55)
P-value vs. Placebo		0.0240	<0.0001
Day 14			
Number of Patients	94	183	203
LS mean Change from Baseline (SE)	-6.7 (1.02)	-10.4 (0.74)	-13.3 (0.70)
P-value vs. Placebo		0.0027	<0.0001
Day 21			
Number of Patients	94	183	203
LS mean Change from Baseline (SE)	-7.8 (1.11)	-11.5 (0.80)	-14.6 (0.76)
P-value vs. Placebo		0.0065	<0.0001

Source: Clinical Study Report A7501004, Table 19 (pg 98)
Note: The reported p-values are nominal and are not adjusted for multiplicity. P-values are based on the difference in the LS means for asenapine and olanzapine treatments versus placebo.

Table 8. Study 1005 YMRS Total Score LS Mean Change from Baseline by Day

Visits	Placebo	Asenapine	Olanzapine
Day 2			
Number of Patients	101	183	182
LS mean Change from Baseline (SE)	-1.5 (0.47)	-3.0 (0.35)	-3.4 (0.35)
P-value vs. Placebo		0.0077	0.0010
Day 4			
Number of Patients	103	189	188
LS mean Change from Baseline (SE)	-3.0 (0.56)	-5.5 (0.41)	-6.6 (0.42)
P-value vs. Placebo		0.0003	<0.0001
Day 7			
Number of Patients	103	189	188
LS mean Change from Baseline (SE)	-3.1 (0.72)	-6.9 (0.53)	-8.2 (0.54)
P-value vs. Placebo		<0.0001	<0.0001
Day 14			
Number of Patients	103	189	188
LS mean Change from Baseline (SE)	-5.1 (0.92)	-9.2 (0.68)	-10.1 (0.69)
P-value vs. Placebo		0.0003	<0.0001
Day 21			
Number of Patients	103	189	188
LS mean Change from Baseline (SE)	-5.5 (1.01)	-10.8 (0.75)	-12.6 (0.76)
P-value vs. Placebo		<0.0001	<0.0001

Source: Clinical Study Report A7501005, Table 19 (pg 92)
Note: The reported p-values are nominal and are not adjusted for multiplicity. P-values are based on the difference in the LS means for asenapine and olanzapine treatments versus placebo.

Sensitivity Analysis

This reviewer conducted sensitivity analysis on the primary efficacy measure. Change from baseline in YMRS Total score was analyzed by mixed effect repeated measures model. The model included therapy, pooled center, visit (day), and interaction of therapy by visit as fixed effects, and baseline as a covariate. The unstructured variance-covariance matrix was used. The results confirmed the results on the primary analysis.

Table 9. Mixed model for repeated measures analysis of change from baseline in YMRS total score

	Study A7501004			Study A7501005		
Visit	**Placebo**	**Asenapine**	**Olanzapine**	**Placebo**	**Asenapine**	**Olanzapine**
	N=94	N=183	N=203	N=103	N=189	N=188
Day 2						
LS Mean Change (SE)	-1.7 (0.55)	-3.2 (0.4)	-4.3 (0.37)	-1.5 (0.47)	-3.1 (0.35)	-3.5 (0.35)
p- value		0.0202	0.0001		0.0054	0.0007
Day 4						
LS Mean Change (SE)	-3.7 (0.66)	-5.8 (0.47)	-7.4 (0.45)	-3.2 (0.56)	-5.7 (0.41)	-6.8 (0.41)
p- value		0.0079	<0.0001		0.0002	<0.0001
Day 7						
LS Mean Change (SE)	-6.2 (0.93)	-8.6 (0.68)	-10.2 (0.62)	-3.8 (0.84)	-7.7 (0.61)	-8.8 (0.6)
p- value		0.0313	0.0003		0.0002	<0.0001
Day 14						
LS Mean Change (SE)	-8.2 (1.06)	-12 (0.76)	-14 (0.69)	-6.9 (0.97)	-10.9 (0.71)	-11.0 (0.69)
p- value		0.0255	0.0003		0.0009	0.0006
Day 21						
LS Mean Change (SE)	-10.8 (1.22)	-14.2 (0.85)	-16.1 (0.77)	-7.4 (1.14)	-13.1 (0.82)	-13.9 (0.78)
p- value		0.0255	0.0003		0.0001	<0.0001

Source: Module 2.7.3 Bipolar Summary of Clinical Efficacy, Table 13 (pg 40)
Note: The reported p-values are nominal and are not adjusted for multiplicity. P-values are based on the difference in the LS means for asenapine and olanzapine treatments versus placebo.

Key Secondary Endpoint

Change from baseline to Day 21 in CGI-BP severity of mania was analyzed by ANCOVA model with treatment and pooled investigative site as fixed effects and baseline as a covariate.
For both studies, improvements in CGI-BP severity of mania from baseline to Day 21 were statistically significantly greater in the asenapine group compared with the placebo group (p=0.0116 in Study 1004, p=0.0017 in Study 1005).

Table 10. CGI-BP Severity Total Score LS mean Change from Baseline to Endpoint (ITT Population)

	Placebo	Asenapine	Olanzapine
Study 1004			
Number of Patients	94	183	203
Baseline Mean (SD)	4.5 (0.79)	4.6 (0.79)	4.6 (0.77)
Day 21 Mean (SD)	3.6 (1.39)	3.3 (1.45)	3.0 (1.24)
Mean Change from Baseline (SD)	-0.8 (1.33)	-1.3 (1.43)	-1.5 (1.28)
LS Mean Change from Baseline (SE)	-0.8 (0.13)	-1.2 (0.10)	-1.5 (0.09)
P-value vs. Placebo		0.0116	<0.0001
Study 1005			
Number of Patients	103	189	188
Baseline Mean (SD)	4.7 (0.79)	4.7 (0.86)	4.6 (0.75)
Day 21 Mean (SD)	4.0 (1.54)	3.5 (1.41)	3.2 (1.16)
Mean Change from Baseline (SD)	-0.7 (1.34)	-1.2 (1.52)	-1.4 (1.20)
LS Mean Change from Baseline (SE)	-0.7 (0.13)	-1.2 (0.10)	-1.4 (0.10)
P-value vs. Placebo		0.0017	<.0001

Source: Clinical Study Report A7501004, Table 22 (pg 101);
Clinical Study Report A7501005, Table 22 (pg 95)
Note: The reported p-values are nominal and are not adjusted for multiplicity.

As an exploratory analysis, this reviewer also considered Cochran-Mantel-Haenszel tests to compare Asenapine versus Placebo. For both studies, improvements in CGI-BP severity of mania from baseline to Day 21 were statistically significantly greater in the asenapine group compared with the placebo group.

Table 11. Cochran-Mantel-Haenszel analysis of change from baseline in CGI-BP Severity Total Score

P-values from Cochran- Mantel-Haenszel Test			
Study 1004		**Study 1005**	
Asenapine vs Placebo	Olanzapine vs Placebo	Asenapine vs Placebo	Olanzapine vs Placebo
0.0117	<0.0001	0.0054	<0.0001

Source: Reviewers results
Note: The reported p-values are nominal p-values and are not adjusted for multiplicity.

2.1.6 REVIEWER'S COMMENTS.

In studies 1004 and 1005, YMRS and CGI-BP total scores were statistically significantly improved (ie, decreased) in the asenapine treatment group compared with the placebo treatment group. Based on the LOCF ANCOVA analysis, the p-values for asenapine vs. placebo with respect to YMRS total score were <0.001 in both studies. The p-values for asenapine vs. placebo with respect to CGI-BP total score were 0.0116 (study 1004) and 0.0017 (study 1005).

One of the inclusion criteria required that to be eligible for the studies a patient had to have YMRS total score ≥20 at screening and at baseline. In both studies there were several patients included in the ITT population with baseline YMRS total score of 18 and 19. However, the primary efficacy results were not affected by the data from these patients.

2.2 EVALUATION OF SAFETY

Not evaluated by this reviewer. Please refer to clinical review of this application for a detailed safety evaluation.

3 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

3.1 GENDER, RACE AND AGE

The reviewer conducted the exploratory analysis for gender and origin subgroups using LOCF ANCOVA model with treatment as a fixed effect and baseline as a covariate. Among all the subgroups, the treatment effect appeared to be numerically in favor of asenapine and olanzapine when compared with placebo. The subgroup analysis by age was not considered since there were too few patients over 65 years of age.

Table 12. Subgroup analysis by gender and race: YMRS total score LS mean change from baseline to endpoint (ITT population)

	Study A7501004			Study A7501005		
	Placebo	**Asenapine**	**Olanzapine**	**Placebo**	**Asenapine**	**Olanzapine**
Gender						
Male; N	47	92	116	51	111	114
Baseline, Mean (SD)	27.5 (5.11)	29.1 (7.03)	29 (6.24)	29.3 (6.74)	27.6 (5.36)	28.4 (5.57)
LS Mean Change from Baseline (SE)	-7.5 (1.53)	-11.1 (1.09)	-15.0 (0.97)	-3.7 (1.31)	-10.6 (0.89)	-12.8 (0.87)
Female; N	47	91	87	52	78	74
Baseline, Mean (SD)	29.1 (7.31)	29.7 (6.41)	30.7 (7.05)	28.8 (5.54)	29.2 (5.67)	28.9 (6.37)
LS Mean Change from Baseline (SE)	-9.2 (1.61)	-12.5 (1.15)	-14.5 (1.18)	-7.1 (1.58)	-10.7 (1.29)	-12.1 (1.33)
Race						
Caucasian; N	52	103	109	58	118	112
Baseline, Mean (SD)	27.0 (5.81)	27.9 (5.59)	28.8 (5.74)	28.0 (5.97)	27.4 (5.22)	28.5 (5.60)
LS Mean Change from Baseline (SE)	-9.7 (1.28)	-10.6 (0.91)	-13.8 (0.89)	-6.9 (1.28)	-9.8 (0.90)	-12.2 (0.92)
Black; N	15	37	40	19	30	31
Baseline, Mean (SD)	30.8 (6.81)	30.1 (6.32)	29.9 (6.64)	27.1 (4.68)	27.9 (3.80)	26.8 (4.58)
LS Mean Change from Baseline (SE)	-8.3 (2.58)	-9.8 (1.64)	-12.8 (1.58)	-6.0 (2.23)	-9.4 (1.78)	-10.1 (1.75)
Asian; N	22	40	44	19	35	34
Baseline, Mean (SD)	30.1 (6.90)	32.5 (8.59)	32.2 (7.94)	34.7 (5.60)	31.3 (7.01)	30.9 (7.73)
LS Mean Change from Baseline (SE)	-4.2 (2.99)	-16.6 (2.21)	-18.6 (2.10)	-0.4 (2.81)	-13.1 (2.04)	-16.3 (2.07)
Others; N	5	3	10	7	6	11
Baseline, Mean (SD)	26.2 (4.09)	30.3 (6.11)	28.0 (7.48)	27.4 (3.69)	29.2 (3.06)	27.0 (2.79)
LS Mean Change from Baseline (SE)	-12.3 (3.90)	-13.5 (5.05)	-16.5 (2.73)	-4.7 (3.49)	-19.6 (3.89)	-10.8 (2.82)

Source: Reviewer's Results
Note: The reported 95% CI's are nominal CI's and are not adjusted for multiplicity.

3.2 OTHER SPECIAL/SUBGROUP POPULATIONS

This reviewer conducted exploratory subgroup analysis of efficacy by principal psychiatric diagnosis and region/country (US, non US) using LOCF ANCOVA model with treatment as a fixed effect and baseline as a covariate. The treatment effect appeared to be numerically in favor of asenapine when compared with placebo except for US patients subgroup in study 1004. For this subgroup, the LS mean changes from baseline in YMRS total score were -10.4 (SE 0.93) in the asenapine arm and -10.5 (SE 1.34) in the placebo arm. For patients randomized to olanzapine the LS mean change was - 14.2 (SE 0.89).

Table 13. Subgroup analysis by psychiatric diagnosis: YMRS total LS mean change from baseline to endpoint

	Study A7501004			Study A7501005		
	Placebo	**Asenapine**	**Olanzapine**	**Placebo**	**Asenapine**	**Olanzapine**
Psychiatric Diagnosis						
Manic; N	63	129	139	68	136	130
Baseline, Mean (SD)	28.0 (5.72)	29.6 (6.83)	30.3 (6.20)	30.2 (6.44)	28.8 (5.66)	29.5 (6.08)
LS Mean Change from Baseline (SE)	-7.9 (1.39)	-12.2 (0.97)	-15.4 (0.93)	-4.9 (1.32)	-10.9 (0.93)	-13.0 (0.95)
Mixed; N	31	54	64	35	53	58
Baseline, Mean (SD)	28.9 (7.46)	28.8 (6.46)	28.4 (7.40)	26.9 (4.90)	26.8 (4.94)	26.6 (4.92)
LS Mean Change from Baseline (SE)	-9.0 (1.81)	-10.8 (1.37)	-13.5 (1.26)	-6.3 (1.47)	-9.9 (1.19)	-11.7 (1.14)

Source: Reviewer's Results
Note: The reported 95% CI's are nominal CI's and are not adjusted for multiplicity.

Table 14. Subgroup analysis by region/country: YMRS total LS mean change from baseline to endpoint

	Study A7501004			Study A7501005		
	Placebo	**Asenapine**	**Olanzapine**	**Placebo**	**Asenapine**	**Olanzapine**
Psychiatric Diagnosis						
US; N	54	112	121	65	118	122
Baseline, Mean (SD)	27.7 (5.94)	28.8 (6.19)	29.2 (6.22)	27.7 (5.32)	27.2 (4.55)	27.4 (4.82)
LS Mean Change from Baseline (SE)	-10.5 (1.34)	-10.4 (0.93)	-14.2 (0.89)	-6.1 (1.13)	-10.4 (0.84)	-11.6 (0.83)
Non US ; N	40	71	82	38	71	66
Baseline, Mean (SD)	29.1 (6.80)	30.3 (7.42)	30.5 (7.18)	31.4 (6.78)	30.06 (6.50)	30.7 (7.04)
LS Mean Change from Baseline (SE)	-5.3 (1.85)	-14.1 (1.39)	-15.6 (1.29)	-4.2 (1.94)	-11.0 (1.41)	-14.2 (1.47)

Source: Reviewer's Results
Note: The reported 95% CI's are nominal CI's and are not adjusted for multiplicity.

4 SUMMARY AND CONCLUSIONS

4.1 STATISTICAL ISSUES AND COLLECTIVE EVIDENCE

In studies 1004 and 1005, YMRS and CGI-BP total scores were statistically significantly improved (ie, decreased) in the asenapine treatment group compared with the placebo treatment group. Based on the LOCF ANCOVA analysis, the p-values for asenapine vs. placebo with respect to YMRS total score were <0.001 in both studies. The p-values for asenapine vs. placebo with respect to CGI-BP total score were 0.0116 (study 1004) and 0.0017 (study 1005).

In study 1004, the observed asenapine treatment effect compared with placebo appears to be mainly driven by the non US patients subgroup (see Table 14). The observed treatment differences between asenapine and placebo for US and non US subgroups were respectively 0.13 (SE 1.63) and -8.73 (SE 2.32). For study 1005, the observed treatment effects appeared to be consistent across the US and non US subgroups.

One of the inclusion criteria required that to be eligible for the studies a patient had to have YMRS total score ≥20 at screening and at baseline. In both studies there were several patients included in the ITT population with baseline YMRS total score of 18 and 19. However, the primary efficacy results were not affected by the data from these patients.

4.2 CONCLUSIONS AND RECOMMENDATIONS

At flexible doses of 5 to 10 mg BID (with 10 mg as the starting dose and the option to downtitrate to 5 mg), the asenapine group was statistically significantly superior to placebo in treatment of patients with manic or mixed episodes associated with Bipolar I as measured by the change from baseline in Y-MRS score on Day 21(primary endpoint, Intent-to-treat population) and CGI-BP severity of mania score on Day 21 (key secondary endpoint, Intent-to-treat population).

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
OFFICE OF TRANSLATIONAL SCIENCES
OFFICE OF BIOSTATISTICS

STATISTICAL REVIEW AND EVALUATION

Carcinogenicity Studies

NDA/Serial Number:	22- 117
Drug Name:	Sycrest (asenapine maleate, Org 5222) Tablets
Indication:	Schizophrenia and Acute Mania Associated with Bipolar Disorder
Applicant:	Organon USA, Inc.
Date:	August 30, 20007
Statistical Reviewer:	Roswitha Kelly, M.S./OTS/OB/DB6
Concurring Reviewers:	Karl Lin, Ph.D.,/OTS/OB/DB6
Medical Division:	Division of Psychiatry Products
Pharm/Tox Team:	Elzbieta Chalecka-Franaszek, Ph.D.
Project Manager:	Keith Kiedrow, Pharm. D.

Keywords: Sycrest, carcinogenicity, mortality, Kaplan-Meier estimates, exact permutation tests, validity

Distribution: NDA 22-117/Sycrest
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1. EXECUTIVE SUMMARY

1.1. Conclusions and Recommendations

There were major modifications to the standard whole-life study in the **Sprague-Dawley Rats**. The female study had to be terminated after 99 weeks of treatment due to high mortality among the vehicle controls, the high dose was increased in both sexes from 3.0 mg/kg/day to 5.0 mg/kg/day of Org 5222 (asenapine) and few tissues were examined for the terminally sacrificed low and mid dose animals. Therefore, pair-wise comparisons between either control and the high dose are the appropriate tests, though they are less powerful than the trend tests. For a few specified tissues all animals were microscopically examined and the trend tests could be employed for them. In general, findings between the reviewer and the sponsor were consistent even though the sponsor presented grouped tumor results per tissue, whereas the reviewer presented the standard individual tumors per tissue results. All treated females experienced better survival than the vehicle control group. Among the male rats, the survival experience of the vehicle control and the high dose was basically identical and the study lasted the full 105 weeks.

Taking intercurrent mortality into account, there were no statistically significant increased tumor findings among the female rats when the vehicle controls were used. When the untreated controls were employed, there were increases in adenocarcinoma in the mammary glands and in benign hair follicle tumors at the injection sites, neither one reaching the standard criteria for significance for common or rare tumors, respectively, much less any more stringent criteria had the added multiplicity been taken into account. Using the same methods of analysis, there were no statistically significant increases in tumors among the male rats, whether employing the vehicle controls or the untreated controls.

Evaluating the validity of the male and female rat studies, it appeared that treatment with Org 5222 did not negatively influence the survival of the high dose animals, but had some effect on average body weight, especially among the male rats. The final decision with respect to the validity of both the male and female rat studies is left to the expertise of the reviewing pharmacologist.

There were similar major modifications to the standard whole-life study in the **CRl:CD-1 ®(IRC)BR Mice**. Both the female and male studies were terminated early due to increased mortality among the treated animals. The original high dose levels had to be reduced and dosing of the mid and high dose groups was stopped before terminal sacrifice. Only the heart and gross lesions and tissue masses were microscopically examined in all animals. The remaining tissues were microscopically examined only for all control and high dose groups as well as for the animals dying on study. Therefore, the pair-wise comparisons between either control and high dose groups are the most appropriate tests for tumor incidences.

Taking intercurrent mortality into account there were statistically significant tumor findings among both genders. However, the significance depended on which control group was employed. Among the female mice, pair-wise comparisons using the vehicle controls reached statistical significance for pleomorphic malignant lymphomas in the hemolymphoreticular system. However, the untreated controls had the same incidence for this tumor as did the high dose group. Among the male mice, interstitial cell adenomas in the testes reached statistical significance using the untreated controls. With the vehicle controls the tumor would no longer be considered rare or statistically significant.

As these tumor findings were not robust, the reviewer evaluated the validity of the male and female mouse studies. From a statistical point of view there were sufficient numbers of animals alive at the time of the early sacrifices. However, as the dosing of the mid and high dose animals stopped even earlier, the expertise of the reviewing pharmacologist is needed to determine whether the length of exposure was adequate. Assuming that the length of exposure was adequate, the reviewer concluded that for both genders the MTD was reached based on reduced average body weights in the high dose animals. Among the female mice, the average body weights did not differentiate themselves from the vehicle controls until late in the first year, but the difference was maintained throughout the remainder of the study. For the male mice, the effect of the drug product on the average body weights of the high dose group seemed clear. The final decision with respect to the validity of both the male and female mouse studies is left to the expertise of the reviewing pharmacologist.

1.2. Brief Overview of Carcinogenicity Studies

The oncogenic potential of Org 5222 was investigated in a whole life study in Sprague-Dawley rats. The compound was subcutaneously administered at levels of 0.3, 1.2 and 3.0 mg/kg/day. After six weeks, the high dose was increased to 5mg/kg/day. In addition, there were also vehicle and untreated control groups. Due to high mortality among the female vehicle control animals, this study was terminated after 99 weeks of treatment. The male rats were treated for 105 weeks. For both genders all tissues were microscopically examined for the two control groups and the high dose groups and all animals dying of study. Terminally sacrificed low and mid dose animals did not have their tissues microscopically examined but for observed gross lesions and tissue masses as well as the thyroid glands, adrenal glands and mammary glands (females only).

The oncogenic potential of Org 5222 was also investigated in a whole life study in Crl:CD-1®(ICR)BR mice. The compound was subcutaneously administered at levels of 0.5, 1.5 and 7.5 (females) or 5.0 (males) mg/kg/day. During week 25 the high doses were decreased to 5.0 and 4.0 mg/kg/day for the females and males respectively. There were also vehicle and untreated control groups. Due to high mortality among the treated animals, the female mice were terminated after 97 weeks of treatment and the male study was terminated after 88 weeks. Dosing was terminated at weeks 95 and 97 for the mid and high dose females, respectively, and at week 88 for the mid and high dose males. For both genders all tissues were microscopically examined for the two control groups

and the high dose groups and all animals dying on study. Terminally sacrificed low and mid dose animals did not have their tissues microscopically examined but for observed gross lesions and tissue masses. The heart was also examined in all animals but only gross histopathology was performed.

1.3. Statistical Issues and Findings

In general one prefers to evaluate tumor findings by trend tests as they are more powerful than pair-wise comparisons. However, if not all tissues of all animals have been examined trend tests may give misleading results. In both the rat and mouse studies, all tissues of the two control groups and the high dose groups were microscopically examined and therefore the pair-wise comparisons between each control and the high dose groups are appropriate for any tumor/tissue combination. For the few tissues which had been specified by the sponsor to be microscopically examined for all low and mid dose animals, i.e. for all animals, the more powerful trend tests can be employed. However, for the terminally sacrificed low and mid dose groups, most tissues were not examined. The reviewer was able to perform approximate trend tests on tumors arising from tissues which were not microscopically examined in all animals, because the software used treated unexamined tissues the same as tissues with no tumor findings. In summary, the trend tests performed provided an approximate evaluation for the tumor/tissue combinations where not all animals were microscopically examined and an appropriate evaluation for the tissues which were examined in all animals. The pair-wise comparisons between either control group and the high dose group were appropriate for all tumor/tissue combinations, but are less powerful tests than the trend tests.

The sponsor presented primarily tumors grouped per tissue. The reviewer analyzed each tumor type per tissue.

The sponsor investigated positive and negative trends in tumor incidences. The reviewer performed only one-sided tests in tumor incidences increasing with dose.

Each study had a vehicle control and an untreated control. The reviewer did not considered these two control groups candidates for pooling and hence all the testing for mortality and tumors was doubled. The reviewer did not further adjust the levels of alpha to compensate for the additional multiplicity, in particular as the false positive rate in tumor findings was not a concern in these studies.

2. INTRODUCTION

2.1. Overview

This review evaluates the carcinogenicity data submitted for one 2-year study in Sprague-Dawley rats and one 2-year study in Crl:CD-1®(ICR)BR mice as well as the final reports written by the contract lab, (b) (4).

Trend tests employing the vehicle control group or the untreated control group and the treated animals were considered statistically significant at α=0.05 (two-sided) for mortality and α=0.025 and α=0.005 (one-sided) for increases in rare or common tumors, respectively. Tumors were considered rare if they occurred in 1% or less of the vehicle control animals and common otherwise. Pair-wise comparisons were statistically significant at α=0.05 (two-sided) for mortality and at α=0.05 and α=0.01 (one-sided) for rare and common tumors respectively.

All statistical analyses were performed by gender. The reviewer presents first the trend results using the vehicle controls and then the pair-wise comparisons between the vehicle controls and the high dose groups. The trend tests using the untreated controls are given in Appendices. These results increase the multiplicity of the tests, but no further adjustments were made to the α-levels. Also, as few tissues were microscopically examined in the low and mid dose terminally sacrificed animals, the trend results are an approximate evaluation for most tumors. The sponsor performed trend tests also with the combined controls, which the reviewer did not.

2.2. Data Sources

The sponsor submitted tumor-rat and tumor-mouse SAS transport files with their Aug. 30, 2007 submission which contained both the tumor and survival data for the rats and for the mice, as well as the final reports. The reviewer used the data as provided by the sponsor in the SAS transport files and the Office of Biostatistics (OB) web-carcin software which has been developed by Dr. Ted Guo and Ms. Feng Zhou of the Division of Biometrics 2. This software is used by most OB statisticians in the evaluation of carcinogenicity data. It provides for survival analyses and mortality-adjusted tumor analyses.

3. STATISTICAL EVALUATION

3.1. Rat Study

The purpose of this study was to determine the oncogenic potential of the test article, Org 5222, via subcutaneous administration to the rat for at least 104 weeks. Due to high mortality among the female vehicle control rats, this study was terminated in weeks 100–102. The male rats were terminated in weeks 106-107. Male and female Sprague Dawley rats were assigned randomly to 60 animals/sex/group. The five groups consisted of a vehicle control group, an untreated control group, and treated groups exposed to 0.3, 1.2, and 3.0 mg/kg/day at a volume-dose of 3 mL/kg. After six weeks of treatment, the high dose was increased to 5 mg/kg/day by increasing the volume-dose from 3 to 5 mL/kg.

Gross lesions and tissue masses and tissues marked in the list given in 'Appendix 3' (b) (4) Study Number 82/74 Amended Final Report, p. 240) were microscopically

examined for both control groups and the high dose animals and all decedents. In addition, thyroid glands and adrenal glands from all low and intermediate dose animals (i.e. from all animals) and mammary glands from all low and intermediate dose females were also microscopically examined. If a treatment related effect was observed in the high dose group, pertinent tissues from one or all lower dose groups were examined after consultation with the sponsor.

3.1.1. Sponsor's Results

The actual microscopic pathology was performed externally by N V Organon, but (b) (4) conducted the study and performed the statistical analyses of the mortality and tumor data. In (b) (4) Study Number 82/74/Amended Final Report, they reported that there was no evidence of increased mortality for either gender in various treated versus control comparisons, including a test for dose response against the vehicle control. Pair-wise comparisons between the female mid- and high-dose groups and the untreated control group against the vehicle control group showed that the vehicle control group experienced significantly higher mortality than these comparison groups. Among the males the mid-dose group experienced significantly lower mortality than the vehicle control group.

(b) (4) listed all benign and malignant tumors per tissue/sex. They noted that the numbers of benign and malignant tumors was significantly lower in both the medium and high dose groups compared to the combined control groups. Histopathology revealed no significant increase in one or more specific tumor types and no rare tumors were attributable to the compound. The incidences of pituitary tumors and mammary gland tumors were lower among the female medium and high dose groups compared to the vehicle controls. Based on survival adjusted methodologies, 'there was statistically no evidence that Org 5222 increased the incidence of any type of tumour significantly.'

The reviewer quotes from p. 20 of the report: 'The overall body weight gain of animals receiving Org 5222 was lower than that of the vehicle control (and the untreated control). The effect was dose-related, with males gaining 73, 63, or 48% and females gaining 91, 79, or 59% that of the vehicle controls, corresponding to dose levels of 0.3, 1.2 or 3.0/5.0 mg/kg/day, respectively'. On p. 22 of the report: 'The overall incidence of tumours was significantly reduced in both the 1.2 mg/kg/day and 5.0 mg/kg/day Org 5222-treated groups. A plausible explanation for this phenomenon is the lower body weight gain in the treated animals'.

3.1.2. Reviewer's Results

3.1.2.1. Female Rats

The reviewer came to the same conclusions as the sponsor using somewhat different approaches. In particular, the reviewer would not accept the analyses of all benign and all malignant tumors per tissues, as the groupings of tumors are selective. In addition, the

standard approach in the Office of Biostatistics is to only test for positive increase in tumor incidences with dose. The many statistically significant negative trends with dose reported by the sponsor were disregarded by this reviewer.

The reviewer observed similar numbers of animals surviving to terminal sacrifice as the sponsor had reported. Only the vehicle control group and the mid-dose group had one more terminally sacrificed animal each than the sponsor had reported. Table 1 gives the number of animals alive at the beginning and end of each time interval as well as the cumulative percent mortality for all groups, including the untreated control animals. The vehicle control group experienced the highest mortality which was the reason that the study was terminated after 99 weeks of treatment. Table 2 shows that the linear trend in mortality when using the vehicle control group (only) was not statistically significant at $\alpha=0.05$ but the significant tests for departure from linear trend and lack of homogeneity reflected the wide variability in mortality experiences of the groups and that the order was not dose-related. These findings are clearly shown by the Kaplan-Meier survival curves (Figure 1).

Most tissues of the terminally sacrificed low- and mid-dose animals were not microscopically examined. However, gross lesions and tissue masses, the thyroid glands, the adrenal glands, and the mammary glands were microscopically examined for all animals. The reviewer performed the trend tests for increasing tumor incidences with dose for all tumor/tissue combinations. For any tumor in the thyroid, the adrenal or mammary glands, these trend tests are most appropriate. For the remaining tissues, where not all low- and mid-dose groups were examined, the results are approximate. Even tumors identified in gross lesions and tissue masses may not be completely enumerated because some may have gone undetected if they were not associated with gross findings. Therefore the results of these approximate trend tests are used only as flags for further investigation. Table 3 gives the p-values for increasing linear trend in tumor incidences. None of the findings approached statistical significance for either rare or common tumors ($\alpha=0.025$ or $\alpha=0.005$, respectively). These findings are broadly consistent with those reported by (b) (4). The difference lies in their grouping of tumors whereas the reviewer analyzed each tumor/tissue combination separately. The sponsor also did not find a statistically significant increase in any tumor.

Table 1: Mortality of Female Rats

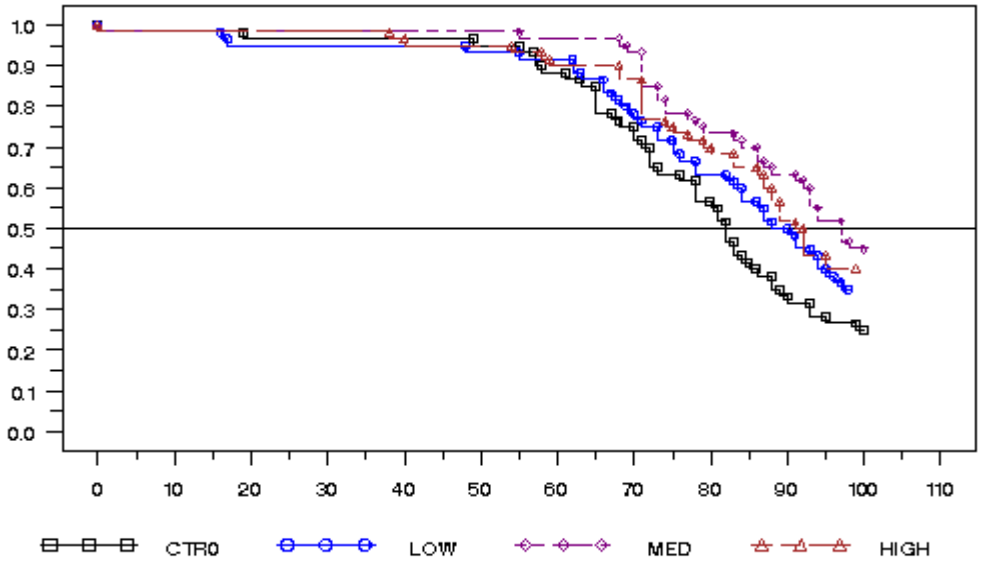
Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
Vehicle CTR	**0-52**	60	2	58	96.7	3.3
	53-78	58	21	37	61.7	38.3
	79-99	37	21	16	26.7	73.3
	FINALKILL100-102	16	16	0		
LOW	**0-52**	60	3	57	95.0	5.0
	53-78	57	17	40	66.7	33.3
	79-99	40	19	21	35.0	65.0
	FINALKILL100-102	21	21	0		
MED	**53-78**	60	14	46	76.7	23.3
	79-99	46	18	28	46.7	53.3
	FINALKILL100-102	28	28	0		
HIGH	**0-52**	60	2	58	96.7	3.3
	53-78	58	14	44	73.3	26.7
	79-99	44	20	24	40.0	60.0
	FINALKILL100-102	24	24	0		
Untreated CTR	**0-52**	60	1	59	98.3	1.7
	53-78	59	11	48	80.0	20.0
	79-91	48	13	35	58.3	41.7
	92-99	35	9	26	43.3	56.7
	FINALKILL100-102	26	26	0		

Table 2: Trend Test in Mortality among Female Rats*

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	7.5904	0.0225	9.0680	0.0107
Depart from Trend				
Dose-Mortality Trend	1.9282	0.1650	2.5624	0.1094
Homogeneity	9.5186	0.0231	11.6304	0.0088

- **Trend for vehicle, low, medium and high dose groups.**

Figure 1: Kaplan Meier Survival Curves for Female Rats*



*** control group is vehicle control only**

Table 3: Tumor Trends in Female Rats Using Vehicle Contol*

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
10000	brain	10028	astrocytoma, malignant (M)	1	0	0	1	0.4715	0.3796
10000	brain	10170	tumor, granular cell, benign (	0	1	0	1	0.3382	0.3148
2000	adrenal glands	2011	adenoma, cortical (B)	2	0	0	2	0.3216	0.2948
2000	adrenal glands	2036	carcinoma, cortical (M)	2	1	0	1	0.7055	0.7001
2000	adrenal glands	2132	pheochromocytoma, benign (B)	2	0	1	0	0.8699	0.8849
2000	adrenal glands	2135	pheochromocytoma, malignant (M	0	1	1	0	0.6144	0.7869
22000	eyes	22092	leiomyoma, iris (B)	0	1	0	0	0.7273	0.8142
27000	haematopoietic system	27104	lymphoma, malignant (S)	2	1	0	0	0.9931	0.9443
27000	haematopoietic system	27147	sarcoma, histiocytic (S)	1	1	1	0	0.8568	0.8782
31000	jejunum	31146	sarcoma, histiocytic (M)	0	1	0	0	0.7308	0.8172
33000	kidney	33021	adenoma, renal tubule (B)	0	0	1	0	0.5843	0.7085
37000	liver	37015	adenoma, hepatocellular (B)	1	2	1	1	0.6898	0.7157
37000	liver	37039	carcinoma, hepatocellular (M)	1	0	0	0	1.0000	0.8538
44000	lymph nodes	44081	haemangioma (B)	0	0	1	1	0.2120	0.2123
46000	mammary glands	46001	adenocarcinoma (M)	23	18	23	25	0.2139	0.2222
46000	mammary	46002	adenocarcinoma in	5	1	1	1	0.9134	0.9120

	glands		adenoma (M)						
46000	mammary glands	46006	adenoma (B)	3	2	4	3	0.5096	0.5494
46000	mammary glands	46072	fibroadenoma (B)	29	17	21	18	0.8767	0.8825
46000	mammary glands	46073	fibroma (B)	1	1	1	0	0.8268	0.8672
53000	oral cavity & related structur	53044	carcinoma, NOS (M)	0	1	0	0	0.7623	0.8216
53000	oral cavity & related structur	53049	carcinoma, squamous cell (M)	0	0	1	0	0.5893	0.7096
54000	ovaries	54189	tumor, sex cord stromal (B)	0	0	0	1	0.2121	0.0529
56000	pancreas	56018	adenoma, islet cell (B)	0	0	1	1	0.2421	0.2266
56000	pancreas	56042	carcinoma, islet cell (M)	1	1	0	1	0.5708	0.5489
59000	parathyroid glands	59006	adenoma (B)	1	0	0	1	0.4523	0.3451
63000	pituitary	63020	adenoma, pars intermedia (B)	0	0	0	1	0.2727	0.0896
63000	pituitary	63045	carcinoma, pars distalis (M)	0	1	1	0	0.6933	0.8142
77000	skin	77090	keratoacanthoma (B)	1	0	0	0	1.0000	0.8749
77000	skin	77173	tumor, hair follicle, benign (	1	0	0	0	1.0000	0.8376
78000	soft tissues	78073	fibroma (B)	0	0	1	0	0.4872	0.6773
78000	soft tissues	78075	fibrosarcoma (M)	0	1	0	0	0.7308	0.8172
78000	soft tissues	78097	lipoma (B)	1	0	2	0	0.6573	0.7890
78000	soft tissues	78151	schwannoma, benign (B)	0	1	0	0	0.7308	0.8172
86000	thymus	86165	thymoma, benign (B)	1	0	2	2	0.1717	0.2004
86000	thymus	86166	thymoma, malignant (M)	0	1	0	0	0.7636	0.8234
87000	thyroid glands	87010	adenoma, C-cell (B)	4	7	3	3	0.8561	0.8657
87000	thyroid glands	87014	adenoma, follicular cell (B)	0	0	1	1	0.2115	0.2135
87000	thyroid glands	87034	carcinoma, C-cell (M)	0	2	1	1	0.5208	0.5916
87000	thyroid glands	87038	carcinoma, follicular cell (M)	1	0	0	0	1.0000	0.8744
90000	injection site(s)	90073	fibroma (B)	0	1	0	1	0.3382	0.3148
90000	injection site(s)	90075	fibrosarcoma (M)	0	1	3	1	0.3785	0.4567
90000	injection site(s)	90088	histiocytoma, fibrous (M)	0	2	0	1	0.4343	0.4765
90000	injection site(s)	90097	lipoma (B)	0	0	0	1	0.2564	0.0784
90000	injection site(s)	90146	sarcoma, histiocytic (M)	2	1	0	0	0.9884	0.9367
90000	injection site(s)	90167	tumor, basal cell, benign (B)	1	0	0	0	1.0000	0.8376
90000	injection site(s)	90173	tumor, hair follicle, benign (	0	0	1	2	0.0859	0.0602
93000	urinary bladder	93130	papilloma, transitional cell (	0	0	1	0	0.4872	0.6773
94000	uterus	94005	adenocarcinoma, endometrial (M	1	0	0	0	1.0000	0.8749
94000	uterus	94013	adenoma, endometrial (B)	0	0	0	1	0.2564	0.0784
94000	uterus	94139	polyp, endometrial stromal (B)	3	1	4	1	0.7968	0.8322
94000	uterus	94140	polyp, glandular,	0	1	0	0	0.8202	0.8413

			benign (B)						
95000	vagina	95157	schwannoma, malignant (M)	0	0	1	0	0.5843	0.7085
95000	vagina	95170	tumor, granular cell, benign (	1	4	3	4	0.2424	0.2706

* Not all tissues of the terminally sacrificed low and medium dosed animals were microscopically examined. Hence these findings may represent an incomplete picture.

For the terminally sacrificed low and mid dose female rats, few tissues were microscopically examined, which makes the trend test results approximate in most cases. However, as all tissues of the vehicle-control and high-dose animals were microscopically examined, their pair-wise comparison is statistical correct and of special importance. One has to keep in mind that pair-wise comparisons are less powerful than trend tests on the same data, so any result close to statistical significance is worth considering. Table 4 and Figure 2 show that the mortality experience of the high-dose female rats was statistically significantly better than that of the vehicle controls. All tumor comparisons were mortality adjusted and one-sided towards an increase with dose. Table 5 shows that no increase approached statistical significance.

Table 4: Pair-wise Comparison in Mortality of Vehicle Control and High-Dose Female Rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Dose-Mortality Trend	3.9420	0.0471	5.2553	0.0219

Figure 2: Kaplan Meier Survival Curves for Female Rats (Vehicle Controls and HD)

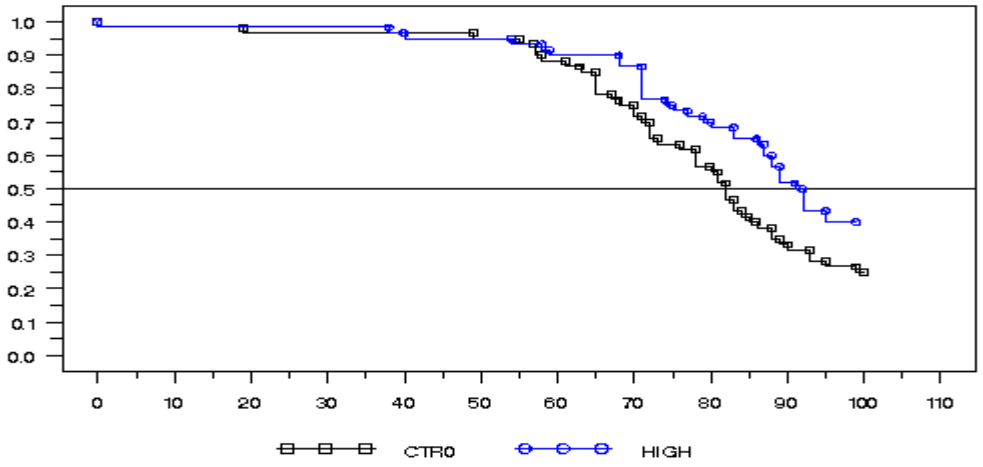


Table 5: Pair-wise Tumor Comparisons between Vehicle Control and HD Female Rats

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
10000	brain	10028	astrocytoma, malignant (M)	1	1	0.8519	0.6841
10000	brain	10170	tumor, granular cell, benign (	0	1	0.6000	0.2701
2000	adrenal glands	2011	adenoma, cortical (B)	2	2	0.8330	0.7023
2000	adrenal glands	2036	carcinoma, cortical (M)	2	1	0.9403	0.8613
2000	adrenal glands	2132	pheochromocytoma, benign (B)	2	0	1.0000	0.9209
27000	haematopoietic system	27104	lymphoma, malignant (S)	2	0	1.0000	0.9617
27000	haematopoietic system	27147	sarcoma, histiocytic (S)	1	0	1.0000	0.8833
37000	liver	37015	adenoma, hepatocellular (B)	1	1	0.8462	0.6696
37000	liver	37039	carcinoma, hepatocellular (M)	1	0	1.0000	0.8802
44000	lymph nodes	44081	haemangioma (B)	0	1	0.4878	0.2048
46000	mammary glands	46001	adenocarcinoma (M)	23	25	0.3595	0.3045
46000	mammary glands	46002	adenocarcinoma in adenoma (M)	5	1	0.9929	0.9773
46000	mammary glands	46006	adenoma (B)	3	3	0.8067	0.6951
46000	mammary glands	46072	f broadenoma (B)	29	18	0.9789	0.9687
46000	mammary glands	46073	f broma (B)	1	0	1.0000	0.8463
54000	ovaries	54189	tumor, sex cord stromal (B)	0	1	0.4000	0.1537
56000	pancreas	56018	adenoma, islet cell (B)	0	1	0.6000	0.2701
56000	pancreas	56042	carcinoma, islet cell (M)	1	1	0.8462	0.6696
59000	parathyroid glands	59006	adenoma (B)	1	1	0.7846	0.5914
63000	pituitary	63020	adenoma, pars intermedia (B)	0	1	0.6000	0.2701
77000	skin	77090	keratoacanthoma (B)	1	0	1.0000	0.9235
77000	skin	77173	tumor, hair follicle, benign (	1	0	1.0000	0.8463
78000	soft tissues	78097	lipoma (B)	1	0	1.0000	0.8802
86000	thymus	86165	thymoma, benign (B)	1	2	0.5513	0.3625
87000	thyroid glands	87010	adenoma, C-cell (B)	4	3	0.8178	0.7210
87000	thyroid glands	87014	adenoma, follicular cell (B)	0	1	0.4878	0.2048
87000	thyroid glands	87034	carcinoma, C-cell (M)	0	1	0.6000	0.2701
87000	thyroid glands	87038	carcinoma, follicular cell (M)	1	0	1.0000	0.9235
90000	injection site(s)	90073	f broma (B)	0	1	0.6000	0.2701
90000	injection site(s)	90075	f brosarcoma (M)	0	1	0.4878	0.2048
90000	injection site(s)	90088	histiocytoma, f brous (M)	0	1	0.4878	0.2048
90000	injection site(s)	90097	lipoma (B)	0	1	0.4878	0.2048
90000	injection site(s)	90146	sarcoma, histiocytic (M)	2	0	1.0000	0.9527
90000	injection site(s)	90167	tumor, basal cell, benign (B)	1	0	1.0000	0.8463
90000	injection site(s)	90173	tumor, hair follicle, benign (	0	2	0.3538	0.1530
94000	uterus	94005	adenocarcinoma, endometrial (M	1	0	1.0000	0.9235

94000	uterus	94013	adenoma, endometrial (B)	0	1	0.4878	0.2048
94000	uterus	94139	polyp, endometrial stromal (B)	3	1	0.9710	0.9246
95000	vagina	95170	tumor, granular cell, benign (	1	4	0.2560	0.1493

3.1.2.2. Male Rats

The reviewer observed basically the same number of animals living until terminal sacrifice as the sponsor had reported. Only the low dose group had one more animal under terminal sacrifice (TS). The vehicle controls and high dose animals had very similar mortality experiences which led to a non-significant test for trend. The fact that the medium dose group had substantially better survival than the other groups was reflected by the statistically significant departure from trend and heterogeneity (Table 7, Figure 3).

Table 6: Mortality of Male Rats

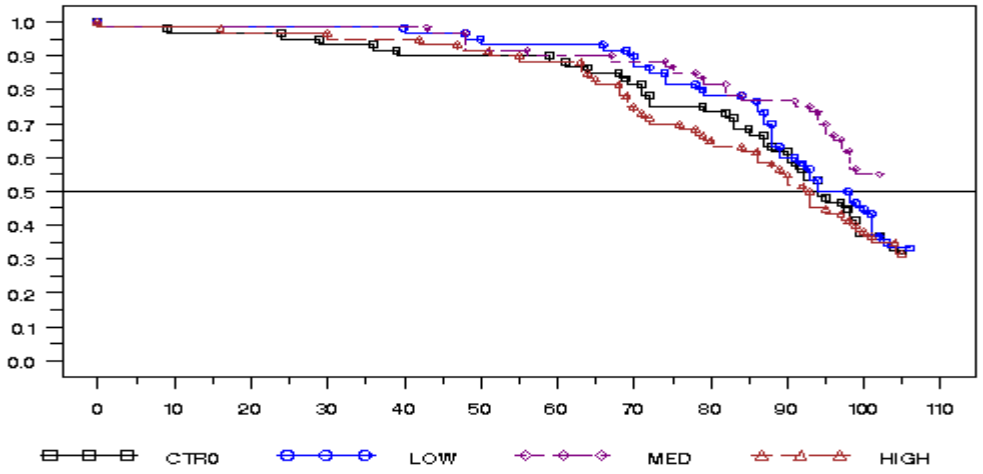
Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
VEHICLE CTR	0-52	60	5	55	91.7	8.3
	53-78	55	8	47	78.3	21.7
	79-91	47	12	35	58.3	41.7
	92-105	35	16	19	31.7	68.3
	FINALKILL106-107	19	19	0		
LOW	0-52	60	3	57	95.0	5.0
	53-78	57	8	49	81.7	18.3
	79-91	49	13	36	60.0	40.0
	92-105	36	15	21	35.0	65.0
	FINALKILL106-107	21	21	0		
MED	0-52	60	2	58	96.7	3.3
	53-78	58	7	51	85.0	15.0
	79-91	51	5	46	76.7	23.3
	92-105	46	13	33	55.0	45.0
	FINALKILL106-107	33	33	0		
HIGH	0-52	60	5	55	91.7	8.3
	53-78	55	14	41	68.3	31.7
	79-91	41	8	33	55.0	45.0
	92-105	33	14	19	31.7	68.3
	FINALKILL106-107	19	19	0		
Untreated CTR	0-52	60	3	57	95.0	5.0
	53-78	57	9	48	80.0	20.0
	79-91	48	7	41	68.3	31.7
	92-105	41	17	24	40.0	60.0
	FINALKILL106-107	24	24	0		

Table 7: Trend Test in Mortality among Male Rats*

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	8.0888	0.0175	7.2470	0.0267
Depart from Trend				
Dose-Mortality Trend	0.9630	0.3264	1.5949	0.2066
Homogeneity	9.0518	0.0286	8.8419	0.0315

* Using vehicle control

Figure 3: Kaplan Meier Survival Curves for Male Rats with Vehicle Controls



As for the female rats, most tissues of the terminally sacrificed low- and mid-dose animals were not microscopically examined. However, gross lesions and tissue masses, the thyroid glands and the adrenal glands were microscopically examined for all groups. For any tumor in the thyroid and the adrenal glands, the trend tests are most appropriate. For the remaining tissues, where not all low- and mid-dose groups were examined, the results are approximate. Even tumors identified in gross lesions and tissue masses may not be completely enumerated. Some may have gone undetected if they were not associated with gross findings. Therefore the results of these approximate trend tests are used only as flags for further investigation. Table 8 shows that none of the findings approached statistical significance for either rare or common tumors at the same levels of significance as mentioned above. These findings are broadly consistent with those reported by [redacted] (b) (4), meaning that the sponsor also did not find a statistically significant increase in any tumor. It is noted that the sponsor grouped all tumors per tissue site whereas the reviewer analyzed each tumor/tissue combination separately.

Table 8: Tumor Trends in Male Rats Using Vehicle Controls*

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
10000	brain	10079	glioma, mixed, malignant (M)	0	0	0	1	0.2510	0.0757
10000	brain	10123	oligodendroglioma, malignant (	1	0	0	0	1.0000	0.8562
10000	brain	10170	tumor, granular cell, benign (	1	0	0	0	1.0000	0.8580
2000	adrenal glands	2011	adenoma, cortical (B)	0	1	1	0	0.6224	0.7795
2000	adrenal glands	2132	pheochromocytoma, benign (B)	7	9	7	3	0.9551	0.9540
2000	adrenal glands	2135	pheochromocytoma, malignant (M	2	0	2	1	0.5153	0.5757
27000	haematopoietic system	27095	leukemia, granulocytic (S)	2	0	0	1	0.5326	0.5166
27000	haematopoietic system	27104	lymphoma, malignant (S)	5	2	0	2	0.6904	0.7475
27000	haematopoietic system	27147	sarcoma, histiocytic (S)	1	1	0	0	0.9451	0.8870
33000	kidney	33021	adenoma, renal tubule (B)	1	0	0	0	1.0000	0.8663
38000	lung & bronchi	38009	adenoma, bronchiolo-alveolar (	1	0	0	0	1.0000	0.8663
44000	lymph nodes	44081	haemangioma (B)	0	2	0	1	0.4369	0.4915
46000	mammary glands	46072	fibroadenoma (B)	0	1	0	0	0.7241	0.8107
53000	oral cavity & related structur	53129	papilloma, squamous cell (B)	1	0	0	0	1.0000	0.8487
56000	pancreas	56018	adenoma, islet cell (B)	2	3	1	1	0.7725	0.7906
56000	pancreas	56042	carcinoma, islet cell (M)	0	0	1	1	0.1906	0.1530
59000	parathyroid glands	59006	adenoma (B)	5	2	1	3	0.4529	0.4950
6000	auditory sebaceous glands	6050	carcinoma, squamo-sebaceous (M	1	1	0	0	0.9404	0.9059
63000	pituitary	63019	adenoma, pars distalis, M (B)	36	48	46	38	0.8785	0.8836
63000	pituitary	63020	adenoma, pars intermedia (B)	0	0	0	1	0.3784	0.1497
69000	prostate gland	69157	schwannoma, malignant (M)	1	0	0	0	1.0000	0.8575
77000	skin	77049	carcinoma, squamous cell (M)	0	1	0	0	0.7241	0.8107
77000	skin	77090	keratoacanthoma (B)	0	1	0	1	0.3840	0.3536
77000	skin	77097	lipoma (B)	1	0	0	0	1.0000	0.8663
77000	skin	77129	papilloma, squamous cell (B)	1	0	0	0	1.0000	0.8854
77000	skin	77167	tumor, basal cell, benign (B)	0	0	1	0	0.4655	0.6658
78000	soft tissues	78073	fibroma (B)	0	1	3	1	0.3379	0.4305
78000	soft tissues	78075	fibrosarcoma (M)	0	2	0	0	0.7368	0.8402
78000	soft tissues	78088	histiocytoma, fibrous (M)	0	1	0	2	0.1329	0.1036
78000	soft tissues	78097	lipoma (B)	0	0	2	1	0.1870	0.2312
78000	soft tissues	78126	osteosarcoma (M)	1	1	0	0	0.9537	0.8917
78000	soft tissues	78146	sarcoma, histiocytic	1	0	0	0	1.0000	0.8854

			(M)						
78000	soft tissues	78157	schwannoma, malignant (M)	0	0	1	0	0.5200	0.6650
85000	testis	85017	adenoma, Leydig cell (B)	2	0	0	0	1.0000	0.9074
85000	testis	85159	seminoma, malignant (M)	1	0	0	0	1.0000	0.8487
87000	thyroid glands	87010	adenoma, C-cell (B)	6	1	8	3	0.6527	0.6870
87000	thyroid glands	87014	adenoma, follicular cell (B)	2	3	1	0	0.9597	0.9455
87000	thyroid glands	87034	carcinoma, C-cell (M)	1	3	0	1	0.6609	0.6909
87000	thyroid glands	87038	carcinoma, follicular cell (M)	1	0	1	0	0.8137	0.8205
87000	thyroid glands	87077	ganglioneuroma (B)	1	0	0	0	1.0000	0.8487
90000	injection site(s)	90073	fibroma (B)	4	2	2	0	0.9868	0.9741
90000	injection site(s)	90075	fibrosarcoma (M)	6	6	5	4	0.6459	0.6746
90000	injection site(s)	90088	histiocytoma, fibrous (M)	7	5	7	2	0.9734	0.9713
90000	injection site(s)	90090	keratoacanthoma (B)	1	1	0	0	0.9424	0.8873
90000	injection site(s)	90097	lipoma (B)	0	2	0	0	0.8117	0.8745
90000	injection site(s)	90146	sarcoma, histiocytic (M)	2	2	0	0	0.9833	0.9528
90000	injection site(s)	90157	schwannoma, malignant (M)	1	1	0	0	0.9129	0.8650
90000	injection site(s)	90173	tumor, hair follicle, benign (	0	0	0	1	0.2088	0.0537

* Not all tissues of the terminally sacrificed low and medium dosed animals were microscopically examined. Hence these findings may represent an incomplete picture.

As all tissues from all control and high dose animals were microscopically examined, the pair-wise comparisons between vehicle-control and high-dose rats are of special importance. One has to keep in mind that pair-wise comparisons are less powerful than trend tests on the same data. Hence, any result close to statistical significance is worth considering. Table 9 and Figure 4 show that the mortality experience of the high-dose male rats was basically identical to that of the vehicle controls. All tumor comparisons are mortality adjusted and one-sided towards an increase with dose. Table 10 shows that no increase in any tumor finding approaches statistical significance.

Table 9: Mortality Comparison between Vehicle Control and High Dose Male Rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Dose-Mortality Trend	0.0227	0.8801	0.2040	0.6515

Figure 4: Kaplan Meier Survival Curves for Vehicle Control and High Dose Male Rats

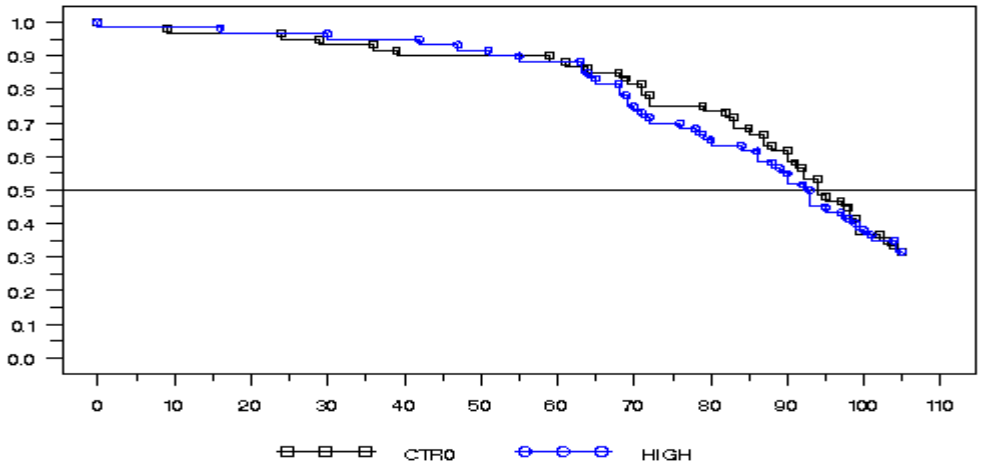


Table 10: Tumor Comparisons between Vehicle Control and High Dose Male Rats

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
10000	brain	10079	glioma, mixed, malignant (M)	0	1	0.5042	0.2143
10000	brain	10123	oligodendroglioma, malignant (	1	0	1.0000	0.8849
10000	brain	10170	tumor, granular cell, benign (	1	0	1.0000	0.8725
2000	adrenal glands	2132	pheochromocytoma, benign (B)	7	3	0.9672	0.9373
2000	adrenal glands	2135	pheochromocytoma, malignant (M	2	1	0.8621	0.7337
27000	haematopoietic system	27095	leukemia, granulocytic (S)	2	1	0.8677	0.7452
27000	haematopoietic system	27104	lymphoma, malignant (S)	5	2	0.9297	0.8748
27000	haematopoietic system	27147	sarcoma, histiocytic (S)	1	0	1.0000	0.8741
33000	kidney	33021	adenoma, renal tubule (B)	1	0	1.0000	0.8849
38000	lung & bronchi	38009	adenoma, bronchiolo-alveolar (	1	0	1.0000	0.8849
44000	lymph nodes	44081	haemangioma (B)	0	1	0.4667	0.1925
53000	oral cavity & related structur	53129	papilloma, squamous cell (B)	1	0	1.0000	0.8720
56000	pancreas	56018	adenoma, islet cell (B)	2	1	0.8851	0.7619
56000	pancreas	56042	carcinoma, islet cell (M)	0	1	0.5000	0.2119
59000	parathyroid glands	59006	adenoma (B)	5	3	0.7852	0.6908
6000	auditory sebaceous	6050	carcinoma, squamo-	1	0	1.0000	0.8720

	glands		sebaceous (M				
63000	pituitary	63019	adenoma, pars distalis, M (B)	36	38	0.6888	0.6306
63000	pituitary	63020	adenoma, pars intermedia (B)	0	1	0.6522	0.3014
69000	prostate gland	69157	schwannoma, malignant (M)	1	0	1.0000	0.8634
77000	skin	77090	keratoacanthoma (B)	0	1	0.6522	0.3014
77000	skin	77097	lipoma (B)	1	0	1.0000	0.8849
77000	skin	77129	papilloma, squamous cell (B)	1	0	1.0000	0.9429
78000	soft tissues	78073	fibroma (B)	0	1	0.5000	0.2119
78000	soft tissues	78088	histiocytoma, fibrous (M)	0	2	0.2092	0.0816
78000	soft tissues	78097	lipoma (B)	0	1	0.5000	0.2119
78000	soft tissues	78126	osteosarcoma (M)	1	0	1.0000	0.8849
78000	soft tissues	78146	sarcoma, histiocytic (M)	1	0	1.0000	0.9429
85000	testis	85017	adenoma, Leydig cell (B)	2	0	1.0000	0.9345
85000	testis	85159	seminoma, malignant (M)	1	0	1.0000	0.8720
87000	thyroid glands	87010	adenoma, C-cell (B)	6	3	0.9253	0.8731
87000	thyroid glands	87014	adenoma, follicular cell (B)	2	0	1.0000	0.9345
87000	thyroid glands	87034	carcinoma, C-cell (M)	1	1	0.7000	0.5000
87000	thyroid glands	87038	carcinoma, follicular cell (M)	1	0	1.0000	0.8849
87000	thyroid glands	87077	ganglioneuroma (B)	1	0	1.0000	0.8720
90000	injection site(s)	90073	fibroma (B)	4	0	1.0000	0.9826
90000	injection site(s)	90075	fibrosarcoma (M)	6	4	0.7420	0.6492
90000	injection site(s)	90088	histiocytoma, fibrous (M)	7	2	0.9943	0.9842
90000	injection site(s)	90090	keratoacanthoma (B)	1	0	1.0000	0.8849
90000	injection site(s)	90146	sarcoma, histiocytic (M)	2	0	1.0000	0.9651
90000	injection site(s)	90157	schwannoma, malignant (M)	1	0	1.0000	0.8720
90000	injection site(s)	90173	tumor, hair follicle, benign (	0	1	0.5000	0.2119

The reviewer considered the findings using the untreated control as secondary. Her results are given in Appendix I and she confirmed the findings the sponsor had reported. In particular, among the female rats the trends in malignant adenocarcinoma in the mammary glands and in benign hair follicle tumors at the injection sites approached statistical significance, but did not reach it at the standard levels, much less at any level of significance adjusted for the further multiplicity. Among the male rats, only statistically significant departure from linear trend and heterogeneity of survival experiences became apparent. No tumor finding approached statistical significance.

The reviewer did not perform the pair-wise comparisons of the two control groups and the tumor trend tests with the combined controls but relied on the results given by the sponsor. For injection site tumors and for tumors where there was a significant difference between the two control groups, the sponsor used the individually appropriate control group. The reviewer generally agreed with the sponsor findings, except that the sponsor reported one more benign hair follicle tumor among the high dose females than was in the data base. Their p-value for trend was 0.0092 which reached statistical significance

for a rare tumor but was labeled 'a weak positive dose-related trend'. From the sponsor's data base, the reviewer observed tumor incidences of 0, 0, 1, 2 (not 3) for vehicle control, low, mid, and high dose groups with a p-value for the exact permutation trend test of 0.0622, which is not close to statistical significance. Using the combined controls, the sponsor observed a positive trend test in adenocarcinoma of the mammary glands (p=0.0368) which the sponsor did not consider a true effect in light of the other findings in the mammary glands. The reviewer observed a p-value of 0.0133 using the untreated controls only, which is considered non-significant for a common tumor. When using the vehicle controls, this tumor was similarly distributed among the groups (n_i = 23, 18, 23, 25 for vehicle control, low, medium and high dose animals, respectively) which resulted in a non-significant p-value. The sponsor did not specifically mention the tumor trend tests for the male rats with the combined controls but stated that there was no evidence that the compound increased the incidence of any type of tumor, since no one-tailed positive trend test was significant.

3.1.2.3. Validity of Male and Female Rat Study

There were no statistically significant tumor findings among either the female or male rats of (b) (4) Study 82/74. Therefore, the validity of the study needs to be evaluated for each gender. Two criteria are considered for this purpose:

i) Were sufficient numbers of animals exposed long enough to allow for late-developing tumors?
ii) Did the high dose provide a sufficient tumor challenge?

The number of animals and length of exposure can be assessed at weeks 52, 80-90, and at termination, but are generally considered adequate if 20-30 animals survive through weeks 80-90. Though the study was terminated early for the female rats, this criterion was met. The vehicle control group experienced the highest mortality and still had 16 animals alive at the beginning of week 100. All other groups had more than 20 animals alive at week 100. For the males this criterion was easily met, as all groups had at least 19 animals surviving to terminal sacrifice at week 106. Hence there were sufficient numbers of animals exposed long enough. In determining whether the high dose provided an adequate tumor challenge, one expects the high dose to be close to the MTD. The following criteria are employed in this assessment:

iii) A dose is considered adequate if there is a detectable reduction in average body weight of up to 10% in a dosed group relative to the controls, or
iv) A dose is considered adequate if the dosed animals show a slightly increased mortality compared to the controls, or
v) A dose is considered an MTD if the dosed animals exhibit severe toxic effects attributed to the chemical. This latter evaluation is performed by the pharmacologist/toxicologist.

The high dose females had body weight averages similar to the vehicle controls' until about week 40. By week 52 they had about 10 percent lower average body weights than the vehicle controls and increased this difference to about 24 percent by week 96, shortly before their terminal sacrifice. Therefore, the compound may have influenced body weight increases and tumor production but not mortality, as the study was terminated early because of high mortality among the vehicle control animals, not because of any of the treated groups.

The high dose male rats experienced 10 percent lower average bodyweights than the vehicle controls by week 9. The difference increased to a maximum of 30 percent (lower) by week 96. Therefore, the compound affected average body weights and probably tumor production, but not mortality, as the high dose male rats had basically an identical mortality experience as their vehicle control group.

In summary, the adequacy of the high dose is in question for both genders. Though the females reached a 10 percent difference in average body weights, it did not occur until the very end of the first year and then continued to increase well beyond the 10 percent criterion. For the males, it is clear that the effect of the compound on body weight averages was early and substantial, which may have in turn influenced tumor formation. The final decision whether the study in either gender can be considered valid in the presence of no statistically significant increases in tumors, is left to the expertise of the reviewing pharmacologist.

3.2. Mouse Study

The purpose of this study was to determine the oncogenic potential of the test article, Org 5222, via subcutaneous administration to the mouse for at least 104 weeks. Due to high mortality among the treated animals, females were terminated during weeks 98/99 and the males during weeks 89/90. Male and female Crl:CD-1®(ICR)BR mice were assigned to five groups (57 animals/sex/group, except 60 animals/sex/high-dose-groups). The animals were house 3 to a cage. Initially each treated group received dose preparations containing the vehicle and Org 5222 at a dose volume of 7.5 mL/kg. This resulted in dose levels of 0.5, 1.5, and 7.5 m/kg/day for the females and of 0.5, 1.5 and 5.0 mg/kg/day for the males. During week 2 the group 5 animals ceased treatment with the vehicle at the sponsor's request and remained untreated controls for the remainder of the study. During week 25, the high dose levels were reduced to 5.0 mg/kg/day for the females and to 4.0 mg/kg/day for the males due to increased morbidity and mortality. For the same reasons dosing ceased for the mid and high dose males at week 88 and for the mid dose females at week 95 and the high dose females at week 97. These animals were maintained treatment–free until their terminal sacrifice.

Tissues marked in the list given in Appendix 3 ((b) (4) Study Number 82/75 Final Report, p. 433) were microscopically examined for both control groups and the high dose animals and all decedents. In addition, the heart from all animals and all gross lesions and tissue masses seen at necropsy were microscopically examined.

The reviewer considered pair-wise comparisons statistically significant at α=0.05 (two-sided) for mortality and at α=0.05 and α=0.01(one-sided) for rare and common tumors respectively. Trend tests employing either the vehicle control group or the untreated control group and the treated animals and were considered statistically significant at α=0.05 (two-sided) for mortality and α=0.025 and α=0.005 (one-sided) for increases in rare or common tumors, respectively. Tumors were considered rare if they occurred in 1% or less of the vehicle control animals and common otherwise. All statistical analyses were performed by gender. The reviewer presents first the trend results using the vehicle controls and then the pair-wise comparisons between the vehicle controls and the high dose group. The trend tests using the untreated controls are given in Appendix II. These results increase the multiplicity of the tests, but no further adjustments to the α-levels were made. Also, one needs to remember that only few tissues were microscopically examined in all animals, hence the trend results are an approximate evaluation for most tumors.

3.2.1. Sponsor's Results

The actual microscopic pathology was performed externally by N V Organon, but (b) (4) conducted the study and performed the statistical analyses of the mortality and tumor data. In (b) (4) Study Number 82/75/Final Report, (b) (4) noted that mortality was investigated for both increasing and decreasing trends with dose. For tumor data, only pair-wise comparisons were performed, namely the vehicle control versus the high dose, the vehicle control versus the untreated control, and the untreated control versus the high dose.

(b) (4) reported a statistically significant increase in mortality with dose for both the female ($p \leq 0.001$) and male ($p=0.007$) mice. With respect to tumor findings, it was reported that no treatment-related increases in tumors in the skin/subcutis or injection sites were observed. No other tumor incidences revealed evidence of systemic oncogenicity. Though there was a statistically significantly higher incidence of lymphomas in the high dose females compared to the vehicle controls ($p<0.001$), the observed incidence was less than the one in the untreated controls.

3.2.2. Reviewer's Results

3.2.2.1. Female Mice

The reviewer used the same approach of analysis as was employed for the rat study. Though the sponsor performed only pair-wise comparisons with the controls and high dose animals, the reviewer performed the trend tests for tumors as an approximate evaluation of all tumor incidences. Also, she provided more detail than the sponsor who grouped all tumor findings by site.

There seemed to be some minor inconsistencies in the sponsor's report. They observed a significant increase in malignant lymphomas in the hemolymphoreticular system of the females, but did not evaluate all tissues of at least one lower group. It is possible, that this was justified because no significance was observed when the untreated controls were used. The lymphoma finding was reported as significant at $p<0.001$ (p. 32 of the Final Report) but on the next page they stated that this finding did not rise to the FDA criterion for pair-wise tests of common tumors, which is $p\leq0.01$. Clearly, the comparison between vehicle control and high dose females qualified as a statistically significant finding.

The mortality analysis of the data provided by the sponsor produced slightly different numbers of animals surviving until the (early) terminal sacrifice (Table 11). However, the observance of the highly significant positive linear trend ($p\leq0.0001$, Table 12) was consistent with the sponsor reported p-value of <0.001. Figure 5 shows that the vehicle controls had clearly the best survival and the high dose the worst. The low and medium dosed female survival curves diverged and crossed a few times which is reflected in the statistically significant departure from linear trend and lack of homogeneity in Table 12.

Table 11: Mortality Table for Female Mice

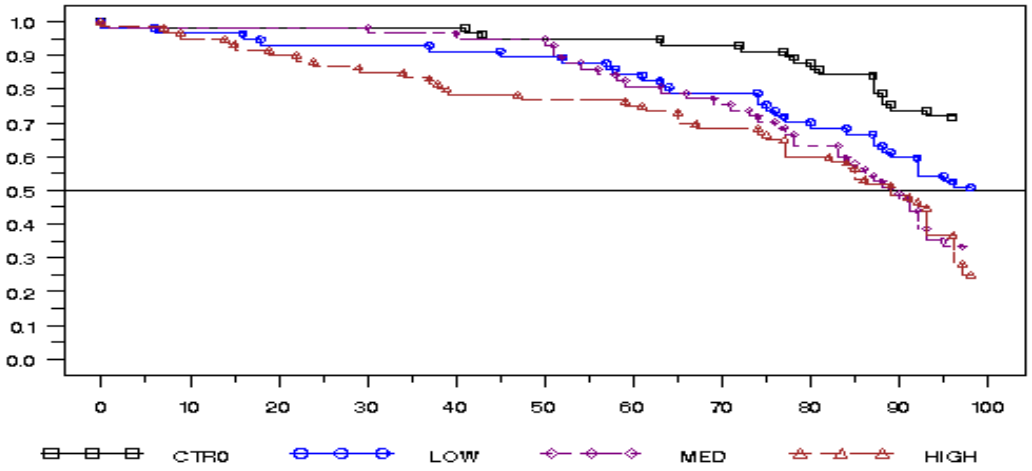
Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
Vehicle CTR	0-52	57	2	55	96.5	3.5
	53-78	55	4	51	89.5	10.5
	79-97	51	10	41	71.9	28.1
	FINALKILL 98-99	41	41	0		
LOW	0-52	57	6	51	89.5	10.5
	53-78	51	10	41	71.9	28.1
	79-97	41	11	30	52.6	47.4
	FINALKILL 98-99	30	30	0		
MED	0-52	57	6	51	89.5	10.5
	53-78	51	13	38	66.7	33.3
	79-97	38	19	19	33.3	66.7
	FINALKILL 98-99	19	19	0		
HIGH	0-52	60	13	47	78.3	21.7
	53-78	47	8	39	65.0	35.0
	79-97	39	22	17	28.3	71.7
	FINALKILL 98-99	17	17	0		
UNTREATED CTR0	0-52	57	7	50	87.7	12.3
	53-78	50	11	39	68.4	31.6
	79-91	39	8	31	54.4	45.6
	92-97	31	5	26	45.6	54.4
	FINALKILL 98-99	26	26	0		

Table 12: Mortality Trend Test for Female Mice*

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	8.4626	0.0145	7.6508	0.0218
Depart from Trend				
Dose-Mortality Trend	16.6253	0.0000	15.2748	0.0001
Homogeneity	25.0879	0.0000	22.9256	0.0000

* Trend for vehicle control, low, medium, and high dose groups.

Figure 5: Kaplan-Meier Survival Graphs for Female Mice Using Vehicle Controls



As always, the trend tests for increasing tumor incidences with dose were mortality adjusted. Though not all tissues for the low and mid dose females were microscopically examined, these findings give a rough idea of the tumorigenic potential of the compound (Table 13). There were no statistically significant increases in any tumors except for pleomorphic malignant lymphomas in the hemolymphreticular system. The trend test for this tumor was significant at p=0.0002. The sponsor had noted similar results (p<0.001) for the pair-wise comparison of all malignant lymphomas in the hemolymphreticular system.

Table 13: Trend Tests for Tumor Incidences for Female Mice Using Vehicle Controls

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AD	ADRENAL	250	SUBCAPSULAR CELL ADENOMA	0	1	0	0	0.8857	0.8343
AD	ADRENAL	535	BENIGN PHAEOCHROMOCYTOMA	2	0	0	1	0.5460	0.5058
BR	BRAIN	733	MALIGNANT	1	0	0	0	1.0000	0.8506

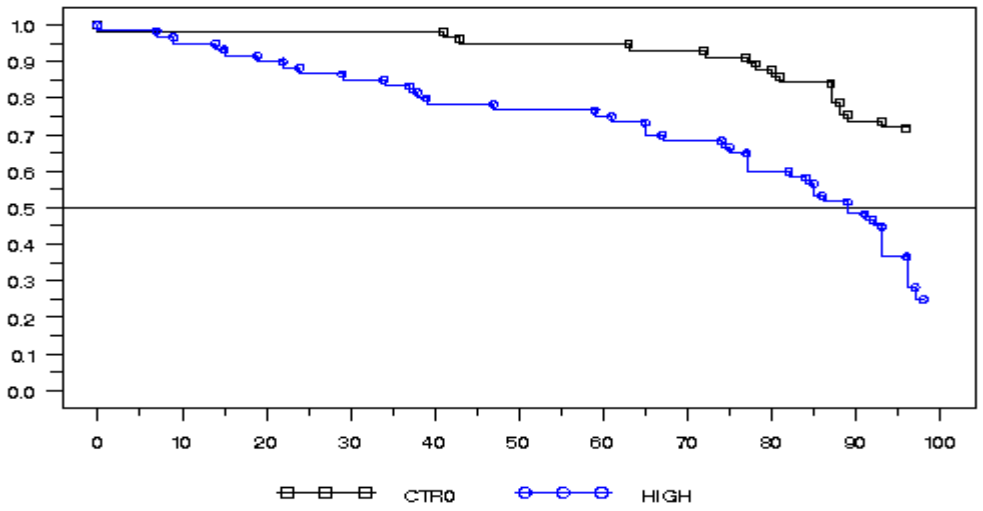
			MENINGIOMA						
EA	EAR	747	NEUROFIBROMA	1	0	0	0	1.0000	0.8150
FE	FEMUR + MARROW	644	HAEMANGIOMA	1	0	0	0	1.0000	0.8225
FE	FEMUR + MARROW	672	HAEMANGIOSARCOMA	1	0	0	0	1.0000	0.8225
HE	HAEMOLYMPHORETICULAR	134	MALIGNANT LYMPHOMA-LYMPHOBLAST	2	0	1	1	0.6215	0.6345
HE	HAEMOLYMPHORETICULAR	141	MALIGNANT LYMPHOMA - PLEOMORPH	2	2	6	14	0.0004	0.0002
HE	HAEMOLYMPHORETICULAR	261	GRANULOCYTIC LEUKAEMIA	2	0	4	1	0.6759	0.6984
HE	HAEMOLYMPHORETICULAR	285	HISTIOCYTIC SARCOMA	1	0	0	0	1.0000	0.9349
HE	HAEMOLYMPHORETICULAR	397	MALIGNANT LYMPHOMA - NOS	0	1	0	0	0.5859	0.8002
HE	HAEMOLYMPHORETICULAR	45	MALIGNANT LYMPHOMA - LYMPHOCYT	3	1	1	5	0.0986	0.1018
HG	HARDERIAN GLAND	503	ADENOMA	0	0	1	0	1.0000	0.8932
IJ1	NECK	490	MALIGNANT FIBROUS HISTIOCYTOMA	0	0	1	0	0.4472	0.6294
IJ2	RIGHT HIP	588	MALIGNANT FIBROUS HISTIOCYTOMA	0	0	0	1	0.1589	0.0251
LI	LIVER	247	HAEMANGIOMA	0	0	1	0	0.6000	0.6847
LI	LIVER	368	HEPATOCELLULAR ADENOMA	1	0	0	0	1.0000	0.8225
LI	LIVER	567	HAEMANGIOSARCOMA	0	1	0	0	0.6500	0.7726
LU	LUNG	221	BRONCHIOLO-ALVEOLAR ADENOMA	10	7	5	5	0.7630	0.7806
LU	LUNG	362	BRONCHIOLO-ALVEOLAR CARCINOMA	1	3	1	0	0.7629	0.8375
MA	MAMMARY GLAND	288	ADENOCARCINOMA	3	2	3	1	0.6866	0.7343
MA	MAMMARY GLAND	601	ADENOMA	0	1	1	0	0.6305	0.7797
OV	OVARY	533	LEIOMYOMA	0	1	0	0	0.8387	0.8617
OV	OVARY	620	CYSTADENOMA	1	1	1	0	0.8836	0.8997
OV	OVARY	673	BENIGN LUTEOMA	3	0	0	0	1.0000	0.9061
OV	OVARY	691	BENIGN SEX CORD STROMAL TUMOUR	1	1	1	0	0.6335	0.7633
OV	OVARY	745	BENIGN SERTOLI CELL TUMOUR	1	0	0	0	1.0000	0.8225
OV	OVARY	765	BENIGN GRANULOSA CELL TUMOUR	0	0	0	1	0.1589	0.0251
PI	PITUITARY	373	ADENOMA	1	0	0	3	0.0590	0.0297
SK	SKIN + SUBCUTIS	356	FIBROSARCOMA	0	0	0	1	0.2288	0.0594
SK	SKIN + SUBCUTIS	415	MALIGNANT FIBROUS HISTIOCYTOMA	1	1	1	1	0.3900	0.4440
SK	SKIN + SUBCUTIS	602	SQUAMOUS CELL PAPILLOMA	0	0	1	0	0.3429	0.5632
SK	SKIN + SUBCUTIS	751	SEBACEOUS CELL ADENOMA	0	0	1	0	0.6613	0.7450
SK	SKIN + SUBCUTIS	757	LEIOMYOSARCOMA	0	1	0	0	0.6585	0.7736
SP	SPLEEN	576	HAEMANGIOMA	1	0	0	1	0.4573	0.3380
SP	SPLEEN	736	HAEMANGIOSARCOMA	0	1	1	0	0.5125	0.7152
ST	STOMACH	744	ADENOMA	0	0	0	1	0.3548	0.1356
UT	UTERUS	332	LEIOMYOMA	2	1	0	2	0.2751	0.2694
UT	UTERUS	442	STROMAL POLYP	1	5	3	2	0.3284	0.3638
UT	UTERUS	557	HISTIOCYTIC SARCOMA	2	1	0	0	0.9463	0.8784
UT	UTERUS	734	HAEMANGIOSARCOMA	0	0	0	1	0.2349	0.0643
UT	UTERUS	766	MALIGNANT SCHWANNOMA	0	1	0	0	0.6982	0.7894

The sponsor did not perform any trend analyses as few tissues were examined for all animals. In their pair-wise comparisons the sponsor grouped all tumors per tissue/organ. The reviewer agrees that the pair-wise comparisons are the appropriate analysis for most tumor/tissue combinations of these studies. However, she performed pair-wise comparisons on each individual tumor/tissue combination. The high dose females experienced much higher mortality than did the vehicle controls (p=0.0000) (Table 14, Figure 6).

Table 14: Mortality Comparison between Vehicle Control and High Dose Female Mice

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
	22.1801	0.0000	21.8393	0.0000
Dose-Mortality Trend				

Figure 6: Kaplan Meier Survival Curves for Vehicle Control and High Dose Female Mice



The pair-wise comparisons in tumors showed no statistically higher incidences among the high dose females versus the vehicle controls except for pleomorphic malignant lymphomas in the hemolymphoreticular system (p=0.0000) (Table 15). The sponsor's comparison was based on all malignant lymphomas in the hemolymphoreticular system and resulted in a similar finding (p-value of < 0.001). As noted by the sponsor and confirmed by the reviewer (Appendix II) this finding disappeared when the untreated controls are used for comparison. In deed the incidence among the untreated controls was equal to the one observed among the high dose animals. It is left to the expertise of the reviewing pharmacologist to determine whether this observed significant finding (using the vehicle control) can be used to establish the validity of the female mouse study.

Table 15: Pair-wise Comparison in Tumor Incidences between Vehicle Control and High Dose Female Mice

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AD	ADRENAL	535	BENIGN PHAEOCHROMOCYTOMA	2	1	0.8473	0.6815
BR	BRAIN	733	MALIGNANT MENINGIOMA	1	0	1.0000	0.8561
EA	EAR	747	NEUROFIBROMA	1	0	1.0000	0.8150
FE	FEMUR + MARROW	644	HAEMANGIOMA	1	0	1.0000	0.7996
FE	FEMUR + MARROW	672	HAEMANGIOSARCOMA	1	0	1.0000	0.7996
HE	HAEMOLYMPHORETICULAR	134	MALIGNANT LYMPHOMA-LYMPHOBLAST	2	1	0.8410	0.7043
HE	HAEMOLYMPHORETICULAR	141	MALIGNANT LYMPHOMA - PLEOMORPH	2	14	0.0000	0.0000
HE	HAEMOLYMPHORETICULAR	261	GRANULOCYTIC LEUKAEMIA	2	1	0.8283	0.6879
HE	HAEMOLYMPHORETICULAR	285	HISTIOCYTIC SARCOMA	1	0	1.0000	0.9975
HE	HAEMOLYMPHORETICULAR	45	MALIGNANT LYMPHOMA - LYMPHOCYT	3	5	0.1746	0.1077
IJ2	RIGHT HIP	588	MALIGNANT FIBROUS HISTIOCYTOMA	0	1	0.2931	0.0874
LI	LIVER	368	HEPATOCELLULAR ADENOMA	1	0	1.0000	0.7996
LU	LUNG	221	BRONCHIOLO-ALVEOLAR ADENOMA	10	5	0.8759	0.8134
LU	LUNG	362	BRONCHIOLO-ALVEOLAR CARCINOMA	1	0	1.0000	0.7996
MA	MAMMARY GLAND	288	ADENOCARCINOMA	3	1	0.8506	0.7301
OV	OVARY	620	CYSTADENOMA	1	0	1.0000	0.7996
OV	OVARY	673	BENIGN LUTEOMA	3	0	1.0000	0.8945
OV	OVARY	691	BENIGN SEX CORD STROMAL TUMOUR	1	0	1.0000	0.7996
OV	OVARY	745	BENIGN SERTOLI CELL TUMOUR	1	0	1.0000	0.7996
OV	OVARY	765	BENIGN GRANULOSA CELL TUMOUR	0	1	0.2931	0.0874
PI	PITUITARY	373	ADENOMA	1	3	0.3333	0.1908
SK	SKIN + SUBCUTIS	356	FIBROSARCOMA	0	1	0.3913	0.1436
SK	SKIN + SUBCUTIS	415	MALIGNANT FIBROUS HISTIOCYTOMA	1	1	0.5697	0.3673

SP	SPLEEN	576	HAEMANGIOMA	1	1	0.7791	0.5428
ST	STOMACH	744	ADENOMA	0	1	0.6875	0.3151
UT	UTERUS	332	LEIOMYOMA	2	2	0.6874	0.5129
UT	UTERUS	442	STROMAL POLYP	1	2	0.3722	0.2096
UT	UTERUS	557	HISTIOCYTIC SARCOMA	2	0	1.0000	0.8551
UT	UTERUS	734	HAEMANGIOSARCOMA	0	1	0.4432	0.1733

3.2.2.2. Male Mice

Table 16 provides the number of male mice alive at each time interval, the number that died during the interval and the percent of cumulative mortality. The vehicle controls experienced the best and the medium dosed animals the worst survival. These findings are reflected in statistically significant tests for linear trend, departure from linearity and homogeneity (Table 17). In addition, Figure 7 demonstrates the relative positions of the Kaplan Meier survival curves.

Table 16: Mortality Table for Male Mice*

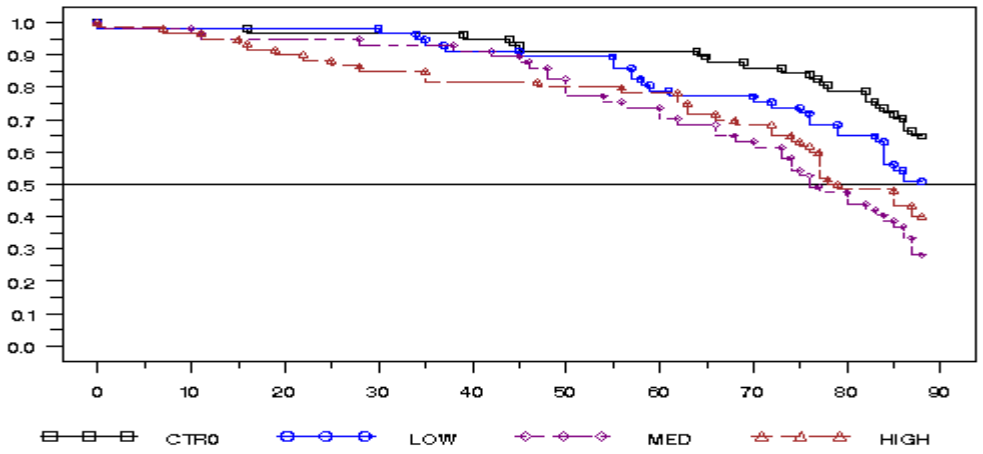
Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
VEHICLE CTR	0-52	57	4	53	93.0	7.0
	53-78	53	7	46	80.7	19.3
	79-88	46	9	37	64.9	35.1
	FINALKILL 89-90	37	37	0		
LOW	0-52	57	5	52	91.2	8.8
	53-78	52	11	41	71.9	28.1
	79-88	41	12	29	50.9	49.1
	FINALKILL 89-90	29	29	0		
MED	0-52	57	10	47	82.5	17.5
	53-78	47	19	28	49.1	50.9
	79-88	28	12	16	28.1	71.9
	FINALKILL 89-90	16	16	0		
HIGH	0-52	60	11	49	81.7	18.3
	53-78	49	18	31	51.7	48.3
	79-88	31	7	24	40.0	60.0
	FINALKILL 89-90	24	24	0		
UNTREATED CTR	0-52	57	5	52	91.2	8.8
	53-78	52	15	37	64.9	35.1
	79-88	37	4	33	57.9	42.1
	FINALKILL 89-90	33	33	0		

Table 17: Mortality Trend in Male Mice*

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	12.0934	0.0024	10.9934	0.0041
Depart from Trend				
Dose-Mortality Trend	6.4304	0.0112	7.2212	0.0072
Homogeneity	18.5238	0.0003	18.2146	0.0004

*Trend with vehicle control, low, medium, and high dose groups.

Figure 7: Kaplan Meier Survival Graphs for Male Mice Using Vehicle Controls



The same caveats as to the validity of the trend tests on tumor incidences apply here as have been explained for the female mice. Among the male mice no statistically significant positive increase in tumor incidences was observed when the vehicle controls were used (Table 18).

Table 18: Tumor Trends with Vehicle Controls for Male Mice

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AB	ABDOMINAL CAVITY	169	OSTEOSARCOMA	1	0	0	0	1.0000	0.8918
AD	ADRENAL	250	SUBCAPSULAR CELL ADENOMA	1	0	2	3	0.0705	0.0749
AD	ADRENAL	443	CORTICAL ADENOMA	1	0	0	0	1.0000	0.8635
BR	BRAIN	481	BENIGN MENINGIOMA	0	0	1	0	0.4750	0.5949

EP	EPIDIDYMIS	369	HAEMANGIOSARCOMA	0	0	0	1	0.2541	0.0912
FE	FEMUR + MARROW	468	OSTEOMA	1	0	0	0	1.0000	0.9352
HE	HAEMOLYMPHORETICULAR	134	MALIGNANT LYMPHOMA-LYMPHOBLAST	1	1	1	2	0.3099	0.3484
HE	HAEMOLYMPHORETICULAR	141	MALIGNANT LYMPHOMA - PLEOMORPH	2	0	2	5	0.0623	0.0635
HE	HAEMOLYMPHORETICULAR	261	GRANULOCYTIC LEUKAEMIA	2	0	0	0	1.0000	0.9230
HE	HAEMOLYMPHORETICULAR	285	HISTIOCYTIC SARCOMA	0	1	1	0	0.6182	0.7695
HE	HAEMOLYMPHORETICULAR	45	MALIGNANT LYMPHOMA - LYMPHOCYT	0	2	1	0	0.7636	0.8474
HG	HARDERIAN GLAND	503	ADENOMA	0	0	0	1	0.6667	0.3226
IJ2	RIGHT HIP	371	FIBROSARCOMA	0	0	1	1	0.1647	0.1659
IJ3	LEFT HIP	737	SARCOMA - NOS	0	1	0	0	0.6509	0.7875
LI	LIVER	247	HAEMANGIOMA	0	0	0	1	0.2264	0.0708
LI	LIVER	368	HEPATOCELLULAR ADENOMA	13	10	4	7	0.7607	0.7808
LI	LIVER	423	HEPATOCELLULAR CARCINOMA	2	0	4	0	0.7577	0.8006
LU	LUNG	221	BRONCHIOLO-ALVEOLAR ADENOMA	15	11	5	9	0.7402	0.7607
LU	LUNG	362	BRONCHIOLO-ALVEOLAR CARCINOMA	4	1	1	2	0.5913	0.6302
PA	PANCREAS	727	ISLET CELL ADENOMA	1	0	0	0	1.0000	0.8635
PI	PITUITARY	373	ADENOMA	0	1	1	0	0.5926	0.7344
PI	PITUITARY	729	CARCINOMA	0	1	0	0	0.6894	0.7991
PR	PROSTATE	724	ADENOMA	1	0	0	0	1.0000	0.8635
RE	RECTUM	456	ADENOMA	0	0	0	1	0.3137	0.1359
RE	RECTUM	754	PAPILLOMA	1	0	0	0	1.0000	0.8635
SK	SKIN + SUBCUTIS	356	FIBROSARCOMA	0	3	1	0	0.7404	0.8185
SK	SKIN + SUBCUTIS	508	OSTEOSARCOMA	0	0	1	0	0.4295	0.6034
SK	SKIN + SUBCUTIS	572	SARCOMA - NOS	0	3	0	0	0.7961	0.8571
SK	SKIN + SUBCUTIS	666	HISTIOCYTIC SARCOMA	1	0	0	0	1.0000	0.8635
SP	SPLEEN	736	HAEMANGIOSARCOMA	0	1	0	0	0.6509	0.7875
ST	STOMACH	738	SQUAMOUS CELL PAPILLOMA	0	1	0	0	0.6509	0.7875
TE	TESTIS	402	INTERSTITIAL CELL ADENOMA	1	0	0	3	0.0638	0.0436

As noted above, the sponsor did not perform any trend analyses, but only pair-wise comparisons since few tissues were microscopically examined for all animals. However, the sponsor grouped all tumors per tissue/organ. The reviewer performed pair-wise comparisons on each individual tumor/tissue combination. The high dose males experienced statistically significantly greater mortality than did the vehicle controls ($p \leq 0.0054$) (Table 19, Figure 8). None of the pair-wise comparisons of tumor incidences between the high dose male mice and the vehicle controls reached statistical significance, (Table 20).

Table 19: Pair-Wise Mortality between Vehicle Control and High Dose in Male Mice

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Dose-Mortality Trend	7.7374	0.0054	9.3376	0.0022

Figure 8: Kaplan Meier Survival Curves for Vehicle Control and High Dose Male Mice

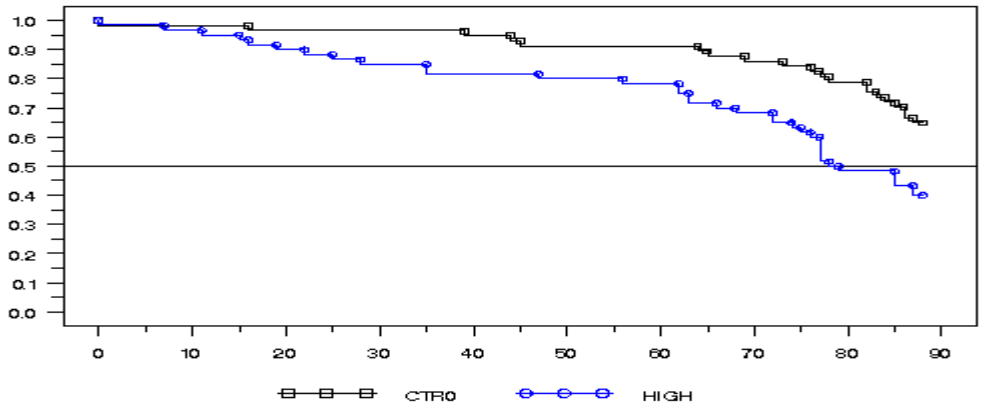


Table 20: Pair-wise Tumor Comparisons between Vehicle Control and High Dose Male Mice

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AB	ABDOMINAL CAVITY	169	OSTEOSARCOMA	1	0	1.0000	0.8858
AD	ADRENAL	250	SUBCAPSULAR CELL ADENOMA	1	3	0.1639	0.0845
AD	ADRENAL	443	CORTICAL ADENOMA	1	0	1.0000	0.8516
EP	EPIDIDYMIS	369	HAEMANGIOSARCOMA	0	1	0.4700	0.2036
FE	FEMUR + MARROW	468	OSTEOMA	1	0	1.0000	0.9687
HE	HAEMOLYMPHORETICULAR	134	MALIGNANT LYMPHOMA-LYMPHOBLAST	1	2	0.4716	0.3054
HE	HAEMOLYMPHORETICULAR	141	MALIGNANT LYMPHOMA - PLEOMORPH	2	5	0.1471	0.0889
HE	HAEMOLYMPHORETICULAR	261	GRANULOCYTIC LEUKAEMIA	2	0	1.0000	0.9196
HG	HARDERIAN GLAND	503	ADENOMA	0	1	0.6667	0.3226

IJ2	RIGHT HIP	371	FIBROSARCOMA	0	1	0.4556	0.1950
LI	LIVER	247	HAEMANGIOMA	0	1	0.3934	0.1578
LI	LIVER	368	HEPATOCELLULAR ADENOMA	13	7	0.8748	0.8280
LI	LIVER	423	HEPATOCELLULAR CARCINOMA	2	0	1.0000	0.9063
LU	LUNG	221	BRONCHIOLO-ALVEOLAR ADENOMA	15	9	0.8501	0.8013
LU	LUNG	362	BRONCHIOLO-ALVEOLAR CARCINOMA	4	2	0.8698	0.7840
PA	PANCREAS	727	ISLET CELL ADENOMA	1	0	1.0000	0.8516
PR	PROSTATE	724	ADENOMA	1	0	1.0000	0.8516
RE	RECTUM	456	ADENOMA	0	1	0.7273	0.3627
RE	RECTUM	754	PAPILLOMA	1	0	1.0000	0.8516
SK	SKIN + SUBCUTIS	666	HISTIOCYTIC SARCOMA	1	0	1.0000	0.8516
TE	TESTIS	402	INTERSTITIAL CELL ADENOMA	1	3	0.3877	0.2388

The reviewer's findings using the untreated control are given in the Appendix II. The female untreated mice had higher mortality than the vehicle controls but there was still a statistically significant trend for increased mortality with dose, but to a lesser degree than when the vehicle controls were employed. Also, the departure from linear trend and lack of homogeneity were no longer significant when employing the untreated controls. None of the tumor findings reached statistical significance when employing the untreated controls. Most noteworthy was the finding for pleomorphic malignant lymphomas in the hemolymphoreticular system which was statistical significant when using the vehicle controls, but was now totally non-significant. The incidences among the untreated controls and the high dose females were identical. In the sponsor's grouping of all malignant lymphomas, the incidence among the untreated controls was numerically even higher than the one among the high dose animals. Performing the tests with the untreated controls increased the multiplicity but no further adjustment in α-level has been made. As no findings approached the standard levels of significance for common and rare tumors, there was no concern of false positive findings.

The mortality trend test with the untreated controls of the male mice was similar to the one with the vehicle controls, but to a lesser degree of statistical significance. With respect to the tumor findings, interstitial cell adenomas in the testes now reached statistically significance for rare tumors. However, in the reviewer's opinion this is a weak finding inasmuch as it depends on zero tumors in the control group, on the assumption that this is a rare tumor in general, and on the recognition that no further adjustments of the α-level have been made for the additional multiplicity.

These findings are consistent with the sponsor's discussion and conclusions.

3.2.2.3. Validity of Male and Female Mouse Study

The single statistically significant finding among each gender depended on which control group was used. Hence these findings are not robust for establishing the validity of the studies. A whole life carcinogenicity study is considered valid despite no significant tumor findings if the following two criteria are met:

vi) Were sufficient numbers of animals exposed long enough to allow for late-developing tumors?
vii) Did the high dose provide a sufficient tumor challenge?

The number of animals and length of exposure can be assessed at weeks 52, 80-90, and at termination, but are generally considered adequate if 20-30 animals survive through weeks 80-90. Though the study was terminated early for both genders, the female mice met the first criterion as there were at least 17 to 41 animals available for terminal sacrifice at week 98. The male mice were terminated earlier at week 89 when the mid dose group had only 16 animals left. However by week 79 there were still 28 animals on study and hence sufficient numbers of animals were exposed in either gender. The exposure to Org 5222 was stopped at weeks 95 and 97 for the mid and high dose females. For the males treatment of the mid and high dose animals was stopped one week before terminal sacrifice. It is left to the expertise of the reviewing pharmacologist to determine whether the length of exposure was also sufficient.

In determining whether the high dose provided an adequate tumor challenge, one expects the high dose to be close to the MTD. The following criteria are employed in this assessment:

viii) A dose is considered adequate if there is a detectable reduction in average body weight of up to 10% in a dosed group relative to the controls, or
ix) A dose is considered adequate if the dosed animals show a slightly increased mortality compared to the controls, or
x) A dose is considered an MTD if the dosed animals exhibit severe toxic effects attributed to the chemical. This latter evaluation is performed by the pharmacologist/toxicologist.

The high dose females had basically the same average body weights as the vehicle controls till about week 40. Thereafter, their average bodyweights were about 5 percent lower than the vehicle controls' through the remainder of the study. This difference may be adequate to establish the high dose as being close to the MTD.

There was a detectable reduction in average body weights of the high dose males versus the vehicle controls. By week 36 the difference was 10 percent and by week 64 it reached a maximum of 13.6 percent. In the reviewer's opinion these findings establish that the high dose was close to the MTD.

The final decision whether the study can be considered valid for either gender is left to the expertise of the reviewing pharmacologist.

4. CONCLUSION

There were major modifications to the standard whole-life study in the **Sprague-Dawley Rats**. The female study had to be terminated after 99 weeks of treatment due to high mortality among the vehicle controls, the high dose was increased in both sexes from 3.0 mg/kg/day to 5.0 mg/kg/day and only selected tissues were microscopically examined for all animals. For the remaining tissues only the pair-wise comparisons, which are less powerful than the trend tests, should be considered primary. In general, findings between the reviewer and the sponsor were consistent even though the sponsor presented grouped tumor results per tissue, whereas the reviewer presented the standard individual tumors per tissue results and analyses.

Taking intercurrent mortality into account, there were no statistically significantly increased tumor findings among the female rats when the vehicle controls were used. When the untreated controls were employed, there were increases in adenocarcinoma in the mammary glands and in benign hair follicle tumors at the injection sites, neither of which reached the standard criteria for significance of common or rare tumors, much less any more stringent criteria had the additional multiplicity been taken into account.

Using the same methods of analysis, there were no statistically significant increases in tumors among the male rats, whether employing the vehicle controls, the untreated controls or performing pair-wise comparisons.

As no tumor findings reached statistical significance, the validity of the male and female rat studies needed to be evaluated. The reviewer concluded that though the female study was terminated early, the criterion for exposing sufficient numbers of animals long enough to allow for late-developing tumors had been met. This was not an issue for the male rats, whose study lasted 105 weeks. The adequacy of the high dose was in question for both genders. Survival was not an indicator, as for the females the vehicle controls had worse survival than the high dose animals and the untreated controls had only slightly better survival (3 percent at the end of the study) than the high dose animals (but slightly worse than the medium dose animals). For the male rats, the vehicle controls and high dose animals had identical survival patterns. The trend test using the vehicle controls was not statistically significant ($p \leq 0.33$). The untreated controls had 8 percent better survival at study end than the high dose group, but was worse than the mid dose group. Among the female rats, the difference in average body weights came late in the first year. The bodyweight averages of the vehicle controls and the high dose were basically identical for the first 40 weeks. By 52 weeks, the high dose females had 10% lower average body weight than the vehicle controls. However, the difference increased to 24% by the end of the study which was week 100. Among the male rats the average body weights diverged almost immediately. By week 9, the high dose animals weighted on the average 10% less than the vehicle controls. The difference increased steadily to a

maximum of 30% by week 96. By the end of the study (week 106), this difference had narrowed somewhat. From these findings it appears that Org 5222 did not negatively influence the survival of the high dose animals, but had some effect on average body weights, certainly with respect to the male rats. The final decision with respect to the validity of both the male and female rat studies is left to the expertise of the reviewing pharmacologist.

There were major modifications to the standard whole-life study in **Crl:CD-1®(IRC)BR Mice**. For both genders the study was terminated early due to increased mortality among the treated animals. The females were terminated after 97 weeks of treatment and the males after 88 weeks of treatment. In addition, the high doses were reduced from 7.5 to 5.0 mg/kg/day for the females and from 5.0 to 4.0 mg/kg/day for the males during week 25 and dosing of the mid and high dose animals ceased 1 – 3 weeks prior to the early terminal sacrifice. Of special importance is that only few selected tissues were microscopically examined in all animals and that for most tissues only the pair-wise comparisons, which are less powerful than the trend tests, are considered primary. In general, findings between the reviewer and the sponsor were consistent even though the sponsor presented only grouped tumor results per tissue site and performed only pair-wise comparisons (which is appropriate), whereas the reviewer presented the standard individual tumors per tissue results for trend tests and pair-wise comparisons. The reviewer considered the findings based on the trend tests as a useful approximation and not as the definitive results.

Taking intercurrent mortality into account, there was a significant trend and pair-wise comparison using the vehicle control for pleomorphic malignant lymphomas in the hemolymphoreticular system among the female mice. However, when using the untreated controls, this finding went away, as they had the same tumor incidence as did the high dose. No other tumor finding approached statistical significance. Among the male mice, the only statistically significant finding was interstitial cell adenomas in the testes using the untreated controls and considering this a rare tumor. There was one such tumor also among the vehicle controls which resulted in non-significant trend or pair-wise comparisons.

As these statistically significant tumor findings were not robust and depended on which control group was used, the reviewer also evaluated the validity of both the male and female mouse study. From a statistical point of view, there were sufficient numbers of animals still available at the time of the early sacrifice. However, as the dosing of the mid and high dose animals stopped even earlier, it is left to the expertise of the reviewing pharmacologist whether the length of exposure was adequate. For the female mice, there was a 5% difference in average body weights between the high dose animals and the vehicle controls after one year of treatment. For the male mice, the difference in average body weights between the high dose animals and vehicle controls seems to indicate that the high dose was close to the MTD. The final decision with respect to the validity of the observed tumor findings, the length of exposure, and the adequacy of the tumor challenge is left to the expertise of the reviewing pharmacologist.

5. APPENDICES

5.1. Rat Findings Using the Untreated Controls

When using the untreated controls there was no significant difference in mortality among the female rats (Table 17, Figure 7). There were suggestions towards statistically significant increases in malignant adenocarcinoma in the mammary glands and benign hair follicle tumors at the injection sites, both of which were also mentioned by the sponsor (Table 18). However, neither reached the standard levels of significance for common and rare tumors respectively, much less any levels of significance further adjusted for the additional multiplicity created by the two control groups.

Table 21: Mortality Tests for Female Rats Using the Untreated Controls

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	2.9837	0.2250	3.7796	0.1511
Depart from Trend				
Dose-Mortality Trend	0.0037	0.9516	0.0021	0.9633
Homogeneity	2.9874	0.3936	3.7817	0.2860

Figure 9: Kaplan Meier Survival Curves for Female Rats Using the Untreated Controls

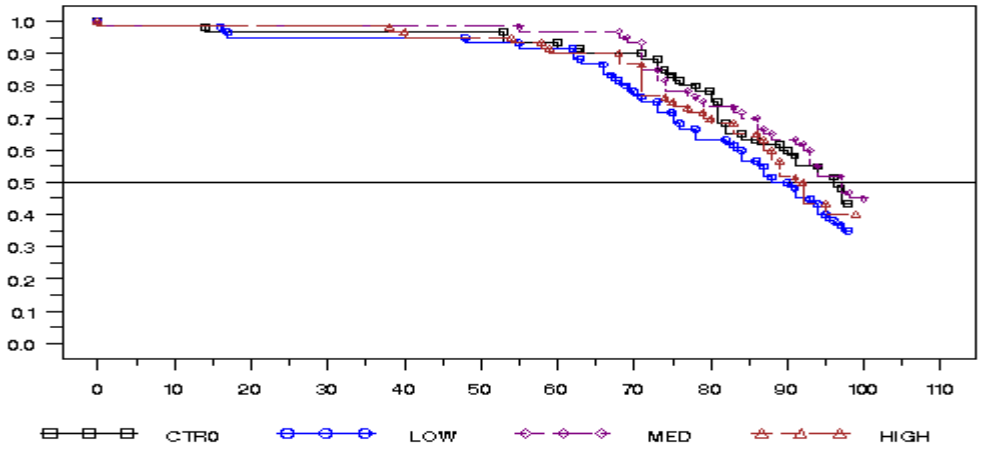


Table 22: Tumor Findings for Female Rats Using the Untreated Controls

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
10000	brain	10028	astrocytoma, malignant (M)	1	0	0	1	0.4277	0.3257
10000	brain	10170	tumor, granular cell, benign (	2	1	0	1	0.6782	0.6754
2000	adrenal glands	2011	adenoma, cortical (B)	3	0	0	2	0.3509	0.3589
2000	adrenal glands	2036	carcinoma, cortical (M)	0	1	0	1	0.3214	0.2956
2000	adrenal glands	2132	pheochromocytoma, benign (B)	3	0	1	0	0.9419	0.9253
2000	adrenal glands	2135	pheochromocytoma, malignant (M	0	1	1	0	0.6162	0.7967
22000	eyes	22092	leiomyoma, iris (B)	0	1	0	0	0.7045	0.8131
27000	haematopoietic system	27104	lymphoma, malignant (S)	1	1	0	0	0.9313	0.8895
27000	haematopoietic system	27147	sarcoma, histiocytic (S)	0	1	1	0	0.6186	0.7824
31000	jejunum	31146	sarcoma, histiocytic (M)	0	1	0	0	0.7353	0.8122
33000	kidney	33021	adenoma, renal tubule (B)	0	0	1	0	0.5253	0.6789
37000	liver	37015	adenoma, hepatocellular (B)	0	2	1	1	0.4218	0.5276
44000	lymph nodes	44081	haemangioma (B)	0	0	1	1	0.1948	0.2081
46000	mammary glands	46001	adenocarcinoma (M)	11	18	23	25	0.0131	0.0133
46000	mammary glands	46002	adenocarcinoma in adenoma (M)	3	1	1	1	0.7413	0.7500
46000	mammary glands	46006	adenoma (B)	2	2	4	3	0.3562	0.3981
46000	mammary glands	46072	fibroadenoma (B)	32	17	21	18	0.9448	0.9473
46000	mammary glands	46073	fibroma (B)	1	1	1	0	0.8266	0.8779
46000	mammary glands	46116	myoepithelioma, malignant (M)	1	0	0	0	1.0000	0.8572
46000	mammary glands	46176	tumor, mixed, malignant (M)	1	0	0	0	1.0000	0.8575
53000	oral cavity & related structur	53044	carcinoma, NOS (M)	0	1	0	0	0.7522	0.8193
53000	oral cavity & related structur	53049	carcinoma, squamous cell (M)	1	0	1	0	0.7591	0.8122
54000	ovaries	54189	tumor, sex cord stromal (B)	1	0	0	1	0.4667	0.3490
56000	pancreas	56007	adenoma, acinar cell (B)	1	0	0	0	1.0000	0.8586
56000	pancreas	56018	adenoma, islet cell (B)	0	0	1	1	0.1954	0.1864
56000	pancreas	56042	carcinoma, islet cell (M)	0	1	0	1	0.2802	0.2616
59000	parathyroid glands	59006	adenoma (B)	0	0	0	1	0.1875	0.0418
63000	pituitary	63020	adenoma, pars intermedia (B)	0	0	0	1	0.2449	0.0727
63000	pituitary	63045	carcinoma, pars distalis (M)	1	1	1	0	0.8304	0.8693
77000	skin	77090	keratoacanthoma (B)	1	0	0	0	1.0000	0.8586
77000	skin	77173	tumor, hair follicle,	1	0	0	0	1.0000	0.8586

			benign (						
78000	soft tissues	78073	fibroma (B)	0	0	1	0	0.5000	0.6567
78000	soft tissues	78075	fibrosarcoma (M)	0	1	0	0	0.7353	0.8122
78000	soft tissues	78097	lipoma (B)	1	0	2	0	0.6667	0.7909
78000	soft tissues	78151	schwannoma, benign (B)	0	1	0	0	0.7353	0.8122
78000	soft tissues	78170	tumor, granular cell, benign (	1	0	0	0	1.0000	0.8586
86000	thymus	86165	thymoma, benign (B)	0	0	2	2	0.0739	0.0874
86000	thymus	86166	thymoma, malignant (M)	0	1	0	0	0.7500	0.8203
87000	thyroid glands	87010	adenoma, C-cell (B)	2	7	3	3	0.6644	0.6946
87000	thyroid glands	87014	adenoma, follicular cell (B)	0	0	1	1	0.1939	0.2091
87000	thyroid glands	87034	carcinoma, C-cell (M)	0	2	1	1	0.4175	0.5182
90000	injection site(s)	90073	fibroma (B)	1	1	0	1	0.4810	0.4659
90000	injection site(s)	90075	fibrosarcoma (M)	0	1	3	1	0.3204	0.4081
90000	injection site(s)	90088	histiocytoma, f brous (M)	0	2	0	1	0.4107	0.4451
90000	injection site(s)	90097	lipoma (B)	0	0	0	1	0.2059	0.0512
90000	injection site(s)	90146	sarcoma, histiocytic (M)	0	1	0	0	0.7430	0.8225
90000	injection site(s)	90173	tumor, hair follicle, benign (	0	0	1	2	0.0622	0.0414
93000	urinary bladder	93130	papilloma, transitional cell (	0	0	1	0	0.5000	0.6567
94000	uterus	94013	adenoma, endometrial (B)	0	0	0	1	0.2889	0.0960
94000	uterus	94139	polyp, endometrial stromal (B)	4	1	4	1	0.8170	0.8455
94000	uterus	94140	polyp, glandular, benign (B)	0	1	0	0	0.7374	0.8217
95000	vagina	95157	schwannoma, malignant (M)	0	0	1	0	0.5253	0.6789
95000	vagina	95170	tumor, granular cell, benign (	7	4	3	4	0.6945	0.7203

Among the male rats, the use of the untreated controls showed no linear trend with dose in mortality, but a statistically significant departure from linearity and from homogeneity (Table 19, Figure 8). The medium dose group had the lowest mortality and the untreated controls experienced the second lowest mortality. None of the tumor findings approached statistical significance for an increase with dose (Table 20).

Table 23: Mortality Tests for Male Rats Using the Untreated Controls

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	5.6378	0.0597	4.6193	0.0993
Depart from Trend				
Dose-Mortality Trend	2.5715	0.1088	3.6169	0.0572
Homogeneity	8.2093	0.0419	8.2362	0.0414

Figure 10: Kaplan Meier Curves for Male Rats Using the Untreated Controls

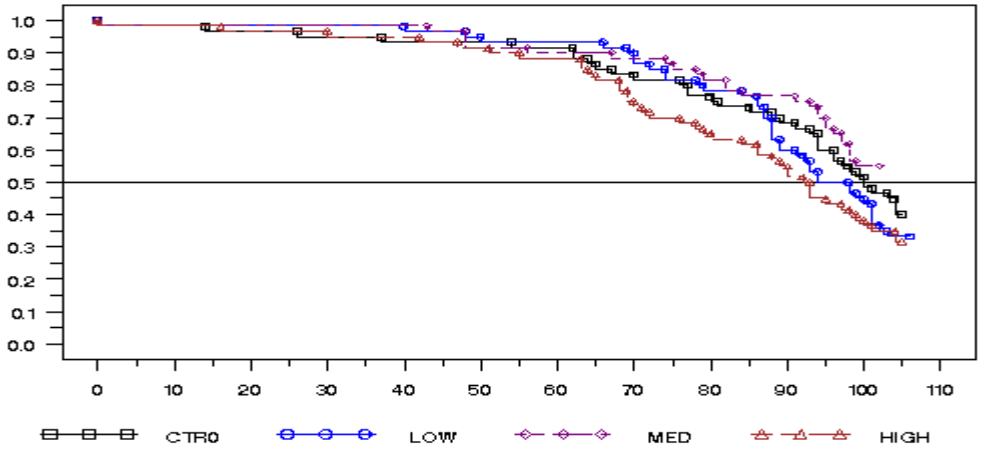


Table 24: Trend in Tumors for Male Rats Using the Untreated Controls

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
10000	brain	10079	glioma, mixed, malignant (M)	0	0	0	1	0.2510	0.0757
10000	brain	10123	oligodendroglioma, malignant (	1	0	0	0	1.0000	0.8561
2000	adrenal glands	2011	adenoma, cortical (B)	0	1	1	0	0.5958	0.7691
2000	adrenal glands	2132	pheochromocytoma, benign (B)	9	9	7	3	0.9673	0.9650
2000	adrenal glands	2135	pheochromocytoma, malignant (M	2	0	2	1	0.4845	0.5488
27000	haematopoietic system	27095	leukemia, granulocytic (S)	0	0	0	1	0.2443	0.0718
27000	haematopoietic system	27104	lymphoma, malignant (S)	1	2	0	2	0.2715	0.2745
27000	haematopoietic system	27147	sarcoma, histiocytic (S)	0	1	0	0	0.7360	0.8109
37000	liver	37039	carcinoma, hepatocellular (M)	1	0	0	0	1.0000	0.8805
44000	lymph nodes	44081	haemangioma (B)	2	2	0	1	0.7470	0.7500
46000	mammary glands	46072	fibroadenoma (B)	0	1	0	0	0.7119	0.8079
53000	oral cavity & related structur	53129	papilloma, squamous cell (B)	1	0	0	0	1.0000	0.8464
56000	pancreas	56003	adenocarcinoma, acinar cell (M	1	0	0	0	1.0000	0.8589
56000	pancreas	56008	adenoma, acinar-islet cell (B)	1	0	0	0	1.0000	0.8589
56000	pancreas	56018	adenoma, islet cell (B)	1	3	1	1	0.6424	0.6961

56000	pancreas	56042	carcinoma, islet cell (M)	1	0	1	1	0.3564	0.3331
59000	parathyroid glands	59006	adenoma (B)	4	2	1	3	0.3782	0.4148
6000	auditory sebaceous glands	6050	carcinoma, squamo-sebaceous (M	1	1	0	0	0.9488	0.9238
62000	pineal gland	62137	pinealoma, malignant (M)	1	0	0	0	1.0000	0.8464
63000	pituitary	63020	adenoma, pars intermedia (B)	0	0	0	1	0.3684	0.1436
70000	rectum	70146	sarcoma, histiocytic (M)	1	0	0	0	1.0000	0.8603
74000	mandibular salivary glands	74116	myoepithelioma, malignant (M)	1	0	0	0	1.0000	0.8816
77000	skin	77044	carcinoma, NOS (M)	1	0	0	0	1.0000	0.8464
77000	skin	77049	carcinoma, squamous cell (M)	0	1	0	0	0.7119	0.8079
77000	skin	77090	keratoacanthoma (B)	1	1	0	1	0.5476	0.5272
77000	skin	77129	papilloma, squamous cell (B)	5	0	0	0	1.0000	0.9748
77000	skin	77167	tumor, basal cell, benign (B)	0	0	1	0	0.4576	0.6615
77000	skin	77168	tumor, basal cell, malignant (	1	0	0	0	1.0000	0.8589
77000	skin	77173	tumor, hair follicle, benign (	1	0	0	0	1.0000	0.8464
78000	soft tissues	78058	chondrosarcoma (M)	1	0	0	0	1.0000	0.8805
78000	soft tissues	78073	fibroma (B)	1	1	3	1	0.4514	0.5350
78000	soft tissues	78075	fibrosarcoma (M)	0	2	0	0	0.7714	0.8511
78000	soft tissues	78088	histiocytoma, f brous (M)	0	1	0	2	0.1250	0.0976
78000	soft tissues	78097	lipoma (B)	1	0	2	1	0.3314	0.3802
78000	soft tissues	78126	osteosarcoma (M)	0	1	0	0	0.7405	0.8110
78000	soft tissues	78146	sarcoma, histiocytic (M)	1	0	0	0	1.0000	0.8805
78000	soft tissues	78151	schwannoma, benign (B)	1	0	0	0	1.0000	0.8464
78000	soft tissues	78157	schwannoma, malignant (M)	0	0	1	0	0.4962	0.6527
85000	testis	85017	adenoma, Leydig cell (B)	1	0	0	0	1.0000	0.8464
87000	thyroid glands	87010	adenoma, C-cell (B)	3	1	8	3	0.3231	0.3572
87000	thyroid glands	87014	adenoma, follicular cell (B)	2	3	1	0	0.9546	0.9431
87000	thyroid glands	87034	carcinoma, C-cell (M)	2	3	0	1	0.7432	0.7758
87000	thyroid glands	87038	carcinoma, follicular cell (M)	0	0	1	0	0.5361	0.6608
90000	injection site(s)	90049	carcinoma, squamous cell (M)	1	0	0	0	1.0000	0.8464
90000	injection site(s)	90073	fibroma (B)	5	2	2	0	0.9912	0.9787
90000	injection site(s)	90075	fibrosarcoma (M)	1	6	5	4	0.3026	0.3327
90000	injection site(s)	90088	histiocytoma, f brous (M)	1	5	7	2	0.7881	0.8136
90000	injection site(s)	90090	keratoacanthoma (B)	0	1	0	0	0.7119	0.8079
90000	injection site(s)	90097	lipoma (B)	0	2	0	0	0.8341	0.8879
90000	injection site(s)	90099	liposarcoma (M)	1	0	0	0	1.0000	0.8420
90000	injection site(s)	90146	sarcoma, histiocytic (M)	1	2	0	0	0.9163	0.9077

90000	injection site(s)	90157	schwannoma, malignant (M)	1	1	0	0	0.9389	0.8744
90000	injection site(s)	90173	tumor, hair follicle, benign (	0	0	0	1	0.1979	0.0476

5.2. Mouse Findings Using Untreated Controls

When using the untreated controls there was a statistically significant trend in mortality among the female mice (Table 25, Figure 11), but it was much weaker than the one observed with the vehicle controls and there were significant departures from linearity or homogeneity. Table 26 gives the - albeit imperfect - trends in tumor incidences. None reached statistical significance. Most noteworthy is the finding for pleomorphic malignant lymphomas in the hemolymphoreticular system which became totally non-significant. The incidences among the untreated controls and the high dose females are identical. In the sponsor's grouping of all malignant lymphomas, the incidence among the untreated controls was numerically even higher than the one among the high dose animals. Performing these tests increased the multiplicity, but no further adjustment in α-level has been made. As no findings approached the standard levels of significance for common and rare tumors, there is no concern of false positive findings.

Table 25: Mortality Tests for Female Mice Using the Untreated Controls

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	2.6671	0.2635	1.6401	0.4404
Depart from Trend				
Dose-Mortality Trend	4.4976	0.0339	4.0182	0.0450
Homogeneity	7.1648	0.0668	5.6583	0.1295

Figure 11: Kaplan Meier Survival Curves for Female Mice Using Untreated Controls

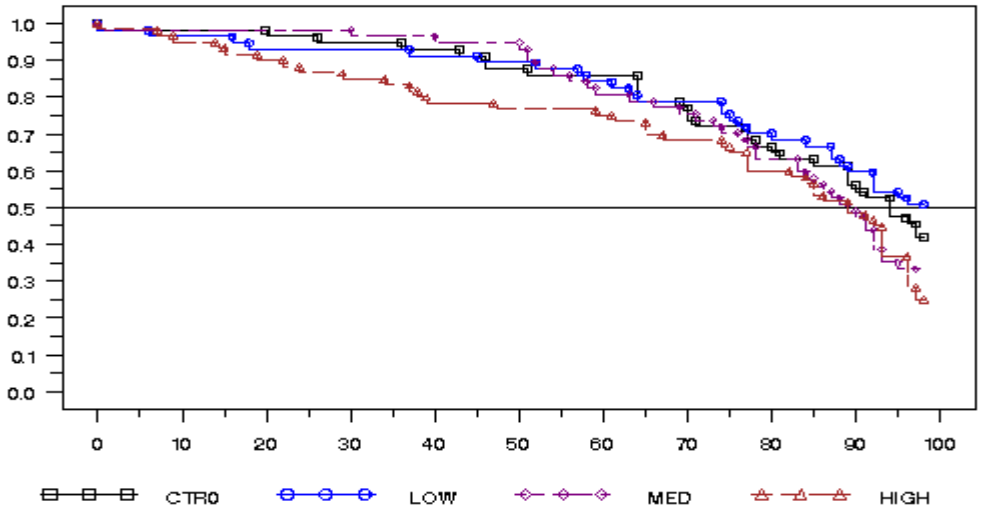


Table 26: Tumor Trends for Female Mice Using Untreated Controls

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AD	ADRENAL	250	SUBCAPSULAR CELL ADENOMA	0	1	0	0	0.7381	0.7490
AD	ADRENAL	394	MALIGNANT PHAEOCHROMOCYTOMA	1	0	0	0	1.0000	0.8910
AD	ADRENAL	535	BENIGN PHAEOCHROMOCYTOMA	0	0	0	1	0.2857	0.1043
EA	EAR	706	SQUAMOUS CELL PAPILLOMA	1	0	0	0	1.0000	0.8210
FE	FEMUR + MARROW	465	CHONDROMA	1	0	0	0	1.0000	0.8949
FE	FEMUR + MARROW	644	HAEMANGIOMA	1	0	0	0	1.0000	0.9332
HE	HAEMOLYMPHORETICULAR	134	MALIGNANT LYMPHOMA-LYMPHOBLAST	3	0	1	1	0.7923	0.8064
HE	HAEMOLYMPHORETICULAR	141	MALIGNANT LYMPHOMA - PLEOMORPH	14	2	6	14	0.3489	0.3584
HE	HAEMOLYMPHORETICULAR	197	MALIGNANT LYMPHOMA - PLASMACYT	1	0	0	0	1.0000	0.8463
HE	HAEMOLYMPHORETICULAR	261	GRANULOCYTIC LEUKAEMIA	0	0	4	1	0.4621	0.4654
HE	HAEMOLYMPHORETICULAR	397	MALIGNANT LYMPHOMA - NOS	2	1	0	0	0.9576	0.9392
HE	HAEMOLYMPHORETICULAR	45	MALIGNANT LYMPHOMA - LYMPHOCYT	2	1	1	5	0.0960	0.0971
HG	HARDERIAN GLAND	503	ADENOMA	0	0	1	0	1.0000	0.9007
IJ1	NECK	490	MALIGNANT FIBROUS HISTIOCYTOMA	0	0	1	0	0.4832	0.6738
IJ2	RIGHT HIP	588	MALIGNANT FIBROUS HISTIOCYTOMA	0	0	0	1	0.1848	0.0442

LI	LIVER	247	HAEMANGIOMA	1	0	1	0	0.7826	0.8226
LI	LIVER	567	HAEMANGIOSARCOMA	1	1	0	0	0.9215	0.8677
LU	LUNG	221	BRONCHIOLO-ALVEOLAR ADENOMA	7	7	5	5	0.6731	0.6921
LU	LUNG	362	BRONCHIOLO-ALVEOLAR CARCINOMA	3	3	1	0	0.9698	0.9592
MA	MAMMARY GLAND	288	ADENOCARCINOMA	0	2	3	1	0.4061	0.4577
MA	MAMMARY GLAND	601	ADENOMA	0	1	1	0	0.6293	0.7404
OV	OVARY	533	LEIOMYOMA	1	1	0	0	0.9354	0.8867
OV	OVARY	620	CYSTADENOMA	0	1	1	0	0.7533	0.8259
OV	OVARY	691	BENIGN SEX CORD STROMAL TUMOUR	0	1	1	0	0.5141	0.6777
OV	OVARY	765	BENIGN GRANULOSA CELL TUMOUR	0	0	0	1	0.1848	0.0442
PI	PITUITARY	373	ADENOMA	2	0	0	3	0.1447	0.1638
SK	SKIN + SUBCUTIS	356	FIBROSARCOMA	0	0	0	1	0.2547	0.0830
SK	SKIN + SUBCUTIS	415	MALIGNANT FIBROUS HISTIOCYTOMA	2	1	1	1	0.6677	0.6927
SK	SKIN + SUBCUTIS	602	SQUAMOUS CELL PAPILLOMA	1	0	1	0	0.7371	0.8092
SK	SKIN + SUBCUTIS	751	SEBACEOUS CELL ADENOMA	0	0	1	0	0.6000	0.7105
SK	SKIN + SUBCUTIS	757	LEIOMYOSARCOMA	0	1	0	0	0.7297	0.7546
SM	STERNUM + MARROW	645	HAEMANGIOMA	1	0	0	0	1.0000	0.8837
SP	SPLEEN	576	HAEMANGIOMA	0	0	0	1	0.2857	0.1043
SP	SPLEEN	736	HAEMANGIOSARCOMA	0	1	1	0	0.5769	0.7251
ST	STOMACH	744	ADENOMA	0	0	0	1	0.4000	0.1721
UT	UTERUS	332	LEIOMYOMA	0	1	0	2	0.0634	0.0280
UT	UTERUS	438	STROMAL SARCOMA	2	0	0	0	1.0000	0.9447
UT	UTERUS	442	STROMAL POLYP	3	5	3	2	0.6760	0.7052
UT	UTERUS	557	HISTIOCYTIC SARCOMA	3	1	0	0	0.9950	0.9571
UT	UTERUS	734	HAEMANGIOSARCOMA	0	0	0	1	0.2532	0.0835
UT	UTERUS	766	MALIGNANT SCHWANNOMA	0	1	0	0	0.7516	0.7663
VA	VAGINA	707	HISTIOCYTIC SARCOMA	1	0	0	0	1.0000	0.8837

When using the untreated controls, the mortality tests for the male mice were similar to the results with the vehicle controls, but to a lesser degree of statistical significance (Table 27, Figure 12). Table 28 gives the - albeit imperfect - trends in tumor incidences. Of these the three interstitial cell adenomas in the testes of the high dose animals now reached statistical significance since the tumor is considered rare with no incidence among the untreated controls. If the tumor were considered common, the observed p-value would be insufficient to declare statistical significance. As no further adjustment of the α-level has been made for the additional multiplicity, this finding ($p=0.0184$) does not appear to be robust.

Table 27: Mortality Trends for Male Mice with Untreated Controls

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	7.9518	0.0188	6.3736	0.0413
Depart from Trend				
Dose-Mortality Trend	3.6311	0.0567	3.6581	0.0558
Homogeneity	11.5829	0.0090	10.0316	0.0183

Figure 12: Kaplan Meier Survival Curves for Male Mice Using Untreated Controls

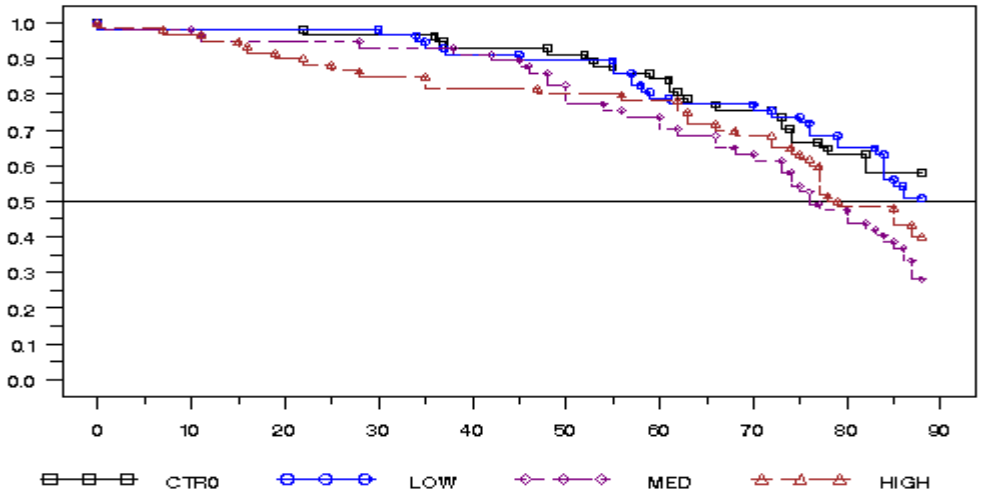


Table 28: Tumor Trends for Male Mice Using Untreated Controls

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AB	ABDOMINAL CAVITY	169	OSTEOSARCOMA	1	0	0	0	1.0000	0.9279
AD	ADRENAL	250	SUBCAPSULAR CELL ADENOMA	2	0	2	3	0.1517	0.1656
AD	ADRENAL	535	BENIGN PHAEOCHROMOCYTOMA	1	0	0	0	1.0000	0.8787
BR	BRAIN	481	BENIGN MENINGIOMA	0	0	1	0	0.5429	0.6395
EP	EPIDIDYMIS	369	HAEMANGIOSARCOMA	0	0	0	1	0.2640	0.1045
EP	EPIDIDYMIS	767	BENIGN SCHWANNOMA	1	0	0	0	1.0000	0.8774
HE	HAEMOLYMPHORETICULAR	134	MALIGNANT LYMPHOMA-LYMPHOBLAST	1	1	1	2	0.3312	0.3693
HE	HAEMOLYMPHORETICULAR	141	MALIGNANT LYMPHOMA - PLEOMORPH	5	0	2	5	0.3434	0.3593
HE	HAEMOLYMPHORETICULAR	197	MALIGNANT LYMPHOMA - PLASMACYT	1	0	0	0	1.0000	0.8673
HE	HAEMOLYMPHORETICULAR	261	GRANULOCYTIC	3	0	0	0	1.0000	0.9681

			LEUKAEMIA						
HE	HAEMOLYMPHORETICULAR	285	HISTIOCYTIC SARCOMA	1	1	1	0	0.8268	0.8855
HE	HAEMOLYMPHORETICULAR	45	MALIGNANT LYMPHOMA - LYMPHOCYT	1	2	1	0	0.8813	0.9155
IJ2	RIGHT HIP	371	FIBROSARCOMA	0	0	1	1	0.1789	0.1789
IJ3	LEFT HIP	737	SARCOMA - NOS	0	1	0	0	0.6765	0.8002
LI	LIVER	247	HAEMANGIOMA	1	0	0	1	0.4181	0.3689
LI	LIVER	368	HEPATOCELLULAR ADENOMA	9	10	4	7	0.6687	0.6874
LI	LIVER	423	HEPATOCELLULAR CARCINOMA	0	0	4	0	0.5141	0.5601
LU	LUNG	221	BRONCHIOLO-ALVEOLAR ADENOMA	9	11	5	9	0.4655	0.4845
LU	LUNG	362	BRONCHIOLO-ALVEOLAR CARCINOMA	5	1	1	2	0.7486	0.7760
PI	PITUITARY	373	ADENOMA	0	1	1	0	0.6538	0.7652
PI	PITUITARY	729	CARCINOMA	0	1	0	0	0.7165	0.8130
RE	RECTUM	456	ADENOMA	0	0	0	1	0.3019	0.1368
SK	SKIN + SUBCUTIS	356	FIBROSARCOMA	0	3	1	0	0.7937	0.8525
SK	SKIN + SUBCUTIS	508	OSTEOSARCOMA	0	0	1	0	0.4527	0.6114
SK	SKIN + SUBCUTIS	572	SARCOMA - NOS	0	3	0	0	0.8132	0.8683
SK	SKIN + SUBCUTIS	587	FIBROMA	1	0	0	0	1.0000	0.8774
SK	SKIN + SUBCUTIS	723	FIBROEPITHELIAL POLYP	1	0	0	0	1.0000	0.8774
SP	SPLEEN	736	HAEMANGIOSARCOMA	0	1	0	0	0.6765	0.8002
ST	STOMACH	738	SQUAMOUS CELL PAPILLOMA	0	1	0	0	0.6765	0.8002
TE	TESTIS	402	INTERSTITIAL CELL ADENOMA	0	0	0	3	0.0184	0.0058

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/s/

Roswitha Kelly
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Concur with review