CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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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 STUDY

IND/NDA Number:	NDA 211855
Drug Name:	Diroximel Fumarate, RDC-5108
Indication(s):	Relapsing Forms of Multiple Sclerosis.
Studies	One Two Year Oral Gavage Carcinogenicity Study in Rats and One Six-Month Oral Gavage Carcinogenicity Study in rasH2 Transgenic Mice.
Applicant:	Sponsor: Alkermes, Inc 852 Winter St Waltham, Massachusetts 024511420 USA Test facility for rat study: (b) (4) (b) (4) Test facility for mouse study: (b) (4) (b) (4)
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Biometrics Division:	Division of Biometrics -VI
Statistical Reviewer:	Malick Mbodj, Ph.D.
Secondary Reviewer:	Hepei Chen
Concurring Reviewer:	Karl Lin, Ph.D.
Medical Division:	Division of Neurology Products
Reviewing Pharmacologist:	Melissa, Banks-Muckenfuss, PhD
Project Manager:	Folkendt, Sandra
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1. Background

In this submission, the sponsor included reports of two animal carcinogenicity studies, one in regular rats and one in transgenic mice. These studies were intended to assess the carcinogenic potential of RDC-5108 in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks in rats and 26 weeks in mice. Results of this review have been discussed with the reviewing pharmacologist Dr. Salicru.

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

2. Rat Study

In this study two separate experiments were conducted, one in male rats and one in female rats. In each of these two experiments there were three treated groups, one water control group and one vehicle control group. Three hundred and thirty Sprague-Dawley rats of each sex were assigned to three treated groups one water control group and one vehicle control group by a stratified randomization scheme designed to achieve similar group mean body weights, as indicated in Table 1. The dose levels for the three treated groups were 15, 50, and 150 mg/kg/day for males and females, for up to 104 weeks. In this review, these dose groups were referred to as the low, medium, and high dose group, respectively, the group size is 70 animals for controls groups and high dose group, and 60 animals for low and medium dose groups. The water control group received distilled water, the vehicle control group received the vehicle [(b) (4) (b) (4) (b) (4) , administered orally by gavage for about 104 weeks in the same manner as the treated groups. Due to early termination criteria in the male and female rats, the study was terminated early based on achieving survival criteria and Sponsor interactions with the US FDA, early final scheduled necropsies were conducted (based on FDA recommendations) at Week 90 and at Week 94 for all surviving male and female rats, respectively.

Table 1: Experimental Design in Rat Study

Group Name	Group N0.	Dose Level (mg/kg/day)		Number of Animal	
		Male	Female	Males	Females
Water Control	1	0	0	70	70
Vehicle Control	2	0	0	70	70
Low	3	15	15	60	60
Medium	4	50	50	60	60
High	5	150	150	70	70

early final scheduled necropsies were conducted during Week 94 (females) and week 90 (males)

During the administration period, all animals were checked for morbidity, mortality, injury, and the availability of food and water twice daily. The animals were removed from the cage, and detailed observations were conducted for each animal prior to randomization on Day -2 and for each surviving main study animal weekly during the study. On occasion, clinical observations were recorded at unscheduled intervals. Detailed examinations for palpable masses were done weekly, the time of onset, location, size, appearance, and progression of each grossly visible or palpable mass, observed in carcinogenicity rats, was recorded weekly any animal showing signs of severe debility or intoxication, and if determined to be moribund or suffering excessively will be euthanized. Observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous

system effects including tremors, convulsions, reactivity to handling, and unusual behavior, and the palpation of masses. Histopathological examinations were performed on all animals found dead or killed moribund or sacrificed at the end of the experiment. Body weights for all animals were measured and recorded at receipt, prior to randomization and once weekly for the first 14 weeks, every two weeks until Week 28, every four weeks thereafter for the duration of the study, and on the day of the scheduled terminal necropsy.

2.1. Sponsor's analyses

2.1.1. Survival analysis

The Kaplan-Meier's curves were presented graphically for male and female rats separately. An overall test for survival was used to compare the homogeneity of survival rates across the groups using a log-rank test at the 0.05 significance level. If the survival rates were significantly different ($p < 0.05$), then a follow up analysis was done where each treatment group was compared to water and vehicle control group using a log-rank test.

Any animal with accidental injury that causes its death, or its unscheduled sacrifice was censored in the estimation. In addition, all animals still alive at the end of the experimental period were censored at the following day. Results of all pair-wise comparisons are reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests.

Sponsor's findings:

Sponsor's analysis showed the numbers of rats surviving to their terminal necropsy were 33 (47%), 20 (29%), 25 (42%), 19 (32%), and 22 (31%) in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 25 (36%), 20 (29%), 16 (27%), 20 (33%), and 24 (34%) in water control, vehicle control, low, medium, and high dose groups, in female rats, respectively. The sponsor's report concluded that, there was a statistically significant difference in mortality across the groups with the overall log rank test $P<0.05$ in male rats. The pairwise comparisons showed a statistically significant difference in mortality between the high dose groups and the water control group in male rats.

2.1.2. Tumor data analysis

Tumor incidence data were analyzed within each sex, using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test was calculated, and Fisher's exact test was used to compare each treatment group with the control groups. The Cochran-Armitage trend test was applied for all control and treatment groups and was arranged in a strictly increasing dose level. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto et al. All tumors in the scheduled terminal sacrifice interval were considered incidental for the purpose of statistical analysis.

The Poly-3 method was used to assess prevalence of tumors. The survival-adjusted rates based on the risk weights were displayed. The tests of significance included both an overall trend and pair-wise comparisons of each treatment group with the corresponding control. All p-values are reported using upper-tailed tests.

Adjustment for the multiplicity:

For multiplicity adjustment, the sponsor used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons. Site-specific background historical control database was used to determine whether the tumors should be designated as rare or common.

Sponsor's findings:

Following the multiple testing adjustment method described above, the sponsor's analysis showed statistically significant increasing overall trend test across the water control or vehicle control and the treated groups of male rats for the incidence of benign adenoma leydig cell in Testes. The pairwise comparisons showed statistically significant increasing in tumor incidences of benign adenoma leydig cell in Testes in the high dose group, when compare to the water control group and the vehicle control group in male rats.

Poly-3 pair-wise comparisons tests showed statistically significant in tumor incidences, of prostatic adenocarcinomas in males at medium dose group when compared with the water controls, and the incidence of subcutaneous fibrosarcomas and hemangiosarcomas in males at low dose group and pancreatic islet cell carcinomas in males at medium dose group when compared to the vehicle control males. All other statistical analyses of the incidence of these four tumors in RDC-5108-treated males compared to male water controls and vehicle controls using the Fisher's exact test, Cochran-Armitage overall trend test, Peto overall test, and Poly-3 overall trend test yielded no statistically significant findings. However, the incidence of these four tumors were not observed in males at 150 mg/kg, and there were no statistically significant increases in the incidence of these tumors in RDC-5108-treated females at any dose level compared to water or vehicle controls in the current study. Therefore, the higher incidences of these four tumors in males in these dose groups compared to water controls or vehicle controls were considered unlikely to be test article related.

No statistically significant findings were noted upon comparison of the incidence of tumors in RDC-5108-treated females to water controls or vehicle controls using statistical analyses that included Fisher's exact test, Poly-3 pair-wise test, Cochran-Armitage overall trend test, Peto overall test, and Poly-3 overall trend test.

2.2 Reviewer's analyses

To verify sponsor's analysis and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed the survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically on December 13, 2018 via SN0001.

It was noticed that male and female rats were dosed for at least up to 90 consecutive weeks or 94 consecutive weeks, respectively. The respective scheduled sacrifice interval was at Week 90 and at Week 94 for all surviving male and female rats, respectively.

2.2.1 Survival analysis

In the reviewer's analysis, intercurrent mortality data were analyzed using the Kaplan-Meier product limit method. The Kaplan-Meier's curves were presented graphically for male and female rats separately. The dose response relationship and homogeneity of survival distributions were tested for the treatment

groups using the Likelihood Ratio test and the Log-Rank test. The intercurrent mortality data are given in Tables 1A and 1B in the appendix for male and female rats, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1A and 1B in the appendix for male and female rats, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 2A and 2B in the appendix for male and female rats, respectively.

Reviewer's findings:

This reviewer's analysis showed the numbers of rats surviving to their terminal necropsy were 33 (47%), 20 (29%), 25 (42%), 19 (32%), and 22 (31%) in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 25 (36%), 20 (29%), 16 (27%), 20 (33%), and 24 (34%) in water control, vehicle control, low, medium, and high dose groups, in female rats, respectively. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three treated groups in either sex of rats. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of rats. However, the pairwise comparisons showed a statistically significant increase in mortality between the water control group and the vehicle control group in male rats.

2.2.2. Tumor data analysis

In the reviewer's analysis, the tumor data were analyzed for dose response relationship across vehicle control group and the treated groups, as well as the pairwise comparisons of vehicle control group with each of the treated groups using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method, an animal that lives the full study period ($w_{\max}$) or dies before the terminal sacrifice with development of the tumor type being tested gets a score of s_h =1. An animal that dies at Week w_h without development of the given tumor type before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}}\right)^k$ <1. The adjusted group size is defined as Σs_h . As an interpretation, an animal with score s_h =1 can be considered as a whole animal, while an animal with score s_h <1 can be considered as a partial animal. The adjusted group size Σs_h is equal to N (the original group size) if all animals live up to the end of the study or if each animal develops the given tumor being tested, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise comparison) tests using the Cochran-Armitage test. 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 [Gebregziabher and Hoel (2009), Moon et al. (2003), Portier, et al. (1986)]. Hence, this reviewer used k=3 for the analysis of the data. Based on the intent to treat (ITT) principle Wmax was considered as 105 for both male and female rats.

For the calculation of p-values, if there were less than 10 tumor bearing animals across all treatment groups for a given tumor type, the exact tests based on the discrete permutation distribution were used, with dose levels (0, 15, 50, and 150 for both male and female rats) as scores, and asymptotic tests were used for tumor types with higher incidences. The tumor rates and the p-values of the tested tumor types are listed in Tables 3A and 3B in the appendix for male rats and female rats, respectively.

Multiple testing adjustments:

Following the FDA more recently revised draft guidance for the carcinogenicity study design and data analysis 2015, for the two-year rat study this reviewer used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

A tumor is defined as a rare tumor if the published spontaneous rate or the spontaneous rate of the vehicle control of the tumor is less than 1%, and a common tumor is defined as one with tumor rate greater than or equal to 1%.

Reviewer's findings:

The tumor types with p-values less than 0.05 for dose response relationship and/or pairwise comparisons of vehicle control and treated groups are reported in Table 2.

Table 2: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons
Treated Groups and Vehicle Control Group in Rats

Sex	Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. W	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med (N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
Male	Liver	Adenoma, Hepatocellular, Benign	5/70 (37) 0.0488*	0/70 (29) 0.4173	2/60 (26) 0.2189	0/60 (24) NC	1/70 (26) 0.4727
	Pancreas	Carcinoma, Islet Cell, Malignant	1/70 (35) 0.5469	0/70 (29) 0.5168	0/60 (25) NC	5/60 (26) 0.0189*	0/70 (26) NC
	Skin, Subcutis	Fibrosarcoma, Malignant	1/70 (35) 0.5469	0/70 (29) 0.8648	4/60 (27) 0.0478*	2/60 (25) 0.2096	0/70 (26) NC
	Testes	Adenoma, Leydig Cell, Benign	2/70 (36) 0.5817	1/70 (29) <0.0001*	0/60 (25) 1.0000	1/60 (24) 0.7054	13/70 (33) 0.0006*
Female	Vagina	Granular Cell Tumor, Benign	0/70 (37) NC	0/70 (34) 0.0075*	0/60 (28) NC	1/60 (30) 0.4687	4/70 (35) 0.0606

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.
*: Statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively.

Following the multiple testing adjustment method described above, this reviewer's analysis showed statistically significant increasing dose response relationships across the vehicle control and the treated groups of male rats for the incidence of benign adenoma leydig cell in Testes and the incidence of benign granular cell tumor in vagina for female rats (p-value <0.0001, and = 0.0075, respectively). The pairwise comparisons test showed statistically significant increase in tumor incidences of malignant fibrosarcoma, in skin subcutis in the low dose group, malignant carcinoma islet cell in pancreas in medium dose group, and benign adenoma leydig cell in Testes in the high dose group, when compare to the vehicle control group in male rats (p-value=0.0478, =0.0189, =0.0006, respectively). Also, the pairwise comparisons test showed statistically significant increase in tumor incidences of benign adenoma, hepatocellular in the water control group, when compare to the vehicle control group in male rats (p-value=0.0488).

No other significant dose response relationship or pairwise comparisons were noted in either sex.

3. Mouse Study

Two separate experiments were conducted, one in male mice and one in female mice. In the experiment in males there were three treated groups (referred them as low, medium and high), one water control group, one vehicle control group, and one positive control group. However, in the experiment in females, there were four treated groups (referred them as low, medium, medium high, and high), one water control group, one vehicle control group, and one positive control group. One hundred and thirty-five Tg rasH2 transgenic male mice were assigned randomly to one of the six groups in the experiment in males, and one hundred sixty female mice were assigned randomly to one of the seven groups in the experiment in females, by a stratified randomization scheme designed to achieve similar group mean body weights in equal size of 25 animals except the positive control group which had 10 animals. The dose levels for treated groups were 30, 100, or 300 mg/kg/day for male mice and 30, 100, 300 or 1000 mg/kg/day for female mice, for up to 26 weeks as indicated in Table 3. In this review, these dose groups would be referred to as the low, medium, and high dose group, respectively, for male mice and the low, medium, medium-high, and high dose group, respectively, for female mice. The positive control mice of both sexes (Group 7) was administered with a total of three intraperitoneal injections of 1000 mg/kg of urethane in saline, once each on Days 1, 3 and 5. This group was included to verify sensitivity of the test system to detect carcinogenicity effect. The water control group received distilled water, the vehicle control group received the vehicle (b) (4) (b) (4), were administered in the same manner as the treated groups. The positive control mice were sacrificed by CO2 overdose on Day 75 (males) and Day 73 (females).

Table 3: Experimental Design in Mouse Study

Males				Females			
Group Name	**Group N0.**	**Dose Level (mg/kg/day)**	**Number of Animal**	**Group Name**	**Group N0.**	**Dose Level (mg/kg/day)**	**Number of Animal**
Water Control	1	0	25	Water Control	1	0	25
Vehicle Control	2	0	25	Vehicle Control	2	0	25
Low	3	30	25	Low	3	30	25
Medium	4	100	25	Medium	4	100	25
High	5	300	25	Meduim-High	5	300	25
Positive control	7	1000(urethane)	10	High	6	1000	25
				Positive control	7	1000(urethane)	10

The positive control was administered with 3 intra-peritoneal injections on Days 1, 3 and 5.
The surviving positive control animals were sacrificed on Day 73 (females), Day 75 (males)

During the study period, all animals were observed for general health/mortality and moribundity twice daily (at least 6 hours apart), abnormal findings were recorded throughout the study. Cage side observations were conducted for each carcinogenicity animal once daily within 2 hours after the last animal was dosed in each group. Detailed observations were conducted for each carcinogenicity animal at least once during the pre-dose phase, prior to dosing on Day 1, and weekly thereafter throughout the dosing phase. Observations will include, but will not be limited to, evaluation for reaction to treatment. The time of onset, location, size, appearance, and progression of each grossly visible or palpable mass, observed in carcinogenicity mice, was recorded at the same intervals as detailed observations, particular

attention being paid to the animals during and for the first hour after dosing. Any animal showing signs of severe debility or intoxication, and if determined to be moribund or suffering excessively will be euthanized. Histopathological examinations were performed on all animals found dead, killed moribund, or sacrificed at the end of the experiment. Body weights of individual animals were recorded pre-dose on Day 1, weekly through Week 13, and biweekly thereafter.

3.1. Sponsor's analyses

3.1.1 Survival analysis

The Kaplan-Meier's curves were presented graphically for male and female mice separately. The generalized Wilcoxon test for survival was used to compare the homogeneity of survival rates across the Vehicle Control (Group 2) and test article groups (Groups 3-5) in the experiment in males; and across the Vehicle Control (Group 2) and test article groups (Groups 3-6) in the experiment in females. The 0.05 significance level was used in the above test. If the survival rates were significantly different, the generalized Wilcoxon test was used to make pairwise comparisons of each treated group with the vehicle control group. Additionally, the Positive Control group (Group 7) and the Water Control group (Group 1) were compared to the Vehicle Control group (Group 2) using the generalized Wilcoxon test.

Survival times in which the status of the animal's death was classified as an accidental death, planned interim sacrifice or terminal sacrifice were considered censored values for the purpose of the Kaplan-Meier estimates and survival rate analyses.

Sponsor's findings:

Sponsor's analysis showed the numbers of mice surviving to their terminal necropsy were 23 (92%), 22(88%), 25 (100%), 25 (100%), and 24 (96%), in water control, vehicle control, low, medium, and high dose groups in male mice, respectively, and 25 (100%), 25 (100%), 24 (96%), 24 (96%), 25 (100%), and 22 (88%), in water control, vehicle control, low, medium, medium-high, and high dose groups in female mice, respectively. Also, the numbers of mice surviving to their interim necropsy for the positive control groups in male and female mice were 9 and 10 respectively. The sponsor's report concluded that there were no statistically significant findings in survival in either sex of mice.

3.1.2 Tumor data analysis

Tumor incidence data were analyzed within each sex, via Peto's mortality-adjusted method, without continuity correction, incorporating the context (incidental or fatal, or mortality-independent) in which tumors were observed. The incidence of each tumor type was analyzed with a one-sided trend test using the positive dose response relationship in tumor occurrence across vehicle control and treated groups. In addition, one-sided pairwise comparisons of vehicle control and treated groups group were conducted. The Vehicle Control group (Group 2) was compared to the Water Control group (Group 1) with a 1-sided test at both the 0.01 and 0.05 levels and all p-values were reported. The following fixed intervals were used for incidental tumor analyses: Days 1 to 120 and Days 121 through. (up to, but not including, scheduled terminal sacrifices), and scheduled terminal sacrifice, for male and female mice. The actual dose levels were used as the scores. Tumors classified as mortality-independent were analyzed with Peto's mortality-independent method incorporating the day of detection. All animals that died or were sacrificed after the first animal of that sex was terminally sacrificed were included in the scheduled terminal sacrifice interval for the incidental finding analyses. All tumors in the scheduled terminal sacrifice interval were considered incidental for the purpose of statistical analysis.

For the calculation of p-values, if there were less than 10 tumor bearing animals across all treatment groups for a given tumor type, the exact tests based on the discrete permutation distribution were used and asymptotic tests were used for tumor types with higher incidences.

Each diagnosed tumor type was analyzed separately. All metastases and invasive tumors will be considered secondary and not statistically analyzed. In addition, tumors were combined for analysis purposes at the discretion of the Study Director.

Tumor incidence in the positive control group will be compared to the water control group with a 1-sided Fisher's exact test at both the 0.01 and 0.05 significance levels.

Only the following tumors will be statistically analyzed: alveolar-bronchiolar adenoma and alveolar-bronchiolar carcinoma.

Multiple testing adjustment:

For multiplicity adjustment testing, no detailed information was provided in the sponsor's report.

Sponsor's findings:

There were no statistically significant tumor findings in the test article groups when compared to the Vehicle Control group in either sex of mice. There were no statistically significant tumor findings when comparing the Vehicle Control group with the Water Control group in either sex of mice. However, there were a statistically significant increase incidence of adenoma alveolar bronchiolar and the combined adenoma alveolar bronchiolar and carcinoma alveolar bronchiolar in the lung with bronchi in the positive control group, when compared to the water control group in both male and female mice (p-value <.0001, <.0001, and <.0001, <.0001, respectively).

3.2 Reviewer's analyses

Similar to the rat study, this reviewer independently performed the survival and tumor data analyses of the mouse study. For the analysis of the survival data and the tumor data of the mouse study, this reviewer used similar methodologies that were used for the analyses of the survival and tumor data of the rat study. Data used in this reviewer's analyses were provided by the sponsor electronically.

3.2.1 Survival analysis

The intercurrent mortality data are given in Tables 4A and 4B in the appendix for male and female mice, respectively. The Kaplan-Meier curves for death rate are given in Figures 2A and 2B in the appendix for male and female mice, respectively. Results for test of dose response relationship and homogeneity of survivals among treatment groups are given in Tables 5A and 5B in the appendix for male and female mice, respectively.

Reviewer's findings:

This reviewer's analysis showed the numbers of mice surviving to their terminal necropsy were 23 (92%), 22(88%), 25 (100%), 25 (100%), and 24 (96%), in water control, vehicle control, low, medium, and high dose groups in male mice, respectively, and 25 (100%), 25 (100%), 24 (96%), 24 (96%), 25 (100%), and 22

(88%), in water control, vehicle control, low, medium, medium-high, and high dose groups in female mice, respectively. Also, the numbers of mice surviving to their interim necropsy for the positive control groups in male and female mice were 9 and 10 respectively. This reviewer's analysis showed a statistically significant decrease mortality in low and medium dose groups when compared to the vehicle control group in male mice (p-value = 0.0384, =0.0384, respectively). Also, this reviewer's analysis showed a statistically significant increase mortality in high dose groups when compared to the vehicle control group in female mice (p-value = 0.0384)

3.2.2 Tumor data analysis

The tumor rates and the p-values of the tumor types tested for dose response relationship and the pairwise comparisons of vehicle control and treated groups are given in Table 6A and 6B in the appendix for male and female mice, respectively.

Multiple testing adjustment:

Also following the same FDA more recently revised draft guidance for the carcinogenicity study design and data analysis 2015, for mouse study the significance levels were 0.05 for all dose response relationship tests and pairwise comparisons, regardless of common or rare tumors.

Reviewer's findings:

The tumor types with p-values less than 0.05 for dose response relationship and/or pairwise comparisons of vehicle control and treated groups are reported in Table 4.

Table 4: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons
Treated Groups and Vehicle Control Group in Mice

Sex	Organ Name	Tumor Name	0 mg Water (N=25) P -VC vs.W	0 mg Vehicle (N=25) P - Trend	30 mg Low (N=25) P - VC vs. L	100 mg Med(N=25) P - VC vs. M	300 mg Med-High (N=25) P - VC vs. MH	1000 mg High (N=25) P - VC vs. C
Female	Thymus	Thymoma	0/25 (25) NC	0/25 (25) 0.0337*	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	2/25 (23) 0.2243

*: statistically significant at 0.05 level for rare and common tumor in dose response relationship and pairwise comparison.

Following the multiple testing adjustment method described above, this reviewer's analyses showed statistically significant increasing dose response relationships across the vehicle control and the treated groups of female mice for the incidence of thymoma, in thymus (p-value = 0.0337). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in RDC-5108 treated groups, when compare to the vehicle control group in either sex of mice.

The pairwise comparisons showed statistically significant increases in the positive control dose group for the incidences of alveolar-bronchiolar adenoma and the combined adenoma alveolar bronchiolar and carcinoma alveolar bronchiolar in lung with bronchi, when compared to the vehicle control group in both male and female mice (p-value <.0001, <.0001, and <.0001, <.0001, respectively).

4. Summary

In this submission, the sponsor included reports of two animal carcinogenicity studies, one in regular rats and one in transgenic mice. These studies were intended to assess the carcinogenic potential of RDC-5108 in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks in rats and 26 weeks in mice.

Rat Study:

In this study two separate experiments were conducted, one in male rats and one in female rats. In each of these two experiments there were three treated groups, one water control group and one vehicle control group. Three hundred and thirty Sprague-Dawley rats of each sex were assigned to three treated groups one water control group and one vehicle control group by a stratified randomization scheme designed to achieve similar group mean body weights, as indicated in Table 1. The dose levels for the three treated groups were 15, 50, and 150 mg/kg/day for males and females, for up to 104 weeks. In this review, these dose groups were referred to as the low, medium, and high dose group, respectively, the group size is 70 animals for controls groups and high dose group, and 60 animals for low and medium dose groups. The water control group received distilled water, the vehicle control group received the vehicle [(b) (4) (b) (4) (b) (4) , administered orally by gavage for about 104 weeks in the same manner as the treated groups. Due to early termination criteria in the male and female rats, the study was terminated early based on achieving survival criteria and Sponsor interactions with the US FDA, early final scheduled necropsies were conducted (based on FDA recommendations) at Week 90 and at Week 94 for all surviving male and female rats, respectively.

This reviewer's analysis showed the numbers of rats surviving to their terminal necropsy were 33, 20, 25, 19, and 22 in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 25, 20, 16, 20, and 24 in water control, vehicle control, low, medium, and high dose groups, in female rats, respectively. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three treated groups in either sex of rats. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of rats. However, the pairwise comparisons showed a statistically significant increase in mortality between the water control group and the vehicle control group in male rats.

For tumor data, following the multiple testing adjustment method described above, this reviewer's analysis showed statistically significant increasing dose response relationships across the vehicle control and the treated groups of male rats for the incidence of benign adenoma leydig cell in Testes and the incidence of benign granular cell tumor in vagina for female rats (p-value <0.0001, and = 0.0075, respectively). The pairwise comparisons test showed statistically significant increase in tumor incidences of malignant fibrosarcoma, in skin subcutis in the low dose group, malignant carcinoma islet cell in pancreas in medium dose group, and benign adenoma leydig cell in Testes in the high dose group, when compare to the vehicle control group in male rats (p-value=0.0478, =0.0189, <0.0006, respectively). Also, the pairwise comparisons test showed statistically significant increase in tumor incidences of benign adenoma, hepatocellular in the water control group, when compare to the vehicle control group in male rats (p-value=0.0488).

No other significant dose response relationship or pairwise comparisons were noted in either sex.

Mouse Study:

Two separate experiments were conducted, one in male mice and one in female mice. In the experiment in males there were three treated groups (referred them as low, medium and high), one water control group, one vehicle control group, and one positive control group. However, in the experiment in females, there were four treated groups (referred them as low, medium, medium high, and high), one water control group, one vehicle control group, and one positive control group. One hundred and thirty-five Tg rasH2 transgenic male mice were assigned randomly to one of the six groups, and one hundred sixty female mice were assigned randomly to one of the seven groups, by a stratified randomization scheme designed to achieve similar group mean body weights in equal size of 25 animals except the positive control group which had 10 animals. The dose levels for treated groups were 30, 100, or 300 mg/kg/day for male and 30, 100, 300 or 1000 mg/kg/day for female mice, for up to 26 weeks as indicated in Table 3. In this review, these dose groups would be referred to as the low, medium, and high dose group, respectively, for male mice and the low, medium, medium-high, and high dose group, respectively, for female mice. The positive control mice of both sexes (Group 7) was administered with a total of three intraperitoneal injections of 1000 mg/kg of urethane in saline, once each on Days 1, 3 and 5. This group was included to verify sensitivity of the test system to detect carcinogenicity effect. The water control group received distilled water, the vehicle control group received the vehicle (b) (4) (b) (4) were administered in the same manner as the treated groups. The positive control mice were sacrificed by CO2 overdose on Day 75 (males) and Day 73 (females).

This reviewer's analysis showed the numbers of mice surviving to their terminal necropsy were 23, 22, 25, 25, and 24, in water control, vehicle control, low, medium, and high dose groups in male mice, respectively, and 25, 25, 24, 24, 25, and 22, in water control, vehicle control, low, medium, medium-high, and high dose groups in female mice, respectively. Also, the numbers of mice surviving to their interim necropsy for the positive control groups in male and female mice were 9 and 10 respectively. This reviewer's analysis showed a statistically significant decrease mortality in low and medium dose groups when compared to the vehicle control group in male mice (p-value = 0.0384, =0.0384, respectively). Also, this reviewer's analysis showed a statistically significant increase mortality in high dose group when compared to the vehicle control group in female mice (p-value = 0.0384)

For tumor data, following the multiple testing adjustment method described above, this reviewer's analyses showed statistically significant increasing dose response relationships across the vehicle control and the treated groups of female mice for the incidence of thymoma, in thymus (p-value = 0.0337). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in RDC-5108 treated groups, when compare to the vehicle control group in either sex of mice.

The pairwise comparisons showed statistically significant increases in the positive control dose group for the incidences of alveolar-bronchiolar adenoma and the combined adenoma alveolar bronchiolar and carcinoma alveolar bronchiolar in lung with bronchi, when compared to the vehicle control group in both male and female mice (p-value <.0001, <.0001, and <.0001, <.0001, respectively).

Malick Mbodj, Ph.D.
Mathematical Statistician

Concur: Karl Lin, Ph.D. Team Leader, DBVI
Hepei Chen, secondary reviewer

cc:
Archival NDA 211855- RDC-5108
Dr. Tsong Ms. Patrician
Dr. Lin Folkendt, Sandra
Dr. Banks-Muckenfuss

5. Appendix

Table 1A: Intercurrent Mortality Rate
Male Rats

	0 mg|kg|day Water Control		0 mg|kg|day Vehicle Control		15 mg|kg|day Low		50 mg|kg|day Medium		150 mg|kg|day High	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 52	2	2.86	11	15.71	10	16.67	8	13.33	12	17.14
53 - 78	17	27.14	21	45.71	17	45.00	24	53.33	29	58.57
79 - 90	18	52.86	18	71.43	8	58.33	9	68.33	7	68.57
Ter. Sac.	33	47.14	20	28.57	25	41.67	19	31.67	22	31.43

Early scheduled sacrifice at Week 90 for all surviving male

Table 1B: Intercurrent Mortality Rate
Female Rats

	0 mg|kg|day Water Control		0 mg|kg|day Vehicle Control		15 mg|kg|day Low		50 mg|kg|day Medium		150 mg|kg|day High	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 52	2	2.86	2	2.86	4	6.67	1	1.67	9	12.86
53 - 78	22	34.29	25	38.57	22	43.33	20	35.00	16	35.71
79 - 94	21	64.29	23	71.43	18	73.33	19	66.67	21	65.71
Ter. Sac.	25	35.71	20	28.57	16	26.67	20	33.33	24	34.29

Early scheduled sacrifice at Week 94 for all surviving female

Table 2A: Intercurrent Mortality Comparison for
Male Rats

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High
Dose-Response (Likelihood Ratio)	0.0142*	0.3578	0.2940	0.9730	0.6573
Homogeneity (Log-Rank)	0.0125*	0.5447	0.2895	0.9726	0.6535

Table 2B: Intercurrent Mortality Comparison for
Female Rats

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High
Dose-Response (Likelihood Ratio)	0.2963	0.4167	0.6603	0.5837	0.5522
Homogeneity (Log-Rank)	0.2894	0.7229	0.6545	0.5785	0.5465

Table 3A: Tumor Rates and P-Values for Dose Response Relationship and the pairwise comparisons

Male Rats Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. W	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med(N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
Adrenal Glands	Adenoma, Cortical, Benign	4/70 (37) 0.6184	3/70 (30) 0.7330	4/60 (27) 0.4393	2/60 (25) 0.7621	2/70 (27) 0.7892
	Pheochromocytoma, Benign	14/70 (41) 0.1875	7/70 (32) 0.5265	8/60 (28) 0.3817	10/60 (28) 0.1842	7/70 (29) 0.5366
	Pheochromocytoma, Malignant	1/70 (35) 0.5469	0/70 (29) 0.1006	1/60 (26) 0.4727	0/60 (24) NC	2/70 (26) 0.2189
	Pheochromocytoma Benign/Malignant	15/70 (41) 0.1350	7/70 (32) 0.3435	9/60 (29) 0.3011	10/60 (28) 0.1842	9/70 (30) 0.3299
Brain	Astrocytoma, Malignant	2/70 (36) 0.7592	2/70 (30) 1.0000	0/60 (25) 1.0000	0/60 (24) 1.0000	0/70 (26) 1.0000
	Ependymoma, Malignant	0/70 (35) 1.0000	1/70 (30) 1.0000	0/60 (25) 1.0000	0/60 (24) 1.0000	0/70 (26) 1.0000
	Granular Cell Tumor, Benign	1/70 (35) 0.5469	0/70 (29) 0.7238	1/60 (26) 0.4727	0/60 (24) NC	0/70 (26) NC
	Oligodendroglioma, Malignant	0/70 (35) 1.0000	1/70 (30) 1.0000	0/60 (25) 1.0000	0/60 (24) 1.0000	0/70 (26) 1.0000
Eyes	Melanoma, Amelanotic, Malignant	0/70 (35) NC	0/70 (29) 0.4808	0/60 (25) NC	1/60 (24) 0.4528	0/70 (26) NC
Kidneys	Fibrosarcoma, Malignant	0/70 (35) 1.0000	1/70 (29) 1.0000	0/60 (25) 1.0000	0/60 (24) 1.0000	0/70 (26) 1.0000
	Polyp, Benign	0/70 (35) NC	0/70 (29) 0.7238	1/60 (26) 0.4727	0/60 (24) NC	0/70 (26) NC
Large Intestine, Cecum	Fibroma, Benign	1/70 (35) 0.5469	0/70 (29) NC	0/60 (25) NC	0/60 (24) NC	0/70 (26) NC
Liver	Adenoma, Hepatocellular, Benign	5/70 (37) 0.0488*	0/70 (29) 0.4173	2/60 (26) 0.2189	0/60 (24) NC	1/70 (26) 0.4727
Multicentric Neoplasm	Hemangiosarcoma, Malignant	1/70 (35) 0.5469	0/70 (29) 0.8715	3/60 (27) 0.1055	0/60 (24) NC	0/70 (26) NC
	Leukemia, Granulocytic, Malignant	0/70 (35) NC	0/70 (29) 0.7238	1/60 (26) 0.4727	0/60 (24) NC	0/70 (26) NC
	Lymphoma, Malignant	4/70 (37) 0.6003	3/70 (31) 0.9939	1/60 (26) 0.9203	0/60 (24) 1.0000	0/70 (26) 1.0000
	Sarcoma, Histiocytic, Malignant	1/70 (36) 0.5538	0/70 (29) 0.7238	1/60 (26) 0.4727	0/60 (24) NC	0/70 (26) NC
Nose	Schwannoma, Malignant	0/70 (35) NC	0/70 (29) 0.2500	0/60 (25) NC	0/60 (24) NC	1/70 (26) 0.4727

Male Rats Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. W	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med(N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
Pancreas	Adenoma, Acinar Cell, Benign	1/70 (35) 0.5469	0/70 (29) NC	0/60 (25) NC	0/60 (24) NC	0/70 (26) NC
	Adenoma, Acinar-Islet Cell, Benign	0/70 (35) NC	0/70 (29) 0.2500	0/60 (25) NC	0/60 (24) NC	1/70 (26) 0.4727
	Adenoma, Islet Cell, Benign	0/70 (35) 1.0000	2/70 (30) 0.5452	0/60 (25) 1.0000	1/60 (24) 0.8363	1/70 (26) 0.8535
	Carcinoma, Islet Cell, Malignant	1/70 (35) 0.5469	0/70 (29) 0.5168	0/60 (25) NC	5/60 (26) 0.0189*	0/70 (26) NC
	Adenoma/Carcinoma, Islet Cell	1/70 (35) 0.9071	2/70 (30) 0.5207	0/60 (25) 1.0000	6/60 (27) 0.0954	1/70 (26) 0.8535
Parathyroid Glands	Adenoma, Benign	0/70 (35) NC	0/70 (29) 0.5904	0/60 (25) NC	3/60 (25) 0.0927	0/70 (26) NC
Pituitary Gland	Adenoma, Pars Distalis, Benign	41/70 (55) 0.8290	41/70 (51) 0.7458	34/60 (44) 0.7341	37/60 (44) 0.4224	32/70 (43) 0.8267
	Adenoma, Pars Intermedia, Benign	0/70 (35) NC	0/70 (29) 0.1830	0/60 (25) NC	1/60 (24) 0.4528	1/70 (27) 0.4821
	Adenoma, Pars Distalis / Pars Intermedia, Benign	41/70 (55) 0.8290	41/70 (51) 0.7247	34/60 (44) 0.7341	38/60 (45) 0.4025	33/70 (44) 0.8105
Preputial Glands	Carcinoma, Squamous Cell, Malignant	0/70 (35) NC	0/70 (29) 0.2500	0/60 (25) NC	0/60 (24) NC	1/70 (26) 0.4727
Prostate Gland	Adenocarcinoma, Malignant	0/70 (35) NC	0/70 (29) 0.4907	0/60 (25) NC	2/60 (25) 0.2096	0/70 (26) NC
Skin	Adenoma, Sebaceous Cell, Benign	1/70 (35) 0.9071	2/70 (30) 1.0000	0/60 (25) 1.0000	0/60 (24) 1.0000	0/70 (26) 1.0000
	Basal Cell Tumor, Benign	2/70 (36) 0.3029	0/70 (29) NC	0/60 (25) NC	0/60 (24) NC	0/70 (26) NC
	Carcinoma, Basal Cell, Malignant	0/70 (35) NC	0/70 (29) 0.4808	0/60 (25) NC	1/60 (24) 0.4528	0/70 (26) NC
	Carcinoma, Undifferentiated, Malignant	1/70 (36) 0.5538	0/70 (29) NC	0/60 (25) NC	0/60 (24) NC	0/70 (26) NC
	Fibrosarcoma, Malignant	0/70 (35) NC	0/70 (29) 0.6005	1/60 (26) 0.4727	1/60 (24) 0.4528	0/70 (26) NC
	Hair Follicle Tumor, Benign	0/70 (35) NC	0/70 (29) 0.2500	0/60 (25) NC	0/60 (24) NC	1/70 (26) 0.4727
	Keratoacanthoma, Benign	3/70 (37) 0.7583	3/70 (30) 0.8739	4/60 (27) 0.4393	3/60 (25) 0.5730	1/70 (26) 0.9254
	Papilloma, Squamous Cell, Benign	0/70 (35) 1.0000	1/70 (30) 0.6569	3/60 (27) 0.2666	3/60 (26) 0.2531	1/70 (26) 0.7175

Male Rats Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. W	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med(N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
	Keratoacanthoma/ Papilloma, Squamous Cell, Benign	3/70 (37) 0.8525	4/70 (31) 0.8647	6/60 (28) 0.3000	4/60 (26) 0.5417	2/70 (27) 0.8685
Skin, Subcutis	Fibroma, Benign	3/70 (36) 0.9699	7/70 (33) 0.4889	1/60 (26) 0.9937	1/60 (24) 0.9916	4/70 (27) 0.8341
	Fibrosarcoma, Malignant	1/70 (35) 0.5469	0/70 (29) 0.8648	4/60 (27) 0.0478*	2/60 (25) 0.2096	0/70 (26) NC
	Lipoma, Benign	3/70 (36) 0.1635	0/70 (29) NC	0/60 (25) NC	0/60 (24) NC	0/70 (26) NC
	Fibroma/Fibrosarcoma	4/70 (37) 0.9366	7/70 (33) 0.7217	5/60 (28) 0.7409	3/60 (25) 0.8999	4/70 (27) 0.8341
Stomach, Nonglandular	Carcinoma, Squamous Cell, Malignant	0/70 (35) NC	0/70 (29) 0.1786	0/60 (25) NC	1/60 (25) 0.4630	1/70 (26) 0.4727
	Papilloma, Squamous Cell, Benign	0/70 (35) NC	0/70 (29) 0.4808	0/60 (25) NC	1/60 (24) 0.4528	0/70 (26) NC
Testes	Adenoma, Leydig Cell, Benign	2/70 (36) 0.5817	1/70 (29) <0.0001*	0/60 (25) 1.0000	1/60 (24) 0.7054	13/70 (33) <0.0006*
Thymus	Thymoma, Malignant	0/70 (35) 1.0000	1/70 (29) 0.8114	1/60 (26) 0.7266	1/60 (25) 0.7163	0/70 (26) 1.0000
Thyroid Gland	Adenoma, C-Cell, Benign	7/70 (38) 0.8134	8/70 (33) 0.9406	4/60 (27) 0.8922	4/60 (26) 0.8787	2/70 (26) 0.9826
	Adenoma, Follicular Cell, Benign	2/70 (36) 0.8737	3/70 (30) 0.3166	1/60 (26) 0.9254	1/60 (24) 0.9133	3/70 (28) 0.6308
	Carcinoma, C-Cell, Malignant	0/70 (35) NC	0/70 (29) 0.7875	2/60 (26) 0.2189	0/60 (24) NC	0/70 (26) NC
	Carcinoma, Follicular Cell, Malignant	0/70 (35) NC	0/70 (29) 0.7238	1/60 (26) 0.4727	0/60 (24) NC	0/70 (26) NC
	Adenoma/Carcinoma C_Cell	7/70 (38) 0.8134	8/70 (33) 0.9644	6/60 (28) 0.7126	4/60 (26) 0.8787	2/70 (26) 0.9826
	Adenoma/Carcinoma Follicular Cell	2/70 (36) 0.8737	3/70 (30) 0.4018	2/60 (26) 0.7762	1/60 (24) 0.9133	3/70 (28) 0.6308
Tongue	Papilloma, Squamous Cell, Benign	0/70 (35) NC	0/70 (29) 0.2500	0/60 (25) NC	0/60 (24) NC	1/70 (26) 0.4727
Zymbal`S Gland	Carcinoma, Zymbals Gland, Malignant	0/70 (35) NC	0/70 (29) 0.1061	1/60 (26) 0.4727	0/60 (24) NC	2/70 (27) 0.2279
	Papilloma, Squamous Cell, Benign	0/70 (35) 1.0000	1/70 (29) 1.0000	0/60 (25) 1.0000	0/60 (24) 1.0000	0/70 (26) 1.0000

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and the pairwise comparisons

Female Rats Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. C	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med(N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
Adrenal Glands	Adenoma, Cortical, Benign	5/70 (39) 0.1342	1/70 (34) 0.4664	0/60 (28) 1.0000	4/60 (31) 0.1501	1/70 (34) 0.7537
	Ganglioneuroma, Benign	0/70 (37) 1.0000	1/70 (34) 1.0000	0/60 (28) 1.0000	0/60 (30) 1.0000	0/70 (34) 1.0000
	Pheochromocytoma, Benign	1/70 (37) 0.8903	2/70 (35) 0.6229	0/60 (28) 1.0000	0/60 (30) 1.0000	1/70 (34) 0.8751
	Pheochromocytoma, Malignant	1/70 (37) 0.5211	0/70 (34) 0.7302	1/60 (28) 0.4516	0/60 (30) NC	0/70 (34) NC
	Pheochromocytoma Benign/Malignant	2/70 (38) 0.7234	2/70 (35) 0.7096	1/60 (28) 0.8352	0/60 (30) 1.0000	1/70 (34) 0.8751
Brain	Astrocytoma, Malignant	0/70 (37) NC	0/70 (34) 0.5118	0/60 (28) NC	1/60 (31) 0.4769	0/70 (34) NC
	Granular Cell Tumor, Benign	1/70 (37) 0.5211	0/70 (34) 0.0701	0/60 (28) NC	1/60 (30) 0.4687	2/70 (34) 0.2463
	Oligodendroglioma, Malignant	2/70 (38) 0.2750	0/70 (34) 0.3177	1/60 (28) 0.4516	1/60 (30) 0.4687	1/70 (35) 0.5072
Esophagus	Carcinoma, Squamous Cell, Malignant	1/70 (37) 0.5211	0/70 (34) NC	0/60 (28) NC	0/60 (30) NC	0/70 (34) NC
Heart	Schwannoma, Endocardial, Benign	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
Kidneys	Nephroblastoma, Malignant	0/70 (37) NC	0/70 (34) 0.7323	1/60 (29) 0.4603	0/60 (30) NC	0/70 (34) NC
Liver	Adenocarcinoma (Primary Site Unknown), M	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
	Cholangioma, Benign	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
Mammary Gland	Adenocarcinoma, Malignant	17/70 (44) 0.9646	26/70 (47) 0.8506	23/60 (40) 0.5053	22/60 (41) 0.6446	21/70 (45) 0.8505
	Adenoma, Benign	2/70 (38) 0.2750	0/70 (34) 0.1231	1/60 (28) 0.4516	0/60 (30) NC	2/70 (35) 0.2536
	Carcinosarcoma, Malignant	0/70 (37) 1.0000	2/70 (35) 1.0000	0/60 (28) 1.0000	0/60 (30) 1.0000	0/70 (34) 1.0000
	Fibroadenoma, Benign	28/70 (48) 0.9217	35/70 (50) 0.9267	24/60 (39) 0.8562	21/60 (39) 0.9630	23/70 (43) 0.9682
	Adenocarcinoma/Adenoma	19/70 (45) 0.9287	26/70 (47) 0.8210	23/60 (40) 0.5053	22/60 (41) 0.6446	22/70 (46) 0.8239

Female Rats Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. C	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med(N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
Multicentric Neoplasm	Lymphoma, Malignant	1/70 (38) 0.7736	1/70 (35) 0.6475	2/60 (29) 0.4288	3/60 (32) 0.2734	1/70 (35) 0.7536
Ovaries	Sarcoma, Histiocytic, Malignant	2/70 (38) 0.5422	1/70 (34) 0.2940	1/60 (28) 0.7033	1/60 (30) 0.7217	2/70 (35) 0.5110
	Sex-Cord/Stromal Tumor, Benign	0/70 (37) 1.0000	1/70 (34) 0.8321	1/60 (28) 0.7033	1/60 (30) 0.7217	0/70 (34) 1.0000
Pancreas	Adenoma, Islet Cell, Benign	1/70 (37) 0.5211	0/70 (34) NC	0/60 (28) NC	0/60 (30) NC	0/70 (34) NC
	Carcinoma, Acinar Cell, Malignant	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
	Carcinoma, Islet Cell, Malignant	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
Parathyroid Glands	Adenoma, Benign	1/70 (37) 0.5211	0/70 (34) 0.5079	0/60 (28) NC	1/60 (30) 0.4687	0/70 (34) NC
Pituitary Gland	Adenoma, Pars Distalis, Benign	57/70 (63) 0.8383	59/70 (63) 0.9480	50/60 (53) 0.5961	50/60 (55) 0.8176	51/70 (59) 0.9507
	Carcinoma, Pars Distalis, Malignant	0/70 (37) 1.0000	2/70 (35) 0.4212	1/60 (28) 0.8352	0/60 (30) 1.0000	2/70 (35) 0.6931
	Adenoma / Carcinoma, Pars Distalis	57/70 (63) 0.9217	61/70 (64) 0.9373	51/60 (54) 0.7373	50/60 (55) 0.9074	53/70 (60) 0.9621
Skin	Carcinoma, Basal Cell, Malignant	0/70 (37) 1.0000	1/70 (35) 1.0000	0/60 (28) 1.0000	0/60 (30) 1.0000	0/70 (34) 1.0000
	Fibrosarcoma, Malignant	0/70 (37) 1.0000	1/70 (35) 1.0000	0/60 (28) 1.0000	0/60 (30) 1.0000	0/70 (34) 1.0000
	Keratoacanthoma, Benign	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
	Trichofolliculoma, Benign	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
Skin, Subcutis	Fibroma, Benign	1/70 (37) 0.7672	1/70 (35) 0.8753	2/60 (29) 0.4288	1/60 (31) 0.7226	0/70 (34) 1.0000
	Fibrosarcoma, Malignant	0/70 (37) NC	0/70 (34) 0.2219	0/60 (28) NC	2/60 (31) 0.2236	1/70 (34) 0.5000
	Granular Cell Tumor, Benign	0/70 (37) NC	0/70 (34) 0.2698	0/60 (28) NC	0/60 (30) NC	1/70 (34) 0.5000
	Lipoma, Benign	2/70 (38) 0.2750	0/70 (34) 0.0744	0/60 (28) NC	0/60 (30) NC	2/70 (35) 0.2536
	Fibroma/Fibrosarcoma	1/70 (37) 0.7672	1/70 (35) 0.6353	2/60 (29) 0.4288	3/60 (32) 0.2734	1/70 (34) 0.7464

Female Rats Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. C	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med(N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
Thymus	Thymoma, Benign	1/70 (37) 0.7742	1/70 (34) 1.0000	0/60 (28) 1.0000	0/60 (30) 1.0000	0/70 (34) 1.0000
Thyroid Gland	Adenoma, C-Cell, Benign	4/70 (38) 0.9128	7/70 (37) 0.9355	3/60 (29) 0.9070	4/60 (32) 0.8546	2/70 (35) 0.9827
	Adenoma, Follicular Cell, Benign	3/70 (38) 0.1414	0/70 (34) 0.4613	2/60 (29) 0.2079	0/60 (30) NC	1/70 (35) 0.5072
	Carcinoma, C-Cell, Malignant	1/70 (37) 0.7742	1/70 (34) 0.4179	0/60 (28) 1.0000	1/60 (31) 0.7303	1/70 (34) 0.7537
	Carcinoma, Follicular Cell, Malignant	0/70 (37) NC	0/70 (34) 0.3281	1/60 (28) 0.4516	0/60 (30) NC	1/70 (35) 0.5072
	Adenoma/Carcinoma C-Cell	5/70 (39) 0.9075	8/70 (37) 0.9559	3/60 (29) 0.9428	5/60 (32) 0.8270	2/70 (35) 0.9912
	Adenoma/Carcinoma Follicular Cell	3/70 (38) 0.1414	0/70 (34) 0.3398	3/60 (29) 0.0920	0/60 (30) NC	2/70 (35) 0.2536
Uterus With Cervix	Adenocarcinoma, Malignant	1/70 (37) 0.5211	0/70 (34) NC	0/60 (28) NC	0/60 (30) NC	0/70 (34) NC
	Carcinoma, Squamous Cell, Malignant	0/70 (37) NC	0/70 (34) 0.7302	1/60 (28) 0.4516	0/60 (30) NC	0/70 (34) NC
	Granular Cell Tumor, Benign	0/70 (37) 1.0000	2/70 (35) 0.5895	0/60 (28) 1.0000	1/60 (30) 0.8502	1/70 (34) 0.8751
	Leiomyoma, Benign	0/70 (37) NC	0/70 (34) 0.5079	0/60 (28) NC	1/60 (30) 0.4687	0/70 (34) NC
	Polyp, Endometrial Stromal, Benign	4/70 (38) 0.7736	5/70 (37) 0.3493	6/60 (30) 0.3498	5/60 (32) 0.5349	7/70 (37) 0.3769
	Sarcoma, Endometrial Stromal, Malignant	0/70 (37) 1.0000	1/70 (35) 1.0000	0/60 (28) 1.0000	0/60 (30) 1.0000	0/70 (34) 1.0000
	Endometrial Stromal Sarcoma/Polyp	4/70 (38) 0.8564	6/70 (37) 0.4302	6/60 (30) 0.4648	5/60 (32) 0.6516	7/70 (37) 0.5000
	Sarcoma, Undifferentiated, Malignant	0/70 (37) NC	0/70 (34) 0.7323	1/60 (29) 0.4603	0/60 (30) NC	0/70 (34) NC
	Schwannoma, Malignant	1/70 (37) 0.7672	1/70 (35) 0.4128	0/60 (28) 1.0000	1/60 (31) 0.7226	1/70 (34) 0.7464
Vagina	Granular Cell Tumor, Benign	0/70 (37) NC	0/70 (34) 0.0075	0/60 (28) NC	1/60 (30) 0.4687	4/70 (35) 0.0606
	Leiomyoma, Benign	0/70 (37) NC	0/70 (34) 0.5118	0/60 (28) NC	1/60 (31) 0.4769	0/70 (34) NC

Female Rats Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=70) P - VC vs. C	0 mg Vehicle Cont (N=70) P - Trend	15 mg Low (N=60) P - VC vs. L	50 mg Med(N=60) P - VC vs. M	150 mg High (N=70) P - VC vs. H
	Polyp, Benign	0/70 (37) NC	0/70 (34) 0.7417	2/60 (29) 0.2079	1/60 (30) 0.4687	0/70 (34) NC
Zymbal`S Gland	Carcinoma, Zymbals Gland, Malignant	0/70 (37) NC	0/70 (34) 0.0744	0/60 (28) NC	0/60 (30) NC	2/70 (35) 0.2536

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

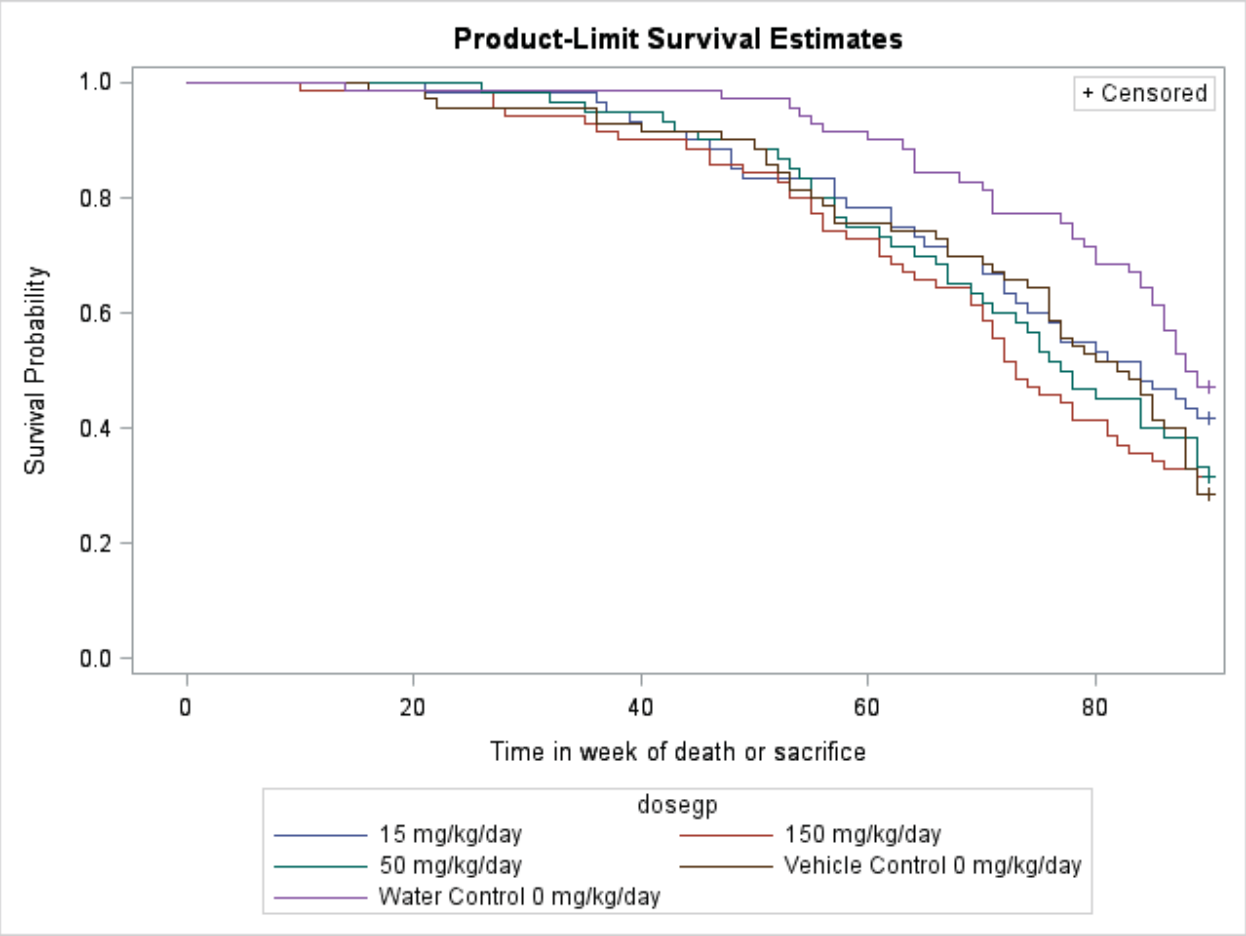


Figure 1B: Kaplan-Meier Survival Curves **for Female Rats**

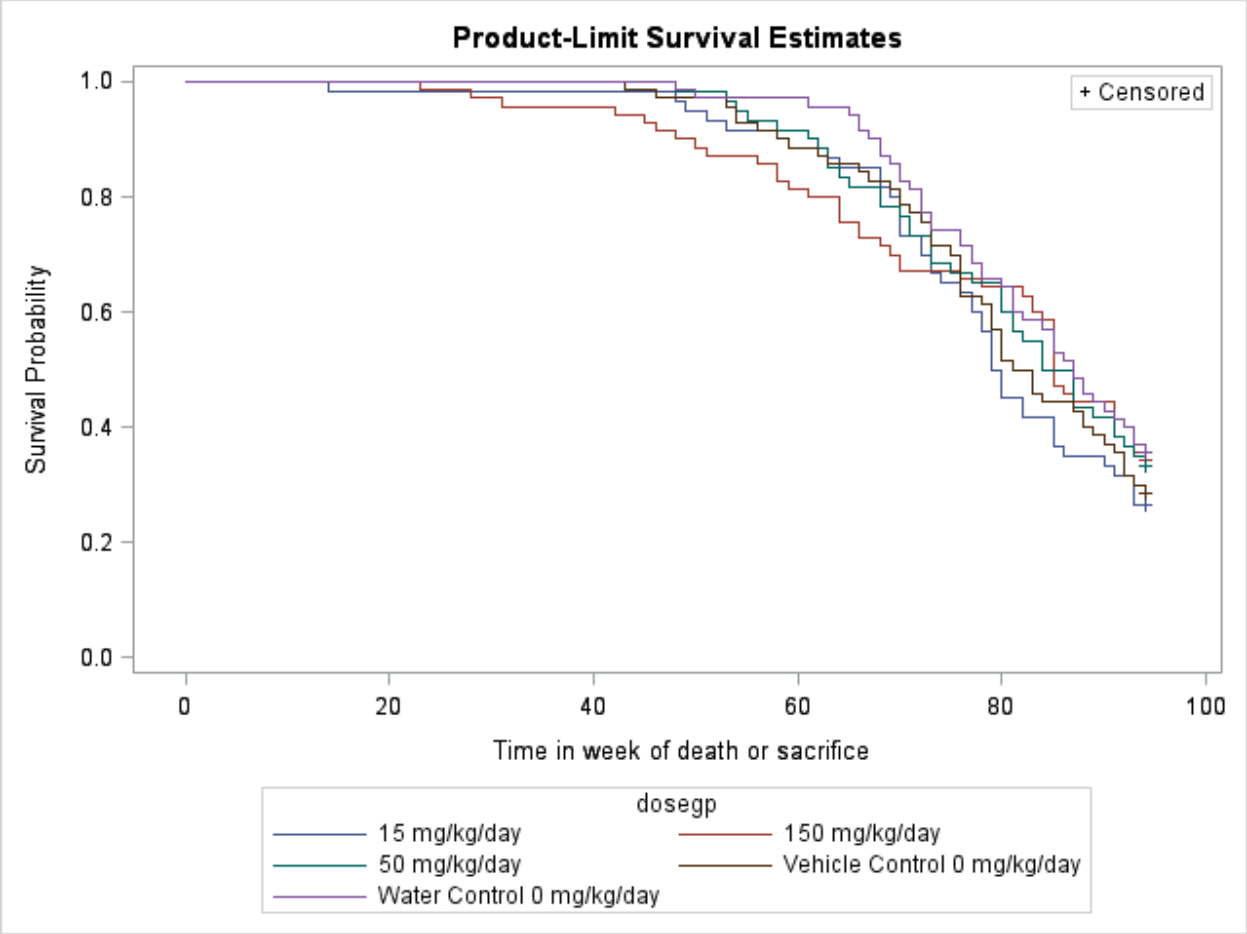


Table 4A: Intercurrent Mortality Rate
Male Mice

	0mg|kg|day Water Control		0mg|kg|day Vehicle Control		30 mg|kg|day Low		100mg|kg|day Medium		300 mg|kg|day High		1000 urethane Positive Control	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 13	.	.	.	.	.	.	.	.	.	.	1	10
IS											9	90
14 - 26	2	8.00	3	12.00	.	.	.	.	1	4.00		
Ter. Sac.	23	92.00	22	88.00	25	100.00	25	100.00	24	96.00		
Total	25	100.00	25	100.00	25	100.00	25	100.00	25	100.00	10	100.00

IS: Interim sacrifice on Day 75

Table 4B: Intercurrent Mortality Rate
Female Mice

	0mg|kg|day Water Control		0mg|kg|day Vehicle Control		30 mg|kg|day Low		100mg|kg|day Medium		300 mg|kg|day Med-High		1000 mg|kg|day High		1000 urethane Positive Control	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 13	.	.	.	.	.	.	.	.	.	.	1	4.00		
IS													10	100.00
14 - 26	.	.	.	.	1	4.00	1	4.00	.	.	2	12.00		
Ter. Sac.	25	100.00	25	100.00	24	96.00	24	96.00	25	100.00	22	88.00	.	.
Total	25	100.00	25	100.00	25	100.00	25	100.00	25	100.00	25	100.00	10	100.00

IS: Interim sacrifice on Day 73

Table 5A: Intercurrent Mortality Comparison for
Male Mice

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High	P-value for Vehicle Cont. vs Positive Cont.
Dose-Response (Likelihood Ratio)	0.6000	0.5594	0.0384*	0.0384*	0.3014	0.1134
Homogeneity (Log-Rank)	0.6007	0.1020	0.0770	0.0770	0.3097	0.1138

* = statistically significant at the 0.05 significance level.

Table 5B: Intercurrent Mortality Comparison for
Female Mice

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs Med-High	P-value for Vehicle Cont. vs High	P-value for Vehicle Cont. vs Positive Cont.
Dose-Response (Likelihood Ratio)	.	0.3724	0.2390	0.2390	.	0.0384*	.
Homogeneity (Log-Rank)	.	0.5681	0.3173	0.3173	.	0.0770	.

* = statistically significant at the 0.05 significance level.

Table 6A: Tumor Rates and P-Values for Dose Response Relationship and the Pairwise Comparisons
Male Mice Using Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=25) P -VC vs. W	0 mg Vehicle Cont (N=25) P - Trend	30 mg Low (N=25) P -VC vs. L	100 mg Med(N=25) P - VC vs. M	300 mg Med High (N=25) P - VC vs. MH	1000 urethane Posi (N=10) P -VC vs. PC
Cavity, Nasal	Hemangiosarcoma	0/25 (24) NC	0/25 (23) 0.3170	1/25 (25) 0.5208	0/25 (25) NC	1/25 (24) 0.5106	NC
Harderian Glands	Adenoma	1/25 (24) 0.5106	0/25 (23) 0.5052	0/25 (25) NC	1/25 (25) 0.5208	0/25 (24) NC	NC
Lungs With Bronchi	Alveolar-Bronchiolar Adenoma	2/25 (24) 0.5163	1/25 (23) 0.6360	5/25 (25) 0.1140	2/25 (25) 0.5319	2/25 (24) 0.5163	10/10 (10) <0.0001*
	Alveolar-Bronchiolar Carcinoma	1/25 (24) 0.5106	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	0/25 (24) NC	0/10 (1) NC
	Alveolar-Bronchiolar Adenoma/Carcinoma	3/25 (24) 0.3206	1/25 (23) 0.6322	5/25 (25) 0.1140	2/25 (25) 0.5319	2/25 (24) 0.5163	10/10 (10) <0.0001*
Multicentric	Mesothelioma	0/25 (24) 1.0000	1/25 (24) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/10 (1) NC
Prostate Gland	Hemangiosarcoma	1/25 (24) 0.5106	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	0/25 (24) NC	NC
Spleen	Hemangiosarcoma	2/25 (25) 0.2660	0/25 (23) 0.7474	2/25 (25) 0.2660	2/25 (25) 0.2660	0/25 (24) NC	NC
Thyroid Glands	Adenoma	1/25 (25) 0.5208	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	0/25 (24) NC	NC
Urinary Bladder	Hemangiosarcoma	0/25 (24) 1.0000	1/25 (23) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	NC

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

Female Mice Using Poly-3 test

Organ Name	Tumor Name	0 mg Water (N=25) P -VC vs.W	0 mg Vehicle (N=25) P - Trend	30 mg Low (N=25) P - VC vs. L	100 mg Med(N=25) P - VC vs. M	300 mg Med-High (N=25) P - VC vs. MH	1000 mg High (N=25) P - VC vs. C	1000 urethane Positive (N=25) P - VC vs. C
Harderian Glands	Adenoma	0/25 (25) NC	0/25 (25) 0.6496	2/25 (25) 0.2449	0/25 (25) NC	2/25 (25) 0.2449	0/25 (23) NC	NC
	Carcinoma	0/25 (25) 1.0000	1/25 (25) 0.3403	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	1/25 (23) 0.7340	NC
	Adenoma/ Carcinoma	0/25 (25) 1.0000	1/25 (25) 0.4474	2/25 (25) 0.5000	0/25 (25) 1.0000	2/25 (25) 0.5000	1/25 (23) 0.7340	0/10 (1) NC
Heart	Sarcoma	0/25 (25) NC	0/25 (25) 0.5935	0/25 (25) NC	1/25 (25) 0.5000	0/25 (25) NC	0/25 (23) NC	NC
Lungs with Bronchi	Alveolar-Bronchiolar Adenoma	0/25 (25) 1.0000	1/25 (25) 0.3194	0/25 (25) 1.0000	1/25 (25) 0.7551	1/25 (25) 0.7551	1/25 (23) 0.7340	10/10 (10) <0.0001*
	Alveolar-Bronchiolar Carcinoma	3/25 (25) 0.1173	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	1/10 (2) NC
	Alveolar-Bronchiolar Adenoma/Carcinoma	3/25 (25) 0.3046	1/25 (25) 0.3194	0/25 (25) 1.0000	1/25 (25) 0.7551	1/25 (25) 0.7551	1/25 (23) 0.7340	10/10 (10) <0.0001*
Spleen	Hemangiosarcoma	0/25 (25) NC	0/25 (25) 0.7401	2/25 (25) 0.2449	1/25 (25) 0.5000	3/25 (25) 0.1173	0/25 (23) NC	NC
Thymus	Thymoma	0/25 (25) NC	0/25 (25) 0.0337*	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	2/25 (23) 0.2243	NC

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

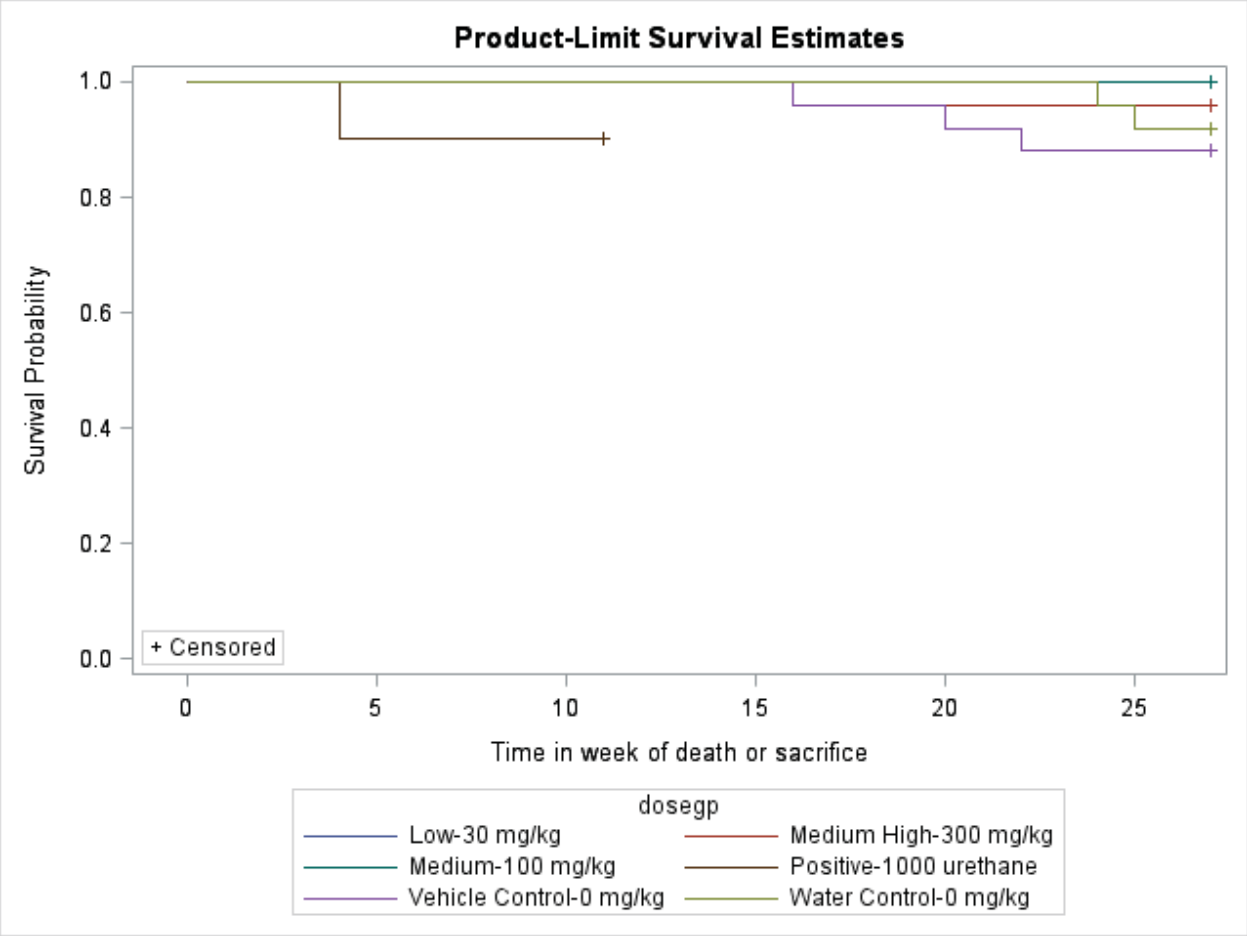
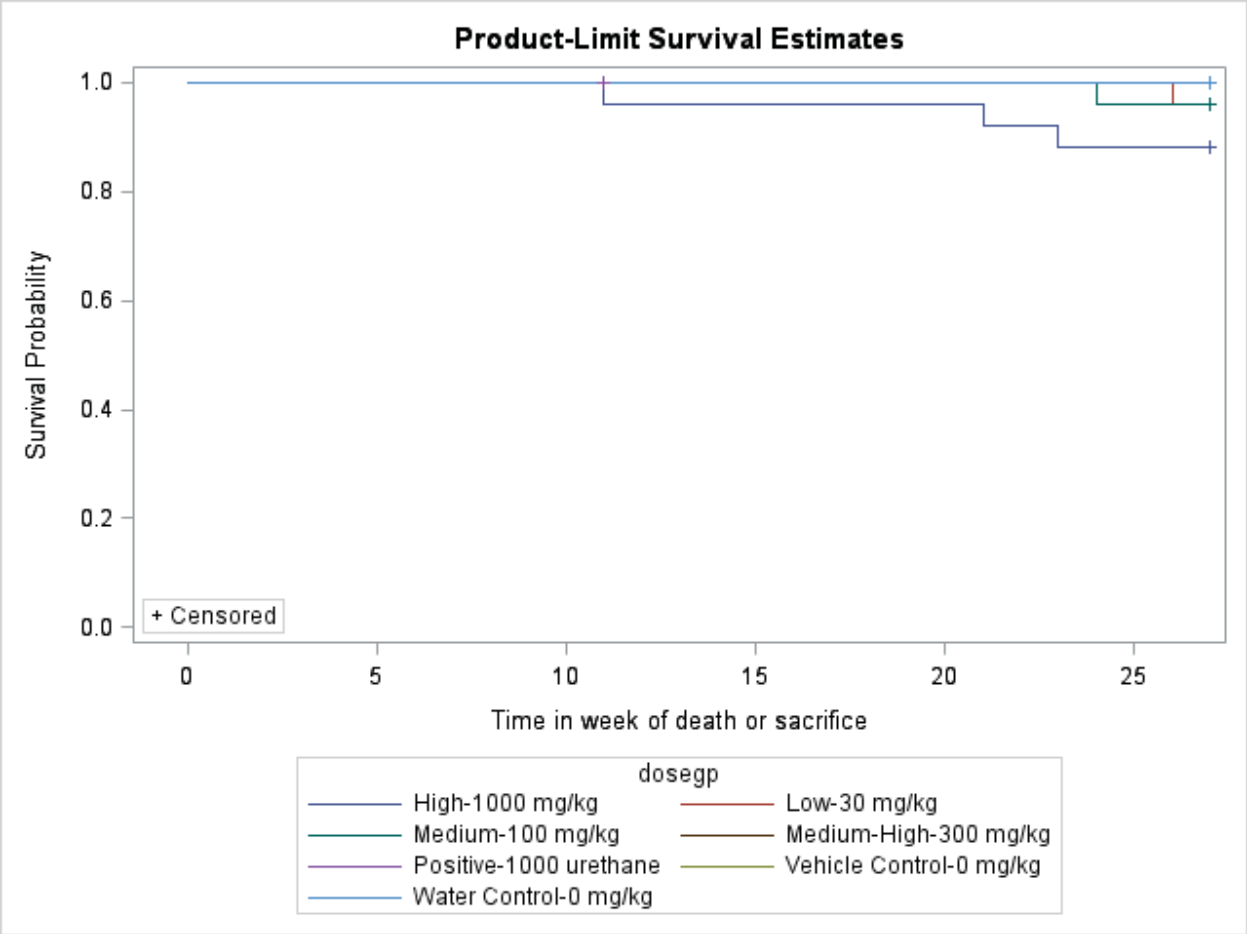


Figure 2B: Kaplan-Meier Survival Curves for
Female Mice



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