Evaluation of Carcinogenicity Study on Female Rats

To evaluate the sponsor's carcinogenicity study on female rats, this reviewer analyzed the sponsor's data. The reviewer's evaluation comprises the following:

- survival-data analysis
- tumor-data analysis

The reviewer's conclusions are summarized at the end of this section.

Survival-Data Analysis

The survival-data analysis determines whether the dose-mortality trend is statistically significant. A positive result indicates that the higher the dose level is, the more deaths are likely to occur.

Table 7 shows the number of female rats by treatment by age group. The dose levels labeled "CTRL1," "CTRL2," "LOW," "MED", "HIGH", and "MAXI" represent 0, 0, 1, 4, 16, and 63 mg/kg/day, respectively. Unlike the male rats, the study of female rats does not have the 8-mg/kg/day dose. The time interval "104-105" represents the terminal-sacrifice week.

Table 7. Number of Female Rats by Treatment and Age Group

Number of Animals
Species: Rat
Sex: Female

	Treatment Group						
	CTRL1	CTRL2	LOW	MED	HIGH	MAXI	Total
	Count	Count	Count	Count	Count	Count	Count
Time Interval							
0-62	3	1	1	2	4	7	18
63-78	7	7	5	7	14	10	50
79-91	8	4	8	5	14	11	51
92-103	10	19	9	15	7	8	68
104-105	31	29	37	31	21	24	173
Total	60	60	60	60	60	60	360

Source: d:\actos\rats\f\XAnimalX.txt

Table 8 describes, for the female rats, the number of deaths, the number at risk, and the cumulate percentages of deaths by treatment and age group.

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Table 8. Cumulative Percentages of Deaths in Female Rats

Analysis of Mortality
Species: Rat
Sex: Female

	Dose																	
	CTRL1			CTRL2			LOW			MED			HIGH			MAXI		
	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died
Time(-wks)																		
0-52	3	60	5.0	1	60	1.7	1	60	1.7	2	60	3.3	4	60	6.7	7	60	11.7
53-78	7	57	16.7	7	59	13.3	5	59	10.0	7	58	15.0	14	56	30.0	10	53	28.3
79-91	9	50	31.7	4	52	20.0	8	54	23.3	5	51	23.3	14	42	53.3	11	43	46.7
92-103	10	41	48.3	19	48	51.7	9	46	38.3	15	46	48.3	7	28	65.0	8	32	60.0
104-105	31	60	51.7	29	60	48.3	37	60	61.7	31	60	51.7	21	60	35.0	24	60	40.0

Source: d:\actos\rate\f\XAnimalX.txt

Figure 3 helps visualize the cumulative percentages of deaths over time by treatment. The HIGH and MAXI groups clearly shows dose-related mortalities compared with the two control groups.

Figure 3. Line Graph of Cumulative Percentages of Deaths in Female Rats

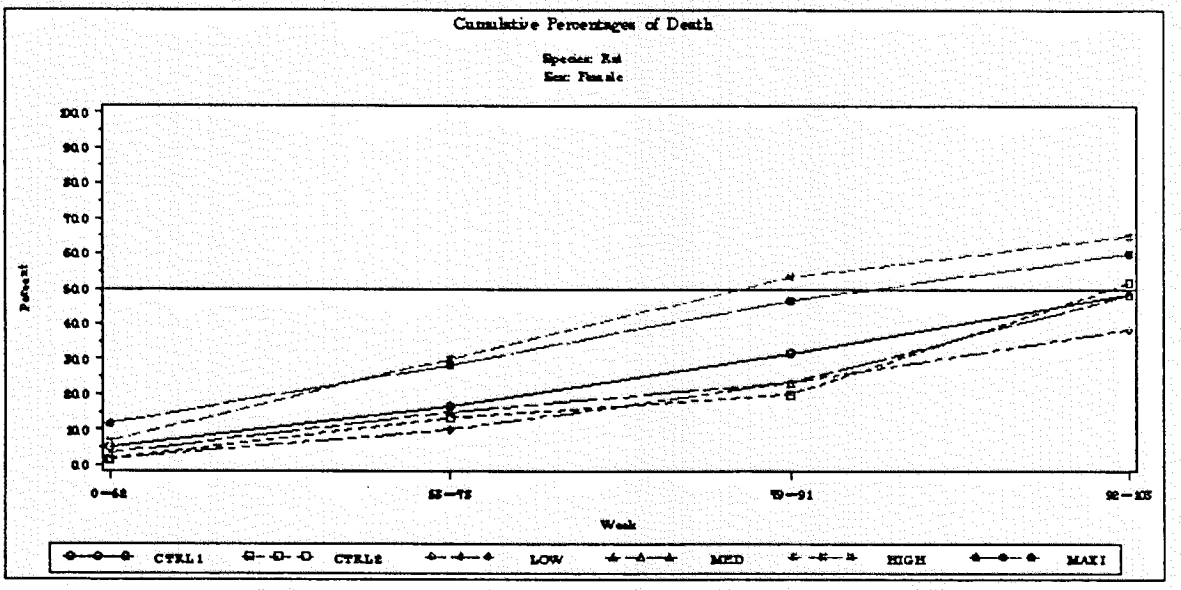
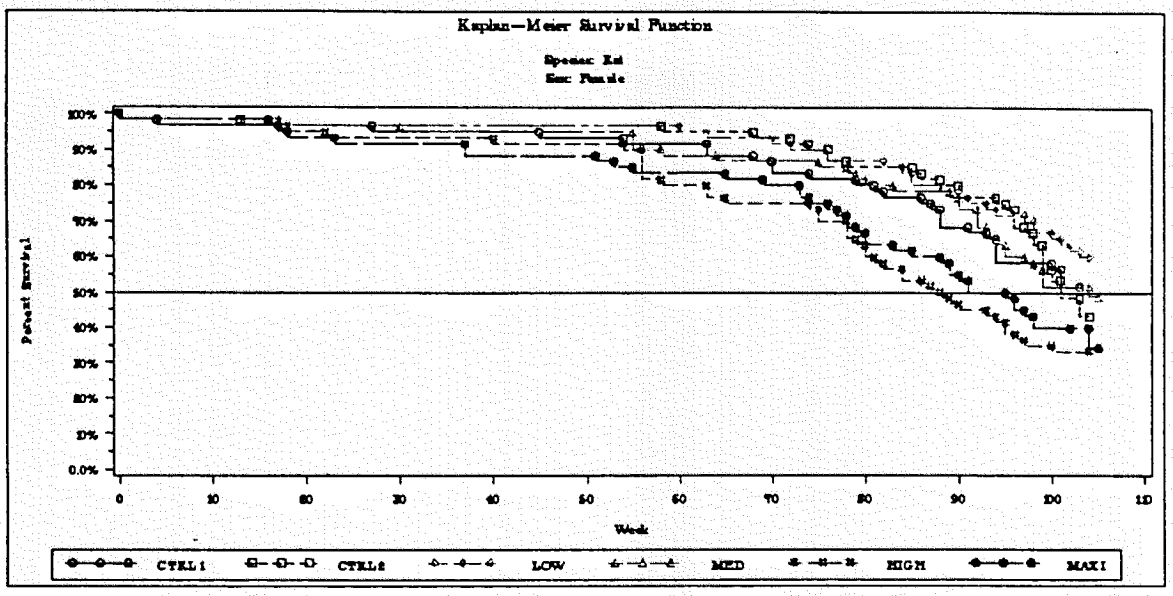


Figure 4 shows the Kaplan-Meier survival functions for female rats. The animals in the HIGH (16 mg/kg/day) and MAXI (63 mg/kg/day) groups died earlier than did those in other groups.

Figure 4. Kaplan-Meier Survival Functions for Female Rats



The test for dose-mortality trend (Table 9) shows significant results based on the Cox test (p=0.0078) and Kruskal-Wallis test (p=0.0022).

Table 9. Dose-Mortality Trend in Female Rats

Dose-Mortality Trend Tests

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Rat
Sex: Female

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	7.08	0.0078
	Depart from Trend	10.35	0.0349
	Homogeneity	17.43	0.0038
Kruskal-Wallis	Dose-Mortality Trend	9.36	0.0022
	Depart from Trend	12.46	0.0143
	Homogeneity	21.82	0.0006

Source: d:\actos\rats\f\XAnimalX.txt

This reviewer's survival-data analysis concludes that the mortality in female rats is positively dose-related. Therefore, the age-adjusted trend test detailed in the following section (Tumor-Data Analysis), is justified.

Tumor-Data Analysis

The tumor-data analysis determines whether the dose-tumor positive linear trend in tumor incidence is statistically significant. This reviewer tests this trend for every organ and tumor. The resulting p-values are

compared against the p-value cutoff points set by the following Agency's procedures. A significant result indicates a dose-tumor positive linear trend.

Statistical Procedure in Evaluation of Tumor-Data Analyses Currently Adopted by CDER Divisions of Biometrics

• For tumors found either fatal or non-fatal to all the animals, the statistical interpretation is based on the **exact test.**
• For tumors found fatal to some, but not to all animals, the statistical interpretation is based on the **asymptotic test,** resulting from the combined test. The asymptotic test uses the Z-statistic, which follows a standard normal distribution.
• To adjust for the effect of multiple testing, one can use a rule proposed by Haseman. A modified rule, proposed by the Divisions of Biometrics, CDER/FDA is applied to the trend tests in the review. In order to keep the overall type-I error at the level of about 0.1, this rule states:
  • Tumors with a spontaneous tumor rate of **1% or less** may be tested at the **0.025** significance level.
  • Otherwise, the **0.005** significance level may be used.

Table 10 quotes the significant trend-tests for female rats. This reviewer informs the reviewing pharmacologist the statistically significant dose-tumor positive linear trend in the female rats.

Table 10. Significant Trend-Tests for Female Rats

Organ	Tumor	Tumor-Bearing Animals	P-value
Skin, Subcutis (333)	Lipoma (508)	0 0 1 0 1 3	=0.000 (P<0.025)

Conclusions on Female-Rat Study

This reviewer informs the reviewing pharmacologist that the Actos™ is potentially carcinogenic (Table 10). Please note that the test could lead to a false conclusion due to chance alone. However, the probability of erroneously concluding a significant test is about 10% or less.

Evaluation of Carcinogenicity Study on Male Mice

To evaluate the sponsor's carcinogenicity study on male mice, this reviewer analyzed the sponsor's data. The reviewer's evaluation comprises the following:

- survival-data analysis
- tumor-data analysis

The reviewer's conclusions are summarized at the end of this section.

Survival-Data Analysis

The survival-data analysis determines whether the dose-mortality trend is statistically significant. A positive result indicates that the higher the dose level is, the more deaths are likely to occur.

Table 11 shows the number of male mice by treatment by age group. The dose levels labeled "CTRL1," "CTRL2," "LOW," "MED," "HIGH," and "MAXI" represent 0, 0, 3, 10, 30, and 100 mg/kg/day, respectively. The time interval "104-106" represents the terminal-sacrifice week.

Table 11. Number of Male Mice by Treatment and Age Group

Number of Animals
Species: Mouse
Sex: Male

	Treatment Group						
	CTRL1	CTRL2	LOW	MED	HIGH	MAXI	Total
	Count	Count	Count	Count	Count	Count	Count
Time Interval							
0-52	6	7	5	6	9	12	45
53-78	6	5	13	7	7	10	48
79-91	10	5	11	7	8	11	52
92-103	11	10	6	7	8	9	51
104-106	27	33	25	33	28	18	164
Total	60	60	60	60	60	60	360

Source: d:\actos\mice\m\XAnimalX.txt

Table 12 describes, for the male mice, the number of deaths, the number at risk, and the cumulate percentages of deaths by treatment and age group.

Table 12. Cumulative Percentages of Deaths in Male Mice

Analysis of Mortality
Species: Mouse
Sex: Male

	Dose																	
	CTRL1			CTRL2			LOW			MED			HIGH			MAXI		
	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died
Time(-wks)																		
0-52	6	60	10.0	7	60	11.7	5	60	8.3	6	60	10.0	8	60	15.0	12	60	20.0
53-78	6	54	20.0	5	53	20.0	13	55	30.0	7	54	21.7	7	51	26.7	10	48	36.7
79-91	10	48	36.7	5	48	28.3	11	42	48.3	7	47	33.3	8	44	40.0	11	38	55.0
92-103	11	38	55.0	10	43	45.0	6	31	58.3	7	40	45.0	8	36	53.3	8	27	70.0
104-106	27	60	45.0	33	60	55.0	25	60	41.7	33	60	55.0	28	60	46.7	18	60	30.0

Figure 5 helps visualize the cumulative percentages of deaths over time by treatment. The MAXI (100 mg/kg/day) group had the highest cumulative percentage of death among all groups.

Figure 5. Line Graph of Cumulative Percentages of Deaths in Male Mice

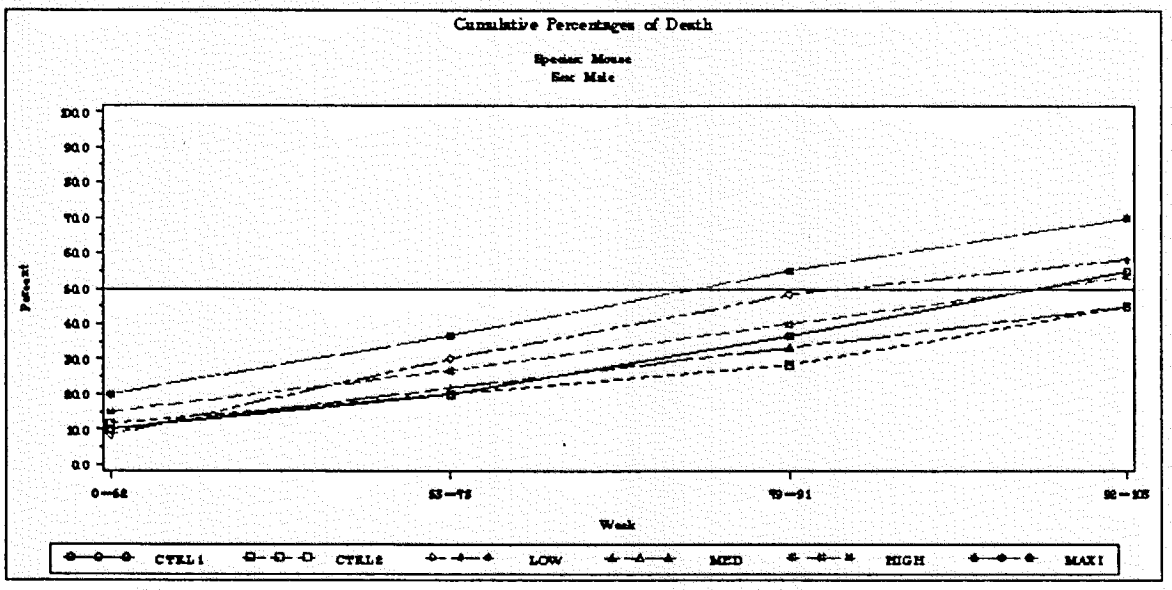


Figure 6 shows the Kaplan-Meier survival functions for male mice. The animals in the MAXI (100 mg/kg/day) group had a slightly lower survival rate than did those in other groups.

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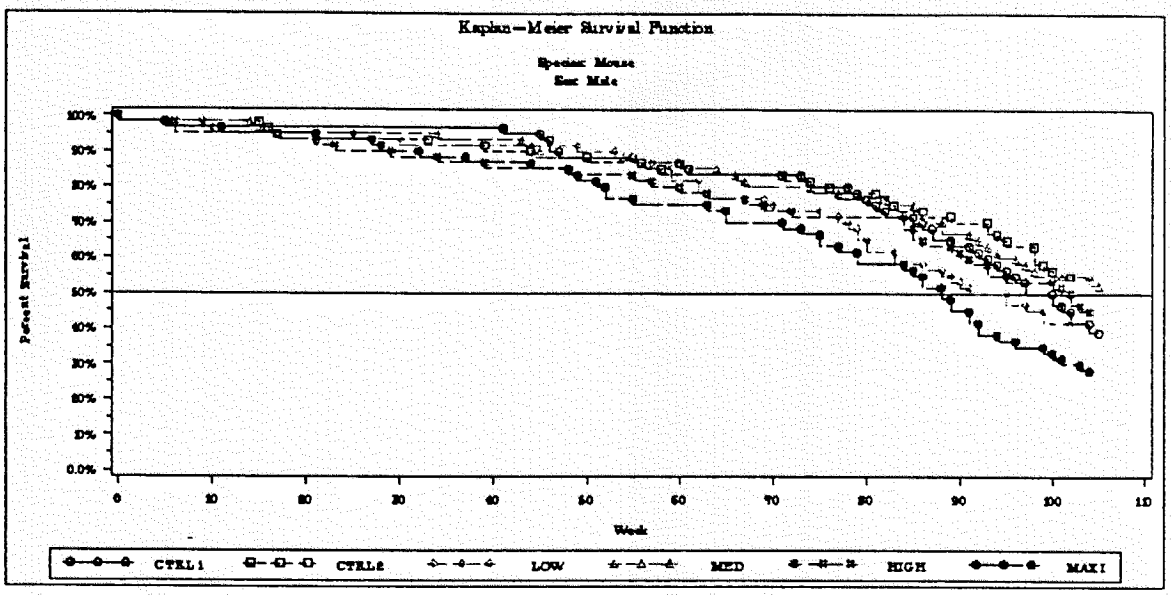
Figure 6. Kaplan-Meier Survival Functions for Male Mice



The test for dose-mortality trend shows significant results based on the Cox test (p=0.0040) and Kruskal-Wallis test (p=0.0044). (See Table 13.)

Table 13. Dose-Mortality Trend in Male Mice

Dose-Mortality Trend Tests

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Mouse
Sex: Male

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	8.31	0.0040
	Depart from Trend	3.77	0.4378
	Homogeneity	12.08	0.0338
Kruskal-Wallis	Dose-Mortality Trend	8.13	0.0044
	Depart from Trend	3.29	0.5112
	Homogeneity	11.42	0.0437

Source: d:\actos\mice\m\XAnimalX.txt

This reviewer's survival-data analysis concludes that the mortality in male mice is positively dose-related. Therefore, the age-adjusted trend test detailed in the following section (Tumor-Data Analysis), is justified.

Tumor-Data Analysis

The tumor-data analysis determines whether the dose-tumor positive linear trend is statistically significant. This reviewer tests this trend for every organ and tumor. The resulting p-values are compared against the

p-value cutoff points set by the following Agency's procedures. A significant result indicates a dose-tumor positive linear trend.

Statistical Procedure in Evaluation of Tumor-Data Analyses Currently Adopted by CDER Divisions of Biometrics

• For tumors found either fatal or non-fatal to all the animals, the statistical interpretation is based on the **exact test.**
• For tumors found fatal to some, but not to all animals, the statistical interpretation is based on the **asymptotic test**, resulting from the combined test. The asymptotic test uses the Z-statistic, which follows a standard normal distribution.
• To adjust for the effect of multiple testing, one can use a rule proposed by Haseman. A modified rule, proposed by the Divisions of Biometrics, CDER/FDA is applied to the trend tests in the review. In order to keep the overall type-I error at the level of about 0.1, this rule states:
  • Tumors with a spontaneous tumor rate of **1% or less** may be tested at the **0.025** significance level.
  • Otherwise, the **0.005** significance level may be used.

This reviewer's analysis does not find any statistically significant dose-tumor positive linear trend in the male mice.

Conclusions on Male-Mouse Study

This reviewer's analysis does not find any statistically significant dose-tumor positive linear trend in the male mice.

Evaluation of Carcinogenicity Study on Female Mice

To evaluate the sponsor's carcinogenicity study on female mice, this reviewer analyzed the sponsor's data. The reviewer's evaluation comprises the following:

- survival-data analysis
- tumor-data analysis

The reviewer's conclusions are summarized at the end of this section.

Survival-Data Analysis

The survival-data analysis determines whether the dose-mortality trend is statistically significant. A positive result indicates that the higher the dose level is, the more deaths are likely to occur.

Table 14 shows the number of female mice by treatment by age group. The dose levels labeled "CTRL1," "CTRL2," "LOW," "MED," "HIGH," and "MAXT" represent 0, 0, 3, 10, 30, and 100 mg/kg/day, respectively. The time interval "104-106" represents the terminal-sacrifice week.

Table 14. Number of Female Mice by Treatment and Age Group

Number of Animals
Species: Mouse
Sex: Female

	Treatment Group						
	CTRL1	CTRL2	LOW	MED	MEDHI	HIGH	Total
	Count	Count	Count	Count	Count	Count	Count
Time Interval							
0-62	3	3	4	2	3	9	24
63-78	11	7	9	9	9	13	58
79-91	19	12	10	12	7	8	69
92-103	7	16	13	6	15	8	65
104-106	20	22	24	31	26	21	144
Total	60	60	60	60	60	60	360

Source: d:\actos\mice\f\XAnimalX.txt

Table 15 describes, for the female mice, the number of deaths, the number at risk, and the cumulate percentages of deaths by treatment and age group.

Table 15. Cumulative Percentages of Deaths in Female Mice

Analysis of Mortality
Species: Mouse
Sex: Female

	Dose																	
	CTRL1			CTRL2			LOW			MED			HIGH			MAXI		
	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died
Time(-wks)																		
0-62	3	60	5.0	3	60	5.0	4	60	6.7	2	60	3.3	3	60	5.0	8	60	15.0
63-78	11	57	23.3	7	57	16.7	9	56	21.7	9	58	18.3	9	57	20.0	13	51	36.7
79-91	19	46	55.0	12	50	36.7	10	47	38.3	12	49	38.3	7	48	31.7	9	38	51.7
92-103	7	27	66.7	16	38	63.3	13	37	60.0	6	37	48.3	15	41	56.7	8	29	65.0
104-106	20	60	33.3	22	60	36.7	24	60	40.0	31	60	51.7	26	60	43.3	21	60	35.0

Figure 7 helps visualize the cumulative percentages of deaths over time by treatment. The doses do not appear to associate the cumulative percentages of deaths.

Figure 7. Line Graph of Cumulative Percentages of Deaths in Female Mice

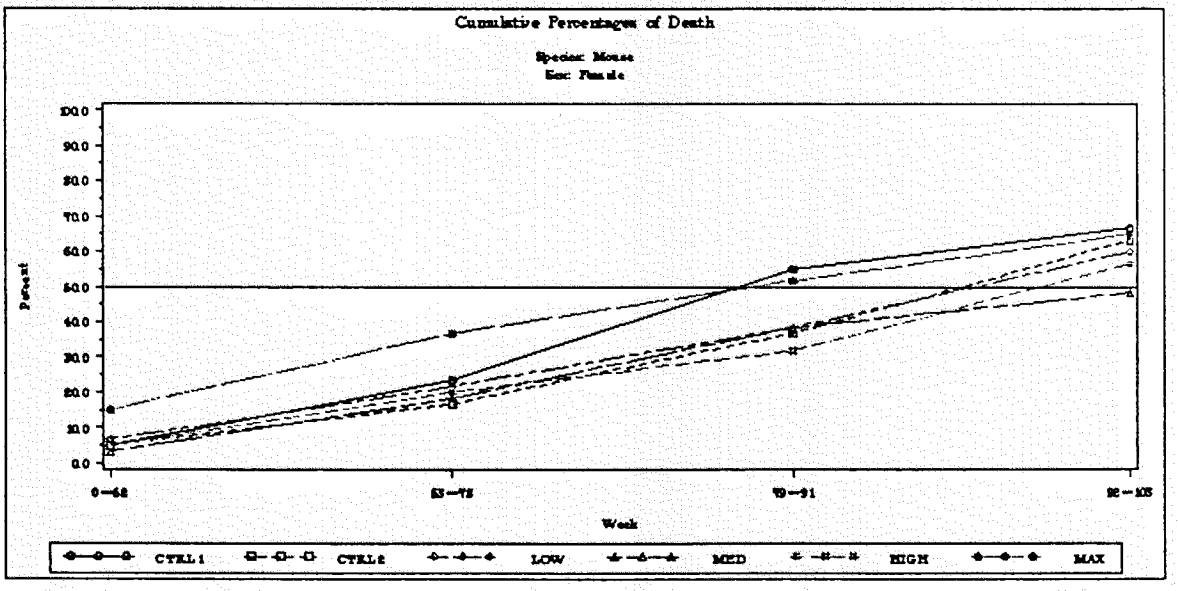
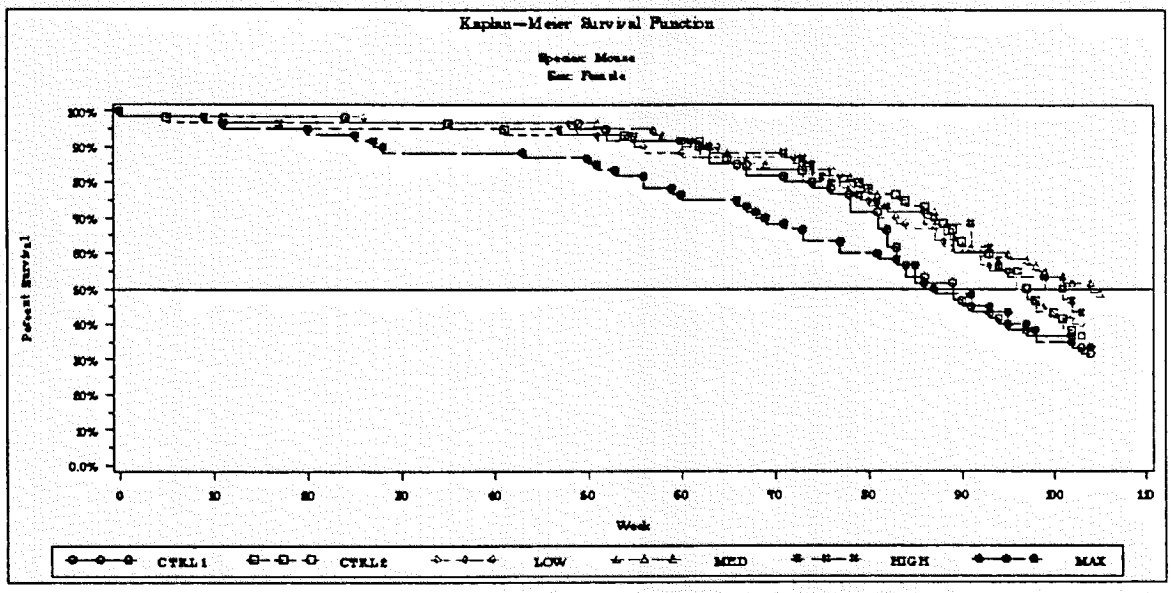


Figure 8 shows the Kaplan-Meier survival functions for female mice. The animals in the MAXI (100 mg/kg/day) group had a lower survival rate than did those in other groups. The MAXI group had more early deaths than did other groups.

Figure 8. Kaplan-Meier Survival Functions for Female Mice



The dose-mortality-trend test in the following Table 16 dose not indicate a significant trend (p>0.05).

Table 16. Dose-Mortality Trend in Female Mice

Dose-Mortality Trend Tests

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Mouse
Sex: Female

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	1.45	0.2280
	Depart from Trend	5.54	0.2362
	Homogeneity	6.99	0.2212
Kruskal-Wallis	Dose-Mortality Trend	3.27	0.0704
	Depart from Trend	5.38	0.2504
	Homogeneity	8.65	0.1237

Source: d:\actos\mice\f\XAnimalX.txt

Tumor-Data Analysis

The tumor-data analysis determines whether the dose-tumor positive linear trend in tumor incidence is statistically significant. This reviewer tests this trend for every organ and tumor. The resulting p-values are compared against the p-value cutoff points set by the following Agency's procedures. A significant result indicates a dose-tumor positive linear trend.

Statistical Procedure in Evaluation of Tumor-Data Analyses Currently Adopted by CDER Divisions of Biometrics

• For tumors found either fatal or non-fatal to all the animals, the statistical interpretation is based on the **exact test**.
• For tumors found fatal to some, but not to all animals, the statistical interpretation is based on the **asymptotic test**, resulting from the combined test. The asymptotic test uses the Z-statistic, which follows a standard normal distribution.
• To adjust for the effect of multiple testing, one can use a rule proposed by Haseman. A modified rule, proposed by the Divisions of Biometrics, CDER/FDA is applied to the trend tests in the review. In order to keep the overall type-I error at the level of about 0.1, this rule states:
  • Tumors with a spontaneous tumor rate of **1% or less** may be tested at the **0.025** significance level.
  • Otherwise, the **0.005** significance level may be used.

This reviewer's analysis does not find any statistically significant dose-tumor positive linear trend in the female mice.

Conclusions on Female-Mouse Study

This reviewer's analysis does not find any statistically significant dose-tumor positive linear trend in the female mice.

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Conclusions

Based on the evaluation of the carcinogenicity studies on rats and mice, this reviewer's concludes that Actos™ is carcinogenic in rats. The probability of erroneously concluding a significant test is about 10% or less.

Signoff Page

Statistical Reviewer: Ji-Yang (Ted) Guo Signature: /S/ Date: 4/21/99

Concur: Karl K. Lin, Ph.D. Signature: /S/ Date: 4/22/99

CC:
Archival NDA 21-073 (Non-Clinical: Carcinogenicity Review)
HFD-510/Division file
HFD-510/HRhee
HFD-510/JWeber
HFD-715/Division file
HFD-715/KLin
HFD-715/Tguo
HFD-715/TSahlroot
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