

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 020895

STATISTICAL REVIEW AND EVALUATION

FEB 4 1998

Statistical Review and Evaluation
Carcinogenicity Review of Sildenafil in Rats and Mice

DATE:

NDA #: 20-895, Animal Carcinogenicity Studies.

DATE BY CDER: October 14, 1997.

DRUG NAME: Sildenafil (Viagra).

SPONSOR: Pfizer Inc.

INDICATION FOR THE HUMAN USE: Male Erectile Dysfunction.

DOSES USED: For the male and female rats; two controls and sildenafil at 1.5, 5 & 60 mg/kg/day.
For the male and female mice; two controls and sildenafil at 3, 10 & 30 mg/kg/day.

DOCUMENTS REVIEWED: Carcinogenicity Volume with Document Identification BS, and the Review of Dr. Thomas Papoian (dated 1/29/98), the pharmacology reviewer in HFD-110.

This review has been discussed with Dr. Thomas Papoian, the pharmacology reviewer and with Dr. Albert Defelice, the pharmacology Team Leader from the Division of Cardio-Renal Drug Products (HFD-110) of the FDA.

Organization

The review's organization consists of **four** sections and each with subsections as follows:

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1. INTRODUCTION

Sildenafil (UK-92, 480) with the trade name of **Viagra** is a competitive and selective inhibitor of cyclic GMP specific phosphodiesterase type-5. For the human use, this drug is being developed for the treatment of male erectile dysfunction.

The Toxicity and Carcinogenicity of sildenafil submission contains: study on male and female Sprague-Dawley rats and study in male and female CD1 Mice.

The aims of the animal studies were to assess the chronic toxicity and potential carcinogenicity of sildenafil when administered orally to the rats and mice for two years.

2. RAT STUDY

2.1. Study Design

The following describes the design and the conduct of the study for both male and female rats.

The study title is "24-Month Oral Toxicity and Carcinogenicity Study in Sprague-Dawley Rats."

The study duration was 24 months and consisted of five treatment groups with 60 animals in each treatment group.

During the study, the rats were observed daily for mortality and clinical signs. Rates were weighed and examined for the presence of palpable masses, once a week. The food consumption was measured weekly for the first six months and monthly there after. Water consumption was measured every two months. Histopathological examinations were performed on numerous tissues recovered from dead animals during the study and those that were sacrificed or killed as moribund.

The terminal sacrifice for the male and female rates started at week 104 and went through week 105.

The study doses were two controls and sildenafil at **1.5, 5.0, and 60.0 mg/kg/day** during the 24 months. According to Dr. Papoian's calculations of the AUC (review was completed on 01/29/98), the dose of 60.0 mg/kg/day is about 36 fold, for the male rats and about 40 fold, for the female rats higher than the human dose. So, the dose level of 60.0 mg/kg/day is high enough to meet the standard for a pharmacokinetics endpoint (25-fold ratio of rodent to human plasma AUC).

2.2. Sponsor's Analysis and Conclusion

The sponsor's analyses include: mortality analysis, tumor analysis, body weight analysis, clinical signs analysis, analysis on clinical laboratory measures, and plasma concentration analysis. Those of concern are discussed below.

2.2.1. Mortality/Survival Analysis

The following table presents the sponsor’s summary of the percent mortality during the study.

Table I_2.2.1: Percent (%) of Mortality/Survival During the Study and Median Survival Time.

		Males				Females			
		Cont. 1+2	1.5 mg	5 mg	60 mg	Cont. 1+2	1.5 mg	5 mg	60 mg
Median Survival Time (Day)		629	652.5 [□]	669.0 [□]	630.0	604.0	583.5	556.5	615.5
Mortality/ Survival (%)	Found Dead	56.7	43.3	30.0	48.3	34.2	33.3	30.0	55.0
	Sacrificed as Moribund	25.0	15.0	35.0	21.7	45.0	41.7	48.3	30.0
	Survived at the End	18.3	41.7	35.0	30.0	20.8	25.0	21.7	15.0
	Total	100	100.0	100.0	100.0	100.0	100.0	100.0	100.0

□: Statistically Significant at $\alpha=0.05$.

For the male rats the survival time for the low and medium doses were statistically significantly higher than that in the controls. However, the sponsor concluded that “in the absence of an effect at the high dose, we consider that overall there was no effect of the treatment on the survival times.”

Comment: As will be seen later, the sponsor’s conclusion is verified from this reviewer’s results.

2.2.2. Tumor Trend Analysis

There was a slight apparent increase in proliferative changes in the thyroid of the males in the sildenafil treated groups, affecting C-cells and follicular cells. This was not seen in treated females. For the male rates the results are summarized in **Table II_2.2.2**.

The sponsor concluded that “The increase in C-cell proliferative changes in treated males was not dose-related and not statistically significant.” The sponsor also stated that the incidence observed is with the range of in-house historical data.

Comment: From the reviewer’s tumor trend (as a function of the dose) analysis, this kind of tumor is categorized as a “common tumor,” with the associated P-values of $P = 0.0266$ for the exact test and $P = 0.0134$ for the asymptotic test. Thus, by the FDA’s rule the trend is not statistically

significant¹.

Table II_2.2.2: Incidences of Thyroid Proliferative Changes in Male Rats

		Cont. 1	Cont. 2	1.5 mg	5 mg	60 mg
C-cell	Hyperplasia	1	5	6	7	3
	Adenoma	2	4	7	9	7
	Carcinoma	0	0	1	1	1
	Combined <input checked="" type="checkbox"/>	3	8	14	15	10
Follicular Cells	Hyperplasia	0	1	3	1	5
	Adenoma	4	0	0	2	5
	Carcinoma	1	1	0	2	0
	Combined <input checked="" type="checkbox"/>	5	2	3	5	10

: Rats bearing more than one of the above are counted only once.

Overall, the sponsor's analyses demonstrated that:

- The mortality/survival analysis has demonstrated that there is no dose-response relationship in survival.
- The tumor trend analysis has demonstrated that there is no dose-response relationship in survival.

2.3. Reviewer's Analysis and Conclusion

The discussion will be presented for male and female rats separately. We also will confine ourselves to the discussion of the results from the mortality/survival and the tumor trend analysis.

¹ - By the FDA's Guidance for Industry on the Statistical Aspects of Design, Analysis, and Interpretation of Animal Carcinogenicity Studies:

- Tumors with the spontaneous incidence rate of $> 1\%$ (based on historical observations or based on observations in the control groups) are considered as **common**. Then, the choice of significance level will be $\alpha' = 0.01$ for the pairwise comparisons and $\alpha' = 0.005$.
- Tumors with the spontaneous incidence rate of $\leq 1\%$ are considered as **rare**. Then, the level of significance should be set at $\alpha' = 0.05$ for the pairwise comparisons and at $\alpha' = 0.025$ for the trend analysis.

2.3.1. Male Rats

2.3.1.1. Mortality/Survival Analysis

Table III_2.3.1.1. displays the distribution of the number and the percent of the male rats which either died or were terminally sacrificed after the week 103, the end of study (during week 104-105).

TABLE III_2.3.1.1. Distribution of Number of Male Rats Died or Terminally Sacrificed

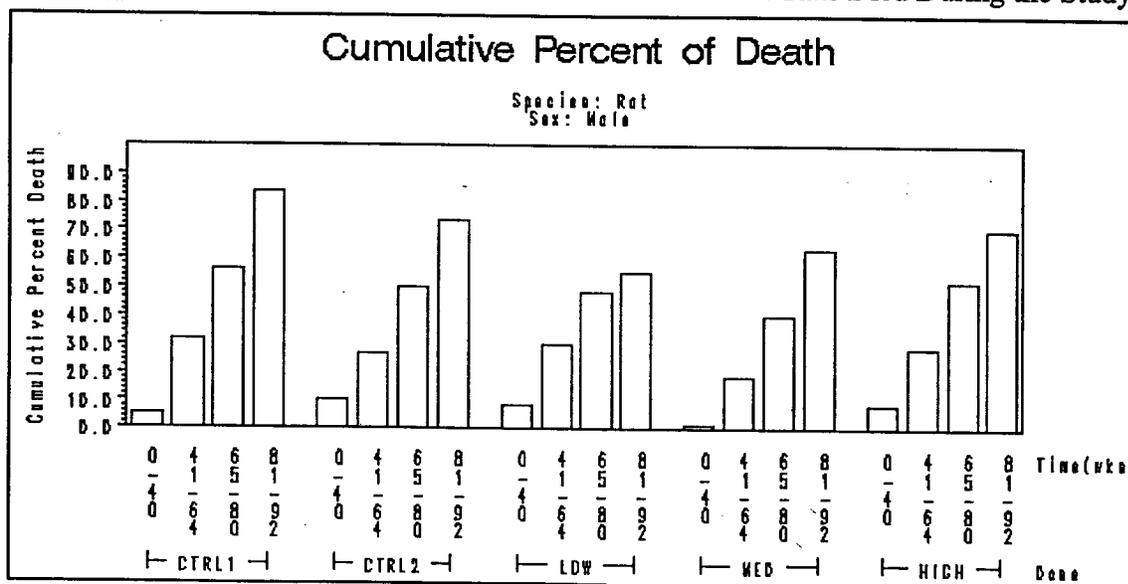
Week	Control 1			Control 2			1.5 mg/kg/day			5 mg/kg/day			60 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-52	60	3	5.0	60	6	10.0	60	5	8.3	60	1	1.7	60	5	8.3	20
53-78	57	16	26.7	54	10	16.7	55	13	21.7	59	10	16.6	55	12	20.0	61
79-91	41	15	25.7	44	14	23.3	42	11	18.3	49	13	21.7	43	14	23.4	67
92-103	26	16	26.2	14	14	23.3	31	4	6.7	36	14	23.3	29	11	18.3	59
Terminal Sacrifice	10	10	16.7	16	16	26.7	27	27	45.0	22	22	36.7	18	18	30.0	93

■: The number of animals terminally sacrificed is the same as the number of animals survived after week 103.

From the last column of the Table III_2.3.1.1., it appears that there is no dose related trend with respect to the animal survival at the end of the study.

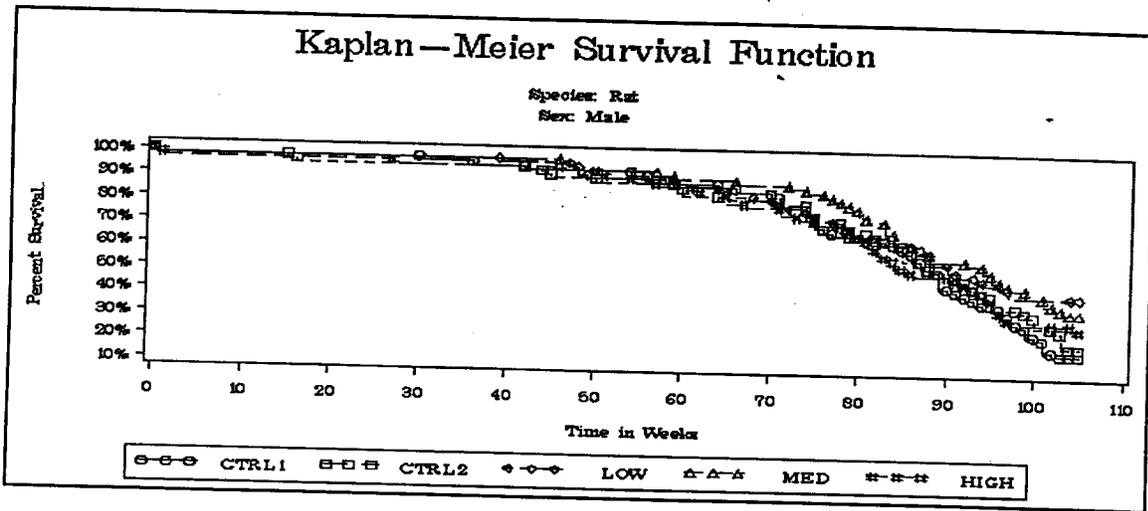
Figure I_2.3.1.1. presents the cumulative distribution of the percent of rat death, up to week 103.

FIGURE I_2.3.1.1. Cumulative Distribution of Percent of Male Rats Died During the Study



Kaplan-Meier estimates of the survival function is presented in **Figure II_2.3.1.1.** The graph shows the homogeneity of the survival distributions in the 5 treatment groups.

FIGURE II_2.3.1.1. Kaplan-Meier Estimate of the Survival Function for the Male Rats



A Dose-Mortality Trend test was also performed, using both Cox Regression and Non-Parametric Kruskal-Wallis methods. The results are summarized in **Table IV_2.3.1.1.** and the resulted P-values indicating of no evidence of statistically significant dose related mortality trend.

TABLE IV_2.3.1.1. Dose Related Mortality Trend Test for Male Rats

Method	Time Adjusted Trend-Test	Statistics	P-Value
Cox	Dose-Mortality Trend	0.08	0.7837
	Departure From Trend	7.47	0.0583
	Homogeneity	7.55	0.1097
Kruskal-Wallis	Dose-Mortality Trend	0.39	0.5332
	Departure From Trend	4.57	0.2061
	Homogeneity	4.96	0.2915

2.3.1.2. Tumor Trend Analysis

The results for the tumor trend analysis are summarized in Tables **I_A-Male-Rats** and **II_A-Male-Rats** of the **Appendix A-Male-Rats**. From the review of the results, the pharmacology reviewer might be interested in the following cases, summarized in **Table V_2.3.1.2.**

TABLE V_2.3.1.2. Noticeable Tumor Trends in Male

ORGAN TYPE	TUMOR TYPE	Number of Incidences Per Treatment					Incidental or Fatal	Rare or Common \square	Trend Analysis P-Values	
		Cot 1	Cot 2	1.5 mg	5 mg	60 mg			Exact	Asymptotic
Kidney	B-Renal Tubule Adenoma	0	0	0	0	2	IN	R	0.0390	0.0023
Skin and Adnexa	B-Lipoma	0	0	0	0	1	IN	R	0.1935	0.0210
Skin and Adnexa	B-Lymphangioma	0	0	0	0	1	IN	R	0.1935	0.0210
Thyroid	B-Follicular Cell Adenoma	4	0	0	0	0	IN	C	0.0265	0.0132

\square : Considered as a common tumor if the incidental rate is > 1% historically or in Control Group.

Now, by the FDA's rule (see Footnote 1 on Page 5), one may draw the following conclusions.

- The tumor observed in the thyroid is of a **common type**, with the $P=0.0266$ for the exact and $P=0.0134$ for the asymptotic test. Thus, as compared to $\alpha = 0.005$, both exact and asymptotic tests suggest that there is no statistically significant dose related trend.
- The tumor observed in the kidney is of **rare type**, with the $P=0.0390$ for the exact and $P=0.0023$ for the asymptotic test. Now, as compared to $\alpha=0.025$ the exact test suggests no statistically significant dose related trend; whereas, the asymptotic test suggests, a statistically significant dose related trend.
- The tumors observed in the skin, for both B-Lipoma and B-Lymphangioma, are of **rare type**, with the $P=0.0193$ for the exact and $P=0.0210$ for the asymptotic test. Now, as compared to $\alpha=0.025$ the exact test suggests no statistically significant dose related trend; whereas, the asymptotic test suggests, a statistically significant dose related trend.

To resolve the contradictory conclusions, for the case of kidney and skin tumors, this reviewer conducted the Fisher Exact Test, comparing the high dose (for which the tumors were observed) with the two controls combined. The resulting P-values are $P=0.110$ for the kidney and $P=0.333$ for the skin tumors. Therefore, one may conclude that: **no statistically significant dose related trend is suggested.**

2.3.2. Female Rat

2.3.2.1. Mortality/Survival Analysis

Table VI_2.3.2.1. displays the distribution of the number and the percent of the female rats that they either died or were terminally sacrificed after the week 103 (during week 104-105, the end of the study).

TABLE VI_2.3.2.1. Distribution of Number of female Rats Died or Terminally Sacrificed

Week	Control 1			Control 2			1.5 mg/kg/day			5 mg/kg/day			60 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-52	60	1	1.7	60	2	13.3	60	4	6.7	60	5	8.3	60	5	8.3	17
53-78	59	17	28.3	58	15	25.0	56	19	31.6	55	21	35.0	55	16	26.7	88
79-91	42	17	28.3	43	19	31.7	37	13	21.7	34	16	26.7	39	16	26.7	81
92-103	25	11	16.4	24	11	18.3	24	8	13.3	18	5	8.3	23	13	21.6	48
Terminal Sacrifice	14	14	23.3	13	13	21.7	16	16	26.7	13	13	21.7	10	10	16.7	66

■: The number of animals terminally sacrificed is the same as the number of animals survived after week 103.

Here as well, the last column of the Table VI_2.3.2.1. shows that there is no dose related trend with respect to the animal survival at the end of the study.

Figure III_2.3.2.1. presents the cumulative distribution of the percent death during the study.

FIGURE III_2.3.2.1. Cumulative Distribution of Percent of Female Rats Died During the Study

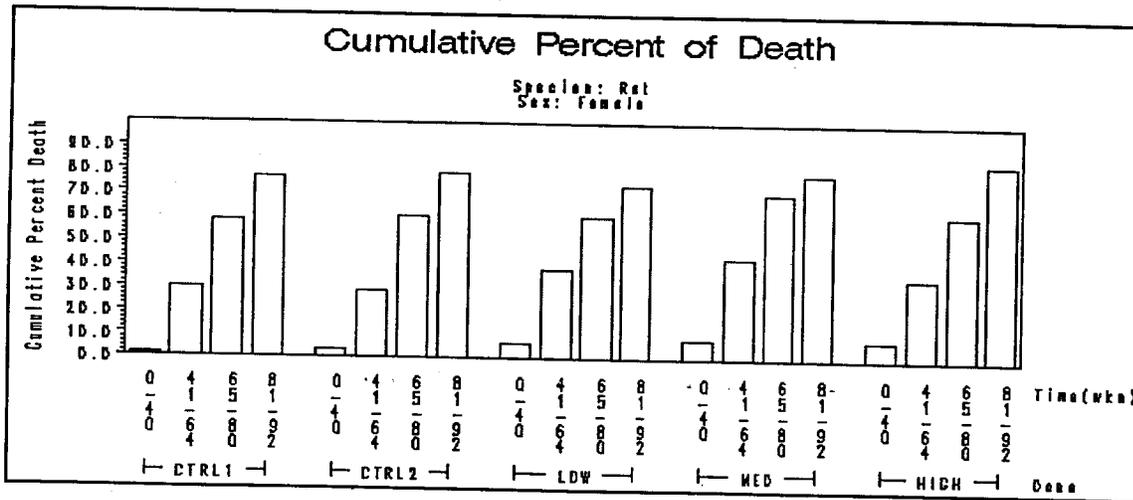
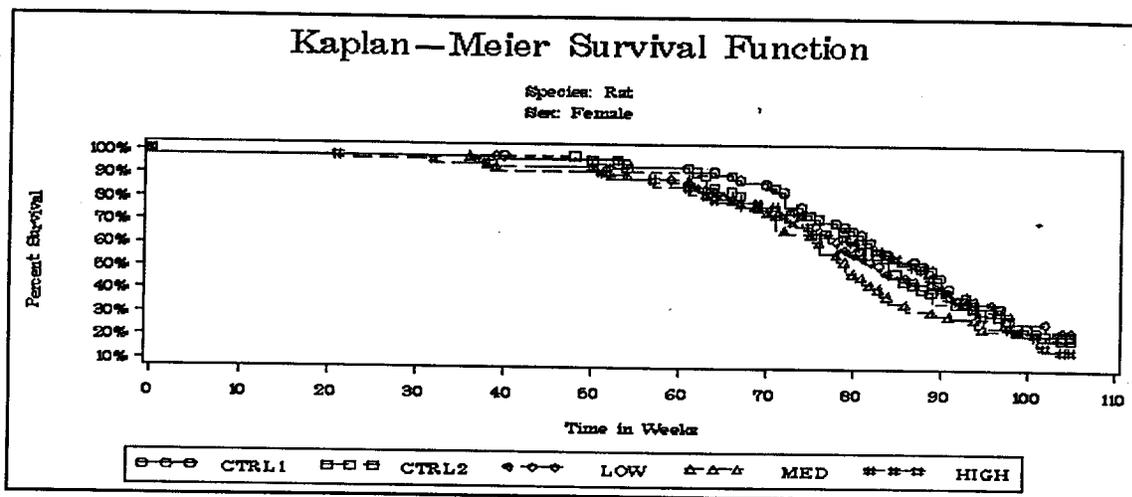


Figure IV_2.3.2.1. shows the Kaplan-Meier estimates of the survival function for the five treatment groups. The graph demonstrates the homogeneity of the five treatment groups with respect to the survival distribution.

FIGURE IV_2.3.2.1. Kaplan-Meier Estimate of the Survival Function for the Female Rats



The results for the Cox Regression and the Non-Parametric Kruskal-Wallis methods on the dose-mortality trend are summarized in Table VII_2.3.2.1. For both methods the resulting high p-values suggest that there is a lack of statistically significant dose related trend.

TABLE VII_2.3.2.1. Dose Related Mortality Trend Test for the Female Rats

Method	Time Adjusted Trend-Test	Statistics	P-Value
Cox	Dose-Mortality Trend	0.35	0.5560
	Departure From Trend	1.39	0.7083
	Homogeneity	1.73	0.7844
Kruskal-Wallis	Dose-Mortality Trend	0.13	0.7234
	Departure From Trend	3.00	0.3921
	Homogeneity	3.12	0.5376

2.3.2.2. Tumor Trend Analysis

The results for the tumor trend analysis are summarized in Tables I_B-Female-Rats and II_B-Female-Rats of the Appendix B-Female-Rats. From the results it appears that no statistically significant dose related trend is suggested.

In conclusion, this reviewer’s results verified the sponsor’s findings for which:

- There is no statistically significant evidence of a dose related mortality trend.

- There is no statistically significant evidence of a dose related tumor trend.

3. MOUSE STUDY

3.1. Study Design

Following is the description of the study design and the conduct of the study for both male and female mice.

The study is titled "24-Month Oral Toxicity and Carcinogenicity Study in CD1 Mice."

The study duration was 24 months and consisted of 5 treatment groups with 55 mice in each treatment group.

During the study, mice were observed daily for mortality and clinical signs. Animals were weighed and examined for the presence of palpable masses once a week. The food consumption was measured weekly for the first six months and monthly then after. Water consumption was measured every two months for about one year. Histopathological examinations were performed on a wide range of tissues recovered from the dead animals during the study and those that were sacrificed or killed as moribund.

The treatment groups consist of two controls and sildenafil at 3, 10.0, and 30.0 mg/kg/day during the study.

Due to high mortality the following procedure was adopted: The mice in the control groups and those in the low and medium dose groups were treated up to 649 to 650 days for the males and up to 558 to 562 for the females. The high dose mice were treated up to day 453 for males and up to day 404 for females.

3.2. Sponsor's Analysis and Conclusion

The sponsor's analyses include: mortality analysis, tumor analysis, body weight analysis, clinical signs analysis, analysis on clinical laboratory measures, and plasma concentration analysis. Those of concern are discussed below.

3.2.1. Mortality/Survival Analysis

The following table presents the sponsor's summary of the percent mortality during the study. From the table it appears that at the high dose (30 mg/kg/day), a compound-related increase in mortality was observed after seven months in males and after four months in female groups. In order to maximize the duration of treatment and ensure a sufficient number of animals for subsequent analyses, when the survival at the high dose fell below 20%, the mice were sacrificed.

For the 10 mg dose groups, a dose related increases in mortality was observed after seven months in female mice, but for the male mice the mortality was the same as those in controls and in the low dose groups.

Table VIII_3.2.1: Percent (%) of Mortality/Survival During the Study and Day of Terminal Sacrifice.

		Males				Females			
		Cont. 1+2	3 mg	10 mg	30 mg	Cont. 1+2	3 mg	10 mg	30 mg
Day of Terminal Sacrifice		650	650	650	454	559	559	559	405
Mortality/ Survival (%)	Found Dead	42.7	45.4	47.3	41.8	30.0	41.8	58.2	50.9
	Sacrificed as Moribund	14.5	12.7	30.9	40.0	15.5	18.2	18.2	36.4
	Survived at the End	42.8	41.9	18.8	9.2	54.5	40.0	23.6	12.7
	Total	100	100.0	100.0	100.0	100.0	100.0	100.0	100.0

□: Statistically Significant at $\alpha=0.05$.

Comment: As will be seen later, these findings are verified from the reviewer’s analyses.

3.2.2. Tumor Trend Analysis

There was no statistically significantly dose related tumor trends, for both male and female mice.

Comment: This assertion is granted from the results of this reviewer’s analysis.

Overall, the sponsor’s analyses demonstrated that:

- The mortality/survival analysis has demonstrated a dose related increases in mortality for the high dose, for both male and female mice and a dose related increases in mortality for the medium dose for the female mice.
- There is no treatment related tumor trend.

3.3. Reviewer’s Analysis and Conclusion

The discussion will be presented for male and female mice separately. For the tumor trend evaluation two sets of analyses will be performed. For the first set, the analysis will include all five dose groups. The second set excludes the high dose (30 mg/kg/day). The rationale for this is that, because of high mortality and, hence, early termination in the high dose group, the potential of observing dose related tumors in the high dose group is eliminated. Thus, the second set of analysis is to determine if there is a dose related trend in tumor growth in the absence of the high dose.

3.3.1. Male Mice

3.3.1.1. Mortality/Survival Analysis

Table IX_3.3.1.1. displays the distribution of the number and the percent of the mice which either died or were terminally sacrificed. The week intervals are chosen because the mice were begun to be terminally sacrificed at week 93 for the animals in the two controls, the low, and the medium dose groups and at the beginning of week 65 for the animals in the high dose group. From the results, it appears that there was a higher mortality in the high dose as compared to the other doses.

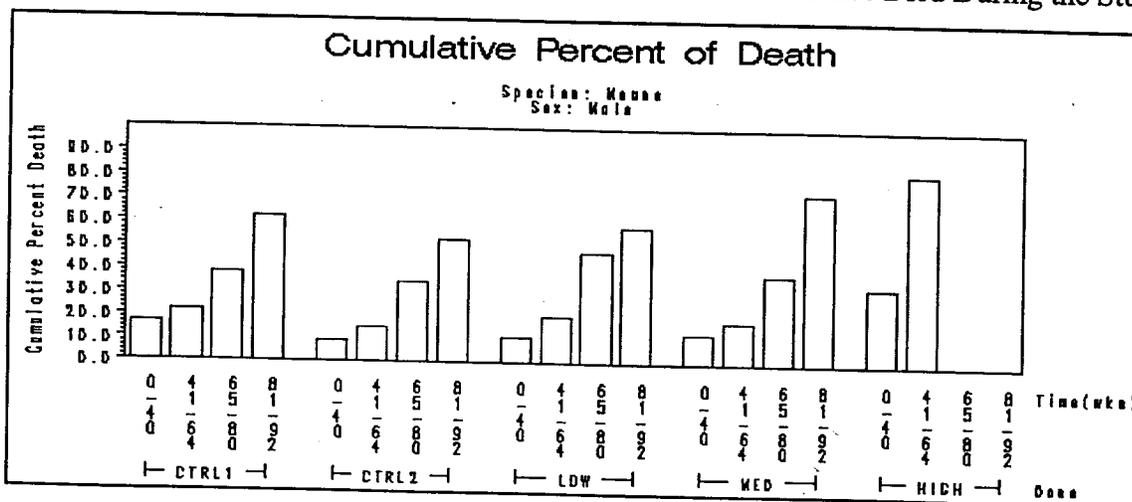
TABLE IX_3.3.1.1. Distribution of Number of Male Mice Died or Terminally Sacrificed

Week	Control 1			Control 2			3 mg/kg/day			10 mg/kg/day			30 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-40	55	9	16.4	55	5	9.1	55	6	10.9	55	7	12.7	55	18	32.7	45
41-64	46	3	5.4	50	3	5.4	49	5	9.1	48	3	5.5	27	27	49.1	41
65-80	43	9	16.4	47	11	20.0	44	15	27.3	45	11	20.0	10♂	10♂	18.2	56
81-92	34	13	23.6	36	10	18.2	29	6	10.9	34	19	34.5	0	0	0	48
Terminal Sacrifice	21	21	38.2	26	26	47.3	23	23	41.8	15	15	27.3	0	0	0	85

■: The number of animals terminally sacrificed is the same as the number of animals survived after week 92.
 ♂: The mice in high dose group were terminally sacrificed at the beginning of week 65.

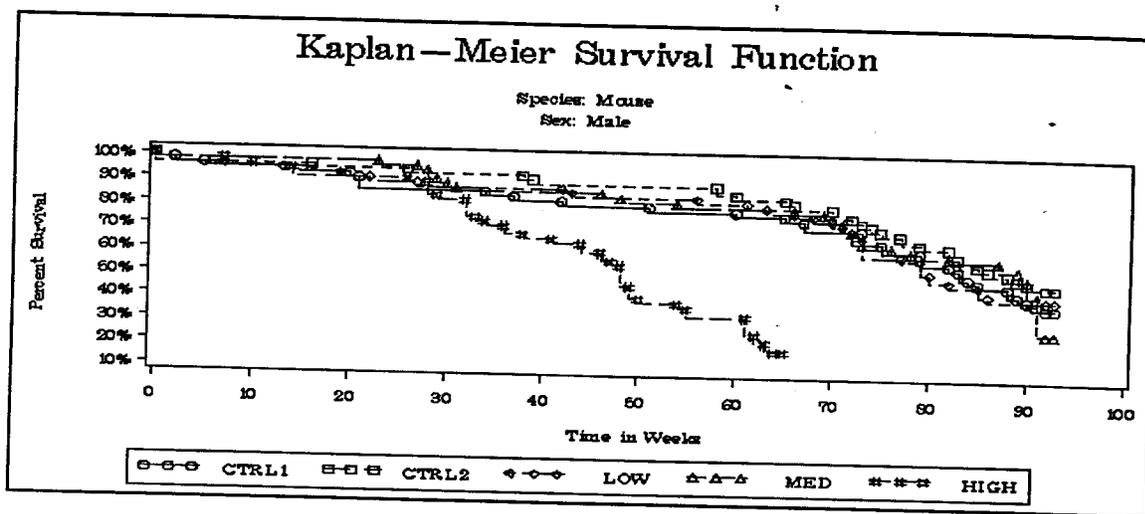
Figure V_3.3.1.1. presents the cumulative distribution of percent death during the study.

FIGURE V_3.3.1.1. Cumulative Distribution of Percent of Male Mice Died During the Study



Further analysis consists of the Kaplan-Meier estimates of the survival function, presented in Figure VI_3.3.1.1.

FIGURE VI_3.3.1.1. Kaplan-Meier Estimate of the Survival Function for the Male Mice



The graph shows that the mortality rate in the high dose is substantially higher than those in the other dose groups.

Dose-Mortality Trend Test was also performed, using both Cox Regression and Non-Parametric Kruskal-Wallis methods. The results are summarized in Table X_3.3.1.1.

TABLE X_3.3.1.1. Dose Related Mortality Trend Test for Male Mice

Method	Time Adjusted Trend-Test		Statistics	P-Value
Cox	Dose-Mortality Trend:	All Doses Included	75.90	0.0000
		High Dose Excluded	1.37	0.2422
	Departure From Trend:	All Doses Included	15.86	0.0012
		High Dose Excluded	0.93	0.6293
Kruskal-Wallis	Dose-Mortality Trend:	All Doses Included	66.85	0.0000
		High Dose Excluded	0.48	0.4880
	Departure From Trend:	All Doses Included	10.56	0.0144
		High Dose Excluded	1.06	0.5893
Homogeneity	All Doses Included	91.76	0.0000	
	High Dose Excluded	2.29	0.5136	
Homogeneity	All Doses Included	77.41	0.0000	
	High Dose Excluded	1.54	0.6734	

The analysis was performed for the case where all doses were included as well as for the case where the high dose was excluded. The results show that the mortality rate in the high dose is statistically significantly higher than the mortality rate in the other doses. After exclusion of the high dose, there is no statistically significant difference among doses with respect to the mortality rate.

The conclusion is that due to the high mortality for the male mice in the 30 mg/kg/day dose group, one may conclude that the dose level of 30.0 mg/kg/day is high enough to meet the standard for a "Maximum Tolerated Dose (MTD)."

3.3.1.2. Tumor Trend Analysis

The results for the tumor trend analysis for the two sets of analyses (with and without exclusion of the high dose from the analysis) are summarized in Tables I_C-Male-Mice, II_C-Male-Mice, III_C-Male-Mice and IV_C-Male-Mice of Appendix C-Male-Mice. From the results presented in those tables one may conclude that:

- When all doses are included, Table I_C-Male-Mice indicates that no statistically significant treatment dependent tumor trend is suggested.
- When the high dose is excluded from the analysis, Table III_C-Male-Mice indicates that, here as well, no statistically significant treatment dependent tumor trend is suggested.

3.3.2. Female Mice

3.3.2.1. Mortality/Survival Analysis

Table XI_3.3.2.1. displays the distribution of the number and the percent of the female mice which either died or were terminally sacrificed.

TABLE XI_3.3.2.1. Distribution of Number of Female Mice Died or Terminally Sacrificed

Week	Control 1			Control 2			3 mg/kg/day			10 mg/kg/day			30 mg/kg/day			Total No. Died
	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	No. At Risk	No. Died	% Died	
0-35	55	1	1.8	55	1	1.8	55	1	1.8	55	11	20.0	55	18	32.7	32
36-57	54	8	14.5	54	4	7.3	54	6	10.9	44	11	20.0	37	28	50.9	57
58-68	46	6	10.9	50	12	21.8	48	7	12.7	33	11	20.0	9♀	9♀	16.4	45
69-79	40	10	18.2	38	8	14.5	41	19	34.5	22	8	14.5				45
Terminal Sacrifice	30	30	54.5	30	30	54.5	22	22	40.0	14	14	25.5				96

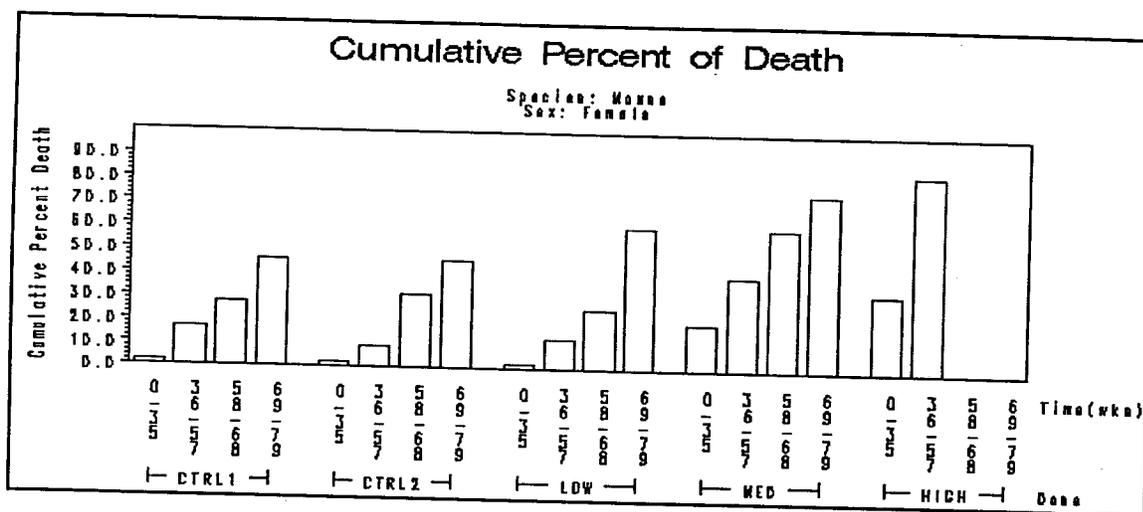
■: The number of animals terminally sacrificed is the same as the number of animals survived after week 79.

♀: The mice in high dose group were terminally sacrificed at the beginning of week 58.

The week intervals in Table XI_3.3.2.1. are chosen because the mice were begun to be terminally sacrificed at week 80 for the animals in the two controls, the low, and the medium dose groups and at beginning of week 58 for the animals in the high dose group. From the table, it appears that there was a higher mortality in the medium and in the high dose groups as compared to the other doses.

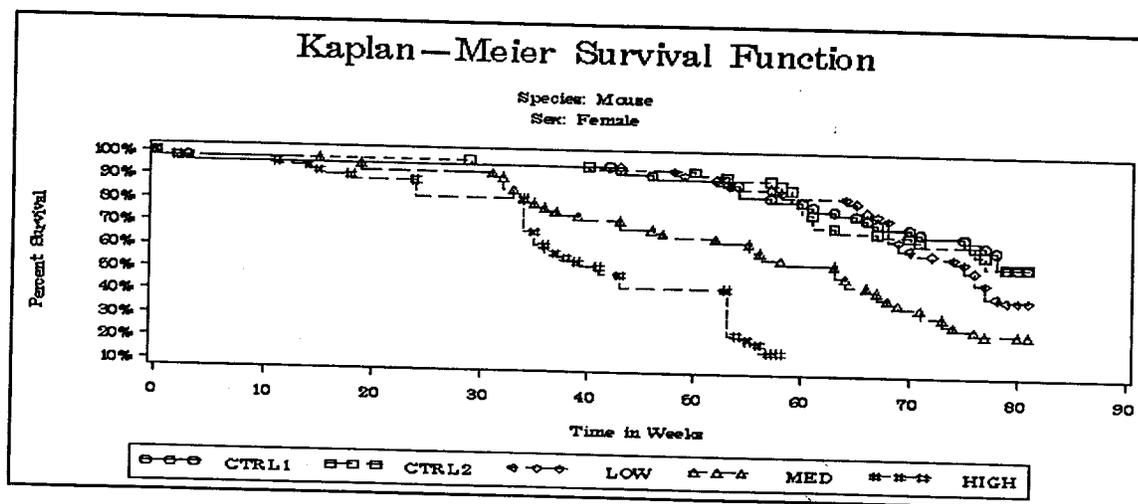
Figure VII_3.3.2.1. presents the cumulative distribution of animals' death during the study with exclusion of the terminal sacrifices.

FIGURE VII_3.3.2.1. Cumulative Distribution of Number of Female Mice Died During the Study



Further analysis consists of Kaplan-Meier estimates of the survival function which is presented in Figure VIII_3.3.2.1..

FIGURE VIII_3.3.2.1. Kaplan-Meier Estimate of the Survival Function for the Female Mice



The graph shows that the mortality rate in the medium and high dose groups was substantially higher than those in the other dose groups.

Dose-Mortality Trend Test was performed, using the Cox Regression and the Non-Parametric Kruskal-Wallis methods. The analysis was carried for the case where all doses were included as well as for the case where the high dose was exclude. The results are summarized in **Table XII_3.3.2.1.**

TABLE XII_3.3.1.1. Dose Related Mortality Trend Test for Female Mice

Method	Time Adjusted Trend-Test		Statistics	P-Value
Cox	Dose-Mortality Trend:	All Doses Included	123.21	0.0000
		High Dose Excluded	22.53	0.0000
	Departure From Trend:	All Doses Included	1.19	0.7559
		High Dose Excluded	0.12	0.9418
	Homogeneity:	All Doses Included	124.4	0.0000
		High Dose Excluded	22.65	0.0000
Kruskal-Wallis	Dose-Mortality Trend	All Doses Included	119.25	0.0000
		High Dose Excluded	26.15	0.0000
	Departure From Trend	All Doses Included	0.596	0.8979
		High Dose Excluded	0.72	0.6960
	Homogeneity	All Doses Included	119.84	0.0000
		High Dose Excluded	26.87	0.0000

The results show that the mortality rates in the high dose, and possibly in the medium dose, are statistically significantly higher than the mortality rate in the other doses. After exclusion of the high dose, the results show that the mortality rate in the medium dose is also statistically significantly higher than the other doses.

One also concludes that due to the high mortality for the female mice in the dose groups of 10 mg/kg/day and 30 mg/kg/day, one may conclude that the dose level of 10 mg and 30 mg are high enough to meet the standard for a "Maximum Tolerated Dose (MTD)."

3.3.2.2. Tumor Trend Analysis

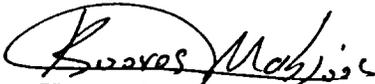
The results for the tumor trend analysis for the two sets of analyses (with and without exclusion of the high dose from the analysis) are summarized in **Tables I_D-Female-Mice, II_D-Female-Mice, III_D-Female-Mice** and **IV_C-Female-Mice** of **Appendix D-Female-Mice**. From the results, one may conclude that:

- When all doses are included, **Table I_D-Female-Mice** indicates that no statistically significant treatment dependent tumor trend is suggested.
- When the high dose is excluded from the analysis, **Table III_D-Female-Mice** indicates that, here as well, no statistically significant treatment dependent tumor trend is suggested.

4. REVIEWER'S CONCLUSION

Overall,

- For both the male and female rats, there was no evidence of a statistically significant treatment related mortality trend.
- For both the male and female rats, there was no evidence of a statistically significant treatment related tumor trend.
- For the male mice, the sildenafil dose of 30 mg/kg/day was toxic and demonstrated a statistically significantly higher mortality rate, as compared to the other doses.
- For the female mice, the sildenafil doses of 10 mg/kg/day and 30 mg/kg/day were toxic and demonstrated a statistically significantly higher mortality rate, as compared to the other doses.
- For both the male and female mice, there was no evidence of a statistically significant treatment related tumor trend. This finding was the same for the analyses with inclusion and without inclusion of the dose 30 mg/kg/day.


Kooros Mahjoob, Ph.D.
Mathematical Statistician

Concur: Dr. Chi


2/4/98

This review consists of 18 pages which include text, 12 tables and 6 Figures. There are 4 appendices attached in the back (Appendix A-Male Rats, 9 pages; Appendix B-Female Rats, 11 pages; Appendix C-Male Mice, 10 pages; and Appendix D-Female Mice, 8 pages).

CC:

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HFD-110
HFD-110/Dr. Defelicy
HFD-110/Dr. Papoian
HFD-110/Mr. Buehler
HFD-344/Dr. Barton
HFD-710/Dr. Chi
HFD-710/Dr. Mahjoob
HFD-710/Chron.

K. Mahjoob: 4-5301:Biometrics 1/Team 1:km.

Statistical Carcinogenicity Reviewer: Kooros Mahjoob