CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-985

STATISTICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

STATISTICAL REVIEW AND EVALUATION

CARCINOGENICITY STUDIES

ADDENDUM

NDA/Serial Number:	21-985 / S_000
Drug Name:	RASILEZ® (aliskiren)
Indication(s):	Treatment of Hypertention
Applicant:	Novartis
Date(s):	Date of Document: February, 10, 2006 PDUFA Due Date: December 10, 2006
Review Priority:	Standard
Biometrics Division:	Biometrics I, HFD-710
Statistical Reviewer:	Ququan Liu, M.D., M.S.
Concurring Reviewers:	Karl Lin, Ph.D., James Hung, Ph.D.
Medical Division:	Division of Cardio-Renal Drug Products, HFD-110
Pharm/Tox Team:	Gowra Jagadeesh Ph.D, Charles Resnick, Ph.D.
Project Manager:	John David
Keywords:	Aliskiren, Carcinogenicity, Hypertension

The purpose of this addendum is to correct a sentence on page 5, and a sentence on page 16 of the original review issued 09/05/2006.

The sentence "All surviving animals were necropsied at week 25 for males and week 22 for females due to high mortality rate in the positive control group" on page 5 should be replaced by "All surviving animals in the positive control groups were necropsied at week 25 for males and week 22 for females. However, the negative control groups and the SPP100 treated groups lasted for 26 weeks" and the sentence "Males were terminated at test week 25 and females at week 22 for the controls and SPP100 treated groups" on page 16 should be replaced by the sentence "Males were terminated at test week 25 and females at week 22 for the positive control groups. However, the negative control groups and the SPP100 treated groups lasted for 26 weeks".

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/s/

Ququan Liu
9/6/2006 11:04:01 AM
BIOMETRICS

Karl Lin
9/6/2006 01:32:56 PM
BIOMETRICS
Concur with review

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Table of Contents

List of Tables

List of Figures

1. EXECUTIVE SUMMARY

1.1 Conclusions

In study 0370063, statistically significant differences in survival were observed among the males, but not the females. There was a negative linear trend in mortality detected in both sexes. No statistically significant positive trend was observed for any tumor types tested for either female or male rats. The validity of the study is not conclusive.

In study 0410091, it appears that a higher mortality rate was observed in the high dose group (1500 mg/kg) among the males. The statistical analysis shows that the difference in mortality in the males was statistically significant across the groups. The death causes for these animals are needed to be further evaluated and confirmed by the pharmacologist/toxicologist. No statistically significant increase in tumor incidence was detected in either female or male animals.

1.2 Brief Overview of Carcinogenicity Studies

Two carcinogenicity studies were included in this NDA, one is a long-term (two-year) study and one is a short-term (26-week) study. The long-term study 0370063 tested Aliskiren (SPP100) at doses of 0, 250, 750 and 1500 mg/kg/day in rats for 104 weeks. The short-term study 0410091 tested Aliskiren (SPP100) at doses of 0, 250, 750 and 1500 mg/kg/day for 26 weeks and N-methyl-N-nitrosourea (MNU) - Isopac® as a positive control at a single dose of 75 mg/kg on test day 1 in CB6F1-TgrasH2 mice.

1.3 Statistical Issues

Long-term study 0370063:

The primary purpose of a long-term rodent carcinogenicity study of a new drug is to evaluate the oncogenic potential of the drug when it is administered to animals for most of their normal life span. The drug, however, may affect the mortality of different treatment groups. Test animals living longer are more likely to develop tumors than those dying early. Therefore, it is essential to adjust differences in mortality among treatment groups in the analysis of tumor data. Two methods, the prevalence and death rate methods based on the contexts of observation of tumors (i.e., if tumors cause animals' death) and described in the paper by Peto et al. (1980), are used to test positive trend in tumor incidence in the study.

Because of the large number of comparisons involved, a great potential exists for finding statistically significant positive trends due to chance alone (i.e., a false positive). Therefore, it is important to make adjustment for multiplicity of

statistical tests of significance. The decision rule is testing the positive trend in incidence rates in rare and common tumors at 0.025 and 0.005 levels of significance, respectively. A tumor type with a background rate of 1 percent or less is classified as rare and common, otherwise.

Short-term study 0410091:

Because of short duration of study, mortality rate is usually very low and only a few types of tumors were developed in study animals. Thus it becomes less important to adjust differences in mortality among treatment groups and to make adjustment for multiplicity in the analysis of tumor data. In this study, the statistical analyses, mainly trend tests were conducted in one single interval (0-26 weeks) without adjusting for mortality differences, and tested at the significance level of 0.05 for both rare and common tumors.

2. INTRODUCTION

2.1 Overview

Aliskiren (SPP100) is a novel anti-hypertensive agent that lowers plasma renin activity (PRA) by inhibiting the enzyme renin. It is intended for use in the treatment of hypertension, alone or in combination with other antihypertensive agents.

In the long-term study, SPP100 was administered orally via feed admixtures to rats for at least 104 weeks. All surviving animals were necropsied at weeks 104-105 for males and week 105 for females. Selected organs/tissues were processed and examined macroscopically and microscopically.

In the short-term study, SPP100 was administered in feed to transgenic (CB6F1-rasH2) mice for 26 weeks. In addition, a group of animals was treated with N-methyl-N-nitrosourea (MNU) - Isopac® as a positive control at a single dose on test day 1. All surviving animals were necropsied at week 25 for males and week 22 for females due to high mortality rate in the positive control group. Selected organs/tissues were processed and examined microscopically.

2.2 Data Sources

The sponsor's SAS datasets were stored in the directory of \\Cdsesub1\n21985\N_000\2006-02-10 of the Center's electronic document room.

3. STATISTICAL EVALUATION

3.1 STUDY A: Long-term study 037006

3.1.1 STUDY DESIGN

Animals were randomly assigned to the following four dose groups shown in Table 1. SPP100 feed admixtures or control was administered orally for at least 104 weeks. Males were terminated at weeks 104-105, and females at week 105.

Table 1 Animal Allocation

Group	Number/Sex
Control (0/mg/kg/day)	60
Low (250/mg/kg/day)	60
Mid (750/mg/kg/day)	60
High (1500/mg/kg/day)	60

3.1.2 SPONSOR'S RESULTS

No test article-related mortality/moribundity was observed based on histopathological assessment. At the study termination, 63% survival was observed in the controls (both sexes); 77-83% in SPP100-treated males and 65-81% in SPP100-treated females at dose levels of 250-1500 mg/kg/day, respectively. There were no statistical differences in the survival between the controls and SPP100-treated animals (Log rank test, p=0.2891, and p=0.2044 for female and male, respectively). There was no statistically significant increase in the incidence of any tumor related to treatment.

3.1.3 STATISTICAL REVIEWER'S RESULTS

The analysis results of the mortality data of the female rats are presented in Tables 2 and 3 and Figure 1. At the study termination, 69% survival was observed in the controls; 70-83% in SPP100-treated females at dose levels of 250-1500 mg/kg/day, respectively. The test of homogeneity of survival distribution is not significant (p=0.2141). There is a linear negative trend in mortality (p=0.0419), indicating that mortality decreases as dose increases. The Kaplan-Meier graph also supports that there is no survival difference across groups.

The tumor findings of the female rats are summarized in Tables 4 and 5. No statistically significant positive linear dose-tumor trend was observed for any tumor types and combined tumor types.

Table 2 Analysis of Mortality Data for Female Rats by Treatment and Time

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
DOSE1	0-52	70	2	68	97.1	2.9
	53-78	68	9	59	84.3	15.7
	79-91	59	4	55	78.6	21.4
	92-104	55	7	48	68.6	31.4
	FINALKILL105-105	48	38	10		
	INTERIM KILL		10			
DOSE2	53-78	70	8	62	88.6	11.4
	79-91	62	8	54	77.1	22.9
	92-104	54	5	49	70.0	30.0
	FINALKILL105-105	49	39	10		
	INTERIM KILL		10			
DOSE3	0-52	70	2	68	97.1	2.9
	53-78	68	5	63	90.0	10.0
	79-91	63	5	58	82.9	17.1
	92-104	58	9	49	70.0	30.0
	FINALKILL105-105	49	40	9		
	INTERIM KILL		9			
DOSE4	0-52	70	2	68	97.1	2.9
	53-78	68	4	64	91.4	8.6
	79-91	64	2	62	88.6	11.4
	92-104	62	4	58	82.9	17.1
	FINALKILL105-105	58	48	10		
	INTERIM KILL		10			

Table 3 Analysis of Dose-Mortality Trend for Female Rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	0.4617	0.7938	0.3387	0.8442
Depart from Trend				
Dose-Mortality Trend	4.2161	0.0400	4.1409	0.0419
Homogeneity	4.6778	0.1970	4.4796	0.2141

Figure 1 Kaplan-Meier Survival Functions for Female Rats

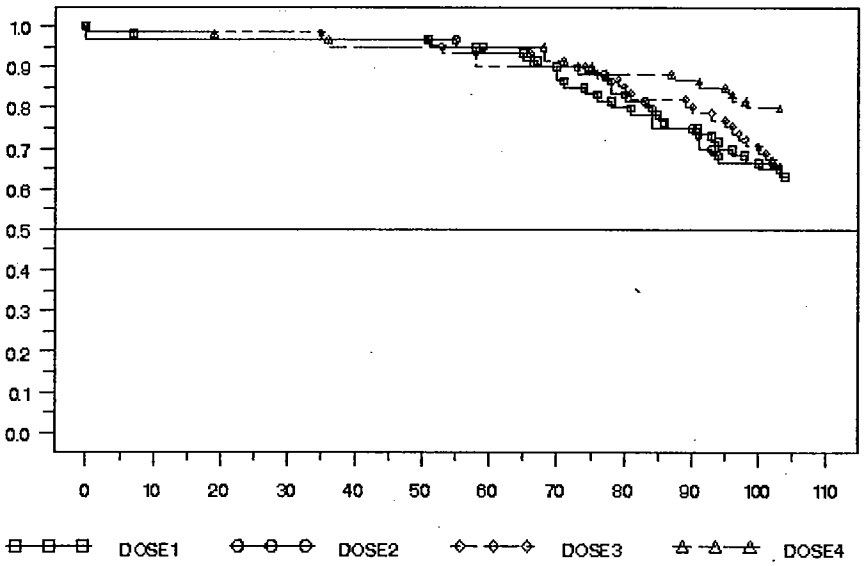

Table 4 Report on Test for Positive Linear Dose-Tumor Trends in Female Rats

Organ Code	Organ Name	Tumor Code	Tumor Name	DOSE1	DOSE2	DOSE3	DOSE4	P-Value (Exact Method)	P-Value (Asymptotic Method)
0100	BRAIN	010003	TUMOR GRANULAR CELL BENIGN	1	0	0	0	1.0000	0.8484
0100	BRAIN	010005	SARCOMA MENINGEAL	1	0	0	0	1.0000	0.8663
0100	BRAIN	010011	ANAPLASTIC GLIOMA	1	0	0	0	1.0000	0.8751
0520	AORTA	052004	HEMANGIOSARCOMA	0	0	1	0	0.5333	0.4512
0900	LUNGS	090009	CARCINOMA BRONCHIOALVEOLAR	0	1	0	0	0.7000	0.6644
0900	LUNGS	090019	ADENOMA BRONCHIOALVEOLAR	0	0	1	0	0.4500	0.3236
1000	ORAL CAV	100001	SQUAMOUS CELL	0	1	0	0	1.0000	0.8418

			CARCINOMA						
1500	STOMACH	150019	LEIOMYOSARCOMA	0	1	1	0	0.6562	0.6515
1500	STOMACH	150027	STROMAL SARCOMA	1	0	0	0	1.0000	0.8729
1602	JEJUNUM	160209	LEIOMYOMA	0	2	0	0	0.8385	0.8484
1703	RECTUM	170305	HEMANGIOSARCOMA	0	1	0	0	0.7697	0.7661
1800	LIVER	180023	ADENOMA	2	0	4	0	0.7566	0.7418
2000	PANCREAS	200015	ADENOMA ISLET CELL	2	0	0	0	1.0000	0.9386
2000	PANCREAS	200018	ISLET CELL CARCINOMA	0	0	0	1	0.1500	0.0259
2100	KIDNEYS	210002	LIPOMA	0	0	1	0	0.5305	0.4477
2300	URINARY BLADDER	230005	PAPILLOMA TRANSITIONAL CELL	2	0	0	0	1.0000	0.9280
3200	OVARIES	320004	CARCINOMA YOLKSAC	1	0	0	0	1.0000	0.8626
3200	OVARIES	320006	CYSTADENOCARCINOMA	1	1	1	0	0.8622	0.8457
3400	UTERUS	340002	POLYP ENDOMETRIAL STROMAL	6	9	6	8	0.5438	0.5315
3400	UTERUS	340006	SARCOMA ENDOMETRIAL STROMAL	0	1	2	2	0.1398	0.1085
3400	UTERUS	340012	ADENOCARCINOMA	0	3	1	4	0.0917	0.0755
3400	UTERUS	340013	LEIOMYOMA	1	0	0	0	1.0000	0.8751
3500	VAGINA	350003	GRANULAR CELL TUMOR BENIGN	0	3	4	1	0.4525	0.4340
3500	VAGINA	350006	POLYP	0	1	0	0	0.7598	0.7575
3600	CERVIX	360003	ADENOCARCINOMA	1	0	0	0	1.0000	0.8751
3600	CERVIX	360007	LEIOMYOSARCOMA	0	0	0	1	0.2909	0.0814
3700	CLITORAL GL	370003	HEMANGIOMA	1	0	0	0	1.0000	0.8765
4100	PITUITARY GLAND	410001	ADENOMA OF PARS DISTALIS	37	33	30	26	0.9864	0.9849
4100	PITUITARY GLAND	410005	ADENOCARCINOMA PARS DISTALIS	2	0	0	0	1.0000	0.9410
4100	PITUITARY GLAND	410009	ADENOMA OF PARS INTERMEDIA	1	1	1	2	0.3115	0.2720
4200	THYROID GLAND	420005	ADENOMA: C-CELL	9	9	7	16	0.0783	0.0717
4200	THYROID GLAND	420007	ADENOMA FOLLICULAR CELL	2	1	4	2	0.4500	0.4273
4300	PARATHYROID GL	430003	ADENOMA	0	1	0	0	0.7610	0.7675
4400	ADRENAL GL	440011	PHEOCHROMOCYTOMA MALIGNANT	0	0	0	1	0.2559	0.0661
4400	ADRENAL GL	440017	PHEOCHROMOCYTOMA BENIGN	1	0	1	0	0.7837	0.7669
4400	ADRENAL GL	440018	ADENOMA CORTICAL	0	0	1	1	0.2253	0.1406
4500	SYSTEMIC	450001	MALIGNANT LYMPHOMA	3	1	2	1	0.8400	0.8236
5000	THYMUS	500005	THYMOMA BENIGN	4	4	2	2	0.9103	0.8986
5100	LYMPH NODES	510010	HEMANGIOMA	1	0	0	0	1.0000	0.8415
5104	MESENTERIC LN	510404	HEMANGIOSARCOMA	1	0	1	0	0.7837	0.7669
5600	MAMMARY GL	560005	FIBROADENOMA	10	17	7	5	0.9906	0.9879
5600	MAMMARY GL	560007	ADENOCARCINOMA	6	1	5	2	0.7944	0.7811
5600	MAMMARY GL	560009	ADENOMA	3	1	3	1	0.7151	0.6975
5700	SKIN	570003	KERATOACANTHOMA	1	0	1	1	0.4624	0.4111
5700	SKIN	570009	PAPILLOMA SQUAMOUS CELL	2	0	0	0	1.0000	0.9306
5700	SKIN	570013	FIBROMA	1	0	0	0	1.0000	0.8484

6830	MESENTERY	683006	HEMANGIOSARCOMA	0	1	0	0	0.6667	0.6945
6900	ZYMBAL'S GL	690001	CARCINOMA SEBACEOUS CELL	1	0	0	1	0.5000	0.1590
6900	ZYMBAL'S GL	690002	CARCINOMA SQUAMOUS CELL	0	0	1	0	1.0000	0.8417

Table 5 Report on Test for Positive Linear Dose-Tumor Trends for Combined Tumors in Female Rats

Organ Name	Tumor Code	Tumor Name	DOSE1	DOSE2	DOSE3	DOSE4	P-Value (Exact Method)	P-Value (Asymptotic Method)
LUNGS +LUNGS	090009+ 090019	CARCINOMA BRONCHIOALVEOLAR + ADENOMA BRONCHIOALVEOLAR	0	1	1	0	0.5077	0.5068
PANCREAS+PANCREAS	200015+ 200018	ADENOMA ISLET CELL + CARCINOMA ISLET CELL	2	0	0	1	0.6200	0.5873
UTERUS+UTERUS	340002+ 340006	POLYP ENDOMETRIAL STROMAL+ SARCOMA ENDOMETRIAL STROMAL	6	10	8	10	0.3378	0.3262
PITUITARY GLAND+PITUITARY GLAND	410001+ 410005	ADENOMA OF PARS DISTALIS + ADENOCARCINOMA PARS DISTALIS	39	33	30	26	0.9936	0.9927
ADRENAL GL+ADRENAL GLANDS	440011+ 440017	PHEOCHROMOCYTOMA MALIGNANT+ PHEOCHROMOCYTOMA BENIGN	1	0	1	1	0.4515	0.3987
STOMACH +JEJUNUM	150019+ 160209	LEIOMYOSARCOMA + LEIOMYOMA	0	3	1	0	0.8586	0.8395
UTERUS+CERVIX	340013+ 360007	LEIOMYOMA + LEIOMYOSARCOMA	1	0	0	1	0.5561	0.4307
UTERUS+VAGINA	340002+ 350006	POLYP ENDOMETRIAL STROMAL+ POLYP	6	10	6	8	0.5851	0.5733
AORTA+RECTUM+LYMPH NODES+MESENT. LYMPH NODES+MESENTERY	052004+ 170305+ 510010+ 510404+ 683006	HEMANGIOSARCOMA	2	2	1	0	0.9417	0.9246

The analysis results of the mortality data of the male rats are presented in Tables 6 and 7 and Figure 2. At the study termination, 66% survival was observed in the controls; 77-86% in SPP100-treated males at dose levels of 250-1500 mg/kg/day, respectively. The test of homogeneity of survival distribution is significant (p=0.0365). There is a linear negative trend in mortality (p=0.0115), indicating that mortality decreases as dose increases. The Kaplan-Meier graph also demonstrates that survival curves are different across the groups.

The tumor findings of the male rats are summarized in Tables 8 and 9. No statistically significant positive linear dose-tumor trend was detected for any tumor types and combined tumor types.

Table 6 Analysis of Mortality Data for Male Rats by Treatment and Time

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
DOSE1	0-52	70	2	68	97.1	2.9
	53-78	68	4	64	91.4	8.6
	79-91	64	7	57	81.4	18.6
	92-103	57	11	46	65.7	34.3
	FINALKILL104-105	46	37	9		
	INTERIM KILL		9			
DOSE2	0-52	70	3	67	95.7	4.3
	53-78	67	3	64	91.4	8.6
	79-91	64	5	59	84.3	15.7
	92-103	59	5	54	77.1	22.9
	FINALKILL104-105	54	46	8		
	INTERIM KILL		8			
DOSE3	53-78	70	4	66	94.3	5.7
	79-91	66	3	63	90.0	10.0
	92-103	63	5	58	82.9	17.1
	FINALKILL104-105	58	48	10		
	INTERIM KILL		10			
DOSE4	0-52	70	2	68	97.1	2.9
	53-78	68	2	66	94.3	5.7
	79-91	66	3	63	90.0	10.0
	92-103	63	3	60	85.7	14.3
	FINALKILL104-105	60	50	10		
	INTERIM KILL		10			

Table 7 Analysis of Dose-Mortality Trend for Male Rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test / Depart from Trend	2.3935	0.3022	2.1238	0.3458
Dose-Mortality Trend	6.7418	0.0094	6.3879	0.0115
Homogeneity	9.1353	0.0275	8.5117	0.0365

Figure 2 Kaplan-Meier Survival Functions for Male Rats

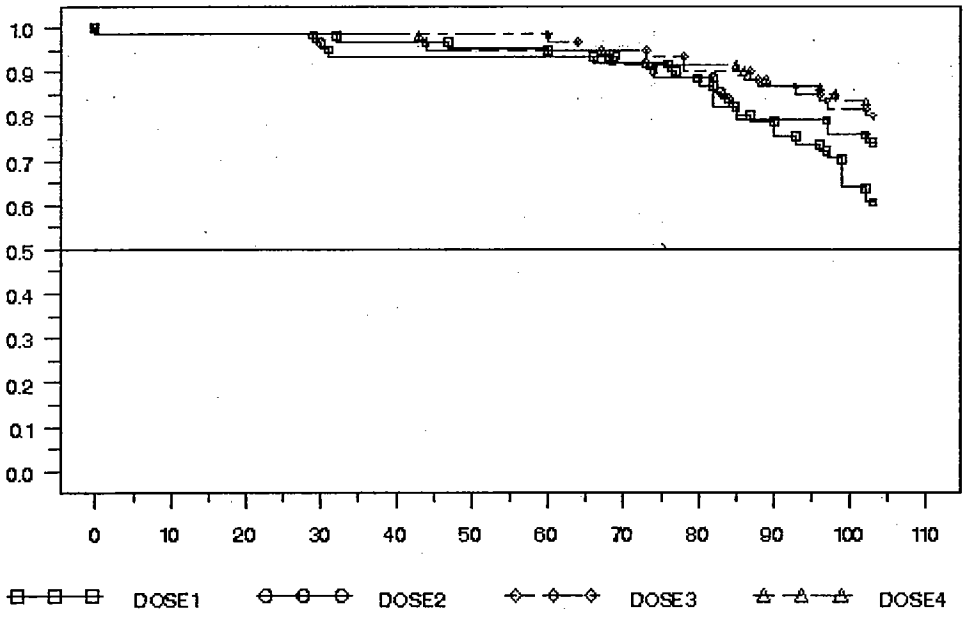

Table 8 Report on Test for Positive Linear Dose-Tumor Trends in Male Rats

Organ Code	Organ Name	Tumor Code	Tumor Name	DOSE1	DOSE2	DOSE3	DOSE4	P-Value (Exact Method)	P-Value (Asymptotic Method)
0100	BRAIN	010003	TUMOR GRANULAR CELL BENIGN	1	0	1	0	0.7893	0.7653
0100	BRAIN	010007	TUMOR GRANULAR CELL MALIGNANT	0	1	0	1	0.3328	0.2896
0100	BRAIN	010012	MENIGNIOMA BENIGN	1	0	0	0	1.0000	0.7962
0100	BRAIN	010013	SCHWANNOMA	0	0	1	0	0.5224	0.4298

0200	SPINAL CORD	020002	MENINGIOMA MALIGNANT	0	1	0	0	0.7956	0.7720
0400	HEART	040007	SCHWANNOMA ENDOCARDIAL MALIGNA	1	1	1	0	0.8726	0.8518
0900	LUNGS	090009	CARCINOMA BRONCHIOALVEOLAR	1	0	0	0	1.0000	0.8441
0900	LUNGS	090019	ADENOMA BRONCHIOALVEOLAR	0	0	1	0	0.4615	0.3270
1500	STOMACH	150027	STROMAL SARCOMA	1	0	0	0	1.0000	0.8735
1701	CECUM	170112	ADENOCARCINOMA	0	0	0	1	0.2762	0.0756
1702	COLON	170211	ADENOMA	0	0	0	1	0.2762	0.0756
1800	LIVER	180015	CHOLANGIOCARCINOMA	0	1	1	0	0.6544	0.6461
1800	LIVER	180023	ADENOMA	2	5	0	0	0.9902	0.9788
1800	LIVER	180032	CARCINOMA HEPATOCELLULAR	2	0	0	0	1.0000	0.9530
2000	PANCREAS	200015	ADENOMA ISLET CELL	3	1	3	3	0.4164	0.3963
2000	PANCREAS	200018	ISLET CELL CARCINOMA	1	0	0	0	1.0000	0.8813
2100	KIDNEYS	210002	LIPOMA	0	1	0	0	0.7568	0.7620
2100	KIDNEYS	210027	ADENOMA RENAL TUBULE	0	0	0	1	0.2762	0.0756
2500	TESTES	250003	ADENOMA INTERSTITIAL LEYDIG CE	0	2	1	1	0.4117	0.4035
2500	TESTES	250007	MESOTHELIOMA	0	1	0	0	0.7956	0.7720
2600	EPIDIDYMIDES	260006	MESOTHELIOMA	0	1	1	1	0.3569	0.3173
2600	EPIDIDYMIDES	260013	SARCOMA NOT OTHERWISE SPECIFIE	0	0	1	0	0.5414	0.4495
2700	PROSTATE	270003	ADENOCARCINOMA	0	1	0	0	0.7578	0.7501
2700	PROSTATE	270005	ADENOMA	1	0	1	0	0.7888	0.7698
4100	PITUITARY GLAND	410001	ADENOMA OF PARS DISTALIS	18	18	16	12	0.9519	0.9475
4100	PITUITARY GLAND	410009	ADENOMA OF PARS INTERMEDIA	1	1	0	0	0.9587	0.9147
4200	THYROID GLAND	420005	ADENOMA: C-CELL	9	12	10	4	0.9573	0.9512
4200	THYROID GLAND	420007	ADENOMA FOLLICULAR CELL	3	6	6	4	0.5977	0.5832
4200	THYROID GLAND	420010	ADENOCARCINOMA FOLLICULAR CELL	0	1	0	0	0.6471	0.6585
4300	PARATHYROID GL	430003	ADENOMA	2	2	1	0	0.9704	0.9562
4400	ADRENAL GL	440011	PHEOCHROMOCYTOMA MALIGNANT	1	0	0	0	1.0000	0.8735
4400	ADRENAL GL	440017	PHEOCHROMOCYTOMA BENIGN	0	1	0	0	0.7956	0.7720
4400	ADRENAL GL	440018	ADENOMA CORTICAL	1	0	0	1	0.5465	0.4284
4500	SYSTEMIC	450001	MALIGNANT LYMPHOMA	0	2	0	0	0.8343	0.8389
4500	SYSTEMIC	450002	HISTIOCYTIC SARCOMA	1	0	1	0	0.7625	0.7405
4500	SYSTEMIC	450003	MYELOID LEUKEMIA	1	0	0	0	1.0000	0.8720
5000	THYMUS	500005	THYMOMA BENIGN	1	1	0	1	0.5519	0.5084
5103	MEDIAST LN	510306	HEMANGIOMA	0	0	0	1	0.2500	0.0632
5104	MESENTERIC LN	510404	HEMANGIOSARCOMA	5	0	3	1	0.9100	0.8957
5104	MESENTERIC LN	510407	HEMANGIOMA	1	0	1	0	0.7911	0.7726
5300	SALIVARY GL	530001	ADENOCARCINOMA	0	1	1	0	0.4874	0.5033
5300	SALIVARY GL	530007	SCHWANNOMA	0	0	1	0	0.3333	0.2418
5600	MAMMARY GL	560010	ADENOLIPOMA	1	0	0	0	1.0000	0.8809

5700	SKIN	570002	BASOSQUAMOUS CARCINOMA	1	0	0	0	1.0000	0.8070
5700	SKIN	570003	KERATOACANTHOMA	1	2	6	0	0.7433	0.7262
5700	SKIN	570004	ADENOMA: SEBACEOUS CELL	1	0	0	0	1.0000	0.7962
5700	SKIN	570009	PAPILLOMA SQUAMOUS CELL	3	0	1	0	0.9743	0.9575
5700	SKIN	570012	FIBROSARCOMA	3	0	0	0	1.0000	0.9486
5700	SKIN	570013	FIBROMA	0	2	1	1	0.3926	0.3853
5700	SKIN	570016	LIPOMA	0	1	1	1	0.3519	0.3136
5700	SKIN	570018	HEMANGIOSARCOMA	1	0	0	0	1.0000	0.8813
5700	SKIN	570020	SCHWANNOMA MALIGNANT	0	1	0	0	0.7956	0.7720
5700	SKIN	570025	TUMOR BASAL CELL BENIGN	0	0	1	0	0.5414	0.4495
5800	SKELETAL MUSCLE	580005	HEMANGIOSARCOMA	1	0	0	0	1.0000	0.8689
6500	EYES	650006	SCHWANNOMA MALIGNANT	1	0	0	0	1.0000	0.8703
6830	MESENTERY	683005	HEMANGIOMA	0	0	1	0	1.0000	0.8417
7100	FEMUR	710004	OSTEOSARCOMA	1	0	0	0	1.0000	0.8819

Table 9 Report on Test for Positive Linear Dose-Tumor Trends for Combined Tumors in Male Rats

Organ Name	Tumor Code	Tumor Name	DOSE1	DOSE2	DOSE3	DOSE4	P-Value (Exact Method)	P-Value (Asymptotic Method)
BRAIN + BRAIN	010003+ 010007	TUMOR GRANULAR CELL BENIGN	1	1	1	1	0.5803	0.5477
LUNGS + LUNGS	090009+ 090019	CARCINOMA BRONCHIOALVEOLAR + ADENOMA BRONCHIOALVEOLAR	1	0	1	0	0.7308	0.6610
LIVER + LIVER	180023+ 180032	ADENOMA + CARCINOMA HEPATOCELLULAR	4	5	0	0	0.9990	0.9954
PANCREAS+PANCREAS	200015+ 200018	ADENOMA ISLET CELL+ ISLET CELL CARCINOMA	4	1	3	3	0.5629	0.5459
PROSTATE+PROSTATE	270003+ 270005	ADENOCARCINOMA + ADENOMA	1	1	1	0	0.8595	0.8394
THYROID GLAND + THYROID GLAND	420007+ 420010	ADENOMA FOLLICULAR CELL +ADENOCARCINOMA FOLLICULAR CELL	3	7	6	4	0.6298	0.6164
ADRENAL GL + ADRENAL GL	440011+ 440017	PHEOCHROMOCYTOMA MALIGNANT + PHEOCHROMOCYTOMA BENIGN	1	1	0	0	0.9538	0.9090
CECUM +COLON	170112+ 170211	ADENOCARCINOMA+ ADENOMA	0	0	0	2*	0.0752	0.0209

*Since the two incidences are incidental and the cell number is small, the exact p-value=0.0752 is used.

3.1.4 EVALUATION OF VALIDITY OF STUDY

Since no statistically significant positive trends in tumor rates were detected in both sexes, it is necessary to further evaluate the validity of the study design to see:

- if there were sufficient numbers of animals living long enough to provide adequate exposure to the chemical tested
- if the doses were adequate to present a reasonable tumor challenge to the tested animal. There are three criteria used in Chu et al paper for checking if the high dose is adequate or not:
  - body weight gain decreases < 10% or
  - slightly higher mortality or
  - clinical signs or severe histopathologic toxic effects caused by the chemical evaluated by the pharmacologist/toxicologist.

Based on the above criteria, the findings of the evaluation of the study are summarized in the followings:

- At week 91, at least 77% females and 81% males were alive in all the dose groups, there are more than 30 animals survived during weeks 80-90. It demonstrates that there are sufficient numbers of animals living enough to provide adequate exposure to the chemical tested.

- Dose-dependent reductions in mean body weight gain decreases were noted in both sexes at all dose levels through the duration of the study (Table 10). Significant reductions were observed in the mid and high dose groups, ranging from 20.8% to 29.6% in the females and 17.8% to 23.6% in the males in the mid dose group; 37.1% to 47.0% in females and 39.1% to 45.3% in males in the high dose group. The relative large magnitude of the decrease in mean body weight gain (greater than 10%) suggests that the mid and high doses are over the maximum tolerated doses (MTD).

Table 10 Mean Body Weight Gain Decreases in SPP100 Treated Rats*

Interval (Week)	Sex	Dose Level (mg/kg/day)		
		250	750	1500
26	M	5.8	17.8	39.1
	F	8.5	20.8	37.1
50	M	6.1	19.8	39.8
	F	8.4	23.7	42.9
104	M	13.1	23.6	45.3
	F	10.1	29.6	47.0

*Values are presented as the mean percent decrease relative to controls

- The body weight gain data seems to suggest that the mid and high doses are over MTD. However, all the treated groups had lower mortalities than the controls. This may suggest that the mid and high doses are under MTD.

Because of the different conclusions on the appropriateness of the doses used basing on body weight gain and mortality data, the pharmacologist/toxicologist should evaluate the appropriateness of the doses used basing on clinical signs and server histopathological toxic effects the tested animals exhibited.

In conclusion, the validity of the study is not conclusive based on the assessment of adequacy of exposure and doses selected in the study.

3.1.5 CONCLUSION

Statistically significant differences in survival were observed among the males, but not the females. There is a negative linear trend in mortality detected in both sexes. No statistically significant positive trend was observed for any tumor types and combined tumor types for either female or male rats. The validity of the study is not conclusive.

3.2 STUDY B: Short-Term Study 0410091

3.2.1 STUDY DESIGN

CB6F1/Jic-TgrasH2 transgenic mice were randomly allocated to the controls, SPP100 dose groups and N-methyl-N-nitrosourea (MNU) positive group shown in Table 11. SPP100 test article was administered in-feed daily for 26 weeks. MNU was single administration on test day one. Males were terminated at test week 25 and females at week 22 for the controls and SPP100 treated groups. The animals in the MNU treated group were terminated when 5 surviving animals were left.

Table 11 Animal Allocation

Group	Number/Sex
Control (0/mg/kg/day)	25
Low (250/mg/kg/day)	25
Mid (250/mg/kg/day)	25
High (1500/mg/kg/day)	25
MNU (75/mg/kg/day)	25

3.2.2 SPONSOR'S RESULTS

Mortality is low in all the groups. Survival rates are 100, 96, 100 and 84% for the controls, 250, 750 and 1500 mg/kg groups among males; and 96, 96, 96 and 100%

among females, respectively. The occurrence of tumors is not considered to be treatment-related. No positive linear dose-tumor trend was detected.

3.2.3 REVIEWER'S RESULTS

It appears that a relative high mortality rate (84%, four deaths) was observed in the high dose (1500/mg/kg) group among the males. Although the sponsor stated that these deaths did not seem to be related to the treatment, the reviewer conducted a statistical analysis to compare mortality rates across the groups. The log-rank test showed that the differences in mortality rates are statistically significant (p=0.0253). The Kaplan-Meier graph in Figure 4 also shows that the survival curves are different across the groups. The permutation trend test indicates a statistically significant positive trend in mortality (p=0.0064). The statistical analysis results are summarized in Table 12.

Table 12 Mortality in Males at the Termination

	Control	Low	Mid	High	P-Value (Log-Rank Test)	P-Value (Trend Test)
Number of Animals	25	25	25	25		
Death at the Termination	0	1	0	4		
Mortality Rate (%)	0	4	0	16	0.0253	0.0064

Neither a statistically significant difference nor a linear trend in mortality was detected among the females (p=0.7978, log-rank test). The Kaplan-Meier graph shown in Figure 3 also suggests that there is no dose-response in mortality among the females.

The tumor findings of mice are summarized in Tables 13 and 14. No statistically significant positive linear dose-tumor trend was detected in tumor types tested for both sexes.

Figure 3 Kaplan-Meier Survival Functions for Female Mice

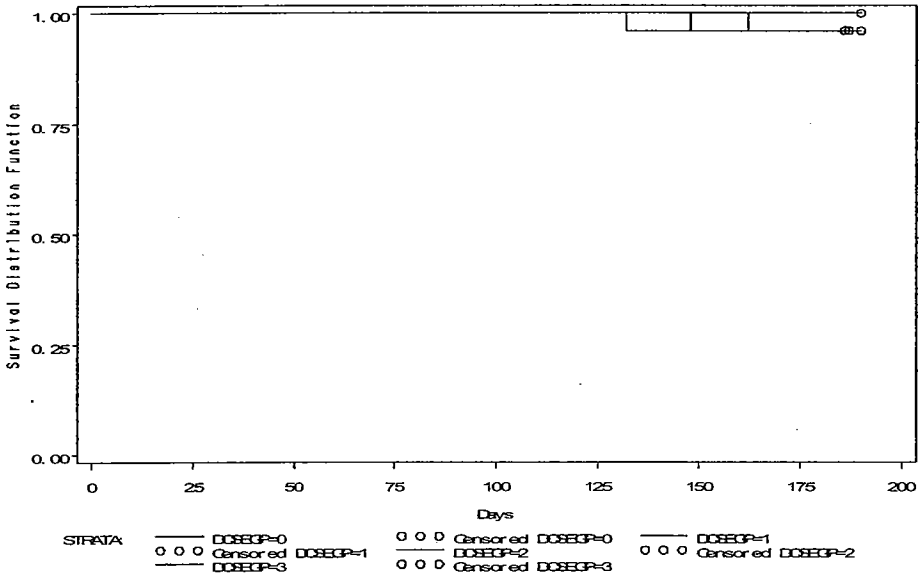

Figure 4 Kaplan-Meier Survival Functions for Male Mice

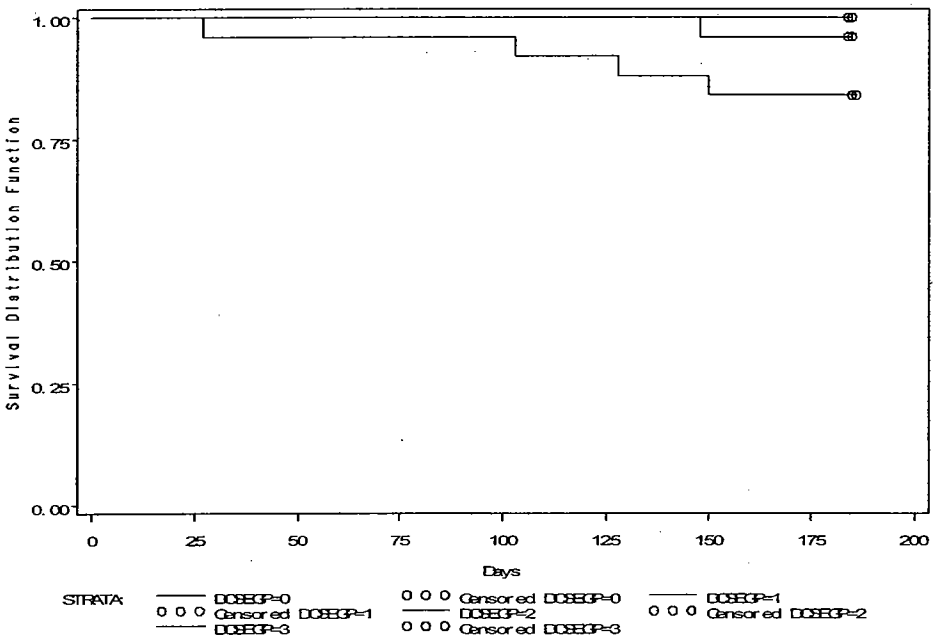

Table 13 Tumor Incidences in Female Mice at Termination

Tumor Code	Tumor Name	Organ	C	L	M	H	Asym. P-value	Exact P-value
560004	Adenocarcinoma	Mammary Area	1	0	0	0	0.1376	0.2500
090006	Adenoma Bronchio Alveolar	Lungs	2	2	1	1	0.2224	0.2659
090012	Carcinoma Bronch Ioalveolar	Lungs	1	0	0	0	0.1376	0.2500
340006	Deciduoma	Uterus	0	1	0	0	0.2563	0.5000
061010	Hemangioma	Nasal Cavity	0	0	0	1	0.0633	0.2500
210010	Hemangioma	Kidneys	0	0	0	1	0.0633	0.2500
460004	Hemangioma	Spleen	0	1	1	0	0.3782	0.5000
710007	Hemangioma	Femur/Marrow	0	1	0	0	0.2563	0.5000
061010	Hemangiosarcoma	Nasal Cavity	0	0	1	0	0.4136	0.5000
460008	Hemangiosarcoma	Spleen	0	1	0	0	0.2563	0.5000

Table 14 Tumor Incidences in Male Mice at Termination

Tumor Code	Tumor Name	Organ	C	L	M	H	Asym. P-value	Exact P-value
540005	Adenoma	Harderian Gland	1	0	0	0	0.1376	0.2500
090006	Adenoma Bronchio Alveolar	Lungs	3	2	2	0	0.0530	0.0589
090012	Carcinoma Bronch Ioalveolar	Lungs	0	1	0	0	0.2563	0.5000
570002	Carcinoma Basal Cell	Uterus	0	0	1	0	0.4136	0.5000
460004	Hemangioma	Spleen	0	1	1	0	0.3782	0.5000
061010	Hemangiosarcoma	Nasal Cavity	1	0	0	0	0.1376	0.2500
460008	Hemangiosarcoma	Spleen	0	0	0	1	0.0633	0.2500

The reviewer also felt that the study should not have been terminated early since the data of the positive control group was not used in the dose trend analysis.

3.2.4 CONCLUSION

Statistically significant differences and a positive trend in mortality were observed among the males, but not the females. No statistically significant positive trend was detected in any tumor types tested for either female or male mice.

4. SUMMARY AND CONCLUSIONS

4.1 Statistical Evaluation of Evidence

It is well-known that trend tests are more powerful than pair-wise comparisons in detecting an effect in a multi-group study. Trend tests are therefore the primary tests evaluated by the reviewer.

The survival analysis conducted among the males in study 0410091 is used only as supportive analysis. The death causes are needed to be further evaluated and confirmed by the pharmacologist/toxicologist.

4.2 Conclusions

In study 0370063, statistically significant differences in survival were observed among the males, but not the females. There is a negative linear trend in mortality detected in both sexes. No statistically significant positive trend was observed for any tumor types and combined tumor types tested for either female or male rats. The validity of the study is not conclusive.

In study 0410091, it appears that a higher mortality rate was observed in the high dose group (1500 mg/kg) among the males. The statistical analysis shows that the difference in mortality in males is statistically significant across the groups. The death causes for these animals are needed to be further evaluated and confirmed by the pharmacologist/toxicologist. No statistically significant increase in tumor incidence was detected in either female or male animals.

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