

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

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SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
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INTRODUCTION

The commercial grade of SC-18862 (L-aspartyl phenylalanine methyl-ester), a nutritive sweetening agent, may contain from 0-1% of a conversion product, SC-19192. This conversion of SC-18862 to SC-19192 also occurs under certain storage conditions. A human population consuming SC-18862 would thus be exposed to varying concentrations of SC-19192, also. The mutagenic potential of this latter agent has been evaluated as part of the comprehensive pre-clinical safety studies program on SC-18862. The present study, a dominant lethal test¹, is one portion of the total mutagenicity test profile. Compound was administered orally to male rats of proven fertility; two equally divided doses were administered on a single day only. Each male rat was then sequentially mated to eight separate groups of untreated females, with each successive group being exposed for a one week period. Dominant lethal mutations induced in the spermatozoa, when present, were detected by observing the number of fetal deaths after 14 days of gestation.

METHODS

Material evaluated.

SC-19192 (diketopiperazine; DKP) is a fine white powder with the chemical

name 5-benzyl-3,6-dioxo-2-piperazineacetic acid. Lot No. A6906 was employed in this study.

Animals, housing and diet.

Six hundred eleven female and 40 male albino rats of the Charles River cd strain were employed. Males were of proven fertility and were approximately 160 days of age at treatment. Females were untreated virgins, ranging from 90 to 130 days of age at initiation of mating.

Males were housed individually in suspended metal cages with wire mesh floors and were acclimated to the laboratory environment for 10 weeks preceding the initiation of the study.

Females were housed in groups of three per wire mesh cage and were acclimated to laboratory conditions for at least three weeks prior to mating.

For mating purposes, each male was housed with two females in a 10 x 11 x 14 inch metal bottom Wahmann breeding cage. Absorbent bedding (Ab-Sorb-Dri, Inc.) was employed. Mated females were housed in groups of two in suspended wire mesh cages.

Male fertility was established prior to treatment in the following manner: one virgin female was housed continuously with one male for one month; all females without litters were then sacrificed, and the uterine horns examined for implantation sites. The presence of a litter or of uterine implantation sites established the fertility of the male.

Animal quarters were air-conditioned with thermostats set to maintain 72°F temperature continuously. Artificial fluorescent lighting was provided as a 14-hour photoperiod daily.

Basal diet [Rockland Mouse/Rat (complete) pelleted form; Teklad, Inc., Winfield, Iowa] and chlorinated tap water were available ad libitum throughout the study.

Experimental design.

Forty males were randomly assigned to the following 3 groups:

Treatment Group	No. of Males	Dosage Level mg/kg
Control (-); vehicle	15	--
SC-19192	15	1000
Control (+); MMS*	10	50

* MMS = Methyl methane sulfonate (Lot No. 120191; Aldrich Chemical Co., Inc., Milwaukee, Wisconsin) is a known mutagen in rodents^{2,3,4}; in the present study this group served as a positive control.

This dominant lethal test with SC-19192 (the present study) was performed in conjunction with a SC-18862 dominant lethal study (P-T 1007S72). Since the two compounds were being evaluated concurrently for their mutagenic potential by the dominant lethal assay, it was felt unnecessary to employ duplicate (-) control and (+) control groups. However, to maintain uniformity with other preclinical safety study reports (one compound - one report), these dominant lethal assays of SC-19192 and SC-18862 are reported as separate documents, containing common (-) and (+) control data.

Compound formulation and administration.

The treated group received SC-19192, administered intragastrically, as a freshly prepared 5% suspension (w/v) in the vehicle indicated below. Two equally divided doses of 2 ml/100 g body weight were given two hours apart on a single day only.

The (-) control group received the vehicle only, a 1% solution of Tween-80 (v/v) in distilled water, administered in comparable volume by the same route and dosage regimen.

The (+) control group received methyl methane sulfonate (MMS) administered intraperitoneally as a 0.7% suspension (w/v) in corn oil (Mazola; Corn Products Co., N. Y.). A single injection was given.

Females were not treated.

Mating procedure.

Immediately following treatment, each male was housed with two untreated, virgin females for a period of one week. Exposure of the males to the females during that week was restricted to four consecutive nights, followed by continuous cohabitation through the mid-seventh day with those females remaining unmated. At this time two new untreated females were presented to each male. This mating schedule was continued for eight consecutive weeks.

Following overnight cohabitation the males were removed from the cage and the females were examined. The presence of a copulatory plug and/or spermatozoa in the vaginal smear was considered evidence of mating and was recorded as gestation day 0. Mated females were subsequently housed in groups of two per suspended wire mesh cage until sacrificed on gestation day 14. Females unmated after one week exposure to a male were caged separately for 12 days, then sacrificed and autopsied.

Observations and examination procedures.

Paternal animal. The general appearance and behavior of each

male were observed daily. Body weight was recorded weekly throughout the entire eight week mating period. Food consumption was not recorded.

Maternal autopsy procedures. Body weight and food consumption were not recorded for females. They were sacrificed by carbon dioxide inhalation, either on gestation day 14 or following the 12 day observation period. The abdominal cavity was opened and the reproductive tract exteriorized. Each implantation along the uterine horns was classified as either: 1) a viable fetal swelling as indicated by general size, clearness of the amnionic fluid and apparent fetal development; 2) late fetal death (LD; a fetal death occurring late in gestation) as indicated by small size, brownish amnionic fluid and slight fetal tissue remnant; or 3) early fetal death (ED; a fetal and/or embryonic death occurring very early in gestation) as indicated by minimal fetal or amnionic development and observed grossly as a deciduoma. The ovaries were then removed, freed of extraneous connective tissue and examined intact under a dissecting microscope (14x). The number of corpora lutea present was recorded.

Statistical procedures.

In this study data were collected and analyzed on a weekly basis. The total number of implants per pregnancy was analyzed in detail to determine the relative contribution of the female, the male and the treatment to the total variation present in this parameter. Differences between females mated to a single male accounted for most, if not all, of the variation; hence, the data were tabulated on a per female basis. The mean number of implants for the SC-19192 and (+) control males was compared to that of the (-) control males, using between-female variation (Student's t-test; $p < 0.05$). Data for corpora lutea and viable fetal swellings were compared in a similar manner.

The distribution of number of fetal deaths (ED and LD combined) was asymmetrical. Even though the basic distribution was Poisson, several outliers were present both in control and treated groups at a few of the intervals. For this reason a distribution-free method (Wilcoxon's rank sum test; $p < 0.05$) was used for comparing mean fetal deaths in the SC-19192 treated and (+) control groups with those of the (-) control group. Chi-square analysis was used to compare the following parameters: paternal survival rates, pregnancy rates and the incidence of pregnant females with fetal deaths.

RESULTS

Paternal animal.

Body weight. Mean body weight data for the various treatment groups are presented in Figure 1. The rate of growth during the eight week mating period was comparable in all groups.

Survival rate. The survival rates for the (-) control, SC-19192 treated and (+) control groups were 87%, 100% and 100%, respectively. Negative control males No. 8 and 9 died 7 and 4 days post-treatment, respectively. A complete gross postmortem examination of these animals was performed. Severe autolysis in animal No. 8 prevented a precise evaluation of lesions. In animal No. 9 no gross lesions attributable to the experimental procedure were detected. Such deaths reduced the total number of (-) control females exposed to males, as depicted in Figure 2.

Maternal animal.

Pregnancy rate data. Group pregnancy rates over the entire eight week mating sequence are presented on page 9. These rates were comparable

Figure 1

SC-19192: AN EVALUATION OF THE MUTAGENIC
POTENTIAL IN THE RAT EMPLOYING
THE DOMINANT LETHAL ASSAY

Mean Paternal Body Weight Data (\pm S.E.)

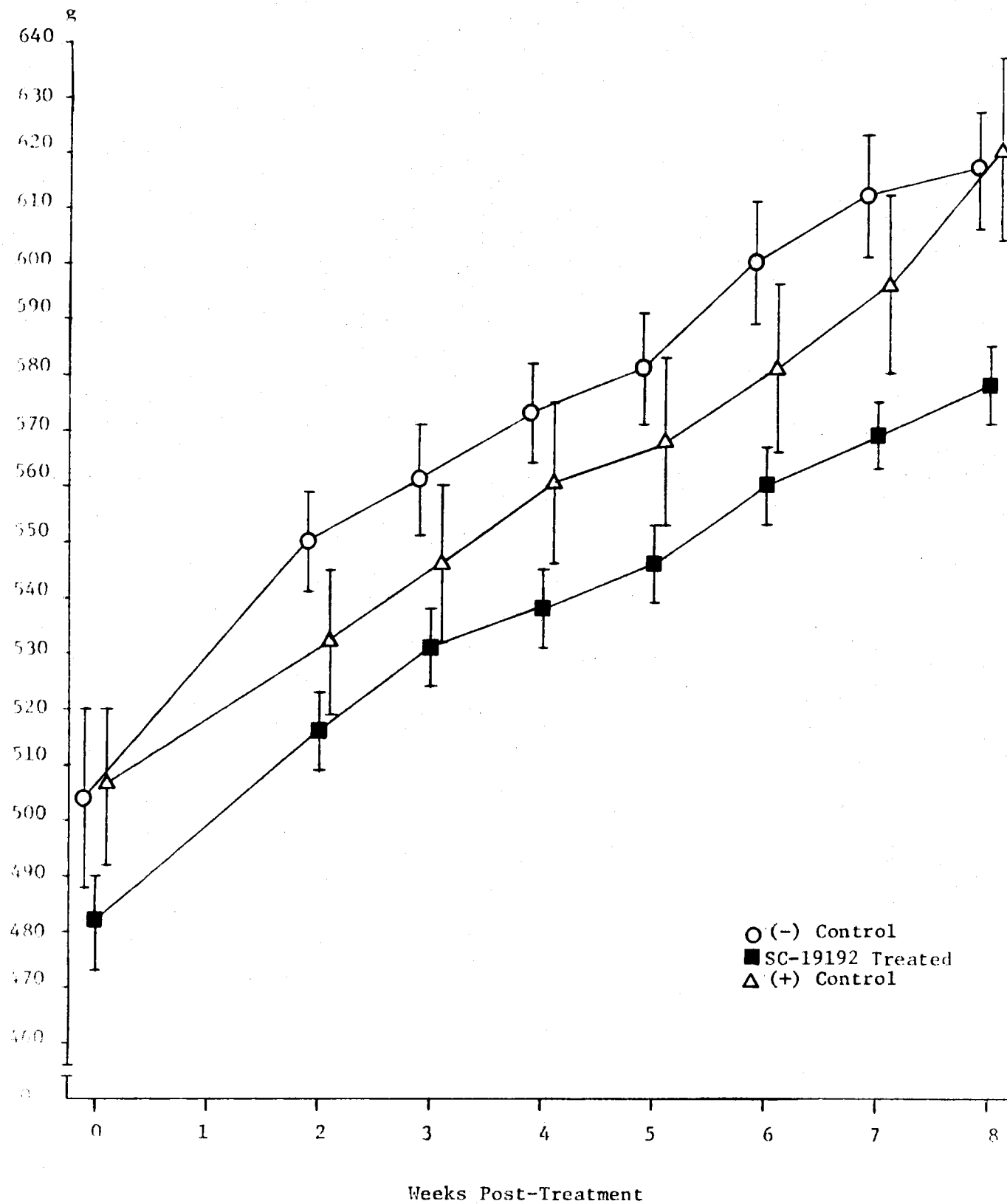
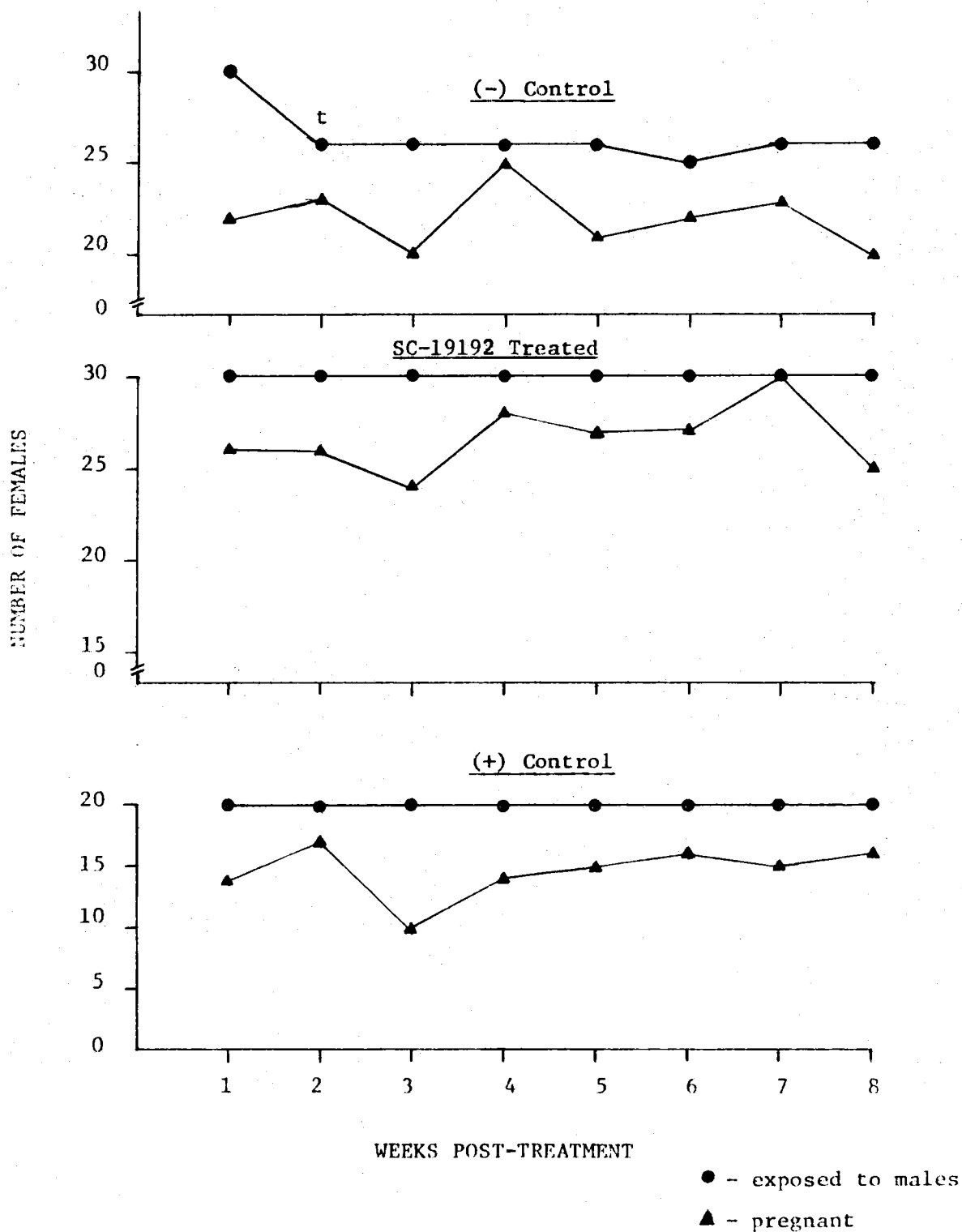


Figure 2

SC-19192: AN EVALUATION OF THE MUTAGENIC
POTENTIAL IN THE RAT EMPLOYING
THE DOMINANT LETHAL ASSAY

Pregnancy Rate Data



t= death of two vehicle control males resulted in reduced total number of (-) control females exposed to males.

between the (-) control and SC-19192 treated groups and significantly reduced in the (+) control group.

Treatment Group	No. of Females		Pregnancy Rate (%) ^b
	Exposed	Pregnant ^a	
(-) Control	211	176	83.4
SC-19192 Treated	240	213	88.8
(+) Control	160	117	73.1*

^a Pregnant: Indicated by the presence of at least one implantation site along either uterine horn at mid-gestation sacrifice.

^b Pregnancy rate: No. of females pregnant/no. of females exposed to males x 100.

* Difference statistically significant ($p < 0.05$).

The pregnancy rates on a week-by-week basis for the (-) control, SC-19192 treated, and (+) control groups (Table 1 and Figure 2) were generally comparable. The sole exception occurred at mating week 4 when the pregnancy rate of the (+) control group was significantly reduced.

Postmortem examination. At sacrifice, in addition to the uterine and ovarian examination, the thoracic cavity of each female was exposed and the lungs were examined grossly. Alterations characterized by a raised, nodular appearing pleural surface and pleural adhesions extending to the thoracic wall and/or diaphragm were observed in 13 of the 611 females examined, as follows: 2 of 211 females from the (-) control group, 11 of 240 females from the SC-19192 group, and 0 of 160 females from the (+) control group. The effect of pulmonary infections on the fertility of the female rat is not clearly understood. Rohrborn⁵ observed a reduction in total implants following maternal infection in mice. As there was no indication in the current rat study that incidence of infection corresponds consistently with an increased

Table 1

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
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Pregnancy Rate Data (%)

Treatment Group	Weeks Post-Treatment							
	1	2	3	4	5	6	7	8
(-) Control	73	88	77	96	81	88	88	77
SC-19192 treated	87	87	80	93	90	90	100	83
(+) Control	70	85	50	70*	75	80	75	80

* Difference statistically significant ($p < 0.05$); during week 4
 (-) control data: 25 females pregnant/26 females exposed
 $\times 100 = 96\%$;
 (+) control data: 14 females pregnant/20 females exposed
 $\times 100 = 70\%$.

or decreased number of implants or fetal deaths, the data from these females were not excluded from the study.

Uterine and ovarian examination data. Results from the uterine and ovarian examinations of females sacrificed during gestation are presented in Table 2; data concerning the females exposed to individual males during each week of the mating period are listed in the Appendix. Each implantation was subclassified as either a fetal death (early or late) or a viable fetal swelling. The mean number of corpora lutea, implantations, fetal deaths, and viable fetal swellings was generally comparable between the (-) control and the SC-19192 group throughout the eight week mating period, with two exceptions. During week 5 a slight, but statistically significant, decrease in the mean number of implantations and viable fetal swellings was observed in the SC-19192 group. Corpora lutea and fetal death data were, however, comparable to the (-) control group during this interval. The slight differences in implantations and viable fetal swellings are not considered biologically meaningful, since both parameters are essentially within the control range for that parameter over the entire eight week period (mean implantation rate for the (-) control group over eight weeks is 13.4 ± 0.5 ; mean value for viable fetal swellings in the (-) control group over eight weeks is 12.6 ± 0.5).

A significant decrease in the mean number of corpora lutea in the SC-19192 treated group was observed during week 7; implantation data during this interval were unremarkable. This alteration in corpora lutea is likewise not biologically meaningful, since it is within the range of weekly mean values recorded for the concurrent (-) control group (mean corpora lutea value over eight weeks is 14.1 ± 0.2 ; weekly values range from 13.0 - 14.9).

Examination of the ovaries and uteri of females mated to (+) control males as compared to the females mated to (-) control males revealed the following significant differences:

Table 2

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
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Summary of Uterine and Ovarian Examination Data

(Mean \pm S.E.)

Parameters	Weeks Post-Treatment			
	1	2	3	4
<u>Vehicle (-) Control Group</u>				
No. of Corpora Lutea	13.0 \pm 0.6	13.8 \pm 0.4	14.0 \pm 0.4	14.9 \pm 0.4
No. of Implantations	11.9 \pm 0.8	13.3 \pm 0.4	13.6 \pm 0.3	14.4 \pm 0.4
No. of Fetal Deaths [†]	1.3 \pm 0.3	1.0 \pm 0.3	0.6 \pm 0.2	1.0 \pm 0.3
No. of Viable Fetal Swellings	10.6 \pm 0.7	12.3 \pm 0.4	13.0 \pm 0.5	13.4 \pm 0.4
<u>SC-19192 Treated Group</u>				
No. of Corpora Lutea	13.5 \pm 0.5	14.5 \pm 0.4	14.0 \pm 0.5	14.4 \pm 0.3
No. of Implantations	12.5 \pm 0.6	13.9 \pm 0.3	13.1 \pm 0.7	13.9 \pm 0.3
No. of Fetal Deaths [†]	0.7 \pm 0.2	1.9 \pm 0.6	1.3 \pm 0.3	0.6 \pm 0.1
No. of Viable Fetal Swellings	11.8 \pm 0.5	12.0 \pm 0.7	11.9 \pm 0.7	13.4 \pm 0.3
<u>MMS (+) Control Group</u>				
No. of Corpora Lutea	13.5 \pm 0.6	9.0 \pm 0.9*	10.5 \pm 0.8*	10.6 \pm 1.4*
No. of Implantations	13.2 \pm 0.6	7.0 \pm 1.0*	1.6 \pm 0.7*	8.4 \pm 1.8*
No. of Fetal Deaths [†]	4.5 \pm 0.6*	6.0 \pm 0.4*	1.6 \pm 0.3*	1.4 \pm 0.3
No. of Viable Fetal Swellings	8.7 \pm 0.8*	1.0 \pm 0.7*	0 *	7.0 \pm 1.7*

[†] Fetal deaths: Early and late deaths combined.

* Difference statistically significant ($p < 0.05$).

Table 2 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
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Summary of Uterine and Ovarian Examination Data

(Mean \pm S.E.)

Parameters	Weeks Post-Treatment			
	5	6	7	8
<u>Vehicle (-) Control Group</u>				
No. of Corpora Lutea	14.9 \pm 0.4	13.7 \pm 0.5	14.4 \pm 0.4	14.2 \pm 0.4
No. of Implantations	14.3 \pm 0.4	13.1 \pm 0.5	13.6 \pm 0.6	13.5 \pm 0.5
No. of Fetal Deaths [†]	0.9 \pm 0.2	0.7 \pm 0.2	0.9 \pm 0.2	0.7 \pm 0.2
No. of Viable Fetal Swellings	13.5 \pm 0.4	12.4 \pm 0.5	12.7 \pm 0.6	12.8 \pm 0.5
<u>SC-19192 Treated Group</u>				
No. of Corpora Lutea	14.1 \pm 0.4	14.2 \pm 0.4	13.3 \pm 0.4*	13.8 \pm 0.5
No. of Implantations	13.0 \pm 0.6*	12.9 \pm 0.7	12.9 \pm 0.5	13.4 \pm 0.6
No. of Fetal Deaths [†]	1.4 \pm 0.4	0.9 \pm 0.2	0.9 \pm 0.2	0.6 \pm 0.1
No. of Viable Fetal Swellings	11.6 \pm 0.8*	11.9 \pm 0.7	12.1 \pm 0.5	12.8 \pm 0.6
<u>MMS (+) Control Group</u>				
No. of Corpora Lutea	13.3 \pm 0.9	13.7 \pm 0.5	13.1 \pm 0.6	14.4 \pm 0.3
No. of Implantations	12.6 \pm 1.0*	12.8 \pm 0.6	12.4 \pm 0.8	13.9 \pm 0.4
No. of Fetal Deaths [†]	2.7 \pm 0.5*	1.1 \pm 0.3	0.7 \pm 0.3	0.9 \pm 0.2
No. of Viable Fetal Swellings	9.9 \pm 1.1*	11.7 \pm 0.7	11.7 \pm 0.8	13.0 \pm 0.5

[†] Fetal deaths: Early and late deaths combined.

* Difference statistically significant ($p < 0.05$).

- 1) Mean number of corpora lutea reduced weeks 2, 3 and 4;
- 2) Mean number of implantation sites reduced weeks 2, 3, 4 and 5;
- 3) Mean number of fetal deaths (ED and LD) increased weeks 1, 2, 3 and 5;
- 4) Mean number of viable fetal swellings reduced weeks 1, 2, 3, 4 and 5.

However, the significant decrease in mean number of implantation sites observed in the (+) control group at weeks 2, 3 and 4 and the reduced number of viable fetal swellings at week 4, in particular, is at least partially attributable to an unexplained decrease in ovulation rate in these animals occurring at these periods (weeks 2, 3 and 4).

Incidence of fetal deaths. Over the entire study fetal deaths (ED and LD combined) were observed among the uterine implantations in 88 of 176 (-) control and 115 of 213 SC-19192 pregnant females, an incidence of 50.0% and 54.0%, respectively, which was comparable (Table 3). Similarly on a week-to-week basis the incidence of pregnant females with at least one fetal death (Figures 3 and 4) was comparable between the (-) control and SC-19192 group throughout the mating period.

In the (+) control group fetal deaths were observed among the uterine implantations in 93 of 117 pregnant females (79.4%). This was a significant increase from the (-) control group. On a week-to-week basis the incidence of pregnant (+) control females with fetal deaths (Figures 3 and 4) was significantly higher at weeks 1, 2, 3 and 4 of mating.

Table 3

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
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Summary of Fetal Death Data

	Treatment Group		
	(-) Control	SC-19192	(+) Control
Total No. of Pregnant Females			
Examined:	176	213	117
With Fetal Deaths:	88	115	93
Incidence of Pregnant Females with Fetal Deaths (%):	50.0	54.0	79.4*
No. of Fetal Deaths Observed			
Total:	154	216	283
Early:	153	211	281
Late:	1	5	2
No. of Pregnant Females with Late Deaths:	1	4	2
Incidence of Pregnant Females with Late Deaths (%):	0.6	1.9	1.7

Figure 3

SC-19192: AN EVALUATION OF THE MUTAGENIC
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Incidence of Pregnant Females with Fetal Deaths

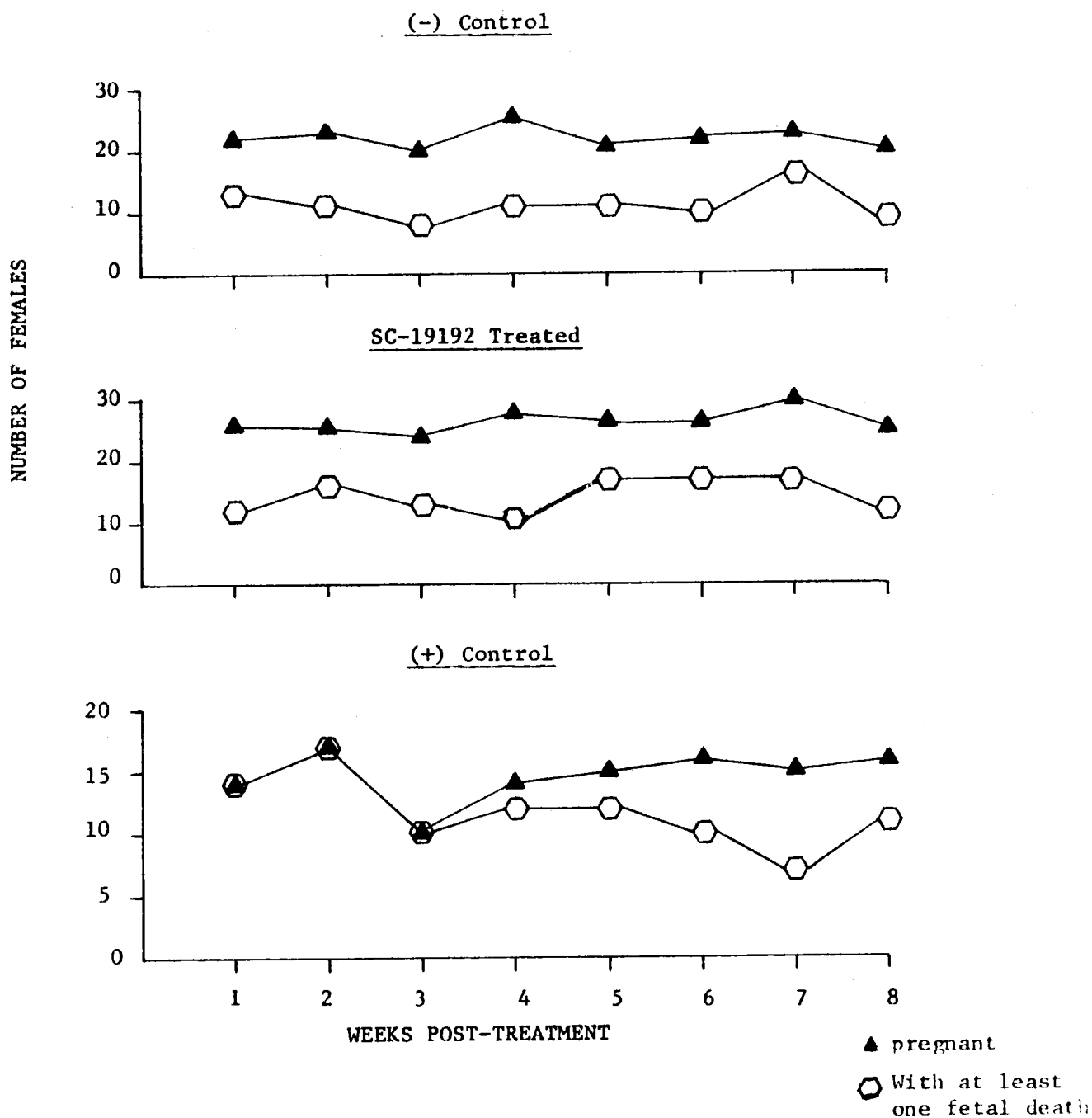
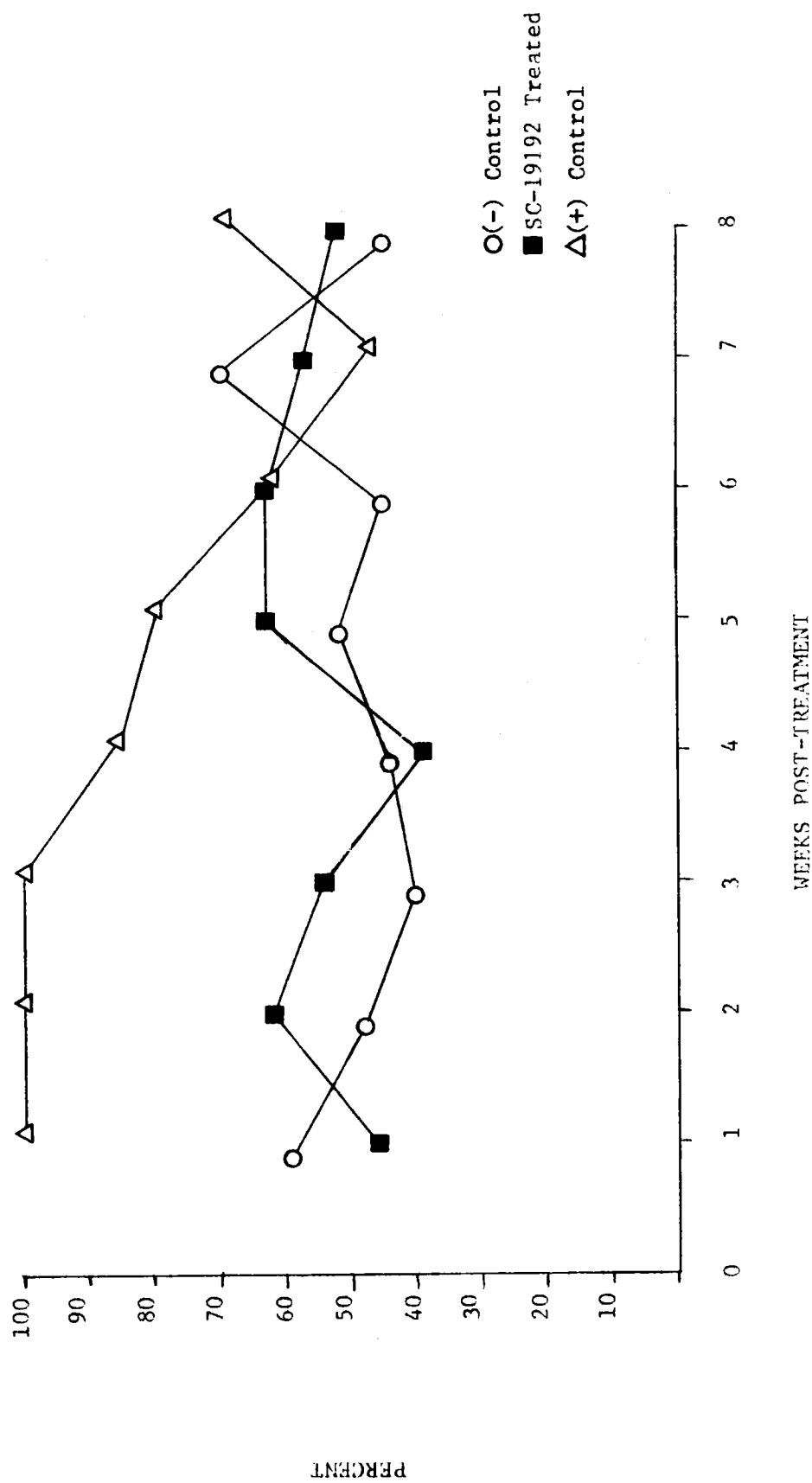


Figure 4

SC-19192: AN EVALUATION OF THE MUTAGENIC
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THE DOMINANT LETHAL ASSAY

Incidence of Pregnant Females with Fetal Deaths



A preponderance of the fetal deaths observed during this study were classified as early deaths. The few late deaths observed were distributed equally among the experimental groups (Table 3).

SUMMARY AND CONCLUSIONS

A dominant lethal mutagenicity test was performed employing intragastric administration of SC-19192 aqueous suspension to 23 week old proven male albino rats of the Charles River cd strain. Compound was administered on a single day at a dosage level of 1000 mg/kg body weight, given in two equally divided doses separated by an interval of two hours. The (-) control males were administered a comparable volume of vehicle only. A third group received methyl methane sulfonate (MMS), a known mutagenic agent, in a single dose of 50 mg/kg, administered intraperitoneally, and served as a (+) control. Female rats were not treated.

Immediately following treatment, each male was mated with two mature virgin females weekly for eight consecutive weeks. On gestation day 14 mated females were sacrificed for ovarian and uterine examinations.

The following parameters were evaluated: paternal growth, maternal pregnancy rate, corpora lutea and implantation sites. The latter were subclassified as viable fetal swellings, early fetal deaths (ED) or late fetal deaths (LD).

Growth of the (-) control, SC-19192 treated and (+) control males was comparable during the eight week post-treatment mating period.

The overall pregnancy rates of the females exposed to the (-) control and SC-19192 treated males were comparable. Similarly, the pregnancy rates compared on a week-to-week basis were comparable between these same two groups.

The overall pregnancy rate of the (+) control group was significantly depressed; however, on a week-to-week basis pregnancy rates were generally comparable with the (-) control group.

The ovarian and uterine examination data for females mated to the (-) control and the SC-19192 treated males were generally comparable over the eight week mating period.

The (+) control group, in accordance with published data⁴, experienced an increase in the mean number of fetal deaths with a corresponding decrease in mean number of viable fetal swellings during weeks 1 to 5 of the post-treatment mating period. However, this same group also experienced a reduced implantation rate during weeks 2 through 5 which was at least partially attributable to an unexplained decrease in ovulation rate occurring in weeks 2, 3 and 4.

The incidence of pregnant females with fetal deaths was comparable between the (-) control and the SC-19192 groups, both for the entire study and on a week-by-week basis. In the (+) control group the incidence of pregnant females with fetal deaths for the entire study was significantly higher, and weekly these incidences were significantly greater at weeks 1 through 4.

Thus, treatment of fertile male rats on a single day with a 1000 mg/kg dosage of SC-19192, administered intragastrically in two equally divided doses separated by an interval of two hours, had no effect on subsequent mating ability or fertility during the ensuing eight week mating period; also, the uterine examination of females mated to SC-19192 treated males

revealed no evidence of a dominant lethal effect. Epstein and co-workers⁶ have indicated that an increase in early fetal deaths is an unequivocal measure of dominant lethal mutations. In the present study fetal death data were comparable between the (-) control and the SC-19192 group throughout the eight week mating period. In sharp contrast, the (+) control group experienced a significant increase in both the mean number of fetal deaths per pregnant female (weeks 1, 2, 3 and 5) and the incidence of pregnant females with fetal deaths in weeks 1, 2, 3 and 4 of mating.

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APPENDIX TABLES OF INDIVIDUAL VALUES

KEY TO APPENDED
TABLES

N.M. = Not mated.

N.P. = Not pregnant; mating indicated by a vaginal plug and/or spermatozoa in the vaginal smear.

N.D. = No data.

* = Female observed with lesions of the pleural tissue.

t = Male died during the study.

Appendix Table 1

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
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Ovarian and Uterine Examination Data for the Vehicle Control Group

Male No.	Female No.	Mating Week 1				Mating Week 2			
		Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings
1	1	12	11	0	11	11	11	1	13
2	2	N.P.	--	--	--	14	13	0	14
3	1	N.M.	--	--	--	14	14	0	8
4	2	N.M.	--	--	--	8	8	0	--
5	1	15	15	0	15	N.P.	--	--	12
6	2	13	13	1	12	12	12	0	11
7	1	22	19	6	13	12	12	1	--
8	2	N.M.	--	--	--	N.M.	--	--	10
9	1	12	11	2	9	14	14	4	9
10	2	N.M.	--	--	--	14	13	4	14
11	1	11	11	0	11	14	14	0	10
12	2	13	12	0	12	12	12	2	13
13	1	15	15	0	15	18	15	2	15
14	2	13	5	0	5	15	15	0	--
15	1	13	13	2	11	N.M.	--	--	--
16	2	N.M.	--	--	--	N.M.	--	--	--
17	1	13	13	1	12	--	--	--	--
18	2	N.M.	--	--	--	--	--	--	--
19	1	6	2	0	2	14	14	1	13
20	2	17	15	1	14	15	15	0	15
21	1	13	12	4	8	16	16	2	14
22	2	13	12	2	10	N.M.	--	--	--
23	1	13	12	0	12	12	12	0	12
24	2	N.M.	--	--	--	15	14	0	14
25	1	12	11	2	9	15	13	2	11
26	2	11	11	1	10	14	14	2	12
27	1	11	11	1	10	15	15	0	15
28	2	12	12	2	10	15	15	0	15
29	1	13*	11	3	8	13	12	0	12
30	2	14	14	0	14	15	14	2	12

Total
 \bar{x}

287
13.0

261
11.9

28
1.3

233
10.6

317.0
13.8

307
13.3

23
1.0

284
12.3

Appendix Table 1 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the Vehicle Control Group

Male No.	Female No.	Mating Week 3				Mating Week 4			
		Corpora Lutea	Implant- ations	Fetal Deaths	Viable Fetal Swellings	Corpora Lutea	Implant- ations	Fetal Deaths	Viable Fetal Swellings
1	1	13	13	0	13	17	17	2	15
2	2	N.M.	--	--	--	13	13	0	13
3	1	N.M.	--	--	--	11	11	4	7
4	2	N.M.	--	--	--	15	15	0	15
5	1	N.P.	--	--	--	13	13	1	12
6	2	14	13	2	11	13	13	0	13
7	1	14	14	0	14	16	16	0	16
8	2	12	12	1	11	15	14	0	14
9	1	N.M.	--	--	--	14	13	0	13
10	2	11	11	0	11	17	17	0	17
11	1	16	16	0	16	17	17	0	17
12	2	N.M.	--	--	--	15	15	0	15
13	1	12	12	0	12	13	13	0	13
14	2	15	15	1	14	14	14	1	13
15	1	13	13	0	13	12	11	0	11
16	2	14	13	1	12	18	16	3	13
17	1	12	11	1	10	13	12	1	11
18	2	16	16	0	16	N.M.	--	--	--
19	1	16	15	1	14	17	15	0	15
20	2	16	15	0	15	19	19	4	15
21	1	13	13	0	13	16	14	3	11
22	2	16	15	0	15	17	16	1	15
23	1	16	15	1	14	12	11	0	11
24	2	13	13	0	13	15	15	2	13
25	1	14	12	4	8	15	14	2	12
26	2	14	14	0	14	15	15	0	15

Total
 \bar{x} 280
14.0271
13.612
0.6259
13.0372
14.9359
14.424
1.0335
13.4

Appendix Table 1 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the Vehicle Control Group

Male No.	Female No.	Mating Week 5				Mating Week 6			
		Corpora Lutea	Implant- ations	Fetal Deaths	Viable etal Swellings	Corpora Lutea	Implant- ations	Fetal Deaths	Viable Feta Swellings
1	1	15	15	0	15	12	11	1	10
2	2	N.M.	--	--	--	14	14	0	14
3	1	N.P.	--	--	--	13	13	1	12
4	2	13	13	2	11	14	13	1	12
5	1	11	11	1	10	15	14	0	14
6	2	15	15	2	13	17	16	1	15
7	1	14	14	0	14	11	11	2	9
8	2	14	13	0	13	N.M.	--	--	--
9	1	N.P.	--	--	--	12	12	0	12
10	2	15	15	2	13	13	12	1	11
11	1	15	15	2	13	17	16	1	15
12	2	15	15	0	15	12	12	0	12
13	1	18	17	1	16	N.P.	--	--	--
14	2	14	14	1	13	16	15	0	15
15	1	14	11	0	11	16	16	0	16
16	2	17	17	0	17	N.D.	--	--	--
17	1	13	13	0	13	14	14	0	14
18	2	19	17	0	17	12	12	0	12
19	1	13	13	1	12	12	12	0	12
20	2	13	13	0	13	8	6	0	6
21	1	18	16	0	16	15	13	0	13
22	2	N.M.	--	--	--	16	16	1	15
23	1	13	13	1	12	14	12	0	12
24	2	17	15	2	13	14	14	2	12
25	1	16	16	3	13	N.P.	--	--	--
26	2	N.M.	--	--	--	15	14	4	10

Total
 \bar{x}

312
14.9

18
0.9

283
13.5

302
13.7

288
13.1

15
0.7

273
12.4

Appendix Table 1 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the Vehicle Control Group

Male No.	Female No.	Mating Week 7				Mating Week 8			
		No. Per Female		Fetal Deaths	Viabie Fetal Swellings	No. Per Female		Fetal Deaths	Viabie Fetal Swellings
		Corpora Lutea	Implantations			Corpora Lutea	Implantations		
1	1	15	15	0	15	13	13	2	11
2	2	15	15	1	14	13	13	0	13
3	1	15	14	2	12	15	15	0	15
4	2	16	15	0	15	14	13	0	13
5	1	16	15	1	14	14	13	1	12
6	2	10	4	1	3	N.P.	--	--	--
7	1	14	14	1	13	13	13	3	10
8	2	13	12	1	11	12	12	1	11
9	1	14	14	0	14	16	16	1	15
10	2	19	19	1	18	N.P.	--	--	--
11	1	16	15	0	15	14	13	0	13
12	2	15	14	0	14	N.P.	--	--	--
13	1	17	17	1	16	12	12	0	12
14	2	N.M.	--	--	--	15	14	0	14
15	1	11	10	1	9	12	12	0	12
16	2	12	11	0	11	16	16	0	16
17	1	13	13	3	10	15	10	0	10
18	2	N.P.	--	--	--	N.P.	--	--	--
19	1	15	15	1	14	17	16	0	16
20	2	N.P.	--	--	--	16	15	0	15
21	1	16	16	1	15	17	16	1	15
22	2	13	11	2	9	N.M.*	--	--	--
23	1	14	13	1	12	13	13	1	12
24	2	15	15	1	14	16	16	3	13
25	1	15	14	1	13	N.P.	--	--	--
26	2	12	12	0	12	11	9	1	8

Total
 \bar{x}

331
14.4

313
13.6

20
0.9

293
12.7

284
14.2

270
13.5

14
0.7

256
12.8

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT EMPLOYING THE DOMINANT LETHAL ASSAY

Male No.	Female No.	Mating Week 1				Mating Week 2			
		Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings
31	1	13	13	0	13	19	13	6	7
32	2	14	14	1	13	N.P.	--	--	--
33	1	16	15	2	13	15	15	1	14
34	2	13	13	1	12	12	12	10	2
35	1	11	11	0	11	10	10	0	10
36	2	14*	12	2	10	15	14	1	13
37	1	12	8	0	8	17	16	1	15
38	2	15	15	0	15	N.P.	--	--	--
39	1	11	11	1	10	14	14	1	13
40	2	N.M.	--	--	--	13	13	7	6
41	1	11	11	0	11	N.P.	--	--	--
42	2	N.M.	--	--	--	15	15	2	13
43	1	11	11	0	11	17	15	0	15
44	2	14	12	0	12	N.P.	--	--	--
45	1	15	12	1	11	14	14	0	14
46	2	12	12	0	12	16	15	0	15
47	1	13	12	3	9	11	11	0	11
48	2	N.M.	--	--	--	11	11	0	11
49	1	9	3	0	3	16	14	12	2
50	2	14	13	1	12	15	15	0	15
51	1	13	13	2	11	14	14	1	13
52	2	N.M.	--	--	--	14	14	1	13
53	1	13	12	1	11	16	16	1	15
54	2	17	17	0	17	15	15	0	15
55	1	16	13	1	12	15	15	1	14
56	2	16	14	0	14	14	14	0	14
57	1	13	13	0	13	15	14	1	13
58	2	12	12	0	12	13	12	2	10
59	1	21	19	2	17	17	15	2	13
60	2	13	13	0	13	15	15	0	15

Total	352.0	324.0	18	306.0	378.0	361.0	50.0	311.0	28
\bar{x}	13.5	12.5	0.7	11.8	14.5	13.9	1.9	12.0	

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Mating Week 3	
No.	Per Female
1	1
2	1
3	1
4	1
5	1
6	1
7	1
8	1
9	1
10	1
11	1
12	1
13	1
14	1
15	1
16	1
17	1
18	1
19	1
20	1
21	1
22	1
23	1
24	1
25	1
26	1
27	1
28	1
29	1
30	1
31	1
32	1
33	1
34	1
35	1
36	1
37	1
38	1
39	1
40	1
41	1
42	1
43	1
44	1
45	1
46	1
47	1
48	1
49	1
50	1
51	1
52	1
53	1
54	1
55	1
56	1
57	1
58	1
59	1
60	1
61	1
62	1
63	1
64	1
65	1
66	1
67	1
68	1
69	1
70	1
71	1
72	1
73	1
74	1
75	1
76	1
77	1
78	1
79	1
80	1
81	1
82	1
83	1
84	1
85	1
86	1
87	1
88	1
89	1
90	1
91	1
92	1
93	1
94	1
95	1
96	1
97	1
98	1
99	1
100	1

Male No.	Female No.	Corpora Lutea	Implantations	Fetal Deaths	Viable Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viable Swellings
31	1	16	16	0	16	12	11	0	11
32	2	15	15	1	14	13	12	2	10
33	1	N.P.	--	--	--	17	17	0	17
34	2	15	14	3	11	13	13	0	13
35	1	14*	14	0	14	12	12	1	11
36	2	16	15	0	15	15*	14	1	13
37	1	16	15	0	15	14	13	1	12
38	2	14	12	0	12	17	17	0	17
39	1	12	12	1	11	N.M.	--	--	--
40	2	18	15	5	10	16	14	0	14
41	1	N.M.	--	--	--	16	16	1	15
42	2	14	1	0	1	15	15	0	14
43	1	18	9	5	4	16	15	2	15
44	2	14	14	0	14	16	14	0	14
45	1	12	12	0	12	16	16	0	16
46	2	14	14	1	13	15	14	0	14
47	1	15	15	5	10	14	12	0	12
48	2	N.M.	--	--	--	14	14	0	14
49	1	15	15	0	15	15	15	1	14
50	2	14	14	1	13	N.M.	--	--	--
51	1	12	12	0	12	12	12	0	12
52	2	13	13	2	11	14	13	0	13
53	1	14	13	1	12	15	15	2	13
54	2	13	13	1	12	15	15	2	13
55	1	N.P.	--	--	--	11	11	0	11
56	2	N.M.	--	--	--	15	15	1	14
57	1	10*	10	0	10	14	14	2	12
58	2	17	14	2	12	16	16	0	16
Total		337	315	30	285	404	390	16	374
\bar{x}		14.0	13.1	1.3	11.9	14.4	13.9	0.6	13.4

Appendix Table 2 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the 19192 Treated Group

Male No.	Female No.	Mating Week 5				Mating Week 6			
		Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings
31	1	17	16	1	15	14	13	2	11
	2	13	13	1	12	15	15	0	15
32	1	11	11	0	11	14	14	1	13
	2	12	12	0	12	15	14	2	12
33	1	15	13	1	12	13	13	1	12
	2	15	15	2	13	11	11	3	8
34	1	11	2	2	0	12	12	0	12
	2	17	15	1	14	12	10	1	9
35	1	17	17	0	17	11	10	1	9
	2	19	18	2	16	17	17	2	15
36	1	15	14	3	11	14	13	0	13
	2	14	12	0	12	N.M.	--	--	--
37	1	9	7	1	6	12	12	2	10
	2	17	17	2	15	14	13	0	13
38	1	N.P.	--	--	--	14	13	1	12
	2	15	12	0	12	10	1	0	1
39	1	N.P.	--	--	--	15	15	1	14
	2	13	13	1	12	15	13	0	13
40	1	16	15	6	9	N.M.	--	--	--
	2	15	14	2	12	15	2	0	2
41	1	16*	15	1	14	N.P.	--	--	--
	2	13	13	0	13	17*	16	0	16
42	1	15	14	0	14	17	16	1	15
	2	14	13	0	13	17	17	1	16
43	1	13	13	1	12	13	12	2	10
	2	13	13	0	13	16	16	0	16
44	1	11	11	0	11	16	16	1	15
	2	N.M.	--	--	--	14	13	2	11
45	1	12*	11	11	0	15	15	0	15
	2	13	12	0	12	15	15	1	14

Total
x381
14.1351
13.0313
11.6383
14.2347
12.925
0.9322
11.9

30

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT EMPLOYING THE DOMINANT LETHAL ASSAY

Male No.	Female No.	Mating Week 7				Mating Week 8				
		Corpora		Implantations	Fetal Deaths	Corpora		Implantations	Fetal Deaths	Viable Fetal Swellings
		Lutea	Viable Swellings			Lutea	Viable Swellings			
31	1	13	13	0		N.P.*	--	--	--	
	2	15	15	0		14	14	0	14	
32	1	14	13	3		15	15	1	14	
	2	13	13	0		N.M.	--	--	--	
33	1	6*	5	0		15	15	0	15	
	2	12*	12	1		N.P.*	--	--	--	
34	1	14	14	2		13	12	0	12	
	2	16	16	0		13	12	0	12	
35	1	14	13	2		13	13	2	11	
	2	14	13	3		14	13	1	12	
36	1	14	14	1		14	14	0	14	
	2	16	16	1		N.P.	--	--	--	
37	1	11	10	0		12	12	0	12	
	2	9	8	4		12	11	1	10	
38	1	14	12	1		16	15	0	15	
	2	13	13	0		14	14	0	14	
39	1	12	12	1		15	15	0	15	
	2	17	17	1		17	17	1	16	
40	1	15	14	1		16	16	1	15	
	2	13	13	0		15	15	1	14	
41	1	13	12	1		13	12	1	11	
	2	15	15	1		13	13	2	11	
42	1	12	12	0		12	12	2	10	
	2	16	16	1		17	15	0	15	
43	1	17	17	0		5	2	1	1	
	2	12	12	0		12	12	0	12	
44	1	12	11	0		13	13	1	12	
	2	14	14	1		16	16	1	15	
45	1	11	11	1		N.P.	--	--	--	
	2	13	13	0		17	17	0	17	

Total	400	388	26	362	346	335	16	319	31
\bar{x}	13.3	12.9	0.9	12.1	13.8	13.4	0.6	12.8	

Appendix Table 3

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the MMS Treated Group

Male No.	Female No.	Mating Week 1				Mating Week 2			
		No. Per Female		No. Per Female		No. Per Female		No. Per Female	
		Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings
46	1	11	11	4	7	11	10	9	1
47	2	N.M.	--	--	--	7	5	5	0
	1	15	15	3	12	8	8	8	0
	2	16	16	10	6	8	5	5	0
48	1	14	14	3	11	12	11	9	2
49	2	N.M.	--	--	--	4	1	1	0
	1	9	9	7	2	8	5	5	0
	2	N.M.	--	--	--	9	4	4	0
50	1	14	14	4	10	13	11	9	2
	2	14	13	2	11	N.M.	--	--	--
51	1	13	13	2	11	11	10	10	0
	2	15	12	4	8	5	2	2	0
52	1	16	16	6	10	6	5	4	1
	2	13	13	7	6	3	3	3	0
53	1	N.M.	--	--	--	10	9	6	3
	2	N.M.	--	--	--	N.M.	--	--	--
54	1	10	10	5	5	7	2	2	0
	2	N.M.	--	--	--	N.M.	--	--	--
55	1	15	15	3	12	16	13	10	3
	2	14	14	2	11	15	15	10	5
Total		189	185	63	122	153	119	102	17
\bar{x}		13.5	13.2	4.5	8.7	9.0	7.0	6.0	1.0

Appendix Table 3 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the MMS Treated Group

Male No.	Female No.	Mating Week 3				Mating Week 4			
		No. Per Female		No. Per Female		No. Per Female		No. Per Female	
		Corpora Lutea	Implantations	Fetal Deaths	Viab Fetal Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viab Fetal Swellings
46	1	16	1	1	0	N.P.	--	--	--
47	2	N.M.	--	--	--	13	12	3	9
	1	10	2	2	0	3	1	1	0
48	2	N.P.	--	--	--	15	15	1	14
	1	9	1	1	0	5	1	1	0
49	2	12	1	1	0	17	17	2	15
	1	12	2	2	0	5	1	1	0
50	2	N.P.	--	--	--	15	15	0	15
	1	10	1	1	0	14	12	4	8
51	2	N.M.	--	--	--	10	6	1	5
	1	N.P.	--	--	--	N.P.	--	--	--
52	2	N.P.	--	--	--	N.P.	--	--	--
	1	N.P.	--	--	--	N.P.	--	--	--
53	2	7	3	3	0	6	3	2	1
	1	8	3	3	0	12	3	0	3
54	2	N.M.	--	--	--	14	14	1	13
	1	N.P.	--	--	--	N.M.	--	--	--
55	2	9	1	1	0	18	16	1	15
	1	12	1	1	0	2	1	1	0
	2	N.P.	--	--	--	N.M.	--	--	--
Total		105	16	16	0	149	117	19	98
\bar{x}		10.5	1.6	1.6	0	10.6	8.4	1.4	7.0

Appendix Table 3 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the MMS Treated Group

Male No.	Female No.	Mating Week 5				Mating Week 6			
		Corpora Lutea	Implantations	Fetal Deaths	Viable Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viable Swellings
46	1	13	12	2	10	N.P.	--	--	--
47	2	N.P.	--	--	--	14	14	1	13
	1	2	1	0	1	13	13	1	12
48	2	13	10	4	6	19	19	1	18
	1	10	10	5	5	11	10	0	10
49	2	12	11	5	6	14	14	1	13
	1	15	15	3	12	14	13	1	12
50	2	N.M.	--	--	--	15	14	3	11
	1	17	16	3	13	N.M.	--	--	--
51	2	N.M.	--	--	--	13	12	0	12
	1	N.P.	--	--	--	14	13	0	13
52	2	14	14	5	9	N.P.	--	--	--
	1	15	15	2	13	12	9	2	7
53	2	15	13	2	11	14	11	5	6
	1	15	15	0	15	11	11	0	11
54	2	16	15	1	14	16	16	0	16
	1	11	11	5	6	14	12	1	11
55	2	N.P.	--	--	--	13	12	0	12
	1	16	16	4	12	12	11	1	10
	2	15	15	0	15	N.P.	--	--	--
Total		199	189	41	148	219	204	17	187
x		13.3	12.6	2.7	9.9	13.7	12.8	1.1	11.7

Appendix Table 3 (cont.)

SC-19192: AN EVALUATION OF THE MUTAGENIC POTENTIAL IN THE RAT
EMPLOYING THE DOMINANT LETHAL ASSAY

Ovarian and Uterine Examination Data for the MMS Treated Group

Male No.	Female No.	Mating Week 7				Mating Week 8			
		Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings	Corpora Lutea	Implantations	Fetal Deaths	Viable Fetal Swellings
46	1	16	15	0	15	14	14	0	14
	2	10	6	0	6	15	15	0	15
47	1	15	14	4	10	14	11	1	10
	2	12	11	2	9	16	16	0	16
48	1	14	14	0	14	11	10	1	9
	2	N.M.	--	--	--	N.M.	--	--	--
49	1	N.P.	--	--	--	17	17	2	15
	2	15	15	1	14	14	13	1	12
50	1	14	13	1	12	13	13	2	11
	2	10	9	1	8	14	14	1	13
51	1	12	12	0	12	14	13	1	12
	2	N.M.	--	--	--	14	14	0	14
52	1	14	13	0	13	14	14	1	13
	2	15	15	1	14	15	15	2	13
53	1	13	12	0	12	14	14	0	14
	2	N.M.	--	--	--	N.M.	--	--	--
54	1	15	15	0	15	16	15	1	14
	2	N.M.	--	--	--	N.M.	--	--	--
55	1	7	7	0	7	16	14	1	13
	2	15	15	1	14	N.M.	--	--	--
Total		197	186	11	175	231	222	14	208
\bar{x}		13.1	12.4	0.7	11.7	14.4	13.9	0.9	13.0