

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

Segment II of the Teratology-Reproduction Profile

R. E. Schroeder, A. Mitchell, K. S. Rao and R. G. McConnell

Department of Biological Research
(Pathology-Toxicology)
Searle Laboratories

January 23, 1973

Pathology-Toxicology
Project No. 997S72

TABLE OF CONTENTS

	PAGE NO.
INTRODUCTION	1
METHODS	1
Material evaluated	1
Animals, housing and diet	2
Chemical reagents	2
Experimental design	2
Mating procedure	3
Compound formulation and administration	4
Observations and records on pregnant females	4
Terminal observations	4
Autopsy procedure	4
Fetal soft tissue examination	5
Fetal skeletal examination	6
Statistical procedures employed	6
RESULTS	7
Maternal pre- and postmortem data	7
Survival rate	7
Conception rate	7
Body weight change	8
Food consumption data	8
SC-19192 intake	8
Hysterotomy data	8
Fetal examination	15
External examination	15
Soft tissue examination	15
Skeletal examination	15

	PAGE NO.
SUMMARY AND CONCLUSIONS	20
REFERENCES	22
APPENDIX TABLES OF INDIVIDUAL VALUES	23

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

R. E. Schroeder, A. Mitchell, K. S. Rao and R. G. McConnell

Department of Biological Research (Pathology-Toxicology)
Searle Laboratories

INTRODUCTION

The commercial grade finished product of SC-18862, a nutritive sweetening agent, may contain from 0-2% of a conversion product, SC-19192. This product is also produced from SC-18862 spontaneously under various laboratory conditions. The human population consuming SC-18862 would also be exposed to varying concentrations of SC-19192; hence, the pre-clinical testing of SC-19192 to evaluate its embryotoxic and teratogenic potential in the rat. In this study SC-19192 was administered orally in the diet to pregnant albino rats from gestation day 6 through 15. A hysterotomy was performed on gestation day 20 and the fetuses were examined for anomalies. A study designed in this manner is commonly referred to as Segment II of the Teratology-Reproduction profile.

METHODS

Material evaluated.

SC-19192 (Diketopiperazine; DKP) is a fine white powder, chemically named 5-benzyl-3, 6-dioxo-2-piperazineacetic acid. Lot No. 232A was employed during this study.

Animals, housing and diet.

One hundred twelve virgin female Charles River cd albino rats were employed. All females were approximately 95 ± 5 days old when mating was initiated. Twenty-eight males of the same strain and of proven fertility, taken from a breeding colony maintained in this laboratory, were used solely for breeding purposes.

Females were housed in groups of two in suspended wire cages and acclimated to the laboratory environment for two weeks prior to use. Animal quarters were air-conditioned, with thermostats set to maintain 72°F temperature continuously; artificial fluorescent lighting was provided on a 12-hour daily photoperiod.

Basal diet [Rockland Mouse/Rat Diet (**complete**) granulated (Teklad, Inc., Monmouth Illinois)] and chlorinated tap water were continuously available.

Chemical reagents.

Tissue fixatives and preservatives: Bouin's solution; 95% aqueous ethyl alcohol; glycerin. Skeletal staining procedure: 5% potassium hydroxide aqueous solution; saturated aqueous solution of Alizarin Red S; graded aqueous dilutions of glycerin.

Experimental design.

One hundred twelve female rats were distributed, employing a simple randomization procedure, into the following groups which received the daily dosages of compound as indicated:

Treatment Group	Number of Females	Daily Dosage Levels ⁺ (g/kg)
Control	28	--
Low	28	0.5
Medium	28	1.0
High	28	2.0

⁺ SC-19192 treated groups received compound-diet from the morning of gestation day 6 to the morning of gestation day 16.

The first 24 rats mated from each group were employed for the remainder of the study. Dietary administration of SC-19192 began on day 6 of gestation and continued through day 15 of gestation, a 10 day period of treatment. On gestation day 20 the females were sacrificed. Uterine horns were exposed and examined for fetal swellings and resorption sites. Fetuses were removed, examined externally and preserved intact to be examined later for soft tissue abnormalities (Wilson's Technique) or skeletal anomalies (Alizarin Red S skeletal staining technique).

Mating procedure.

Four females, one from each of the four dosage groups, were housed together in a breeding cage. At 4:30 p.m. on weekdays except Friday one male of proven fertility was placed into each cage; he was removed at 8:30 a.m. the following morning. At that time females were examined for a copulatory vaginal plug and/or spermatozoa in the vaginal smear. Observations of either of these signs indicated mating; this was designated day 0 of pregnancy. Such females were removed from the breeding cages and housed individually.

This procedure was continued until at least 24 females from each group were mated. For this particular study mating was completed over an 11 day period.

Compound formulation and administration.

Diet for treated groups was prepared by admixing SC-19192 into the basal granulated diet on a weight-per-weight basis by thorough dry mixing in a Hobart Model V-1401 Mixer. Concentrations used for the low, medium and high dose groups were 0.6%, 1.2% and 2.4%, respectively. These concentrations were based on the mean maternal body weight of all females (regardless of treatment group) which mated during the first 3 days of the mating period and an assumed mean food consumption of 20 g/day/rat. These concentrations of compound-diet remained unchanged throughout the study.

Control animals received basal (compound-free) diet throughout gestation. Treated animals received basal diet from gestation day 0 to the morning of gestation day 6, respective compound-diet thereafter to the morning of gestation day 16 and basal diet thereafter.

Observations and records on pregnant females.

The general appearance and behavior of each mated female was observed daily while determining food consumption. Body weights were measured and recorded periodically throughout pregnancy.

Terminal observations.

Autopsy procedure. Females were euthanized in a carbon dioxide chamber on day 20 of gestation. The abdominal cavity was opened,

the uterus exposed, and a count of fetal swellings and resorption sites in the intact uterus was performed. The relative positions of such fetal swellings and resorptions were recorded. The uterus was promptly incised and fetal viability determined on the basis of respiratory movements, skin color and movements of the extremities and head.

All fetuses were weighed to the nearest 0.1 gram, sexed, crown-rump distance (CRD) measured, and then examined externally for gross malformations prior to being euthanized and preserved intact in fixative solution.

Fetal soft tissue examination. Approximately one-third of the fetuses were fixed in Bouin's solution for subsequent examination by the free-hand sectioning technique of Wilson¹, as follows:

Head: Five tissue slices, approximately 1 mm thick, were prepared to reveal abnormalities of the palate, nasal cavities, eyes and brain.

Thorax: Four slices, somewhat thinner than 1 mm, were made starting at the shoulder joint and proceeding caudally to the diaphragm to reveal abnormalities of the thymus, heart, lungs, esophagus, trachea, diaphragm and major blood vessels.

Abdomen: One slice was made approximately 2 mm caudal to the diaphragm and was examined for abnormalities of the liver, aorta, esophagus and spinal cord. The next slice was made at the level of the stomach and was examined for anomalies of the spinal cord, aorta and intestinal tract. A final slice was made at the level of the renal pelvis, and examined for anomalies of the spinal cord, aorta and kidneys. Structures found in the

pelvic cavity (i.e., ureters, bladder and reproductive tract) were examined in situ after removal of the remaining intestines.

Such tissue slices were routinely examined under a dissecting microscope; all tissue slices from treated fetuses and from control fetuses with anomalies were transferred to glass vials for temporary storage.

Fetal skeletal examination. The remaining fetuses were preserved in 95% alcohol for subsequent skeletal staining by the Alizarin Red S staining technique². Such preserved fetuses were eviscerated, soft tissues macerated in 5.0% aqueous potassium hydroxide solution, skeletons stained with Alizarin Red S, and subsequently stored in 100% glycerin (to which several crystals of thymol were added to reduce microbial growth). Such preparations were examined by naked eye and/or dissecting microscope for anomalies, e.g., absence or incomplete ossification of the cranial bones, incomplete ossification or abnormal shape of the vertebrae, long bones, ribs, etc. Certain parameters were also recorded, e.g., number and size of sternal ossification centers, degree of closure of cranial ossifications, number of metacarpals, metatarsals and corresponding phalanges, etc. All treated and control skeletal preparations were placed in temporary storage.

Statistical procedures employed.

The means and standard errors of various measured parameters were calculated for each treatment group. The significance of differences between control and treated group means was tested using Student's t-test with $p < 0.05$. The 2 x 2 contingency tables were employed to compare conception rates and survival rate data ($p < 0.05$).

RESULTS

Maternal pre- and postmortem data.

Survival rate. Maternal survival rates to gestation day 20 were 96.0%, 96.0%, 100.0% and 100.0% in the control, low, medium and high dose groups, respectively. In the control group 1 of 24 mated females died; control female No. 9 was found dead in its cage on gestation day 20. An immediate autopsy revealed a gastric obstruction "hair ball" at the level of the cardiac orifice; the esophagus of this female was distended with diet and the stomach was empty. The other fatality was observed in the low dose group as 1 of 24 mated females died; low dose female No. 36 was found dead in its cage on gestation day 19. An autopsy of this animal was performed; no gross lesions could be detected.

Conception rate. Maternal conception rates as presented below were comparable between control and treated groups.

Treatment Group	No. of Females		Conception Rate (%) ^b
	Mated	Pregnant ^a	
Control	24	20	83
Low	24	20	83
Medium	26	20	77
High	27	18	67

^a Pregnant: as indicated by the presence of at least one uterine implantation site at term sacrifice.

^b Conception rate: number of pregnant females divided by the number of females mated x 100.

Body weight change. Group mean maternal body weights for the control and treated groups are presented in Table 1. These weights were comparable between control and treated groups throughout the gestation period.

Food consumption data. Group mean food consumption for the control and treated groups during gestation is presented in Table 2. Food consumption was generally comparable between control and treated groups, except on gestation day 10 when there was a significant depression in mean food consumption in the medium dose group. Food consumption data for individual females from the control and treated groups for representative days are presented in the Appendix.

SC-19192 intake.

The amounts of SC-19192 ingested by the treated groups during the 10 day treatment period (Table 3) closely approximated the planned daily dosages of 0.5, 1.0 and 2.0 g/kg for the low, medium and high dose groups, respectively.

Hysterotomy data.

The ovaries and uteri of all control and treated rats were grossly unremarkable at sacrifice with one exception. The right uterine horn of control female No. 4 was unremarkable and contained 4 viable, normal appearing fetuses and 2 early resorption sites; the left horn, however, appeared slightly distended and upon incision exuded yellowish-white, purulent material. All implantations along the left horn were undergoing resorption and the number of implantation sites was not distinguishable.

Uterine examination of the two females which died during the study (see Maternal pre- and postmortem data, page 7) revealed the following:

Table 1

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

Maternal Body Weight Data (g)
(Mean \pm S. E.)

Treatment Group	No. Females Pregnant	Gestation Day				
		1	6	10	15	20
Control	20	242.9 ± 4.1	262.3 ± 5.3	285.5 ± 5.6	308.7 ± 7.7	372.2 ± 12.5
Low	20	233.8 ± 3.5	254.6 ± 4.0	272.5 ± 4.3	297.2 ± 4.4	365.2 ± 6.0
Medium	20	241.3 ± 4.1	262.4 ± 5.2	279.1 ± 4.7	305.2 ± 5.1	379.4 ± 7.7
High	18	240.2 ± 3.3	263.3 ± 4.8	285.6 ± 4.3	311.3 ± 5.1	383.3 ± 6.3

Table 2

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

Maternal Food Consumption (g/kg/day)
(Mean \pm S. E.)

Treatment Group	No. Females Pregnant	Gestation Day			
		1	6	10	15
Control	19†	120.9 \pm 8.0	81.1 \pm 4.0	86.4 \pm 2.6	81.3 \pm 1.4
Low	20	130.9 \pm 7.9	81.8 \pm 2.6	83.3 \pm 2.6	79.5 \pm 3.9
Medium	20	110.4 \pm 6.3	84.2 \pm 2.3	77.4 \pm 3.5*	76.0 \pm 4.1
High	18	109.3 \pm 6.4	90.4 \pm 5.0	81.8 \pm 2.5	82.3 \pm 2.5

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

† Maternal food consumption of control female No. 9 was not included in the evaluation of group mean food consumption.

Table 3

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

Compound Consumption (g/kg/day)
(Mean \pm S.E.)

Treatment Group	No. of Females	Gestation Day			Mean of Day 6, 10 and 15
		6 \pm S.E.	10 \pm S.E.	15 \pm S.E.	
Low	20	0.49 (\pm 0.02)	0.50 (\pm 0.02)	0.48 (\pm 0.02)	0.49
Medium	20	1.01 (\pm 0.03)	0.93 (\pm 0.04)	0.91 (\pm 0.05)	0.95
High	18	2.17 (\pm 0.12)	1.96 (\pm 0.06)	1.98 (\pm 0.06)	2.04

Control female No. 9: pregnant; uterus contained 11 implantation sites, all of which were non-viable fetuses in initial stages of autolysis. Uterus and ovaries grossly unremarkable.

Low dose female No. 36: pregnant; uterus contained 13 implantations, all being early resorptions. Uterus and ovaries grossly unremarkable.

Results of the uterine implantation examinations are presented in Table 4. Data for individual females from the control and treated groups are presented in the Appendix. Mean number of fetuses per pregnant female was comparable between control, low and medium dose groups and significantly larger in the high dose group. This was attributed to a somewhat higher resorption rate observed in the concurrent control group.

Excluding those females which died prior to gestation day 20 sacrifice, none of the litters examined -- control or treated -- contained non-viable term fetuses; however, fetal resorption occurred in all experimental groups and, with one exception, involved fetuses in an early stage of development. One late resorption was observed in the in utero litter of medium dose female No. 61; thirteen normal appearing viable fetuses completed the litter. The incidence of litters containing at least one resorption site was 67%, 63%, 45% and 39% in the control, low, medium and high dose groups, respectively. Mean number of resorption sites per pregnant female was comparable between control, low and medium dose groups and significantly reduced in the high dose group (Table 4); this is considered incidental and not treatment-related.

Group mean fetal body weights and crown-rump distance data (both sexes) were comparable between control and treated groups (Table 5). Similarly, the sex distributions in the treated groups were comparable to those observed in the concurrent control group (Table 5).

Table 4

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

Summary of Uterine Examination Data

Treatment Group	No. of Pregnant Females	No. per pregnant female	
		Fetuses $\bar{x} \pm \text{S.E.}$	Resorptions $\bar{x} \pm \text{S.E.}$
Control	20 ^a	12.0 \pm 0.6	1.4 \pm 0.4
Low	20 ^b	12.1 \pm 0.5	0.9 \pm 0.2
Medium	20	12.2 \pm 0.6	0.8 \pm 0.2
High	18	13.2 \pm 0.4 [*]	0.4 \pm 0.1 [*]

^a Data for control females Nos. 4 and 9 were not included in evaluation of implantation data (see appended Hysterotomy Data).

^b Data for low dose female No. 36 were not included in evaluation of implantation data (see Maternal pre- and postmortem data, page 7).

^{*} Difference statistically significant ($p < 0.05$).

Table 5

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

Summary of Sex Distribution, Fetal Weight and Crown-Rump Distance (CRD)

Treatment Group	No. of Fetuses		Fetal Body Weight† (g)		Fetal CRD† (cm)	
	Male	Female	Male	Female	Male	Female
Control	112	104	4.1±0.06	3.9±0.06	3.8±0.02	3.7±0.04
Low	104	126	4.1±0.07	3.9±0.07	3.7±0.03	3.7±0.03
Medium	125	119	4.1±0.06	3.9±0.06	3.8±0.02	3.7±0.02
High	112	125	4.3±0.06	4.1±0.06	3.8±0.02	3.7±0.02

† Mean ± S.E.

Fetal examination.

External examination. No external irregularities were observed among the 220 control, 230 low, 244 medium and 237 high dose fetuses examined.

Soft tissue examination. Examination of soft tissues by the Wilson's technique did not reveal any anomalies in the 81 control, 89 medium or 83 high dose fetuses. However, one of 84 fetuses (1.2%) in the low dose group, when examined under the bioscope, had soft "spongy" appearing lenses (bilateral). Microscopic examination of a cross-sectional head segment containing both eyes from this fetus revealed bilateral disintegration of lenticular fibers in the cortex and nucleus (affecting approximately 85% of the lens structure). The affected fetus was one of four fetuses processed for soft tissue examination from the litter of low dose female No. 32. The lens lesion described above has a low incidence of spontaneous occurrence in this laboratory; over the last 12 months such a lesion occurred in 1 of 235 control fetuses, an incidence of 0.4%.

Skeletal examination. Results of the skeletal examination are presented in Table 6. No skeletal anomalies were observed in the 139 control, 146 low, 155 medium or 154 high dose fetuses examined. Evaluation of the several skeletal parameters indicative of fetal development (Table 6) revealed they were comparable between control and treated groups.

Table 6

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RAT

Skeletal Examination Data

PARAMETERS	Control No. Fetuses Total Fetuses %	Low Dose No. Fetuses Total Fetuses %	Medium Dose No. Fetuses Total Fetuses %	High Dose No. Fetuses Total Fetuses %
Total Fetuses Examined	139	146	155	154
Skull				
Closure Grading #0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	139	146	155	154
4	0	0	0	0
Occipitals Poorly Ossified	0	0	1	0
Interparietals Poorly Ossified	0	0	1	0
Axial Skeleton				
Ribs: 12 pairs	0	0	1	0
13 pairs	139	146	153	154
14 pairs	0	0	1	0

*Skull Closure Grading: 0 - Lack of Skull Ossification. 1 - 25% of Skull Ossified.
2 - 50% of Skull Ossified. 3 - 75% of Skull Ossified.
4 - 100% of Skull Ossified.

Table 6 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RATSkeletal Examination Data

PARAMETERS	Control No. Fetuses	% of Total Fetuses	Low Dose No. Fetuses	% of Total Fetuses	Medium Dose No. Fetuses	% of Total Fetuses	High Dose No. Fetuses	% of Total Fetuses
Total Fetuses Examined	139	—	146	—	155	—	154	—
Rudimentary Structures**	12	8.6	21	14.4	14	9.0	23	14.9
Sternum Ossifications Absent	0	0.0	0	0.0	0	0.0	0	0.0
Center No. 1	0	0.0	0	0.0	0	0.0	1	0.6
2	0	0.0	0	0.0	0	0.0	0	0.0
3	0	0.0	0	0.0	0	0.0	0	0.0
4	0	0.0	0	0.0	0	0.0	0	0.0
5	8	5.7	20	13.7	12	7.7	9	5.8
6	3	2.1	6	4.1	5	32.2	2	1.3
Sternum Ossifications Small	0	0.0	0	0.0	0	0.0	0	0.0
Center No. 1	8	5.7	12	8.2	9	5.8	6	3.9
2	0	0.0	1	0.7	0	0.0	0	0.0
3	3	2.1	2	1.4	2	1.3	1	0.6
4	85	61.1	86	58.9	101	65.2	98	63.6
5	18	12.9	34	23.3	20	12.9	12	7.8
6								

**Rudimentary Structures: Unilateral or bilateral ossification centers positioned adjacent to the fourteenth thoracic vertebrae.

Table 6 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RATSkeletal Examination Data

PARAMETERS	Control No. Fetuses	% of Total Fetuses	Low Dose No. Fetuses	% of Total Fetuses	Medium Dose No. Fetuses	% of Total Fetuses	High Dose No. Fetuses	% of Total Fetuses
Total Fetuses Examined	139	—	146	—	155	—	154	—
Vertebrae								
Unossified centrum	120	86.3	110	75.3	117	75.5	121	78.6
cervical	0	0.0	0	0.0	0	0.0	0	0.0
thoracic	0	0.0	0	0.0	0	0.0	0	0.0
lumbar	0	0.0	0	0.0	0	0.0	0	0.0
sacral	0	0.0	0	0.0	0	0.0	0	0.0
caudal	139	100.0	146	100.0	155	100.0	154	100.0
Split thoracic centra	3	2.1	4	2.7	0	0.0	1	0.6
Pelvic centers								
Unossified	0	0.0	0	0.0	0	0.0	0	0.0
right ischium	1	0.7	0	0.0	0	0.0	0	0.0
right pubis	0	0.0	0	0.0	0	0.0	0	0.0
right ilium	1	0.7	0	0.0	0	0.0	0	0.0
left ischium	1	0.7	0	0.0	0	0.0	0	0.0
left pubis	1	0.7	0	0.0	1	0.6	1	0.6
left ilium	1	0.7	0	0.0	0	0.0	0	0.0
Appendicular Skeleton								
Forelimbs (bilat.)	139	100.0	146	100.0	155	100.0	154	100.0
Carpals unossified	0	0.0	0	0.0	0	0.0	0	0.0
Metacarpals unossified	12	8.6	26	17.8	26	16.8	13	8.4
1-6 ossifications	127	91.4	120	82.2	129	83.2	141	91.6
7-8 ossifications								

Table 6 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RATSkeletal Examination Data

PARAMETERS	Control No. Fetuses	% of Total Fetuses	Low Dose No. Fetuses	% of Total Fetuses	Medium Dose No. Fetuses	% of Total Fetuses	High Dose No. Fetuses	% of Total Fetuses
Total Fetuses Examined	139	—	146	—	155	—	154	—
Phalanges Unossified	6	4.3	8	5.5	9	5.8	2	1.3
1-10 ossifications	74	53.2	78	53.4	89	57.4	72	46.7
11-16 ossifications	59	42.5	60	41.1	57	36.8	80	52.0
Hindlimbs (bilat.)								
Tarsals unossified	139	100.0	146	100.0	155	100.0	154	100.0
Metatarsals unossified	0	0.0	0	0.0	0	0.0	0	0.0
1-6 ossifications	0	0.0	0	0.0	0	0.0	0	0.0
7-8 ossifications	139	100.0	146	100.0	155	100.0	154	100.0
Phalanges Unossified	24	17.3	27	18.5	38	24.5	19	12.3
1-10 ossifications	115	82.7	119	81.5	117	75.5	135	87.7

SUMMARY AND CONCLUSIONS

This study was conducted to evaluate the potential of SC-19192 for embryotoxic and/or teratogenic effects employing continuous dietary administration of the compound from gestation day 6 through 15 to primigravid Charles River cd rats. The compound was administered at mean daily intake levels of 0.5, 1 and 2 g/kg \pm 10%. Prior to day 6 and subsequent to day 15, all SC-19192 treated group rats received the basal diet (compound-free) only. A concurrent control group consumed basal diet throughout the gestation period. All females were sacrificed on gestation day 20 and uterine examinations performed; term fetuses were removed and examined.

Parameters evaluated include maternal survival and conception rate, body weight, food consumption and necropsy findings (reproductive organs); in utero litter size (viable fetuses), resorptions, non-viable fetuses; and fetal sex distribution, size, gross appearance and examination of the soft tissues and skeletal systems.

Maternal survival, conception and body weight change were comparable between control and treated groups. Food consumption was generally comparable between control and treated groups with the exception of gestation day 10 when food consumption of the medium dose group was significantly depressed.

Mean number of fetuses per pregnant female was comparable between control, low and medium dose groups and significantly larger in the high dose group. The mean number of resorption sites was comparable between the control, low and medium dose groups and significantly reduced in the high dose group. This latter finding was probably related to the somewhat higher than usual resorption rate exhibited by the concurrent control group. The incidence of litters with at least one resorption site was comparable between the control and

treated groups.

Sex distribution, fetal body weights and crown-rump distance data were comparable between control and treated groups.

No evidence of treatment induced anatomical alterations was observed in the 57 treated litters (711 term fetuses) examined. The sole abnormality observed among the term fetuses recovered from SC-19192 treated females was bilateral cataractous lesions observed in 1 of 84 low dose fetuses (1.2%) examined for soft tissue abnormalities. In this laboratory the above eye lesion has a spontaneous incidence of 0.4% in a limited historical control group.

It is concluded that continuous dietary administration of SC-19192 to primigravid rats at dosages up to 2 g/kg daily, from gestation day 6 through 15, was neither embryotoxic nor teratogenic in the developing rat fetus.

REFERENCES

1. Wilson, J. G. (1965). Teratology Principles and Techniques. (J. G. Wilson and J. Warkany, eds.), pp. 271-277. Univ. of Chicago Press, Chicago, Illinois.
2. Dawson, A. B. (1926). Note on the staining of the skeleton of cleared specimens with Alizarin Red S. Stain Tech. 1, 123.

APPENDIX TABLES OF INDIVIDUAL VALUES

Appendix Table 1

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RATMaternal Food Consumption for the Control Group
(Individual Values (g/kg))

Animal No.	Day 1	Day 6	Day 10	Day 15	Day 19
1	86.4	101.3	122.5	78.4	76.3
2	N.M.	—	—	—	—
3	130.9	71.4	85.6	84.3	62.8
4	82.6	81.0	75.3	87.2	73.6
5	N.P.	—	—	—	—
6	99.0	64.1	81.4	76.9	64.9
7	N.M.	—	—	—	—
8	145.6	94.6	92.1	80.1	76.7
9†	N.R.	N.R.	N.R.	N.R.	N.R.
10	216.5	92.1	93.5	80.7	78.0
11	140.9	91.6	85.9	76.6	68.1
12	80.7	21.2	68.9	95.5	109.0
13	N.P.	—	—	—	—
14	172.4	87.3	80.9	92.4	76.7
15	123.8	76.9	88.0	75.8	81.4
16	N.M.	—	—	—	—
17	75.3	78.6	90.9	80.0	69.5
18	130.0	85.1	73.3	81.2	73.9
19	131.8	80.5	90.5	78.0	75.6
20	102.3	78.5	87.5	74.9	67.1
21	N.M.	—	—	—	—
22	133.6	85.9	80.9	80.0	65.1
23	130.2	89.4	96.2	87.6	73.4
24	N.P.	—	—	—	—
25	89.8	91.8	86.8	71.4	76.7
26	116.6	95.4	87.2	82.0	71.6
27	108.6	74.3	74.8	81.1	74.4
28	N.P.	—	—	—	—
Totals	2297.0	1541.0	1642.2	1544.1	1414.8
Means	120.89	81.10	86.43	81.27	74.46

†Food consumption not recorded due to gastric blockage at the cardiac orifice.

N.M. - Not mated

N.P. - Not pregnant

N.R. - Not recorded

Appendix Table 1 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RATMaternal Food Consumption for the Low Dose Group

(Individual Values (g/kg))

Animal No.	Day 1	Day 6	Day 10	Day 15	Day 19
29	N.M.	—	—	—	—
30	134.1	78.3	87.5	76.9	63.6
31	97.6	79.3	74.0	68.0	72.8
32	79.8	80.0	77.4	69.9	56.1
33	168.1	85.2	82.7	89.1	74.3
34	150.0	119.4	115.0	76.6	64.1
35	N.M.	—	—	—	—
36	158.7	83.3	86.2	20.8	N.R.
37	N.M.	—	—	—	—
38	N.M.	—	—	—	—
39	120.3	83.3	79.5	67.3	69.2
40	N.P.	—	—	—	—
41	N.P.	—	—	—	—
42	118.1	66.1	90.5	70.9	66.8
43	100.0	70.9	68.0	75.0	64.6
44	130.4	75.1	83.3	89.1	69.4
45	173.9	64.5	100.0	90.9	43.4
46	N.P.	—	—	—	—
47	141.0	82.7	80.2	88.5	72.0
48	206.1	78.7	87.1	89.7	78.1
49	136.3	82.6	91.2	98.9	79.1
50	N.P.	—	—	—	—
51	128.5	74.5	85.4	76.9	72.5
52	92.9	81.7	67.8	85.5	68.0
53	66.1	87.5	68.6	73.5	73.8
54	181.8	91.6	90.9	90.2	77.1
55	123.9	96.2	81.4	87.0	74.2
56	111.1	74.8	69.5	104.3	80.5
Totals	2618.7	1635.7	1666.2	1589.0	1319.6
Means	130.94	81.78	83.31	79.45	69.45

N.M. - Not mated

N.P. - Not pregnant

N.R. - Not recorded

Appendix Table 1 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RATMaternal Food Consumption for the Medium Dose Group

(Individual Values (g/kg))

Animal No.	Day 1	Day 6	Day 10	Day 15	Day 20
57	57.6	79.2	54.3	49.8	77.1
58	129.4	99.1	97.1	84.4	63.5
59	N.P.	—	—	—	—
60	N.M.	—	—	—	—
61	139.8	88.0	82.0	79.3	64.0
62	N.M.	—	—	—	—
63	130.4	88.0	71.4	72.8	75.2
64	113.8	90.9	74.0	80.6	62.8
65	110.1	101.6	100.7	81.9	73.9
66	107.7	86.9	78.7	89.2	67.6
67	65.2	77.7	39.2	36.3	65.8
68	N.P.	—	—	—	—
69	105.0	80.7	82.4	96.3	76.7
70	81.6	89.8	85.0	91.4	73.8
71	N.P.	—	—	—	—
72	120.9	58.8	76.6	63.0	66.0
73	60.8	70.4	53.1	50.8	81.8
74	129.4	85.9	87.5	92.7	76.2
75	137.1	86.0	92.7	50.3	72.7
76	150.4	88.7	81.4	109.2	77.3
77	N.P.	—	—	—	—
78	120.6	85.2	80.8	77.4	73.8
79	N.P.	—	—	—	—
80	N.P.	—	—	—	—
81	135.1	65.3	N.R.	93.8	72.6
82	136.3	93.2	91.2	79.8	72.0
83	94.8	88.4	70.9	70.4	52.0
84	82.7	79.8	72.0	69.8	71.4
Totals	2208.7	1683.6	1471.0	1519.2	1416.2
Means	110.44	84.18	77.42	75.96	70.81

N.M. - Not mated

N.P. - Not pregnant

N.R. - Not recorded

Appendix Table 1 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC
AND TERATOGENIC POTENTIAL IN THE RATMaternal Food Consumption for the High Dose Group
(Individual Values (g/kg))

Animal No.	Day 1	Day 6	Day 10	Day 15	Day 19
85	86.7	37.4	98.4	87.8	81.9
86	N.P.	—	—	—	—
87	56.4	78.9	80.8	74.8	63.8
88	96.0	73.9	80.6	82.8	67.4
89	N.P.	—	—	—	—
90	83.3	91.2	76.6	89.1	75.3
91	165.2	84.6	78.5	79.3	72.5
92	160.0	103.8	86.8	79.3	75.3
93	86.6	87.1	84.1	85.3	72.6
94	N.P.	—	—	—	—
95	115.3	119.0	87.3	70.3	80.9
96	N.P.	—	—	—	—
97	122.9	85.1	87.4	80.5	73.9
98	125.0	94.6	65.2	62.7	68.0
99	N.P.	—	—	—	—
100	77.2	72.5	60.1	83.3	57.5
101	N.P.	—	—	—	—
102	122.7	103.0	69.9	93.5	66.1
103	N.P.	—	—	—	—
104	118.3	92.1	80.6	102.2	69.9
105	121.7	133.9	100.0	95.2	74.7
106	100.9	94.6	88.7	78.4	67.1
107	N.M.	—	—	—	—
108	N.P.	—	—	—	—
109	115.3	115.3	96.4	95.8	77.9
110	100.7	82.7	73.2	72.8	75.2
111	N.P.	—	—	—	—
112	112.5	78.3	78.5	68.9	74.9
Totals	1966.7	1628.0	1473.1	1482.0	1294.9
Means	109.26	90.44	81.84	82.33	71.94

N.M. - Not mated

N.P. - Not pregnant

Appendix Table 2

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC AND
TERATOGENIC POTENTIAL IN THE RAT

Hysterotomy Data for the Control Group

Animal No.	No. of Fetuses		Resorptions		Sex		Body Wt. (g)		CRD (cm)		Fetal Examination	
	Right ^a	Left ^b	Right ^a	Left ^b	Male	Female	Male	Female	Male	Female	Soft tissue (sectioned)	Skeletal (cleared)
1	5	8	2	3	5	8	4.1	3.8	3.6	3.5	5	8
3	5	7	1	0	5	7	4.2	3.9	3.8	3.2	4	8
4 ^c	4	0	2	0	3	1	4.7	4.5	4.0	3.9	2	2
6	8	4	0	0	5	7	3.7	3.6	3.7	3.5	4	8
8	6	4	0	0	4	6	3.9	3.7	3.7	3.6	4	6
9 ^d	0	0	5	6	0	0	0.0	0.0	0.0	0.0	0	0
10	5	5	1	0	3	7	4.1	4.2	3.8	3.7	4	6
11	6	10	0	1	10	6	4.1	3.9	3.7	3.6	6	10
12	7	6	0	0	5	8	4.1	4.0	3.7	3.7	5	8
14	1	5	0	0	3	3	4.5	4.0	3.8	3.8	2	4
15	6	3	2	0	1	8	4.6	4.3	3.8	3.9	3	6
17	3	9	1	2	7	5	4.1	3.9	3.7	3.7	4	8
18	3	4	2	2	5	2	3.8	3.3	3.8	3.6	3	4
19	9	5	0	0	9	5	4.0	3.9	3.7	3.8	5	9
20	5	8	0	0	7	6	4.3	4.1	3.9	3.8	5	8
22	7	8	0	1	10	5	4.2	4.0	3.8	3.6	5	10
23	6	8	1	1	6	8	3.8	3.9	3.8	3.7	5	9
25	5	8	0	3	5	8	4.4	3.9	3.7	3.7	5	8
26	6	8	0	1	12	2	4.3	4.1	3.9	3.9	5	9
27	9	4	1	0	10	3	4.1	4.0	3.8	3.7	5	8
Subtotal	102	114	11	14	112	104	74.3	70.5	67.7	66.0	79	137
Totals	216	216	25	25	216	216	74.3	70.5	67.7	66.0	216	216
Means	12.0	11.4	1.4	1.4	11.2	10.4	4.1	3.9	3.8	3.7	3.7	3.7

^a Right uterine horn

^b Left uterine horn

^c Female whose left uterine horn was distended and upon incision exudent a purulent material;
values listed are not included in the sub-totals, totals or means.

^d Female observed with hairball in esophagus on gestation day 20; values listed in the resorption column are not included in the sub-totals, totals, or means.

Appendix Table 2 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC AND
TERATOGENIC POTENTIAL IN THE RAT

Hysterotomy Data for the Low Dose Group

Animal No.	No. of Fetuses		Resorptions		Sex		Body Wt. (g)		CRD (cm)		Fetal Examination	
	Right ^a	Left ^b	Right ^a	Left ^b	Male	Female	Male	Female	Male	Female	Soft tissue (sectioned)	Skeletal (cleared)
30	6	10	0	0	8	8	3.9	3.6	3.7	3.6	6	10
31	7	4	2	1	2	9	3.8	3.4	3.6	3.5	4	7
32	8	4	0	1	6	6	4.0	3.8	3.6	3.6	4	8
33	3	9	1	0	5	7	4.0	3.8	3.7	3.7	4	8
34	9	5	0	0	4	10	4.1	3.7	3.7	3.6	5	9
36 ^c	0	0	4	9	0	0	0.0	0.0	0.0	0.0	0	0
39	1	8	0	2	5	4	4.1	3.7	3.8	3.6	3	6
42	4	6	0	0	7	3	4.3	3.9	3.8	3.7	4	6
43	9	4	0	1	6	7	4.3	3.8	3.9	3.7	5	8
44	9	1	1	0	6	4	3.7	3.6	3.6	3.6	4	6
45	5	7	1	0	6	6	4.3	4.1	3.8	3.7	4	8
47	8	5	1	0	5	8	3.7	3.6	3.6	3.6	5	8
48	9	2	0	0	7	4	4.4	4.0	3.9	3.8	4	7
49	2	8	0	1	3	7	4.5	4.2	3.8	3.8	4	6
51	6	4	0	3	4	6	3.8	3.7	3.6	3.6	4	6
52	7	6	1	1	3	10	4.4	4.1	3.8	3.8	5	8
53	9	9	0	1	7	11	3.8	3.6	3.6	3.6	6	12
54	3	7	0	0	6	4	4.6	4.5	3.9	3.9	4	6
55	8	6	0	0	9	5	4.2	3.9	3.8	3.8	5	9
56	6	6	0	0	5	7	4.7	4.6	4.0	3.8	4	8
Subtotal:	119	111	7	11	104	126	78.6	73.6	71.2	70.0	84	146
Totals:	230		18		230		4.1		3.7		230	
Means:	12.1		0.9									

^a Right uterine horn

^b Left uterine horn

^c Female found dead on gestation day 19; values listed for this female are not included in the sub-totals, totals, or means.

Appendix Table 2 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC AND
TERATOGENIC POTENTIAL IN THE RAT

Hysterotomy Data for the Medium Dose Group

Animal No.	No. of Fetuses		Resorptions		Sex		Body Wt. (g)		CRD (cm)		Fetal Examination	
	Right ^a	Left ^b	Right ^a	Left ^b	Male	Female	Male	Female	Male	Female	Soft tissue (sectioned)	Skeletal (cleared)
57	5	6	0	2	8	3	4.3	4.2	3.8	3.8	4	7
58	6	6	0	1	6	6	3.9	3.6	3.8	3.7	4	8
61	7	6	1	0	10	3	4.2	3.8	3.8	3.6	5	8
63	6	4	2	2	7	3	3.9	3.6	3.8	3.6	4	6
64	7	4	1	0	5	6	4.0	3.8	3.7	3.6	4	7
65	6	7	0	0	5	8	4.2	3.9	3.8	3.7	5	8
66	4	10	0	0	8	6	4.3	4.0	3.6	3.6	5	9
67	7	9	0	0	9	7	4.0	4.0	3.6	3.6	6	10
69	7	5	0	0	4	8	4.3	3.9	3.7	3.9	4	8
70	7	7	0	0	5	9	4.3	3.6	3.6	3.6	5	9
72	12	2	0	0	5	9	3.9	3.8	3.8	3.7	5	9
73	0	4	0	0	1	3	4.3	4.2	3.6	3.7	2	2
74	7	4	0	0	6	5	4.3	4.1	3.8	3.8	4	7
75	8	2	1	0	4	6	4.4	4.2	3.8	3.8	4	6
76	9	3	0	0	7	5	4.2	3.9	3.9	3.8	4	8
78	6	5	0	0	6	5	4.5	4.2	4.0	3.8	4	7
81	6	7	2	0	7	6	3.7	3.4	3.7	3.6	5	8
82	5	7	1	0	6	6	4.5	4.4	3.9	3.8	4	8
83	8	7	0	0	8	7	4.0	3.8	3.7	3.7	5	10
84	6	10	2	0	8	8	3.5	3.3	3.7	3.6	6	10
Subtotal	129	115	10	5	125	119	82.7	77.7	75.1	74.0	89	155
Totals	244		15		244		4.1		3.8		244	
Means	12.2		0.8									

^a Right uterine horn

^b Left uterine horn

Appendix Table 2 (cont.)

SC-19192: AN EVALUATION OF THE EMBRYOTOXIC AND
TERATOGENIC POTENTIAL IN THE RAT

Hysterotomy Data for the High Dose Group

Animal No.	No. of Fetuses			Resorptions		Sex		Body Wt. (g)		CRD (cm)		Fetal Examination	
	Right ^a	Left ^b	Total	Right ^a	Left ^b	Male	Female	Male	Female	Male	Female	Soft tissue (sectioned)	Skeletal (cleared)
85	8	5	13	0	0	8	5	4.3	4.2	3.7	3.7	5	8
87	5	9	14	1	0	7	7	4.1	3.7	3.6	3.6	5	9
88	6	11	17	0	0	11	6	4.6	4.1	3.8	3.7	6	11
90	6	6	12	1	0	7	5	4.4	4.3	3.7	3.7	4	8
91	12	3	15	0	0	7	8	4.0	3.7	3.8	3.7	5	10
92	7	6	13	0	1	5	8	4.1	4.1	3.7	3.7	5	8
93	8	4	12	0	1	5	7	4.8	4.5	4.0	3.8	4	8
95	8	4	12	0	0	5	7	4.3	3.8	3.8	3.7	4	8
97	5	7	12	0	0	8	4	4.6	4.3	3.9	3.8	4	8
98	7	7	14	0	0	6	8	4.2	4.1	3.9	3.7	5	9
100	7	5	12	0	0	2	10	4.7	4.4	3.9	3.8	4	8
102	9	3	12	0	0	6	6	4.0	3.8	3.8	3.8	4	8
104	5	10	15	1	0	9	6	4.2	4.0	3.9	3.8	5	10
105	5	8	13	0	0	5	8	4.0	3.8	3.8	3.8	5	8
106	6	9	15	0	0	7	8	4.1	4.1	3.7	3.7	5	10
109	6	5	11	0	0	4	7	4.3	4.1	3.8	3.6	4	7
110	6	8	14	0	1	6	8	4.2	4.2	3.8	3.9	5	9
112	5	6	11	0	1	4	7	4.6	4.5	3.9	3.9	4	7
Subtotal	121	116	237	3	4	112	125	77.5	73.7	68.5	67.4	83	154
Totals		237		7			237						237
Means		13.2		0.4		4.3	4.1	3.8		3.7			

^a Right uterine horn

^b Left uterine horn