

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS


THIS STUDY IS NOT INTENDED TO SUPPORT APPLICATIONS FOR
RESEARCH OR MARKETING PERMITS FOR PRODUCTS REGULATED
BY GOVERNMENTAL AGENCIES. THIS IS AN EXPLORATORY/
RANGE-FINDING STUDY AND IS NOT WITHIN THE SCOPE OF
GOOD LABORATORY PRACTICE REGULATIONS.

James W. Noveroske and Gary Chmielewski


Safety Assessment Project Number 2552

Department of Product Safety Assessment
G. D. Searle & Co.
Skokie, IL


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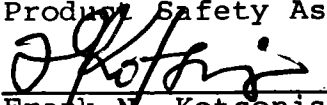
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 Mar. 1, 1985

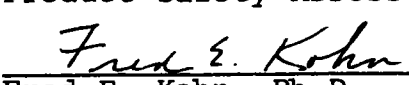
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TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
SUMMARY	1
INTRODUCTION	2
MATERIALS AND METHODS	2
RESULTS AND DISCUSSION	5
COMPLIANCE STATEMENT	6
Table 1 Maternal Body Weights	7
Table 2 Reproductive Status of Females at Sacrifice	8
Table 3 Maternal Food Consumption	9
Table 4 Actual Average Maternal Dosage Levels	10
Table 5 Individual Female Body Weights	11
Table 6 Individual Fetal Data	17
Table 7 Individual Average Daily Food Consumption	23
Table 8 Individual Female Dosage Levels	29
Appendix A Protocol and Amendment	A1-A6
Appendix B Analytical Data	B1-B3

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DEPARTMENT OF PRODUCT SAFETY ASSESSMENT

G. D. Searle & Co.
Skokie, IL

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Author: James W. Noveroske and Gary Chmielewski

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Date: March 1, 1985

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Summary:

SC-19200 was administered by diet admix to ten rats per group at intended dosage levels of 125, 250, 500, 750 and 1000 mg/kg/day for 10 consecutive days (days 6 through 15 of gestation). A control group received the standard diet.

Actual average dosage levels of SC-19200 from days 6 through 15 of gestation were 127, 254, 494, 755 and 981 mg/kg/day. No compound-related deaths or clinical signs occurred at any dosage level.

Average maternal body weight gain and food consumption were unaffected at all dosage levels.

Examination of the reproductive status of females at sacrifice revealed no adverse effects of SC-19200 on average numbers of implantations, resorptions, and live or dead fetuses per litter at any dosage level.

S.A. 2552

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

INTRODUCTION

This study was conducted to determine potential toxic effects of SC-19200 as evidenced by clinical signs, body weights, fetal viability, and to provide a basis for dosage level selection in teratology/reproduction studies.

MATERIALS AND METHODS

Sixty virgin female rats (Charles River COBS CD strain, Portage, MI) approximately fifteen weeks of age and weighing 228 to 303 g, were divided into 6 groups of 10 rats each for this study.

Female rats were placed with males (1 female/male) of the same strain. Each morning during the breeding period, a vaginal lavage was taken from the females. Upon detection of spermatozoa in the vaginal lavage (day 1 of gestation) the mated females were randomly assigned to treatment groups and given an ear tag with a unique number. The females were then housed in individual suspended, stainless steel, wire mesh cages, and given free access to Certified Purina Rat Chow Meal # 5002 and to municipally supplied tap water.

The animal room was set to maintain a $72^{\circ} \pm 5^{\circ}\text{F}$ temperature and 25% or greater relative humidity with a 12-hour light and 12-hour dark cycle. The study was started on January 9, 1985 and terminated on January 31, 1985.

SC-19200, N-L- β -aspartyl-L-phenylalanine (Lot #CD141-197), was given to the 5 groups of rats for 10 consecutive days. SC-19200/Certified Purina Rat Chow Meal # 5002 diet admix preparations were made at levels of 125, 250, 500, 750 and 1000 mg/kg/day. Actual average dosage levels (Tables 4 and 8) were 127, 254, 494, 755 and 981 mg/kg/day. Dosage calculations (Days 6-13, and 13-16) were based upon cumulative average food consumption and body weights of the first animals to reach appropriate stages of gestation (Days 5 and 12, respectively). Historical body weight data was also used to anticipate maternal weight gains. The control group received the standard diet ad libitum.

The identity, strength, purity, and composition of the test article were determined before and after use in this study. The results of the test article analyses are shown in Appendix B.

Body weights of rats were recorded on days 1, 6, 8, 10, 12, 14, 16, and 21 of gestation. The rats were examined daily for adverse clinical signs throughout the study, sacrificed by CO₂ inhalation on day 21 of gestation, and examined to obtain the numbers of corpora lutea, implantations, resorptions, and live or dead fetuses.

Maternal body weights, body weight changes, and food consumption were analyzed using a one-way analysis of variance, and if the ANOVA F-test was significant at the 5% level, Student's t-tests (using the pooled error variance from the one-way analysis of variance) of control versus the other dose groups were performed. A Bartlett-Box test for homogeneity of variance was done. The Kruskal-Wallis test

was used to analyze the following variables: numbers of implantations, resorptions, and live fetuses per litter. If significant at the 5% level, then the Mann-Whitney U test was used to compare the control to each compound-treated group. Numbers of corpora lutea were not analyzed or tabulated in this report. All t-tests were two-tailed and significance levels achieved have been reported for 5% for t-tests, Bartlett-Box tests, and Mann-Whitney U tests.

The study was conducted at G. D. Searle & Co., and the final report, protocol, raw data, and supporting documents are on file at G. D. Searle & Co., Skokie, Illinois.

A list of the study professionals that participated in the study is as follows:

Laboratory Animal Resources	J. Erickson
Analytical Coordinator	K. Pilipauskas
Biostatistics	P. Sanders
Teratology	G. Chmielewski
Product Development	J. Jiu
Analytical Department	

RESULTS AND DISCUSSION

From days 6 to 16 gestation, actual average dosage levels of SC-19200 (Tables 4 and 8) administered by diet admix were 127, 254, 494, 755 and 981 mg/kg/day. No compound-related deaths or clinical signs occurred at any dosage level.

Average maternal body weight gain and food consumption were unaffected at all dosage levels (Tables 1, 3, 5 and 7).

Examination of the reproductive status of females at sacrifice (Tables 2 and 6) revealed no adverse effects on average numbers of implantations, resorptions, and live or dead fetuses per litter.

COMPLIANCE STATEMENT

Although this is a range-finding study and not within the scope of Good Laboratory Practice regulations, the laboratory phase was conducted with the intention of complying with the GLP regulations. Two known deviations occurred as follows:

1. Animal room humidity and temperature levels were not recorded January 9 - 23, 1985.
2. Study animals weighed between 228 - 303 grams, and were approximately fifteen weeks old at the start of the study.

However, these deviations did not affect the quality or integrity of the study and this report accurately reflects the data obtained during the performance of the study.

TABLE 1

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Maternal Body Weights

		(mg/kg/day)				
	Control	125	250	500	750	1000
Average Body Weight (g)						
Day 1	253	256	259	257	263	257
Day 6	270	274	281	274	281	277
Day 8	276	279	289	280	286	282
Day 10	285	288	299	287	297	290
Day 12	293	295	304	292	304	298
Day 14	298	299	308	298	309	303
Day 16	314	313	325	317	326	318
Day 21	372	368	378	375	388	374 ^a
Change						
Days 1-6	+17	+18	+22	+17	+18	+20
Days 6-16	+44	+39	+44	+44	+45	+41
Days 16-21	+58	+55	+53	+58	+62	+57 ^a
Days 1-21	+120	+112	+119	+118	+125	+118 ^a

^aExcludes Female No. 85-155

TABLE 2
A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS
Reproductive Status Of Females At Sacrifice

		(mg/kg/day)				
	Control	125	250	500	750	1000
Females						
Total No.	10	10	10	10	10	10
No. Live Pregnant	10	10	10	8	9	9
No. Live Not Pregnant	0	0	0	2	1	1
Implantations						
Total No.	144	150	134	124	140	124
No./Pregnant Female	14.4	15.0	13.4	15.5	15.6	13.8
Resorptions						
Total No.	9	13	14	8	8	7
No./Pregnant Female	0.9	1.3	1.4	1.0	0.9	0.8
Fetuses						
Total No.	135	137	120	116	132	117
No. Live	135	137	120	116	132	117
No. Live/Pregnant Female	13.5	13.7	12.0	14.5	14.7	13.0

TABLE 3
A Range-Finding Study of SC-19200 in Pregnant Rats
Maternal Food Consumption

	Control	mg/kg/day				
		125	250	500	750	1000
Average Daily Food Consumption (g)						
Days 1-6	17.8	19.5	18.6	18.6	19.4	19.1
Days 6-13	20.9	21.1	21.7	20.0	21.7	20.3
Days 13-16	20.6	21.0	21.9	21.8	21.3	21.3
Days 6-16	20.8	21.1	21.8	20.5	21.5	20.6
Days 16-21	23.3	22.8	23.2	23.5	23.6	22.5

TABLE 4

A Range-Finding Study of SC-19200 in Pregnant Rats

Actual Average Maternal Dosage Levels

	Control	mg/kg/day				
		125	250	500	750	1000
Average Dosage Levels (mg/kg/day)						
Days 6-16	0	127	254	494	755	981

TABLE 5

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Female Body Weights (g)

Control Group

Female	Reproductive Status	Gestation Day							
		1	6	8	10	12	14	16	21
85-101	Pregnant	285	305	307	316	330	336	348	417
85-102	Pregnant	270	297	303	307	318	326	329	400
85-103	Pregnant	243	270	278	287	289	293	312	361
85-104	Pregnant	230	256	259	268	276	281	301	366
85-105	Pregnant	252	268	278	283	289	295	309	366
85-106	Pregnant	280	300	305	316	323	328	343	400
85-107	Pregnant	229	249	249	262	266	271	286	326
85-108	Pregnant	238	259	263	272	281	279	302	353
85-109	Pregnant	237	257	262	269	280	283	299	351
85-110	Pregnant	262	239	258	270	277	289	310	383

TABLE 5 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Female Body Weights (g)

125 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		1	6	8	10	12	14	16	21
85-111	Pregnant	228	249	259	263	270	282	294	361
85-112	Pregnant	232	249	254	262	272	276	286	330
85-113	Pregnant	247	259	259	270	279	280	291	346
85-114	Pregnant	275	299	306	320	322	330	342	399
85-115	Pregnant	302	319	325	333	346	350	371	430
85-116	Pregnant	258	271	274	282	289	293	307	357
85-117	Pregnant	268	283	291	302	309	310	324	382
85-118	Pregnant	258	274	277	286	287	289	306	354
85-119	Pregnant	233	258	262	277	281	284	301	367
85-120	Pregnant	256	275	278	287	293	296	307	354

TABLE 5 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Female Body Weights (g)

250 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		1	6	8	10	12	14	16	21
85-121	Pregnant	242	282	296	306	319	323	330	392
85-122	Pregnant	250	267	272	281	291	296	306	349
85-123	Pregnant	246	259	267	275	283	289	311	384
85-124	Pregnant	252	288	296	309	315	320	351	401
85-125	Pregnant	259	274	283	293	301	303	319	377
85-126	Pregnant	288	309	314	324	327	327	346	377
85-127	Pregnant	267	291	301	313	314	321	333	385
85-128	Pregnant	265	293	295	304	301	300	320	350
85-129	Pregnant	264	281	288	300	306	309	329	397
85-130	Pregnant	254	267	277	281	284	292	304	366

TABLE 5 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Female Body Weights (g)

500 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		1	6	8	10	12	14	16	21
85-131	Not Pregnant	303	311	315	318	325	326	322	314
85-132	Pregnant	249	272	274	275	255	281	293	362
85-133	Not Pregnant	256	284	291	299	306	298	301	303
85-134	Pregnant	263	279	285	291	299	301	319	369
85-135	Pregnant	247	268	274	282	291	292	314	369
85-136	Pregnant	282	288	296	305	317	325	347	407
85-137	Pregnant	265	281	290	292	299	303	318	367
85-138	Pregnant	244	256	259	270	279	283	304	368
85-139	Pregnant	277	300	311	321	331	331	353	400
85-140	Pregnant	230	245	250	258	263	270	289	361

TABLE 5 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Female Body Weights (g)

750 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		1	6	8	10	12	14	16	21
85-141	Pregnant	267	279	286	297	309	317	329	401
85-142	Pregnant	280	295	299	310	319	320	327	383
85-143	Not Pregnant	252	252	262	254	263	256	259	263
85-144	Pregnant	275	278	284	292	299	306	326	392
85-145	Pregnant	282	315	318	331	337	347	364	432
85-146	Pregnant	262	280	286	301	303	310	330	390
85-147	Pregnant	232	257	263	273	280	282	297	350
85-148	Pregnant	255	271	277	286	290	293	310	362
85-149	Pregnant	244	266	275	282	289	293	317	383
85-150	Pregnant	266	285	282	300	307	309	331	397

TABLE 5 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Female Body Weights (g)

1000 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		1	6	8	10	12	14	16	21
85-151	Pregnant	260	284	286	295	309	316	325	406
85-152	Pregnant	243	259	264	264	279	280	289	351
85-153	Pregnant	258	289	297	305	315	317	325	385
85-154	Not Pregnant	294	292	283	296	292	287	296	300
85-155	Pregnant	272	288	289	300	308	311	330	323 ^a
85-156	Pregnant	280	289	295	303	305	311	330	373
85-157	Pregnant	249	278	284	290	299	306	331	400
85-158	Pregnant	248	270	272	285	287	292	310	377
85-159	Pregnant	248	263	273	287	293	299	316	365
85-160	Pregnant	259	273	279	284	289	295	304	332

^aExcluded from calculations - considered to be invalid number as female had 17 fetuses

TABLE 6

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Fetal Data

Control Group

Animal Number	Reproductive Status	Number of			
		Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-101	Pregnant	14	0	14	0
85-102	Pregnant	16	3	13	0
85-103	Pregnant	15	3	12	0
85-104	Pregnant	15	0	15	0
85-105	Pregnant	13	1	12	0
85-106	Pregnant	16	0	16	0
85-107	Pregnant	13	1	12	0
85-108	Pregnant	14	1	13	0
85-109	Pregnant	13	0	13	0
85-110	Pregnant	15	0	15	0

TABLE 6 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Fetal Data

125 mg/kg/day Group

Animal Number	Reproductive Status	Number of			
		Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-111	Pregnant	14	0	14	0
85-112	Pregnant	14	3	11	0
85-113	Pregnant	15	2	13	0
85-114	Pregnant	17	0	17	0
85-115	Pregnant	17	1	16	0
85-116	Pregnant	14	0	14	0
85-117	Pregnant	16	3	13	0
85-118	Pregnant	13	1	12	0
85-119	Pregnant	15	1	14	0
85-120	Pregnant	15	2	13	0

TABLE 6 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Fetal Data

250 mg/kg/day Group

Animal Number	Reproductive Status	Number of			
		Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-121	Pregnant	15	2	13	0
85-122	Pregnant	12	3	9	0
85-123	Pregnant	16	0	16	0
85-124	Pregnant	16	2	14	0
85-125	Pregnant	14	1	13	0
85-126	Pregnant	7	1	6	0
85-127	Pregnant	16	2	14	0
85-128	Pregnant	7	1	6	0
85-129	Pregnant	17	2	15	0
85-130	Pregnant	14	0	14	0

TABLE 6 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Fetal Data

500 mg/kg/day Group

Animal Number	Reproductive Status	Number of			
		Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-131	Not Pregnant	0	0	0	0
85-132	Pregnant	16	1	15	0
85-133	Not Pregnant	0	0	0	0
85-134	Pregnant	14	0	14	0
85-135	Pregnant	14	0	14	0
85-136	Pregnant	18	2	16	0
85-137	Pregnant	15	2	13	0
85-138	Pregnant	17	2	15	0
85-139	Pregnant	13	1	12	0
85-140	Pregnant	17	0	17	0

TABLE 6 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Fetal Data

750 mg/kg/day Group

Animal Number	Reproductive Status	Number of			
		Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-141	Pregnant	17	1	16	0
85-142	Pregnant	16	4	12	0
85-143	Not Pregnant	0	0	0	0
85-144	Pregnant	16	0	16	0
85-145	Pregnant	16	1	15	0
85-146	Pregnant	16	0	16	0
85-147	Pregnant	14	1	13	0
85-148	Pregnant	15	1	14	0
85-149	Pregnant	15	0	15	0
85-150	Pregnant	15	0	15	0

TABLE 6 (cont.)

A RANGE-FINDING STUDY OF SC-19200 IN PREGNANT RATS

Individual Fetal Data

1000 mg/kg/day Group

Animal Number	Reproductive Status	Number of			
		Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-151	Pregnant	14	0	14	0
85-152	Pregnant	13	0	13	0
85-153	Pregnant	15	3	12	0
85-154	Not Pregnant	0	0	0	0
85-155	Pregnant	17	0	17	0
85-156	Pregnant	14	1	13	0
85-157	Pregnant	15	1	14	0
85-158	Pregnant	15	0	15	0
85-159	Pregnant	15	2	13	0
85-160	Pregnant	6	0	6	0

TABLE 7

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Average Daily Food Consumption (g)

Control Group

Female	Reproductive Status	Gestation Period (days)			
		1-6	6-13	13-16	16-21
85-101	Pregnant	21.4	23.4	24.3	26.6
85-102	Pregnant	21.0	21.3	18.7	24.0
85-103	Pregnant	21.8	21.7	20.3	22.6
85-104	Pregnant	21.2	21.9	21.3	25.8
85-105	Pregnant	16.0	18.7	20.0	23.4
85-106	Pregnant	19.4	21.7	20.7	23.6
85-107	Pregnant	18.0	19.0	18.7	20.2
85-108	Pregnant	17.8	20.3	19.0	21.8
85-109	Pregnant	18.4	19.7	20.0	21.2
85-110	Pregnant	3.2	21.6	22.7	24.0

TABLE 7 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Average Daily Food Consumption (g)

125 mg/kg/day Group

Female	Reproductive Status	Gestation Period (days)			
		1-6	6-13	13-16	16-21
85-111	Pregnant	21.2	21.7	23.7	23.6
85-112	Pregnant	20.0	21.7	20.0	20.2
85-113	Pregnant	18.2	18.6	19.3	22.4
85-114	Pregnant	22.6	24.7	23.0	24.8
85-115	Pregnant	20.0	24.1	24.0	25.4
85-116	Pregnant	19.6	20.0	19.3	21.8
85-117	Pregnant	16.0	20.6	19.7	22.8
85-118	Pregnant	18.8	19.4	20.3	23.0
85-119	Pregnant	22.0	20.6	21.7	24.2
85-120	Pregnant	16.8	19.9	19.3	19.6

TABLE 7 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Average Daily Food Consumption (g)

250 mg/kg/day Group

Female	Reproductive Status	Gestation Period (days)			
		1-6	6-13	13-16	16-21
85-121	Pregnant	21.4	23.3	23.3	22.8
85-122	Pregnant	22.2	21.9	23.3	23.2
85-123	Pregnant	10.6	22.1	21.7	23.4
85-124	Pregnant	20.4	23.0	22.7	24.8
85-125	Pregnant	18.4	20.7	20.3	22.6
85-126	Pregnant	20.8	23.0	25.7	24.6
85-127	Pregnant	20.4	21.9	21.7	21.6
85-128	Pregnant	21.0	20.0	21.7	24.2
85-129	Pregnant	18.4	21.6	19.0	22.8
85-130	Pregnant	12.4	19.7	20.0	21.8

TABLE 7 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Average Daily Food Consumption (g)

500 mg/kg/day Group

Female	Reproductive Status	Gestation Period (days)			
		1-6	6-13	13-16	16-21
85-131	Not Pregnant	19.8	21.6	19.0	15.0
85-132	Pregnant	20.8	12.9	23.0	25.2
85-133	Not Pregnant	20.4	23.6	20.0	18.2
85-134	Pregnant	16.4	19.9	19.7	24.0
85-135	Pregnant	17.0	18.3	19.3	21.8
85-136	Pregnant	18.6	24.0	25.0	24.6
85-137	Pregnant	18.2	19.9	20.0	21.0
85-138	Pregnant	20.2	20.7	22.3	23.4
85-139	Pregnant	21.0	26.4	25.3	26.4
85-140	Pregnant	16.2	18.0	19.7	21.4

TABLE 7 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Average Daily Food Consumption (g)

750 mg/kg/day Group

Female	Reproductive Status	Gestation Period (days)			
		1-6	6-13	13-16	16-21
85-141	Pregnant	20.2	22.9	23.0	25.6
85-142	Pregnant	20.2	22.9	20.7	23.0
85-143	Not Pregnant	16.2	17.1	14.7	15.4
85-144	Pregnant	15.4	18.9	19.7	23.4
85-145	Pregnant	23.6	25.4	24.0	26.2
85-146	Pregnant	18.0	21.1	22.3	22.2
85-147	Pregnant	19.4	21.7	21.0	22.8
85-148	Pregnant	18.2	19.1	18.7	20.6
85-149	Pregnant	18.4	20.9	21.0	23.0
85-150	Pregnant	21.6	22.0	21.0	25.2

TABLE 7 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Average Daily Food Consumption (g)

1000 mg/kg/day Group

Female	Reproductive Status	Gestation Period (days)			
		1-6	6-13	13-16	16-21
85-151	Pregnant	19.2	20.0	22.0	26.2
85-152	Pregnant	16.0	17.3	18.7	19.6
85-153	Pregnant	23.2	22.6	22.0	24.6
85-154	Not Pregnant	15.8	17.0	16.0	17.4
85-155	Pregnant	17.0	18.6	20.3	14.6
85-156	Pregnant	14.8	20.6	21.7	22.2
85-157	Pregnant	23.6	21.9	23.7	27.6
85-158	Pregnant	19.6	19.7	20.0	23.0
85-159	Pregnant	19.4	21.1	21.3	22.4
85-160	Pregnant	19.2	21.0	21.7	22.6

TABLE 8

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Female Dosage Levels

125 mg/kg/day Group

Animal #	Average Body Wt. (g) Days 6-16	Average Daily Compound Intake (mg)			Average Dose (mg/kg/day) 6-16
		6-13	13-16	6-16	
85-111	270	37.5	43.1	39.1	145
85-112	267	37.5	36.4	37.1	139
85-113	273	32.1	35.1	33.0	121
85-114	320	42.6	41.8	42.4	133
85-115	341	41.6	43.6	42.2	124
85-116	286	34.5	35.1	34.7	121
85-117	303	35.6	35.8	35.6	118
85-118	287	33.5	36.9	34.5	120
85-119	277	35.6	39.5	36.7	132
85-120	289	34.3	35.1	34.6	119

TABLE 8 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Female Dosage Levels

250 mg/kg/day Group

Animal #	Average Body Wt. (g) Days 6-16	Average Daily Compound Intake (mg)			Average Dose (mg/kg/day) 6-16
		6-13	13-16	6-16	
85-121	309	80.4	84.7	81.7	264
85-122	286	75.6	84.7	78.3	274
85-123	281	76.3	78.9	77.1	275
85-124	313	79.4	82.5	80.3	257
85-125	296	71.5	73.8	72.2	244
85-126	325	79.4	93.4	83.6	258
85-127	312	75.6	78.9	76.6	245
85-128	302	69.0	78.9	72.0	238
85-129	302	74.6	69.1	72.9	241
85-130	284	68.0	72.7	69.4	244

TABLE 8 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Female Dosage Levels

500 mg/kg/day Group

Animal #	Average Body Wt. (g) Days 6-16	Average Daily Compound Intake (mg)			Average Dose (mg/kg/day) 6-16
		6-13	13-16	6-16	
85-131	320	149.1	138.2	145.9	457
85-132	275	89.1	167.3	112.5	409
85-133	297	163.0	145.5	157.7	532
85-134	296	137.4	143.3	139.2	471
85-135	287	126.4	140.4	130.6	455
85-136	313	165.7	181.8	170.6	545
85-137	297	137.4	145.5	139.8	471
85-138	275	142.9	162.2	148.7	540
85-139	325	182.3	184.0	182.8	563
85-140	263	124.3	143.3	130.0	495

TABLE 8 (cont.)

A Range-Finding Study of SC-19129 in Pregnant Rats

Individual Female Dosage Levels

750 mg/kg/day Group

Animal #	Average Body Wt. (g) Days 6-16	Average Daily Compound Intake (mg)			Average Dose (mg/kg/day) 6-16
		6-13	13-16	6-16	
85-141	303	237.2	250.9	241.3	797
85-142	312	237.2	225.8	233.8	750
85-143	258	177.1	160.4	172.1	668
85-144	298	195.7	214.9	201.5	677
85-145	335	263.1	261.8	262.7	783
85-146	302	218.5	243.3	226.0	749
85-147	275	224.7	229.1	226.0	821
85-148	288	197.8	204.0	199.7	694
85-149	287	216.5	229.1	220.2	767
85-150	302	227.9	229.1	228.2	755

TABLE 8 (cont.)

A Range-Finding Study of SC-19200 in Pregnant Rats

Individual Female Dosage Levels

1000 mg/kg/day Group

Animal #	Average Body Wt. (g) Days 6-16	Average Daily Compound Intake (mg)			Average Dose (mg/kg/day) 6-16
		6-13	13-16	6-16	
85-151	303	276.2	320.0	289.3	956
85-152	273	238.9	272.0	248.8	913
85-153	308	312.1	320.0	314.5	1021
85-154	291	234.8	232.7	234.2	805
85-155	304	256.9	295.3	268.4	882
85-156	306	284.5	315.6	293.8	962
85-157	298	302.4	344.7	315.1	1057
85-158	286	272.1	290.9	277.7	971
85-159	289	291.4	309.8	296.9	1029
85-160	287	290.0	315.6	297.7	1036

APPENDIX A

PROTOCOL

1. Study Title: A Range-Finding Study of SC-19200 in Pregnant Rats

THIS STUDY IS NOT INTENDED TO SUPPORT APPLICATIONS
FOR RESEARCH OR MARKETING PERMITS FOR PRODUCTS
REGULATED BY GOVERNMENTAL AGENCIES. THIS IS AN
EXPLORATORY/RANGE-FINDING STUDY AND IS NOT WITHIN
THE SCOPE OF GOOD LABORATORY PRACTICE REGULATIONS.

2. Study Sponsor: G. D. Searle & Co.
3. Facility: G. D. Searle & Co., 4901 Searle Parkway, Skokie, Illinois 60077.
4. Proposed Dates:
 - A. Initiate Breeding: January 9, 1985
 - B. Initiate Dosing: January 15, 1985
 - C. Initiate Day 21 Sacrifice: January 30, 1985
5. Purpose: To determine potential toxic effects as evidenced by clinical signs, body weights, and fetal viability, and to provide a basis for dosage level selection in teratology/reproduction studies.
6. Overview of Study Design:

<u>Group</u>	<u>Treatment</u>	<u>Dosage Level (mg/kg/day)</u>	<u>Number of Females/Group</u>
1	Control	0	10
2	SC-19200	125	10
3	SC-19200	250	10
4	SC-19200	500	10
5	SC-19200	750	10
6	SC-19200	1000	10
7. Laboratory Procedures: This is an exploratory/range-finding study and is not within the scope of Good Laboratory Practice Regulations.

8. Proposed Use:

9. Test Article:

- A. Chemical Name: N-L- β -aspartyl-L-phenylalanine.
- B. Formulation: The appropriate amount of test article will be mixed with diet.
- C. Administration:
 - 1. Route: By diet admix.
 - 2. Duration: The females will receive the SC-19200/diet admix ad libitum from day 6* through day 15 of gestation. The concentration of SC-19200 in the diet will be adjusted on day 13 of gestation based on average group body weights from day 12 of gestation.
- D. Analysis
 - 1. Test Article
 - a. Identity, strength, purity and composition: Will be determined before use.
 - b. Stability: Will be reported if available.
 - 2. Test Article Carrier Mixture:
 - a. Stability: Will be reported if available.
- E. Storage
 - 1. Test Article: Will be stored in a well-closed, light-resistant container at controlled room temperature.
 - 2. Test article carrier mixture: Will be stored in an appropriate container at controlled room temperature.
- F. Estimated Test Article Requirements: 300 g

*NOTE - Day 6 and day 13 SC-19200/diet concentrations will be based on food consumption and body weight data obtained on day 5 and day 12 respectively, from the first rats to reach this stage of gestation.

10. Study Design Conditions:

- A. Animals: Sixty virgin female rats of the Charles River COBS CD strain (Portage, MI.) will be used in this study. The rat is widely used for teratogenic studies, and as such a vast amount of historical control data is available. The rats will be approximately 2 to 3 months of age and weigh 170-270 grams at the start of the study. The rats will be allowed approximately two weeks acclimatization prior to the start of the study.
- B. Husbandry and Diet: Rats will be housed (2 or 3 females/box) in polycarbonate shoe boxes prior to the start of the study. Following mating, the female rats will be housed individually in suspended, stainless steel, wire mesh cages for the remainder of the study. The rats will have free access to a Certified Purina Rat Chow Diet #5002 and have free access to municipally supplied tap water throughout the study. It is considered that there are no known interfering contaminants in the diet or water. Animal room temperature will be 72° + 5°F and relative humidity will be 25% or greater; both parameters will be monitored. A 12-hour light/12-hour dark cycle will be used throughout the study.
- C. Breeding Procedure: Female rats will be grouped with breeder colony males (1 or 2 females/male) of the same strain and source. Each morning a vaginal lavage will be prepared from each female and examined for the presence of spermatozoa. The presence of sperm in the lavage will indicate a successful mating. The day this occurs will be designated as day 1 of gestation. Once mating has occurred, the females will be randomly assigned to treatment groups by using a block design of random permutation and be given their unique identification numbers using ear tags.

11. Maternal Observations:

- A. Clinical Signs: Animals checked at least once a day and all remarkable signs observed will be recorded.
- B. Mortality: Any rats that die will be examined to verify reproductive status and to possibly determine cause of death.

C. Body Weight: Females will be weighed on gestation days 1, 6, 8, 10, 12, 14, 16, and 21.

D. Feeder Weight: Measured on days 1, 6, 13, 16, and 21 of gestation.

12. Caesarean Section:

On day 21 of gestation, all females will be sacrificed by CO₂ inhalation. The uterus will be exposed and the numbers of corpora lutea, implantations, resorptions, and live or dead fetuses recorded.

13. Statistical Procedures:

The mean values and standard deviations of each variable will be determined. Food consumption, maternal body weights, and body weight changes will be analyzed by a one-way analysis of variance, Student's t-tests (using the pooled error variance from the one-way analysis of variance) of control vs. the other dose groups (if the F ratio among treatments is significant at the 5% level), and the Bartlett-Box test for homogeneity of variance. All t-tests will be two-tailed. The Kruskal-Wallis test will be used to analyze the following variables: number of implantations, resorptions, live or dead fetuses per litter. If significant at the 5% level, then the Mann-Whitney U test will be used to compare each drug-treated group to the control group. Significance levels achieved will be reported for 5% for t-tests, Mann-Whitney U tests and Bartlett-Box test.

14. Archiving of Materials:

All raw data, supporting documents, protocol, specimens, and the final report will be transferred to the R&D Central File.

15. Protocol Approval

A. J. W. Noveroske, Ph.D.
Study Director
Product Safety Assessment:

J. W. Noveroske 1/7/85
Date

B. F. N. Kotsonis, Ph.D.
Diplomate, A.B.T.
Director, Toxicology
Product Safety Assessment:

F. N. Kotsonis 1/7/85
Date

C. F. E. Kohn, Ph.D.
Senior Director,
Product Safety Assessment:

F. E. Kohn 1/7/85
Date

PROTOCOL AMENDMENT
January 10, 1985

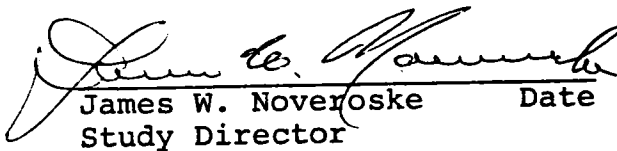
Protocol Amendment #1
S.A. 2552
A Range-Finding Study of SC-19200 in Pregnant Rats

The following is a change to the approved protocol:

1. Page 2, Sect. 9.D.1. - change "before use" to
"before and after use".

Reason for change - Stability of compound unavailable
at this time.

Approval:

 1/11/85
James W. Noveroske Date
Study Director

S.A. 2552

APPENDIX B

R&D PRODUCT DEVELOPMENT FUNCTION
REPORT REVIEW AND RELEASE

Page 1 of 3

DEPARTMENT: Product Development Analytical

DOCUMENT NUMBER: F-314-035-01

TITLE OF REPORT: SC-19200

TYPE OF REPORT: Analytical Summary in Support of Product Safety
Assessment Study Number 2552

AUTHOR(S):	DATE	REVIEWER(S):	DATE
<i>James Dwyer</i>	<i>8/2/85</i>	<i>Daniel L. Sweeney</i>	<i>2-25-85</i>
.....
.....

APPROVAL:	DATE
<i>James Dwyer</i>	<i>25-Feb '85</i>
.....
.....

TECHNICAL WRITER: Michele Newcomb *Michele Newcomb*

APPROVAL FOR RELEASE:

<i>R. Baum</i>	<i>2/27/85</i>	<i>L. Hansen</i>	<i>2/28/85</i>
.....
R. Baum, Director	Date	L. Hansen,	Date
Analytical Development		Senior Director	
		Product Development	

NORTH AMERICAN PRECLINICAL RESEARCH AND DEVELOPMENT
SEARLE PHARMACEUTICALS AND CONSUMER PRODUCTS
SKOKIE, ILLINOIS

S.A. 2552

ANALYTICAL SUMMARY

Product Development Analytical Department

Page 2 of 3

Subject: SC-19200

Summary Number: F-314-035-01

Applicable to SA Study Number: 2552

Test Article Characterization and Stability

Lot CD141-197 was analyzed using the release method of testing, released against the current specifications (NS-S85-001-A), and given a re-evaluation period of one year prior to use in this study.

Summary of the significant results used to characterize the SC-19200 is presented in Table 1.

Table 1

	Pre-Study	Post-Study
Lot Designation	CD141-197	CD141-197
Analysis Report #	84N1194	85N0068
Completion Date	12/18/84	02/13/85
Identity (IR)	Conforms to standard spectrum	Conforms to standard spectrum
Assay (titration)	101.3% n = 3 s = 0.2	100.4% n = 3 s = 0.1
Loss on Drying	0.05%	0.01%

These results and all other results, coupled with the use of lot CD141-197 within its re-evaluation period indicate that lot CD141-197 of SC-19200 was suitable for use in this study.

ANALYTICAL SUMMARY

Product Development Analytical Department

Page 3 of 3

Subject: SC-19200

Summary Number: F-314-035-01

Applicable to SA Study Number: 2552

GLP Compliance Statement

To the best of our knowledge, the support activities provided by the Product Development Analytical Department for this study were conducted in compliance with the Good Laboratory Practices Regulations, as set forth in part 58, 21 CFR.

S.A. 2552

B-3