

CONFIDENTIAL

85/SEA047/509

SEGMENT II TERATOLOGY EVALUATION
OF SC-19129 IN RABBITS, S.A. NO. 2643

To:
G.D.Searle and Company
4901 Searle Parkway
Skokie
Illinois 60077
U S A

From:
J.M. Tesh
F.W. Ross
T.J. Wightman
O.K. Wilby
Life Science Research
Eye
Suffolk IP23 7PX
England
6 January 1986





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LSR Report No: 85/SEA047/509

We, the undersigned, hereby declare that the report following constitutes a true and faithful account of the procedures adopted, and the results obtained, in the performance of this study.

J.M. Tesh, B.Pharm., Ph.D., M.P.S., C.Biol., M.I.Biol.
(Director, Reproductive Studies)

.....*J.M. Tesh*.....
Date:8 Jan '86.....

F.W. Ross, B.Sc., C.Biol., M.I.Biol.
(Study Director)

.....*F.W. Ross*.....
Date:8 Jan '86.....

T.J. Wightman, B.Sc.
(Staff Reproductive Biologist)

.....*T.J. Wightman*.....
Date:8 Jan '86.....

O.K. Wilby, B.Sc., D.Phil., C.Biol., M.I.Biol.
(Staff Reproductive Biologist)

.....*O.K. Wilby*.....
Date:8 Jan '86.....

S.A. Tesh, B.Sc., C.Biol., M.I.Biol.
(Consultant Reproductive Biologist)

.....*Shaila A. Tesh*.....
Date:8 Jan '86.....





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LSR Report No : 85/SEA047/509
QUALITY ASSURANCE INSPECTIONS

DATES	DAY/MONTH/YEAR	
Inspection	Report to Study Director	Report to Management

PROTOCOL

Inspection of protocol was made in accordance with LSR Standard Operating Procedure QAU/020. Dates for inspection of protocol amendments in accordance with this S.O.P. are not quoted

22/4/85	22/4/85	22/4/85
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DATA

Inspection of data generated on this study was made in accordance with LSR Standard Operating Procedure QAU/050

6/6/85	7/6/85	7/6/85
23/9/85	23/9/85	23/9/85

PROCEDURES

Inspection of procedures on this type of study was made in accordance with LSR Standard Operating Procedure QAU/040

11/3/85	14/3/85
22/3/85	25/3/85
1/5/85	1/5/85
1/5/85	1/5/85
1/5/85	1/5/85
1/5/85	1/5/85
2/5/85	2/5/85

Other routine procedures used in this type of study, and facilities were inspected regularly and reports made in accordance with LSR Standard Operating Procedures QAU/040.

This report has been reviewed by the LSR Quality Assurance Unit employing methods laid down in LSR Standard Operating Procedure QAU/060. The reported methods and procedures were found to describe those used and the results to constitute an accurate representation of the data recorded.

This review was completed on: 7 January 1986

PP D.J. Ford, B.Sc., Ph.D.,
(Head of Quality Assurance Unit).

.....
.....7 January 1986.....



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1. SUMMARY

1.1 Procedures

SC-19129 was administered by gavage to pregnant New Zealand White rabbits during organogenesis from Day 6 to Day 19 of gestation inclusive, at dosages of 250, 500 or 750 mg/kg/day. A fourth group, serving as controls, received the vehicle 0.5% (w/v) methylcellulose and 0.1% (w/v) Tween 80 at the same volume-dosage during the same treatment period. On Day 29 of gestation, females were killed to allow examination of their uterine contents.

1.2 Results

- 1.2.1 Females receiving 750 mg/kg/day showed a reduction in food intake and faecal output and three females also exhibited a post-dosing increase in respiration rate on the first day of treatment only.
- 1.2.2 Five of fifteen females receiving 750 mg/kg/day died or were killed in extremis; two of five necropsied females showed respiratory problems and most had gastro-intestinal tract disorders.
- 1.2.3 There was no indication of any adverse treatment-related effect upon maternal bodyweight performance.
- 1.2.4 No adverse treatment-related effects upon litter parameters and foetal morphogenesis were observed.

2. INTRODUCTION

The aim of this investigation was to examine the effects of repeated oral administration of SC-19129 upon the progress and outcome of pregnancy in the rabbit.

The rabbit was selected because it meets the requirements of the regulatory authorities. The New Zealand White rabbit in particular was selected because of the background data available on this strain of rabbit in these laboratories.

SC-19129 was administered by gavage. Dose levels were based on information provided by the Sponsor.

The study commenced on 22 April 1985 at Life Science Research, Eye, Suffolk, England, and live animal work was completed on 23 May 1985. Laboratory studies were completed by 17 August 1985.

Original data pertaining to this study and the final report are stored in the archives of Life Science Research.

3. MATERIAL

Part of a 12 kg sample of SC-19129 (Lot No. 84K-047-101) received on 29 October 1984 was used throughout the investigation.

The material was a fine white powder and was stored at ambient temperature protected from light.

Unused material was returned to the Sponsor on 17 July 1985.

4. METHODS

4.1 Design conditions

4.1.1 Animals

Sexually mature virgin female New Zealand White rabbits from an accredited closed colony (Ranch Rabbits, Crawley Down, Sussex, England), were used in the investigation.

The animals were approximately 18-24 weeks old on arrival and were within the weight range 2.70 - 3.66 kg. Shortly after arrival, oestrus was synchronised by intravenous injection of 25 i.u. luteinising hormone (Pregnyl, Organon). The animals were allowed a minimum of three weeks acclimatisation during which time they were examined daily to check their physical condition. At commencement of the study they were in the bodyweight range of 3.45 - 5.02 kg.

4.1.2 Environmental control

The animals were housed in a limited access rabbit facility.

The rabbitry had its own supply of filtered air which was not re-circulated, providing approximately 17 to 20 room air changes per hour. The temperature and relative humidity in the rabbitry were recorded daily and the records retained. Mean recorded values were as follows:

		Mean	95% range
Temperature	min °C	17	+ 3
	max °C	19	+ 2
Relative humidity %		55	+ 8

A 14-hour light : 10-hour dark cycle operated throughout.

4.1.3 Water supply

Tap water from the local domestic mains was supplied to the cages via an automatic system. The East Anglian Water Company monitor the quality of water supplies at intervals and copies of their most recent analyses have been filed with the raw data.

4.1.4 Basal diet

A commercially-available certified laboratory animal diet, S.Q.C. Standard Rabbit Diet (813 181W; Special Diet Services Limited, Witham, Essex, England) was fed ad libitum throughout the study. The manufacturers supply a Certificate of Analysis with every batch, copies of which have been filed with the raw data.

4.1.5 Contaminants

No contaminants in either the diet or the water were reasonably expected to be present at levels known to be capable of interfering with the purpose or outcome of this study.

4.1.6 Cage type and number of rabbits per cage

Rabbits were housed singly in galvanised steel caging, (Cope and Cope Limited, Reading, Berkshire, England), and were randomly distributed in order to equalise, as far as possible, environmental influences amongst the groups.

The cages had mesh floors, and the trays beneath the cages were scraped and flushed down each day.

4.1.7 Insemination procedures

Females were artificially inseminated with pooled semen from New Zealand White bucks of established fertility. Following insemination, each female was injected intravenously with 25 i.u. of luteinising hormone (Pregnyl, Organon) to ensure successful ovulation. The day of insemination was designated Day 0 of gestation.

4.1.8 Treatment

Females were uniquely identified by ear-tags on arrival. They were randomly allocated to four treatment groups in order of insemination so that females inseminated on any one day were evenly distributed amongst the groups.

The four groups were treated as follows:

<u>Group</u>	<u>Treatment</u>	<u>Dose level</u> (mg/kg/day)	<u>Number per group</u>
1	Control	0	15
2	SC-19129	250	15
3	SC-19129	500	15
4	SC-19129	750	15

The test compound was formulated freshly each day in 0.5% (w/v) methylcellulose and 0.1% (w/v) Tween 80.

Animals were dosed daily by gavage from Day 6 to Day 19 inclusive of gestation at a volume-dosage of 4 ml/kg. Control animals received the vehicle at the same volume-dosage during the same treatment period. The volume administered daily to each animal was based on the animal's bodyweight on that day and was recorded.

4.1.9 Compound identity

The batch identification, information on the chemical identity, purity and stability in the vehicle of the experimental compound supplied for the study, and on the absorption of the compound from the gastro-intestinal tract, were the responsibility of the Sponsor.

Before commencement of treatment, a 10 g reserve sample of the experimental compound was taken and stored under the conditions specified for storage of the bulk supply of the experimental compound.

Samples of the test suspensions (all concentrations) were taken prior to commencement of the study for determination of homogeneity by the Sponsor and once during the period of dosing for analysis of test chemical concentration by the Sponsor. The remaining test article was returned to the Sponsor at the end of the study.

4.2 Serial observations

4.2.1 Maternal signs and bodyweight

All animals were weighed and examined daily throughout the study and any visible signs of reaction to treatment were recorded with details of type, severity, time of onset and duration.

4.2.2 Mortality

Animals killed in extremis or found dead were subjected to a thorough macroscopic examination of the visceral organs with the object of identifying the cause of their condition. Specimens of any abnormal tissues were retained.

4.2.3 Abortion

Animals that aborted were killed by intravenous injection of Pentobarbitone sodium on the same day that the abortion was detected. The females were subjected to a detailed macroscopic examination and the numbers of corpora lutea and implantation sites were recorded. Where possible the foetuses were examined.

4.3 Terminal studies

4.3.1 Litter responses

On Day 29 of gestation the females were killed by intravenous injection of Pentobarbitone sodium B.Vet.C. (Abbott Laboratories, Queenborough, Kent), for examination of their uterine contents. Each animal was first examined macroscopically for evidence of disease or adverse reaction to treatment and specimens of tissues considered abnormal were retained in an appropriate fixative. The reproductive tract, complete with ovaries, was dissected out and the following recorded:

- a) Number of corpora lutea in each ovary;
- b) Number of implantation sites. In apparently non-pregnant animals, presence of implantation sites was checked using a staining technique (Salewski, E.; Arch. Exp. Pathol. Pharmacol., 247, 367, 1964);
- c) Number of resorption sites (classified as early or late);
- d) Number and distribution of live and dead foetuses in each uterine horn.

4.3.2 External examination

The following were recorded:

- a) Weight of individual fetuses;
- b) Weight of individual placentae;
- c) External abnormalities of individual fetuses and placentae.

4.3.3 Internal examination

All fetuses were killed by subcutaneous injection of Pentobarbitone sodium. The neck and the thoracic and abdominal cavities of all fetuses from each litter were dissected, the contents examined and sex recorded. Following examination and evisceration, approximately one third of the fetuses from each litter were decapitated, and the heads fixed in Bouin's fluid for subsequent free-hand serial sectioning and examination. Decapitated fetuses and the remaining intact fetuses were placed in industrial methylated spirit (74 o.p.)

4.3.4 Skeletal examinations

The eviscerated fetuses were processed using a modification of the Dawson Alizarin staining technique (Tesh, J.M., Some effects of ageing in spermatozoa on fertility, Ph.D. Thesis, Faculty of Veterinary Science, University of Liverpool, 1968), and the skeletons were examined.

4.4 Treatment of data

Data were expressed as means with standard deviations (S.D.) calculated according to the formula:

$$S.D. = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

unless otherwise indicated.

4.4.1 Maternal bodyweight

Group mean values (\pm S.D.) were calculated on Days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 28 of gestation. Weight changes were plotted graphically with respect to Day 6 of gestation.

4.4.2 Litter responses

- 4.4.2.1 Group mean values (\pm S.D.) were calculated from numbers of corpora lutea, implantations, resorptions (early, late and total) and live young, (male, female and total) at Day 29 of gestation.

Resorptions approximate to a Poisson distribution and their standard deviations were calculated as:

$$\sqrt{\bar{x}}$$

- 4.4.2.2 Pre-natal losses were considered separately for the pre- and post-implantation phases.

a) Pre-implantation loss

Pre-implantation loss included losses due to non-fertilisation of ova, and early post-implantation deaths (i.e. those occurring up to Days 10-11 of gestation), in addition to true pre-implantation loss. It was calculated from the formula:

$$\frac{\text{No. corpora lutea} - \text{No. implantations}}{\text{No. corpora lutea}} \times 100$$

b) Post-implantation loss

Post-implantation loss covered only the period between Days 10 and 29 of gestation; it did not include the first 3-4 days post-implantation as any deaths that occurred in this phase would leave no remains visible at Day 29. It was calculated from the formula:

$$\frac{\text{No implantations} - \text{No. live fetuses}}{\text{No. implantations}} \times 100$$

- c) Group values for pre- and post-implantation losses were calculated as a mean of the individual litter values.

4.4.2.3 Group mean foetal and placental weights (\pm S.D.) were calculated for each group as:

$$\frac{\text{Total of individual litter mean foetal/placental weights}}{\text{Number of litters}}$$

4.4.3 Foetal observations

Group values for foetal observations at necropsy, skeletal evaluation or following free-hand serial sectioning of foetal heads were calculated as:

$$\frac{\text{No. fetuses/heads with a particular observation}}{\text{No. fetuses/heads examined}} \times 100$$

The number of litters in which a particular observation occurred has also been presented for each group.

4.4.4 Statistical evaluation

Visual appraisal of intergroup differences revealed no evidence of any treatment attributable effects/trends and were consequently not considered to warrant further analysis through the use of statistical tests.

5. RESULTS

5.1 Maternal observations

5.1.1 General condition and mortality (Tables 1 and 2; Appendix 1)

An increased incidence of females in Group 4 (750 mg/kg/day) showed reduced food intake and faecal output, in addition, three Group 4 females showed an isolated occurrence of increased respiration rate on Day 6 of gestation. In all other respects, the general condition of the treated females was essentially similar to that of the controls.

A total of six females died or were killed in extremis, one in Group 3 (500 mg/kg/day) and five in Group 4 (750 mg/kg/day). Necropsy revealed respiratory disorder in the one Group 3 animal (500 mg/kg/day) and in two of the five Group 4 animals (750 mg/kg/day). In addition, gastro-intestinal tract disorders were observed in most of the necropsied females.

5.1.2 Maternal bodyweight (Figure 1; Table 3; Appendix 3)

Marked inter- and intra-group variations in maternal bodyweight gain were recorded in all groups, however, there were no indications of any effects that could be attributed to treatment.

5.1.3 Necropsy findings (maternal)

At termination on Day 29, no macroscopic changes in maternal condition were recorded that could be attributed to treatment with SC-19129.

5.1.4 Abortion (Appendix 2)

One female in each of Groups 2 (250 mg/kg/day), 3 (500 mg/kg/day) and 4 (750 mg/kg/day) aborted and in each case the event was preceded by a period of weightloss. In view of the isolated nature of these abortions and their occurrence within the previously recorded background control range, involvement of the test compound was considered unlikely.

All the other females carried their young successfully to term.

5.2 Litter responses (Table 4; Appendix 4)

Litter responses as assessed by the numbers of implantations and viable young, the extent of pre- and post-implantation losses, and foetal and placental weights showed some inter-group variation, but no treatment-related changes were apparent.

5.3 Foetal examination (Tables 5-7; Appendices 4-6)

Examination of foetuses at necropsy, following skeletal examination and examination of foetal heads subsequent to free-hand serial sectioning, revealed a number of observations, the majority of which were of types and incidences previously recorded in this strain of rabbit in these laboratories.

In one Group 4 litter (750 mg/kg/day), all foetuses were small and exhibited a degree of skeletal development typical of immaturity. Similar changes were not seen in foetuses from other litters in this group.

Bodyweight change (kg) of females during gestation

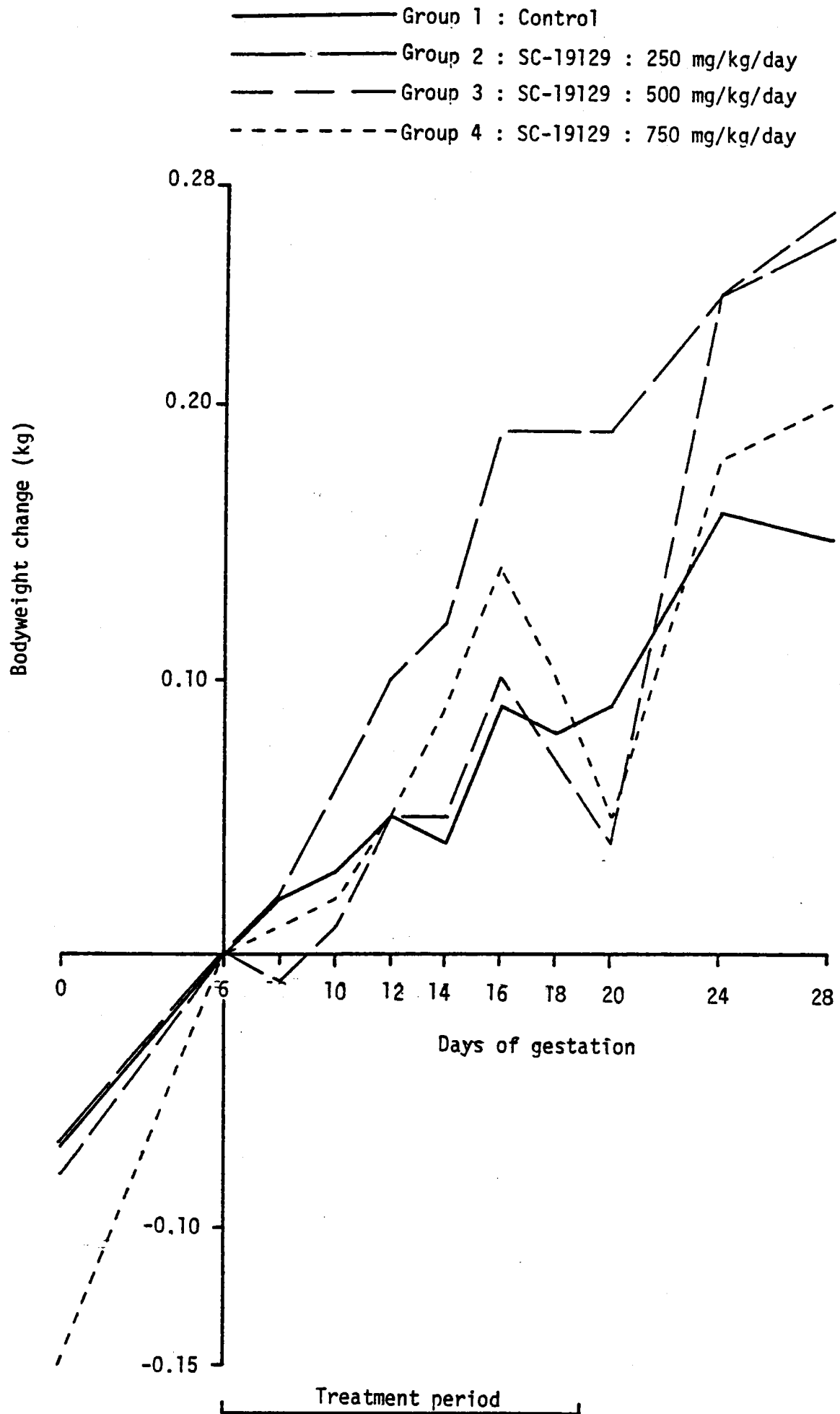


TABLE 1

Disposition of animals

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4
Number inseminated	15	15	15	15
Mortality	-	-	1	5
Not pregnant	1	1	1	-
Abortion	-	1	1	1
Pregnant to term with viable young	14	13	12	9

TABLE 2

Summary of clinical signs observed in females from commencement of treatment - incidence expressed as a percentage of "animal days"

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4
Number of females ⁺	14	14	14	14
<u>Clinical signs: % incidence</u>				
Little diet eaten	14.0	7.5	10.7	21.9
Few faeces in undertray	12.7	4.4	10.7	21.5

+ Excludes non-pregnant animals.

Clinical signs observed prior to Day 7 of gestation are excluded.

TABLE 3

Group mean bodyweights (kg) of females during gestation

Group : 1 2 3 4
 Compound : Control --- SC-19129 ---
 Dosage (mg/kg/day) : 0 250 500 750

Group	Number of pregnant animals	Day of gestation											
		0	6	8	10	12	14	16	18	20	24	28	
1	14	Mean S.D.	4.18 0.32	4.27 0.32	4.28 0.33	4.30 0.33	4.29 0.34	4.34 0.35	4.33 0.38	4.34 0.39	4.41 0.39	4.40 0.41	
2	13	Mean S.D.	4.35 0.44	4.44 0.43	4.48 0.45	4.52 0.47	4.54 0.44	4.61 0.44	4.61 0.44	4.61 0.46	4.66 0.45	4.68 0.47	
3	12	Mean S.D.	4.17 0.42	4.24 0.42	4.26 0.46	4.30 0.47	4.30 0.43	4.35 0.47	4.32 0.48	4.29 0.49	4.49 0.49	4.52 0.50	
4	9	Mean S.D.	4.39 0.33	4.54 0.35	4.55 0.36	4.56 0.39	4.63 0.41	4.68 0.41	4.64 0.42	4.59 0.45	4.72 0.44	4.74 0.43	

S.D. Standard deviation.

TABLE 4

Group mean litter data - females killed on Day 29 of gestation

Group : 1 2 3 4
 Compound : Control --- SC-19129 ---
 Dosage (mg/kg/day) : 0 250 500 750

Group	Number of pregnant animals	% Abortion	Corpora lutea count	Implan- tations	Viable young			Resorptions			Implantation loss (%)		Foetal weight (g)	Placenta weight (g)
					M	F	Total	Early	Late	Total	Pre-	Post-		
1	14	Mean S.D.	12.7 2.6	10.7 3.0	4.2 2.2	4.9 2.1	9.1 2.4	0.5 0.7	1.1 1.0	1.6 1.3	15.9	13.6	36.1 7.2	5.3 1.1
2	13 ⁺	Mean S.D.	12.2 3.9	8.9 4.0	4.3 2.1	3.8 2.3	8.2 3.4	0.4 0.6	0.4 0.6	0.8 0.9	23.8	6.7	40.2 6.0	6.1 2.0
3	12 ^Δ	Mean S.D.	11.2 2.7	8.5 3.1	3.3 2.5	3.8 1.6	7.2 3.2	0.7 0.8	0.7 0.8	1.3 1.1	22.8	18.0	41.1 5.2	5.9 1.3
4	9 ^Ω	Mean S.D.	12.3 2.5	11.0 2.6	4.9 2.1	5.0 2.2	9.9 2.1	0.3 0.5	0.8 0.9	1.1 1.1	10.7	9.3	37.2 7.3	5.3 1.2
Background data	Overall mean		11.1	9.0	4.0	4.0	8.0	0.4	0.6	0.9	18.8	10.5	41.5	5.9
from 87 studies	Recorded ranges		9.3-13.5	6.5-11.0	2.3-5.9	2.5-5.5	5.5-9.8	0.0-1.1	0.0-1.4	0.1-1.7	4.7-35.7	1.0-20.5	37.0-46.9	5.0-7.2

+ Excludes Female 16TU 417 aborted on Day 25 after insemination.

Δ Excludes Female 16TU 403 aborted on Day 27 after insemination.

Ω Excludes Female 16TU 439 aborted on Day 21 after insemination.

TABLE 5

Summary of foetal observations at necropsy

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4	Control data
Number of foetuses (litters):	127 (14)	106 (13)	86 (12)	89 (9)	from 87 studies
Observations:% incidence ^σ (number of litters)					Mean Study range
Abnormal foetus I (See page 33)	0.8 (1)	-	-	-	0.20 0.0 - 2.0
Abnormal foetus II (See page 33)	-	0.9 (1)	-	-	0.05 0.0 - 1.3
Depression in skull	-	-	-	1.1 (1)	0.02 0.0 - 1.1
Bilateral forelimb flexure	0.8 (1)	-	-	-	0.24 0.0 - 3.3
Eyes contain small opaque discs	-	-	-	1.1 (1)	0.01 0.0 - 1.1
Punctate dark area on midline of palate	-	1.9 (1)	-	-	0.06 0.0 - 3.0
Left thyroid gland dark and enlarged	0.8 (1)	-	-	-	0.09 0.0 - 1.4
Thymus gland haemorrhagic	-	0.9 (1)	2.3 (1)	-	0.25 0.0 - 2.5
Agnesis of median lung lobe	0.8 (1)	-	1.2 (1)	-	0.15 0.0 - 2.0
Haemorrhage on wall of aorta and pulmonary artery	0.8 (1)	-	-	-	π
Free/clotted red serous fluid/blood in abdominal cavity	1.6 (2)	-	1.2 (1)	2.2 (2)	0.23 0.0 - 3.7
Free clear fluid in abdominal cavity	-	0.9 (1)	1.2 (1)	-	0.09 0.0 - 6.0
Stomach contains gas/distended with gas	1.6 (2)	1.9 (1)	3.5 (3)	7.9 (3)	3.41 0.0 - 12.5
Stomach contents dark	-	0.9 (1)	-	-	0.03 0.0 - 1.9
Pale area(s) on liver	1.6 (1)	-	-	-	1.17 0.0 - 5.6

σ One foetus may have more than one observation.

π No previous record in background control data.

TABLE 5 - continued

Summary of foetal observations at necropsy

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4	Control data
Number of foetuses (litters):	127 (14)	106 (13)	86 (12)	89 (9)	from 87 studies
Observations: % incidence ^σ (number of litters)					Mean Study range
Liver congested and thickened					π
Gall bladder variants	18.1 (11)		1.2 (1)		19.19 1.0 - 42.7
Raised haemorrhagic area on kidney	0.8 (1)	8.5 (5)	11.6 (5)	10.1 (5)	0.01 0.0 - 0.9
Unilateral hydronephrosis	0.8 (1)	-	-	-	0.03 0.0 - 3.6
Bilateral hydronephrosis	3.1 (1)	-	-	1.1 (1)	0.07 0.0 - 1.2
Unilateral hydroureter	0.8 (1)	-	-	-	0.03 0.0 - 2.0
Ovaries congested ^φ			2.2 (1)		0.02 0.0 - 2.2
Congested blood supply to one or both testes ^φ	1.7 (1)	7.1 (1)	2.5 (1)	4.5 (1)	π
Fluid filled vesicle adjacent to ovary ^φ		4.0 (1)	-	-	0.07 0.0 - 4.3
Small foetus (less than 32.0 g)	32.3 (9)	17.0 (7)	11.6 (6)	16.9 (3)	13.4 0.0 - 30.4
Amniotic fluid dark in colour		4.7 (1)	-	-	0.31 0.0 - 6.7
Pale area on placenta	0.8 (1)	-	-	-	1.17 0.0 - 16.3
Pale placenta	0.8 (1)	3.8 (2)	-	-	1.17 0.0 - 16.3

σ One foetus may have more than one observation.

φ Expressed as a percentage of male or female foetuses.

π No previous record in background control data.

TABLE 6

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4	Control data	
Number of foetuses (litters) examined:	90 (14)	75 (13)	60 (12)	61 (9)	8407 foetuses	86 studies
Observations: % incidence ^σ (number of litters)					Mean	Study ranges
Head						
Size of anterior fontanelle	23.3 (9) 74.4 (14) 2.2 (1)	25.3 (9) 74.7 (13)	30.0 (7) 70.0 (11)	21.3 (6) 65.6 (8) 13.1 (1)	23.39 72.84 3.50	4.4 - 57.3 38.5 - 93.8 0.0 - 21.3
Incomplete ossification of supraoccipital bone	8.9 (6)	4.0 (3)	8.3 (5)	6.6 (3)	4.28	0.0 - 13.0
Incomplete ossification of interparietal bone	1.1 (1)	2.7 (2)	1.7 (1)	-	2.32	0.0 - 9.5
Interparietal bone reduced in size or absent	7.8 (5)	5.3 (3)	-	-	2.16	0.0 - 17.0
Small discrete unossified areas in parietal bones	-	2.7 (1)	-	-	0.25	0.0 - 3.1
Large unossified areas in parietal bones	-	-	-	4.9 (1)	π	0.0 - 5.8
Small discrete unossified areas in frontal bones	1.1 (1)	-	-	-	0.24	0.0 - 10.2
Small additional suture in cranial bones	1.1 (1)	2.7 (2)	-	1.6 (1)	3.13	0.0 - 46.5
Irregular ossification of frontal suture	10.0 (7)	10.7 (7)	8.3 (4)	3.3 (2)	12.54	0.0 - 1.4
Frontal suture enlarged at frontal/nasal junction	1.1 (1)	1.3 (1)	-	3.3 (1)	0.01	0.0 - 1.1
Lacrimal fossa enlarged	1.1 (1)	-	-	-	0.06	0.0 - 1.1

σ One foetus may have more than one observation.
π No previous record in background control data.

TABLE 6 - continued

Summary of foetal observations at skeletal examination

Group : 1 2 3 4
 Compound : Control --- SC-19129 ---
 Dosage (mg/kg/day) : 0 250 500 750

Group	1	2	3	4	Control data	
Number of foetuses (litters) examined:	90 (14)	75 (13)	60 (12)	61 (9)	8407 foetuses	86 studies
<u>Observations: % incidence^σ (number of litters)</u>						
Head - continued					Mean	Study ranges
Posterior fontanelle enlarged	12.2 (6)	9.3 (4)	1.7 (1)	9.8 (1)	1.95	0.0 - 22.8
Anterior fontanelle negligible, size of suture lines only	-	-	3.3 (2)	-	0.24	0.0 - 4.2
Anterior fontanelle extended anteriorly into frontal suture	1.1 (1)	2.7 (2)	-	-	0.05	0.0 - 2.0
Anterior fontanelle extended laterally into frontal bones	-	1.3 (1)	-	-	π	π
Additional fissures from anterior fontanelle into frontal bones	1.1 (1)	-	-	-	π	π
Incomplete ossification or absence of hyoid body	30.0 (10)	25.3 (9)	26.7 (9)	42.6 (8)	16.49	0.0 - 48.0
Cornua of hyoid bent inwards arch flattened	1.1 (1)	1.3 (1)	1.7 (1)	1.6 (1)	0.38	0.0 - 4.2
One cornua of hyoid bent outwards	-	2.7 (2)	-	-	1.47	0.0 - 12.7

^σ One foetus may have more than one observation.

π No previous record in background control data.

TABLE 6 - continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4	Control data	
Number of foetuses (litters) examined:	127 (14)	106 (13)	86 (12)	89 (9)	8655 foetuses	86 studies
Observations: % incidence ^σ (number of litters)						
Rib cage and vertebral column:						
Number of ribs	52.0 (14) 13.4 (10) 34.6 (11) 1.6 (2) 0.8 (1)	55.7 (12) 17.9 (9) 26.4 (11) 6.6 (5) 0.9 (1)	60.5 (11) 14.0 (6) 25.6 (9) 5.8 (4) -	59.6 (8) 4.5 (4) 36.0 (6) 5.6 (3) -	51.82 12.14 35.91 2.43 0.10 0.06	28.6 - 81.0 6.0 - 20.6 11.9 - 61.0 0.0 - 12.8 0.0 - 1.8 0.0 - 1.7
One or more ribs thickened at costal cartilage						
Additional (cervical) ribs on 7th cervical vertebra						
Right 5th and 6th ribs fused at costal cartilage						
Incomplete ossification of sternbrae:						
Number of bones affected	46.5 (14) 12.6 (7) 0.8 (1) 0.8 (1) 2.4 (3) 1.6 (2) 0.8 (1)	41.5 (12) 5.7 (3) 1.9 (1) - 1.9 (2) - 0.9 (1)	62.8 (12) 7.0 (4) - - - - -	52.8 (9) 9.0 (4) 2.2 (1) 2.2 (1) - - 2.2 (2)	57.38 9.79 0.96 0.15 0.65 0.61 0.44	20.4 - 79.2 1.4 - 26.8 0.0 - 8.5 0.0 - 2.4 0.0 - 3.3 0.0 - 5.3 0.0 - 6.3
One or more sternbrae offset						
Two or more sternbrae fused						
Small additional sternbral bone between 5th sternbra and xiphisternum						
Xiphisternum bifurcated						
	-	-	1.2 (1)	-	0.19	0.0 - 3.5

σ One foetus may have more than one observation.

TABLE 6 - continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4	Control data		
Number of foetuses (litters) examined:	127 (14)	106 (13)	86 (12)	89 (9)	8655 foetuses	86 studies	
Observations: % incidence ^σ (number of litters)					Mean	Study ranges	
Rib cage and vertebral column - continued							
Number of pre-sacral vertebrae (26)	75.6 (14)	80.2 (12)	84.9 (11)	74.2 (9)	80.80	55.7 - 92.8	
Incomplete ossification of one or more cervical vertebral centra ^z (27)	24.4 (12) 2.2 (1)	19.8 (10) 4.0 (2)	15.1 (8) 3.3 (2)	25.8 (6) 11.5 (2)	18.12 2.11	7.2 - 44.3 0.0 - 9.9	
Incomplete ossification of one or more sacral vertebrae, less than 16 ossified	-	-	-	1.1 (1)	-	-	
Incomplete ossification of one or more thoracic vertebral centra	0.8 (1)	-	2.3 (2)	2.2 (1)	0.75	0.0 - 11.5	
Incomplete ossification of one or more thoracic vertebral arches	-	-	-	1.1 (1)	0.02	0.0 - 0.9	
Incomplete or asymmetric ossification of costal elements of sacral vertebrae	4.7 (4)	8.5 (6)	3.5 (2)	4.5 (3)	4.58	0.0 - 15.2	
Anomalous vertebrae:- right hemicentrum and hemivertebral arch of 3rd lumbar vertebra absent, left hemicentrum fused to centrum of 2nd lumbar vertebra, left hemivertebral arch of 2nd lumbar vertebra displaced caudally, enlarged; left scoliosis	-	-	1.2 (1)	-	0.13	0.0 - 2.7	

^z Expressed as a percentage of intact foetuses.
^σ One foetus may have more than one observation.

TABLE 6 - continued

Summary of foetal observations at skeletal examination

Group : 1 2 3 4
 Compound : Control --- SC-19129 ---
 Dosage (mg/kg/day) : 0 250 500 750

Group	1	2	3	4	Control data	
Number of foetuses (litters) examined:	127 (14)	106 (13)	86 (12)	89 (9)	8655 foetuses	86 studies
Observations: % incidence ^σ (number of litters)						
<u>Ribcage and vertebral column - continued</u>						
Anomalous foetus:- spina bifida; hemivertebral arches of 6th and 7th lumbar vertebrae reduced in size, spacing between hemivertebral arches of 5th lumbar and 1st sacral vertebrae increased, open pore through to spinal canal at 6th and 7th lumbar vertebra	-	0.9 (1)	-	-	0.02	0.0 - 1.0
<u>Limbs, pectoral and pelvic girdles</u>						
Number with heads of long bones unossified	78.0 (13)	70.8 (12)	62.8 (11)	75.3 (9)	36.18	1.9 - 66.3
One or both olecranon processes ossified	0.8 (1)	-	1.2 (1)	1.1 (1)	2.53	0.0 - 11.7
Incomplete ossification or absence of one or both centrals	3.9 (2)	0.9 (1)	1.2 (1)	10.1 (1)	0.81	0.0 - 3.2
Incomplete ossification of metacarpals and/or phalanges	22.8 (8)	13.2 (8)	27.9 (7)	25.8 (6)	11.91	1.9 - 53.2
Abnormal forelimb flexure	1.6 (2)	-	-	3.4 (3)	0.31	0.0 - 3.3
Incomplete ossification of astragalus	0.8 (1)	-	-	-	π	π
Asymmetric pelvis, ilia associated with different sacral vertebrae	3.1 (4)	5.7 (5)	3.5 (2)	2.2 (2)	3.54	0.0 - 8.9
Double association pelvis, ilia associated with both sacral vertebrae	2.4 (3)	1.9 (2)	-	-	1.04	0.0 - 7.8
Incomplete ossification or absence of pubic bones	7.1 (4)	1.9 (2)	2.3 (1)	12.4 (1)	0.39	0.0 - 4.0

^σ One foetus may have more than one observation.

π No previous record in background control data.

TABLE 7

Summary of observations following freehand serial sectioning of foetal heads

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	1	2	3	4
Number of heads (litters) examined*	37 (14)	31 (12)	26 (11)	28 (9)
Observations: % foetal incidence (number of litters) affected				
Upper and lower incisors erupted	81.1 (14)	93.5 (12)	80.8 (11)	67.9 (7)
Lower incisors erupted	13.5 (5)	6.5 (2)	11.5 (3)	25.0 (4)
Incisors not erupted	5.4 (2)	-	7.7 (2)	7.1 (1)
Unilateral dilated orbital sinus	2.7 (1)	-	-	-
Bilateral dilated orbital sinus	-	3.2 (1)	11.5 (3)	-
Unilateral folded retina	5.4 (2)	3.2 (1)	7.7 (2)	3.6 (1)
Bilateral folded retina	5.4 (2)	-	3.8 (1)	-
Blood in nasal sinuses	2.7 (1)	-	3.8 (1)	3.6 (1)
Blood in nasopharynx	2.7 (1)	3.2 (1)	7.7 (2)	7.1 (2)
Blood in cochlea(s)	18.9 (5)	12.9 (4)	19.2 (4)	25.0 (4)
Cystic dilatation of the brain	10.8 (4)	12.9 (4)	23.1 (6)	3.6 (1)
Crystalline deposits	35.1 (10)	41.9 (9)	42.3 (9)	28.6 (6)
Dent in right parietal region of head	-	-	-	3.6 (1)

* One foetus may have more than one observation.

APPENDIX 1

Summary of mortality

Group : 1 2 3 4
Compound : Control --- SC-19129 ---
Dosage (mg/kg/day) : 0 250 500 750

Group	Animal number	Day of death (after insemination)	History and circumstances of death	Summary of necropsy findings
3	16TU 473	27	KIE Fur matted on nares and forelimbs and around left eye. Nasal discharge. Laboured breathing. Eyes dark. Reduced food intake and faecal output. Weightloss.	Approximately 20 ml free fluid in thoracic cavity. Pericardium thickened. Cranial, cardiac and caudal lung lobes consolidated and covered with pale purulent material. Cut surface reveals same. Left lung lobe dark and congested. Stomach gas distended. Gall bladder enlarged (x 2). Yellow/green mucoid material in small intestine. Caecal contents fluid. No faecal pellet formation. Pregnant.
4	16TU 374	8	FDC Fur matted on nares and forelimbs. Respiratory noise. Laboured breathing. Reduced food intake and faecal output. Weightloss.	Approximately 2/3 of left lung lobe dark and consolidated, caudal pole necrotic. Stomach contents fluid. Small intestine contents fluid. No faecal pellet formation.

KIE Killed in extremis.
FDC Found dead in cage.

APPENDIX 1 - continued

Summary of mortality

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	Animal number	Day of death (after insemination)	History and circumstances of death	Summary of necropsy findings
4	16TU 409	17	FDC Fur matted and stained red on forelimbs and nares. Inactive. Reduced food intake and faecal output. Weightloss. Thin.	Animal autolysing. Stomach contains fur. Numerous ulcerations on stomach wall. Small intestine generally devoid of content. Caecal contents fluid. Caecal wall slightly reddened. Pregnant.
4	16TU 426	27	FDC Fur matted on nares and forelimbs. Furlloss from head. Tail and perianal region soiled. Gelatinous discharge from anus. Reduced food intake and faecal output. Inactive Weightloss.	All lung lobes dark and congested. Gall bladder enlarged (x 3) contents solid. Cut surface reveals firm dark material. Stomach and intestines distended with gas (x 2). Contents fluid. Pregnant. All implantations resorbing.

FDC Found dead in cage.

APPENDIX 1 - continued

Summary of mortality

Group : 1 2 3 4
 Compound : Control --- SC-19129 ---
 Dosage (mg/kg/day) : 0 250 500 750

Group	Animal number	Day of death (after insemination)	History and circumstances of death	Summary of necropsy findings
4	16TU 529	15	KIE Fur matted on nares and around eyes. Nasal discharge. Fur loss and scabs on neck. Mass of dried faeces around anus. Unsteady on feet. Reduced food intake and faecal output. Thin. Weightloss.	Very little food material in stomach. Small and large intestines almost devoid of contents. Caecum gas distended. No faecal pellet formation in rectum. Pregnant. All implantations resorbing.
4	16TU 546	24	KIE Fur matted on nares and forelimbs. Nasal discharge. Laboured breathing. Eyes dark. Reduced food intake and faecal output. Weightloss.	All lung tissue congested. Cranial portion of left lung lobe partially consolidated. Cardiac lobe consolidated. Cut surface of consolidated tissue reveals pale coloured purulent material. Stomach contains gas. Contents reduced. Caecal contents reduced. Area of haemorrhage in fat (approximately 2 x 3 cms) surrounding right kidney. Pregnant. All implantations resorbing.

KIE Killed in extremis.

APPENDIX 2

Abortion

Group : 1 2 3 4
Compound : Control --- SC-19129 ---
Dosage (mg/kg/day) : 0 250 500 750

Group	Animal number	Abortion (Day after insemination)	Corpora lutea	Implantations	Summary of necropsy findings
2	16TU 417	25	10	10	Approximately 25 ml free clear fluid in thoracic cavity. Left lung lobe congested. Liver pale Gall bladder enlarged (x 2). Small intestine devoid of food material but containing mucus. Caecum contains alternating areas of fluid and hardened material. No faecal pellets in rectum.
3	16TU 403	27	8	8	Approximately 10 ml pale serous fluid in thoracic cavity. Approximately 33% of left and 20% of median lung lobe congested and consolidated. Pale consolidated areas throughout approximately 75% of caudal lung lobe. Cut surface reveals same. Liver dark and consolidated with pale areas.
4	16TU 439	21	12	8	NAD All implantations resorbing.

NAD No abnormality detected.

APPENDIX 3

Individual bodyweights (kg) of females after insemination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	Animal number	Days after insemination										
		0	6	8	10	12	14	16	18	20	24	28
1	16TU 109	3.72	3.73	3.73	3.72	3.74	3.79	3.83	3.85	3.89	3.98	4.05
	16TU 411	4.43	4.37	4.38	4.33	4.27	4.13	4.08	4.02	4.02	3.87	3.67
	16TU 429	4.54	4.74	4.80	4.83	4.90	4.96	5.01	5.02	5.07	5.20	5.03
	16TU 430	4.52	4.47	4.54	4.55	4.62	4.56	4.78	4.82	4.88	4.78	4.94
	16TU 455	4.25	4.38	4.40	4.42	4.40	4.49	4.51	4.46	4.49	4.60	4.68
	16TU 472	3.86	4.04	4.02	4.06	4.10	4.04	4.14	4.12	4.05	4.26	4.32
	16TU 475	4.10	4.20	4.18	4.20	4.24	4.11	4.32	4.30	4.26	4.27	4.28
	16TU 492	4.02	4.16	4.20	4.22	4.23	4.25	4.33	4.36	4.31	4.36	4.42
	16TU 521	3.97	3.96	3.98	3.98	4.02	4.11	4.07	4.12	4.10	4.36	4.23
	16TU 531	4.20	4.26	4.23	4.21	4.25	4.24	4.35	4.34	4.43	4.55	4.60
	16TU 533	3.84	4.07	4.10	4.11	4.12	4.18	4.15	4.16	4.19	4.20	4.22
	16TU 554	4.91	4.88	4.95	4.95	4.96	5.00	4.93	5.03	5.04	5.03	5.00
	16TU 556	4.10	4.10	4.14	4.15	4.20	4.17	4.07	3.97	3.98	4.12	3.97
	16TU 557	4.09	4.12	4.10	4.13	4.15	4.08	4.14	4.11	4.10	4.09	4.12
	16TU 376 ^D	4.32	4.62	4.69	4.72	4.74	4.70	4.76	4.83	4.75	4.79	4.81

D Not pregnant - excluded from group mean values.

APPENDIX 3 - continued

Individual bodyweights (kg) of females after insemination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	Animal number	Days after insemination										
		0	6	8	10	12	14	16	18	20	24	28
2	16TU 379	4.31	4.45	4.47	4.50	4.56	4.53	4.68	4.65	4.64	4.73	4.76
	16TU 389	4.63	4.53	4.71	4.73	4.75	4.87	4.89	4.97	5.08	5.08	5.04
	16TU 407	3.62	3.66	3.69	3.71	3.72	3.76	3.76	3.78	3.77	3.84	3.93
	16TU 419	3.86	4.02	4.04	4.09	4.12	4.08	4.16	4.12	4.12	4.22	4.24
	16TU 456	4.89	5.12	5.14	5.22	5.24	5.07	5.12	5.22	5.22	5.35	5.43
	16TU 465	5.02	4.86	4.71	4.78	4.88	4.90	4.93	4.97	5.01	5.08	5.18
	16TU 519	4.16	4.06	4.04	3.98	3.99	4.17	4.22	4.26	4.23	4.22	4.24
	16TU 536	4.67	4.82	4.78	4.81	4.89	4.93	4.98	4.99	4.99	4.86	4.94
	16TU 537	4.08	4.26	4.26	4.27	4.38	4.29	4.52	4.44	4.42	4.49	4.39
	16TU 541	4.88	4.98	5.06	5.09	5.16	5.18	5.21	5.14	5.16	5.17	5.24
	16TU 545	4.45	4.44	4.60	4.70	4.73	4.76	4.86	4.78	4.75	4.75	4.77
	16TU 551	3.93	4.10	4.10	4.11	4.12	4.20	4.28	4.29	4.26	4.36	4.29
	16TU 558	4.08	4.16	4.18	4.22	4.22	4.26	4.27	4.30	4.31	4.39	4.40
	16TU 417 ^A	4.25	4.34	4.32	4.30	4.28	4.24	4.40	4.38	4.36	4.07	
	16TU 542 ^D	4.04	4.12	4.20	4.19	4.20	4.13	4.17	4.19	4.20	4.25	4.30

All annotated animals excluded from group mean values.

A Aborted Day 25 after insemination.

D Not pregnant.

APPENDIX 3 - continued

Individual bodyweights (kg) of females after insemination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	Animal number	Days after insemination											
		0	6	8	10	12	14	16	18	20	24	28	
3	16TU 340	4.72	4.79	4.68	4.84	4.80	4.84	4.97	4.88	4.71	4.93	4.98	
	16TU 412	3.80	3.87	3.86	3.73	3.74	3.85	3.95	3.93	3.97	4.00	4.04	
	16TU 436	4.71	4.74	4.73	4.77	4.80	4.75	4.90	4.82	4.84	4.90	4.90	
	16TU 497	3.98	3.94	3.90	3.88	3.92	3.94	3.95	4.03	4.00	4.04	3.97	
	16TU 512	3.68	3.89	3.86	3.82	3.92	4.08	4.09	4.06	4.15	4.24	4.36	
	16TU 514	3.77	3.88	3.86	3.84	3.86	3.90	3.91	3.88	3.84	3.98	3.94	
	16TU 526	4.24	4.18	4.27	4.36	4.42	4.32	4.14	4.03	3.90	4.34	4.52	
	16TU 530	4.33	4.36	4.39	4.40	4.42	4.44	4.32	4.15	3.98	4.79	4.64	
	16TU 532	4.60	4.58	4.46	4.53	4.56	4.53	4.66	4.70	4.74	4.86	4.99	
	16TU 543	4.40	4.64	4.68	4.71	4.80	4.78	4.84	4.86	4.88	5.06	5.07	
	16TU 555	3.45	3.52	3.51	3.57	3.55	3.54	3.61	3.54	3.54	3.68	3.74	
	16TU 567	4.34	4.58	4.64	4.65	4.75	4.59	4.84	4.92	4.92	5.00	5.08	
	16TU 403 ^B	4.20	4.26	4.24	4.24	4.30	4.25	4.20	4.20	4.09	3.77		
	16TU 539 ^D	3.90	3.83	3.82	3.81	3.80	3.66	3.84	3.90	3.93	3.98	4.03	
	16TU 473 ^E	4.23	4.32	4.30	4.32	4.34	4.32	4.35	4.42	4.45	4.28		

All annotated animals excluded from group mean values.

B Aborted Day 27 after insemination.

D Not pregnant.

E Killed in extremis Day 27 after insemination.

APPENDIX 3 - continued

Individual bodyweights (kg) of females after insemination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Group	Animal number	Days after insemination										
		0	6	8	10	12	14	16	18	20	24	28
4	16TU 393	4.34	4.40	4.38	4.43	4.41	4.44	4.44	4.44	4.46	4.51	4.54
	16TU 398	4.66	4.96	4.98	5.05	5.04	5.08	5.15	5.12	5.14	5.22	5.18
	16TU 432	3.93	4.06	4.05	4.00	4.07	4.07	4.14	4.01	3.85	4.10	4.25
	16TU 442	4.14	4.19	4.22	4.15	4.08	4.17	4.27	4.25	4.25	4.30	4.42
	16TU 444	3.92	4.16	4.18	4.20	4.27	4.27	4.32	4.31	4.17	4.33	4.36
	16TU 535	4.64	4.82	4.83	4.87	4.90	4.95	4.86	4.77	4.66	4.70	4.50
	16TU 549	4.60	4.74	4.73	4.78	4.84	4.92	5.00	5.03	5.10	5.27	5.30
	16TU 552	4.44	4.54	4.52	4.51	4.57	4.60	4.63	4.62	4.66	4.82	4.79
	16TU 559	4.82	4.98	5.02	5.03	5.12	5.16	5.27	5.17	5.04	5.20	5.36
	16TU 439 ^C	3.94	4.05	4.02	3.98	3.82	3.61	3.54	3.53	3.34		
	16TU 374 ^F	3.82	4.00	3.76								
	16TU 409 ^G	4.16	4.27	4.30	4.23	3.95	3.76	3.62				
	16TU 426 ^H	4.80	4.95	4.98	4.98	5.04	4.91	5.00	5.04	4.72	4.49	
	16TU 529 ^I	4.26	4.32	4.19	3.95	3.75	3.57					
	16TU 546 ^J	4.45	4.40	4.44	4.43	4.50	4.39	4.20	4.04	3.92	3.93	

All annotated animals excluded from group mean values.

C Aborted Day 21 after insemination.

F Died Day 8 after insemination.

G Died Day 17 after insemination.

H Died Day 27 after insemination.

I Killed in extremis Day 15 after insemination.

J Killed in extremis Day 24 after insemination.

APPENDIX 4

Individual litter data - females killed on Day 29 of gestation

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Key to observations

- a) Abnormal foetus I. Opaque ring around perimeter of eyes. Punctate aperture in centre of left eyelid. Heart and major vessels enlarged.
- b) Abnormal foetus II. Soft tissue protruding through aperture on dorsal surface (spina bifida). Hindlimbs bowed.
- c) Depression in skull.
- d) Bilateral forelimb flexure.
- e) Eyes contain small opaque discs.
- f) Punctate dark area on midline of palate.
- g) Left thyroid gland dark and enlarged (x 1½).
- h) Thymus gland haemorrhagic.
- j) Agenesis of median lung lobe.
- k) Haemorrhage on wall of aorta and pulmonary artery.
- l) Free/clotted red serous fluid/blood in abdominal cavity.
- m) Free clear fluid in abdominal cavity.
- n) Stomach contains gas.
- p) Stomach gas distended.
- q) Stomach contents dark.
- r) Pale area(s) on liver.
- s) Liver congested and thickened.
- t) Haemorrhage on gall bladder.
- u) Gall bladder enlarged (x 2).
- v) Gall bladder bilobed.
- w) Gall bladder rudimentary.
- x) Raised haemorrhagic area on kidney.
- y) Unilateral hydronephrosis.
- z) Bilateral hydronephrosis.
- A) Unilateral hydroureter.
- B) Ovaries congested.
- C) Congested blood supply to one or both testes.
- D) Fluid-filled vesicle adjacent to ovary.
- E) Small foetus (less than 32.0 g).
- F) Amniotic fluid dark in colour.
- G) Pale area on placenta.
- H) Pale placenta.

APPENDIX 4 - continued

Individual litter data - females killed on Day 29 of gestation

Group 1 : Control

Animal number	Corpora lutea count	Implan- tations	Viable young		Resorptions		Implantation loss (%)		Foetal weight (g)		Placental weight (g)		Observations	
			M	F	Total	Early	Late	Total	Pre-	Post-	Mean	S.D.		
														Mean
16TU 109	11	9	4	5	9	0	0	0	18.2	0.0	42.0	5.3	1.3	1t
16TU 411	11	9	2	4	6	1	2	3	18.2	33.3	19.9	6.0	0.7	1a1EH,3tE,2E
16TU 429	11 ^W	12	8	3	11	1	0	1	0.0	8.3	35.0	7.6	1.0	1nE,1t,3tz,1tCE,1xy,1z,1E
16TU 430	12	4	3	1	4	0	0	0	66.7	0.0	49.1	1.6	1.3	1t
16TU 455	18	16	8	6	14	1	1	2	11.1	12.5	31.8	5.4	1.2	3tE,7E
16TU 472	17	15	7	3	10	2	3	5	11.8	33.3	38.8	2.6	0.7	1dt
16TU 475	12	12	2	8	10	0	2	2	0.0	16.7	34.4	5.3	1.0	1g,1AE,3E,1G
16TU 492	13	10	6	3	9	0	1	1	23.1	10.0	35.8	4.8	0.9	1jr,1rE,1t,2E
16TU 521	12	11	2	8	10	1	0	1	8.3	9.1	37.9	2.6	0.7	1kl,2t
16TU 531	10	10	3	6	9	1	0	1	0.0	10.0	38.3	5.1	0.7	2E
16TU 533	9	9	3	6	9	0	0	0	0.0	0.0	37.7	6.6	0.7	1u,1E
16TU 554	16	12	4	3	7	0	5	5	25.0	41.7	42.7	6.3	1.1	-
16TU 556	14	13	4	7	11	0	2	2	7.1	15.4	25.2	4.4	0.8	1wE,9E
16TU 557	12	8	3	5	8	0	0	0	33.3	0.0	37.2	6.9	1.4	1nt,1t,1tE,1v,1E
16TU 376		Not pregnant.												

W Number of implantations substituted in calculation of pre-implantation loss.

APPENDIX 4 - continued

Individual litter data - females killed on Day 29 of gestation

Group 2 : SC-19129 : 250 mg/kg/day

Animal number	Corpora lutea count	Implan- tations	Viable young		Resorptions		Implantation loss (%)		Foetal weight (g)		Placental weight (g)		Observations		
			Total		Early	Late	Total	Pre-	Post-	Mean	S.D.	Mean		S.D.	
			M	F											
16TU 379	14	12	8	4	12	0	0	0	14.3	0.0	34.5	5.8	4.9	1.0	4E
16TU 389	11	5	3	2	5	0	0	0	54.5	0.0	48.9	2.3	8.4	1.4	1t
16TU 407	9 ^W	10	4	4	8	2	0	2	0.0	20.0	36.3	6.9	4.2	1.0	3E
16TU 419	14	11	5	5	10	1	0	1	21.4	9.1	38.5	10.1	5.5	1.3	1mE,2D,1H
16TU 456	14	11	6	4	10	0	1	1	21.4	9.1	38.8	4.6	6.0	1.0	1b,1p,1pE
16TU 465	22	1	1	0	1	0	0	0	95.5	0.0	50.6	-	9.8	-	-
16TU 519	11	9	5	4	9	0	0	0	18.2	0.0	37.6	4.0	4.9	0.4	1tC,1tE,3C
16TU 536	11	9	6	1	7	1	1	2	18.2	22.2	42.6	2.7	6.5	1.1	2t
16TU 537	8	7	1	6	7	0	0	0	12.5	0.0	40.6	4.5	9.5	1.8	1qF,3F,1FH,2H
16TU 541	14	14	5	8	13	0	1	1	0.0	7.1	35.8	6.0	4.3	0.7	2f,2E
16TU 545	15	15	5	7	12	1	2	3	0.0	20.0	29.7	4.6	3.7	0.6	1t,6E
16TU 551	7	4	2	2	4	0	0	0	42.9	0.0	47.6	3.5	6.0	1.0	1h
16TU 558	9	8	5	3	8	0	0	0	11.1	0.0	40.9	3.9	5.5	0.6	3t
16TU 417			Aborted Day 25 after insemination.												
16TU 542			Not pregnant.												

W Number of implantations substituted in calculation of pre-implantation loss.

APPENDIX 4 - continued

Individual litter data - females killed on Day 29 of gestation

Group 3 : SC-19129 : 500 mg/kg/day

Animal number	Corpora lutea count	Implan- tations	Viable young		Resorptions		Implantation loss (%)		Foetal weight (g)		Placental weight (g)		Observations
			M	F	Total	Early	Late	Total	Pre-	Post-	Mean	S.D.	
16TU 340	12	7	0	5	5	2	0	2	41.7	28.6	47.6	5.2	1p
16TU 412	6	2	1	1	2	0	0	0	66.7	0.0	53.7	4.6	-
16TU 436	9	9	3	3	6	0	3	3	0.0	33.3	40.6	7.2	1n,1t,1E
16TU 497	15	3	0	1	1	2	0	2	80.0	66.7	44.6	-	-
16TU 512	15	11	3	4	7	3	1	4	26.7	36.4	39.8	7.3	11t,1t,1B,2E
16TU 514	9	9	2	5	7	1	1	2	0.0	22.2	38.9	3.7	1tC
16TU 526	9	8	3	5	8	0	0	0	11.1	0.0	36.8	3.7	-
16TU 530	12	11	7	4	11	0	0	0	8.3	0.0	38.1	4.8	-
16TU 532	12	11	7	4	11	0	0	0	8.3	0.0	39.0	9.3	2E
16TU 543	11	10	7	3	10	0	0	0	9.1	0.0	40.1	5.2	1n,1E
16TU 555	10	10	3	6	9	0	1	1	0.0	10.0	34.8	6.9	1mSE,1t,2E
16TU 567	14	11	4	5	9	0	2	2	21.4	18.2	39.6	4.8	1h,1ht,1j,4t,1E
16TU 403			Aborted Day 27 after insemination.										
16TU 539			Not pregnant.										
16TU 473			Killed in <u>extremis</u> Day 27 after insemination.										

APPENDIX 4 - continued

Individual litter data - females killed on Day 29 of gestation

Group 4 : SC-19129 : 750 mg/kg/day

Animal number	Corpora lutea count	Implan- tations	Viable young		Resorptions		Implantation loss (%)		Foetal weight (g)		Placental weight (g)		Observations
			M	F	Total	Early	Late	Total	Pre-	Post-	Mean	S.D.	
16TU 393	12	8	7	1	8	0	0	0	33.3	0.0	38.7	1.9	2n,1p
16TU 398	11	11	5	5	10	1	0	1	0.0	9.1	41.2	4.3	11,1t
16TU 432	9	9	1	6	7	0	2	2	0.0	22.2	38.6	6.2	1t
16TU 442	11	10	3	7	10	0	0	0	9.1	0.0	42.2	2.3	3p
16TU 444	12	9	3	5	8	1	0	1	25.0	11.1	38.9	4.0	1E
16TU 535	14	14	6	7	13	0	1	1	0.0	7.1	18.1	3.4	1ceE,1ze,11E
16TU 549	17	15	6	5	11	1	3	4	11.8	26.7	40.1	3.6	1t
16TU 552	10	9	7	2	9	0	0	0	10.0	0.0	39.5	5.3	11,2t
16TU 559	15	14	6	7	13	0	1	1	6.7	7.1	37.3	5.8	1n,3t,1tE,2C
16TU 439	Aborted	Day 21 after insemination.											
16TU 374	Died	Day 8 after insemination.											
16TU 409	Died	Day 17 after insemination.											
16TU 426	Died	Day 27 after insemination.											
16TU 529	Killed	in <u>extremis</u> Day 15 after insemination.											
16TU 546	Killed	in <u>extremis</u> Day 24 after insemination.											

APPENDIX 5

Individual foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Key to observations

- a) Incomplete ossification of supraoccipital bone.
- b) Incomplete ossification of interparietal bone.
- c) Small additional sutures in cranial bones.
- e) Interparietal bone reduced in size or absent.
- f) Irregular ossification of frontal suture.
- g) Incomplete ossification or absence of hyoid body.
- h) Cornua of hyoid bent inwards, arch flattened.
- i) One cornu of hyoid bent outwards.
- j) One or more sternebrae offset.
- k) Two or more sternebrae fused.
- m) One or more ribs thickened at costal cartilage.
- n) One or both olecranon processes ossified.
- p) Asymmetric pelvis, ilia associated with different sacral vertebrae.
- q) Incomplete or asymmetric ossification of costal elements of sacral vertebrae.
- r) Incomplete ossification of one or more cervical vertebral centra.
- s) Incomplete ossification of sacral vertebrae, less than 16 ossified.
- t) Incomplete ossification of one or more thoracic vertebral centra.
- u) Incomplete ossification or absence of one or more centrales.
- v) Incomplete ossification or absence of pubic bones.
- w) Incomplete ossification of metacarpals and/or phalanges.
- x) Abnormal forelimb flexure.
- y) Small additional sternbral bone between 5th sternebra and xiphisternum.
- z) Posterior fontanelle enlarged.
- A) Anterior fontanelle negligible, size of suture lines only.
- B) Small discrete unossified areas in frontal bones.
- C) Frontal suture enlarged at frontal/nasal junction.
- D) Double association pelvis, ilia associated with both sacral vertebrae.
- E) Lacrymal fossa enlarged.
- F) Incomplete ossification of astralagus.
- G) Anterior fontanelle extended anteriorly into frontal suture.
- H) Additional (cervical) ribs on 7th cervical vertebra.
- I) Additional fissures from anterior fontanelle into frontal bones.
- J) Anterior fontanelle extended laterally into frontal bones.
- K) Right 5th and 6th ribs fused at costal cartilage.

APPENDIX 5 - continued

Individual foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Key to observations - continued

- L) Anomalous foetus:- spina bifida; hemivertebral arches of 6th and 7th lumbar vertebrae reduced in size, spacing between hemivertebral arches of 5th lumbar and 1st sacral vertebrae increased, open pore through to spinal canal at 6th and 7th lumbar vertebra.
- M) Small discrete unossified area in parietal bones.
- N) Xiphisternum bifurcated.
- O) Anomalous vertebrae:- right hemicentrum and hemivertebral arch of 3rd lumbar vertebra absent, left hemicentrum fused to centrum of 2nd lumbar vertebra, left hemivertebral arch of 2nd lumbar vertebra displaced caudally, enlarged; left scoliosis.
- P) Large unossified areas in parietal bones.
- Q) Incomplete ossification of one or more thoracic vertebral arches.

APPENDIX 5 - continued

Individual foetal observations at skeletal examination

Group 1 : Control

Animal number	Number of foetuses examined	Incomplete ossification of sternebrae: number of bones affected										Number of ribs			Pre-sacral vertebrae		Number with heads of long bones unossified	Size of anterior fontanelle		Observations
		Total	Intact	1	2	3	4	5	6	12	12	12	13	13	26	27		Small	Medium	Large
16TU 109	9	6	2	-	-	-	-	-	-	1	2	6	8	1	4	1	5	1	-	1aw,2gw,1w,1wpq,1wy,1wD
16TU 411	6	4	1	4	-	-	-	-	-	3	-	3	4	2	6	2	2	-	2	1agruwzB,1agruwzCEF
16TU 429	11	8	2	4	-	-	-	-	-	10	1	-	11	-	11	-	7	1	-	1guvz,1gz,1v
16TU 430	4	3	1	-	-	-	-	-	-	1	1	2	3	1	-	-	3	-	-	1az,2w
16TU 455	14	11	10	1	-	-	-	-	-	10	1	3	13	1	13	-	7	4	-	1en,1epq,1eg
16TU 472	10	7	3	1	-	-	-	-	-	8	-	2	7	3	7	-	7	-	-	1agvw,1aw,1c,2gw,1pq
16TU 475	10	8	4	3	-	-	-	-	-	5	2	3	7	3	10	-	6	-	-	1qD,1w
16TU 492	9	6	6	-	-	-	-	-	-	2	3	4	2	7	8	-	6	-	-	leg,1f,1gj,1gwz,1gx
16TU 521	10	7	6	-	-	-	-	-	-	2	-	8	8	2	5	-	7	-	-	le,1f,1jk
16TU 531	9	6	5	2	-	-	-	-	-	1	-	8	5	4	9	-	2	-	-	legz,1fgwz,1k,1wz
16TU 533	9	6	6	1	-	-	-	-	-	7	2	-	9	-	6	-	3	-	-	1a,1t,1x
16TU 554	7	5	3	-	-	-	-	-	-	6	1	-	4	3	6	-	1	-	-	1fw,1gw,1jw,1wv
16TU 556	11	7	8	-	1	-	-	-	-	4	3	4	9	2	11	-	6	-	-	3g,1H
16TU 557	8	6	2	-	-	-	1	-	-	6	1	1	6	2	4	-	5	-	-	1f,1g,1gm
16TU 376	Not pregnant.																			1af,1bgwvz,1f,1g,1uwv,1wv,3w
																				1eqzDI,1f,1fg,1g,1gm,1gpq

Individual foetal observations at skeletal examination

Animal number	Number of foetuses examined	Incomplete ossification of sternebrae:								Number of ribs				Pre-sacral vertebrae		Number with heads of long bones unossified	Size of anterior fontanelle		Observations
		Total	Intact	1	2	3	4	5	6	12	12/13	13	26	27	Small		Medium	Large	
16TU 379	12	8	4	-	-	-	-	-	-	6	5	1	12	-	11	1	7	1awz,1fc,3g,lgw,1w,1zg	
16TU 389	5	4	1	-	-	-	-	-	-	3	-	2	2	3	2	-	4	lcfm,lg,lgul,jm	
16TU 407	8	6	2	-	-	-	-	-	-	2	-	6	4	4	6	1	5	lf,lg,lpq,lwz,lk	
16TU 419	10	7	4	-	-	-	-	-	-	4	2	4	7	3	7	-	7	labguvwz,le,lepq,lg	
16TU 456	10	7	3	-	-	-	-	-	-	6	1	3	9	1	3	3	4	lcw,lf,lg,li,jm,igl	
16TU 465	1	1	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	lh	
16TU 519	9	6	2	2	-	-	-	-	-	5	1	3	8	1	7	1	5	lew,jm,lpq	
16TU 536	7	5	5	-	-	-	-	-	-	3	3	1	5	2	4	1	4	lgmpq,li,lpq,ly	
16TU 537	7	5	6	1	-	-	-	-	-	5	1	1	6	1	4	1	4	la,lb,lf,lg,lgw	
16TU 541	13	9	11	-	-	-	-	-	-	5	3	5	10	3	11	6	3	lgr,lj,lw	
16TU 545	12	8	4	3	2	-	-	-	-	10	1	1	10	2	12	-	8	legar,z,lg,lgjqwD,lgwzm, lgw,lpqrwz,2w	
16TU 551	4	3	1	-	-	-	-	-	-	2	2	-	4	-	2	1	2	2f	
16TU 558	8	6	1	-	-	-	-	-	-	8	-	-	8	-	6	4	2	lf,jm,lqd	
16TU 417																			
16TU 542																			

Aborted Day 25 after insemination.
Not pregnant.

APPENDIX 5 - continued

Individual foetal observations at skeletal examination

Group 3 : SC-19129 : 500 mg/kg/day

Animal number	Number of foetuses examined	Incomplete ossification of sternebrae: number of bones affected										Number of ribs			Pre-sacral vertebrae	Number with heads of long bones unossified	Size of anterior fontanelle			Observations
		Total	Intact	1	2	3	4	5	6	12	12/13	13	26	27	Small		Medium	Large		
16TU 340	5	4	2	-	-	-	-	-	-	-	-	5	2	3	2	1	3	-	1ag,1gm,1m	
16TU 412	2	1	2	-	-	-	-	-	-	1	-	1	1	1	-	1	-	-	1ghw	
16TU 436	6	4	2	-	-	-	-	-	-	2	1	3	6	-	6	-	4	-	1fgw,1pq	
16TU 497	1	1	1	-	-	-	-	-	-	1	-	-	-	1	1	-	1	-	1ant0	
16TU 512	7	5	6	1	-	-	-	-	-	5	1	1	6	1	3	2	3	-	1rw,4w	
16TU 514	7	5	7	-	-	-	-	-	-	5	-	2	6	1	4	3	2	-	1f,1g,1rt,1A	
16TU 526	8	6	4	1	-	-	-	-	-	1	2	5	5	3	6	1	5	-	1ag,1g	
16TU 530	11	8	8	3	-	-	-	-	-	6	3	2	11	-	8	6	2	-	1f,1fgw,1gmw,2w,2w,1A	
16TU 532	11	7	9	-	-	-	-	-	-	9	-	2	10	1	5	4	3	-	1a,1bgw,1f,1n,1w	
16TU 543	10	7	3	-	-	-	-	-	-	9	-	1	10	-	7	-	7	-	1pq,1pqw,2w	
16TU 555	9	6	8	1	-	-	-	-	-	5	4	-	7	2	3	-	6	-	1agwz,2g,1uw,2w	
16TU 567	9	6	2	-	-	-	-	-	-	8	1	-	9	-	9	-	6	-	1g,2gw,1mw	
16TU 403																				
16TU 539																				
16TU 473																				

16TU 403 Aborted Day 27 after insemination.

16TU 539 Not pregnant.

16TU 473 Killed in extremis Day 27 after insemination.

APPENDIX 5 - continued

Individual foetal observations at skeletal examination

Group 4 : SC-19129 : 750 mg/kg/day

Animal number	Number of foetuses examined	Incomplete ossification of sternebrae:											Number of ribs				Pre-sacral vertebrae	Number with heads of long bones unossified	Size of anterior fontanelle			Observations																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
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Aborted Day 21 after insemination.

Died Day 8 after insemination.

Died Day 17 after insemination.

Died Day 27 after insemination.

Killed in extremis Day 15 after insemination.

Killed in extremis Day 24 after insemination.

APPENDIX 6

Individual observations following free-hand serial sectioning of foetal heads

Group	:	1	2	3	4
Compound	:	Control	---	SC-19129	---
Dosage (mg/kg/day)	:	0	250	500	750

Key to observations

- a) Lower incisors erupted.
- b) Blood in nasal sinuses.
- c) Upper and lower incisors erupted.
- d) Incisors not erupted.
- e) Crystalline deposits.
- f) Unilateral dilated orbital sinus.
- g) Bilateral dilated orbital sinus.
- h) Cystic dilatation of the brain.
- j) Unilateral folded retina.
- k) Bilateral folded retina.
- m) Blood in cochlea(s).
- n) Blood in nasopharynx.
- p) Dent in right parietal region of head.

APPENDIX 6 - continued

Individual observations following
free-hand serial sectioning of foetal heads

Group 1 : Control

Animal number	Number of heads examined	Observations
16TU 109	3	1am,1c,1cef
16TU 411	2	1a,1ch
16TU 429	3	3c
16TU 430	1	1ce
16TU 455	3	1ae,1c,1ck
16TU 472	3	3ce
16TU 475	2	1ce,1cem
16TU 492	3	1c,1cehjm,1cm
16TU 521	3	1amn,1cjm,1ck
16TU 531	3	2c,1ce
16TU 533	3	1bdh,1c,1ce
16TU 554	2	1c,1cehm
16TU 556	4	1a,2c,1d
16TU 557	2	1c,1ce

16TU 376 Not pregnant.

APPENDIX 6 - continued

Individual observations following
free-hand serial sectioning of foetal heads

Group 2 : SC-19129 : 250 mg/kg/day

Animal number	Number of heads examined	Observations
16TU 379	4	3c,1ce
16TU 389	1	1ceh
16TU 407	2	1a,1c
16TU 419	3	1c,1cem,1ch
16TU 456	3	2c,1cem
16TU 519	3	2ce,1cem
16TU 536	2	1c,1ceh
16TU 537	2	1ce,1ceg
16TU 541	4	2c,1ce,1cej
16TU 545	4	1a,2c,1ch
16TU 551	1	1cemn
16TU 558	2	2c

16TU 417 Aborted Day 25 after insemination.
16TU 542 Not pregnant.

APPENDIX 6 - continued

Individual observations following
free-hand serial sectioning of foetal heads

Group 3 : SC-19129 : 500 mg/kg/day

Animal number	Number of heads examined	Observations
16TU 340	1	1ce
16TU 412	1	1ceh
16TU 436	2	1c,1cejm
16TU 512	2	1cegm,1ceh
16TU 514	2	2c
16TU 526	2	1c,1ce
16TU 530	3	1bcghn,1cem,1cm
16TU 532	4	3c,1dh
16TU 543	3	1ajm,1ce,1cegh
16TU 555	3	1ae,1c,1dh
16TU 567	3	1ak,1ce,1cn

16TU 403 Aborted Day 27 after insemination.

16TU 539 Not pregnant.

16TU 473 Killed in extremis Day 27 after insemination.

APPENDIX 6 - continued

Individual observations following
free-hand serial sectioning of foetal heads

Group 4 : SC-19129 : 750 mg/kg/day

Animal number	Number of heads examined	Observations
16TU 393	3	3c
16TU 398	3	1c,1ce,1ch
16TU 432	2	2a
16TU 442	3	1ae,1ce,1cn
16TU 444	2	1a,1ae
16TU 535	5	2a,1cp,1d,1dm
16TU 549	3	1cem,1cj,1cm
16TU 552	3	1c,1ce,1cem
16TU 559	4	1bcmn,1c,1cem,1cm

16TU 439 Aborted Day 21 after insemination.
16TU 374 Died Day 8 after insemination.
16TU 409 Died Day 17 after insemination.
16TU 426 Died Day 27 after insemination.
16TU 529 Killed in extremis Day 15 after insemination.
16TU 546 Killed in extremis Day 24 after insemination.

ADDENDUM 1

Protocol and amendments



RECEIVED
27 MAR 1985
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LSR Schedule No. SEA047

LSR Enquiry No. ZZZ/0380 A

SEGMENT II TERATOLOGY EVALUATION OF
SC-19129 IN RABBITS, S.A. NO. 2643

Protocol prepared for
G.D. SEARLE AND COMPANY

by

Life Science Research
Eye, Suffolk, England

6 March 1985

MANAGEMENT OF STUDY

<u>Study director</u>	:	Frank W. Ross, B.Sc., M.I.Biol.
<u>Staff reproductive biologist</u>	:	Timothy J. Wightman, B.Sc.
<u>Sponsor</u>	:	G.D. Searle and Company 4901 Searle Parkway Skokie Illinois 60077 U.S.A.
<u>Monitor</u>	:	Dr. J.W. Noveroske

PROTOCOL APPROVAL

For LIFE SCIENCE RESEARCH

Issued by : *At the Faculty* Date : ... *6/3/85*

Released by : *Smith* Date : ... *6 March 85*

For G.D. SEARLE AND COMPANY

This protocol is accepted without revision and my signature authorises the study to proceed as described in this document. The document becomes the FINAL PROTOCOL for the study, and will be reproduced in the final report.

Approved by *John C. Munn* Date : ... *Mar. 15, 1985*

Study Co-ordinator

..... *John C. Munn* Date : ... *3/15/85*

Director of Toxicology

..... *Frederic E. Kohn* Date : ... *3/15/85*

Senior Director, Product Safety Assessment

STUDY DIRECTOR

The Sponsor has approved the initiation of the study according to the procedures described in this document. My signature below denotes that I have read and agreed the contents of this document.

..... *John C. Munn*
(Study Director)

Date : ... *7 March 85*

TERATOLOGY STUDY IN THE RABBIT1. INTRODUCTION1.1 Objective

To assess the influence of SC-19129 upon the organogenesis phase of pregnancy in the rabbit.

1.2 Choice of species

The rabbit is selected because of the requirements for the use of a non-rodent species by regulatory authorities. The New Zealand White rabbit in particular is selected because of the background data available on this strain of rabbit in these laboratories.

1.3 Choice of route of administration and treatment levels

SC-19129 will be administered by oral gavage to simulate the route of human exposure.

Dosages will be based on information provided by the Sponsor, and by reference to the expected levels of human exposure.

1.4 Location of study

: Life Science Research
Eye
Suffolk IP23 7PX
England

Telephone: Diss (0379) 4122
Telex : 975389

2. SCHEDULED TIME PLAN (to be decided)

2.1 Insemination commences :

2.2 Draft report to Sponsor :

3. DESIGN CONDITIONS

3.1 Animals

Sexually mature virgin female New Zealand White rabbits from an accredited closed colony (Morton Rabbitries, Stansted, Essex, England) are used in the investigation.

The animals will be in the approximate weight range of 3.0-3.75 kg on arrival. Shortly after arrival, oestrus will be synchronized by intravenous injection of 25 i.u. luteinising hormone (Pregnyl, Organon). The animals will be allowed a minimum of three weeks acclimatisation during which time they will be examined daily to check their physical condition.

3.2 Environmental control

The animals will be housed in a limited access, rabbit facility. All personnel entering the facility are required to wash exposed skin and wear protective clothing.

The rabbitry has its own supply of filtered air which is not re-circulated, providing approximately 17 to 20 room air changes per hour. The temperature and relative humidity in the rabbitry are recorded daily and the records retained. A 14-hour light : 10-hour dark cycle operates throughout.

A stand-by power supply is automatically brought into operation should the mains supply fail.

3.3 Water supply

Tap water from the local mains is supplied to the cages via an automatic system. The East Anglian Water Company monitor the quality of the water at approximately six-monthly intervals and copies of the relevant analyses will be lodged with the raw data.

3.4 Basal diet

A commercially-available laboratory animal diet, Beta Rabbit Standard Diet (813 181W; Special Diet Services Limited, Witham, Essex) will be fed ad libitum throughout the study. The manufacturers supply a certificate of analysis with each batch of diet; copies of the relevant analyses will be lodged with the raw data.

3.5 Contaminants

There are no contaminants in either the diet or the water that are reasonably expected to be present at levels that are known to be capable of interfering with the purpose or conduct of the study.

3.6 Cage type and number of rabbits per cage

Rabbits will be housed singly in galvanised steel caging, (Cope and Cope Limited, Reading, Berkshire, England) and will be evenly distributed in order to minimise the effects of environmental influences.

The cages have mesh floors, and the trays beneath the cages will be scraped and flushed down each day.

3.7 Insemination procedure

Females will be mated naturally with fertile males of the same strain, or artificially inseminated. Following insemination, each female will be injected intravenously with 25 i.u. of luteinising hormone (Pregnyl, Organon) to ensure successful ovulation. The day of insemination will be designated Day 0 of gestation.

3.8 Test substance

3.8.1 Identification

SC-19129.

3.8.2 Lot number

84K-047-101

3.8.3 Characterisation

Pre-study analysis of the identity, purity, strength and composition of SC-19129 and its stability in 0.5% (w/v) methylcellulose and 0.1% (w/v) Tween 80 has been determined by the Sponsor.

Homogeneity of the suspensions as prepared by Life Science Research will be determined before the start of the study by the Sponsor. In addition, test article concentrations will be determined once during the period of dosing, and a post-study analysis to confirm identity, purity, strength and composition of the compound will be performed by the Sponsor. All suspension samples will be deep frozen for despatch to the Sponsor.

3.8.4 Reserve sample

At or before initiation of the study, a 10 g reserve sample of the test substance will be taken and stored in a well-closed glass container under the conditions specified for storage of the bulk supply of the test substance.

3.8.5 Storage conditions

In a light-resistant container at room temperature.

3.8.6 Safety precautions

No special safety precautions needed.

3.8.7 Absorption of compound

The assessment of the absorption of the compound in the vehicle is the responsibility of the Sponsor.

3.9 Treatment

Females will be identified by ear-tags on arrival. They will be randomly allocated to four treatment groups in order of insemination so that females inseminated on any one day are evenly distributed amongst the groups.

The four groups will be treated as follows:

<u>Group</u>	<u>Treatment</u>	<u>Dose level</u> (mg/kg/day)	<u>Number per group</u>
1	Control	0	15
2	SC-19129	250	15
3	SC-19129	500	15
4	SC-19129	750	15

The compound will be prepared freshly each day as a suspension in 0.5% (w/v) methylcellulose and 0.1% (w/v) Tween 80. Animals will be dosed daily by oral gavage from Day 6 to Day 19 inclusive of gestation at a volume-dosage of 4 ml/kg. Control animals will receive the vehicle at the same volume-dosage during the same treatment period. The dose administered daily to each animal will be based on the animal's bodyweight on that day and the individual volume-dosage will be recorded.

4. SERIAL OBSERVATIONS

4.1 Maternal signs

All animals will be examined daily throughout the study and any visible signs of reaction to treatment will be recorded with details of type, severity, time of onset and duration.

4.2 Mortality

Any animals found dead or killed in extremis will be subjected to a thorough macroscopic examination of the visceral organs with the object of identifying the cause of death. Specimens of abnormal tissue will be retained.

4.3 Maternal bodyweight

Animals will be weighed daily throughout gestation.

5. TERMINAL STUDIES

- 5.1 On Day 29 of gestation the females will be killed by intravenous injection of Pentobarbitone sodium B.Vet.C. (Abbott Laboratories, Queenborough, Kent), for examination of their uterine contents. Each animal will first be examined macroscopically for evidence of disease or adverse reaction to treatment. Any tissues considered abnormal will be retained. The reproductive tract, complete with ovaries, will be dissected out and the following recorded:
- a) Number of corpora lutea in each ovary;
 - b) Number of implantation sites. In apparently non-pregnant animals, presence of implantation sites will be checked using the Salewski staining technique (Salewski, E.; Arch. Exp. Pathol. Pharmacol., 247, 367, 1964);
 - c) Number of resorption sites (classified as early or late);
 - d) Number and distribution of live and dead fetuses in each uterine horn;
 - e) Weight and sex of individual fetuses;
 - f) Individual placental weights;
 - g) External abnormalities of individual fetuses.

5.2 Internal and skeletal examinations

All fetuses will be killed by a subcutaneous injection of Pentobarbitone sodium. The neck and thoracic and abdominal cavities of all fetuses from each litter will be dissected and their contents examined. Low-power magnification will be used if necessary. Following examination one third of the fetuses in each litter will be decapitated and the heads fixed in Bouin's Fluid for subsequent examination following free hand serial sectioning. Torsos and the remaining intact fetuses will then be eviscerated and placed in industrial methylated spirit (74 o.p.) before processing, which utilises a modification of the Dawson staining technique (Tesh, J.M., Ph.D. Thesis, Faculty of Veterinary Science, University of Liverpool, 1968), and subsequent examination.

5.3 Abortions

Any animals that abort will be killed by intravenous injection of Pentobarbitone Sodium on the same day that the abortion was detected. The females will be subjected to a detailed macroscopic examination and the numbers of corpora lutea and implantation sites will be recorded. Where possible the fetuses will be examined.

6. TREATMENT OF DATA

Data are expressed as means with standard deviations of the mean (S.D.) calculated according to the formula:

$$S.D. = \sqrt{\frac{\sum(x - \bar{x})^2}{n-1}}$$

unless otherwise indicated.

6.1 Maternal bodyweight

Group mean values (\pm S.D.) will be calculated on Days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 28 of gestation. Weight changes will be plotted graphically with respect to Day 6 of gestation.

6.2 Group mean values (\pm S.D.) will be calculated for numbers of corpora lutea, implantations, resorptions (early, late and total) and viable young (male, female and total) at Day 29 of gestation. The standard deviations for resorptions will be calculated as:

$$\sqrt{\bar{x}}$$

6.3 Pre-natal losses will be considered separately for the pre- and post-implantation phases.

a) Pre-implantation loss

Pre-implantation loss includes losses due to non-fertilisation of ova and very early post-implantation deaths (i.e. those occurring up to Days 10-11 of gestation), in addition to true pre-implantation loss. It will be calculated from the formula:

$$\frac{\text{No. of corpora lutea} - \text{No. implantations}}{\text{No. corpora lutea}} \times 100$$

Group values will be calculated as a mean of the individual litter values.

b) Post-implantation loss

Post-implantation loss covers only the period between Day 10 and 29 of gestation; it does not include the first 3-4 days post-implantation as any deaths that occur in this phase leave no remains that may be detected at Day 29. It will be calculated from the formula:

$$\frac{\text{No. implantations} - \text{No. viable fetuses}}{\text{No. implantations}} \times 100$$

Group values will be calculated as a mean of the individual litter values.

6.4 Group mean foetal and placental weights (\pm S.D.) will be calculated for each group as:

$$\frac{\text{Total of individual litter mean foetal/placental weights}}{\text{Number of litters}}$$

6.5 Observations on fetuses at examination post mortem and at skeletal evaluations will be calculated on a group basis for each abnormality observed as:

$$\frac{\text{No. fetuses with a particular abnormality}}{\text{Total No. fetuses examined in each group}} \times 100$$

The number of litters in which an observation is recorded is also reported.

6.6 Statistical evaluation

The significance of suggestive inter-group differences will be tested using appropriate statistical tests, each of which will be specified where significance is found.

The following tests are used:

One-way analysis of variance
and/or t-test

Bodyweights
Bodyweight change
Foetal weight
Placental weight
Litter size

Mann-Whitney U-test

Corpora lutea count
Implantation count
Resorption count

Chi-squared test, Fisher's
Exact Probability test or
Mann-Whitney U-test

Pre-implantation loss
Post-implantation loss

7. REPORTING

Short status summaries will be submitted monthly.

The information and data required in Section 58.185 of the Good Laboratory Practice Regulations published by the U.S. Food and Drug Administration in the Federal Register (Vol. 43. No.247, 22 December 1978) are included in the final report.

8. RECORDS

All raw data, original records, slides, blocks and any wet tissues will be retained until management decides they should be discarded, and such action has been agreed by the Sponsor.

Documents and samples to be stored.

1. Compound : reserve samples, analytical certificates and records of use.
2. Environment : records of temperature and humidity of animal room.
3. Animals : records of supplier and identification numbers.
4. Cages : cage plan and position of animals and groups on rack.

5. Physical examination records.
6. Dosing records.
7. Bodyweight : weight records for each animal.
8. Terminal findings, including foetal responses.

All records will be inspected by the Responsible Scientist and lodged with central records at Life Science Research.



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27 JUN 1985

LSR Schedule No.

SEA/047/SC-19129

LSR Enquiry No.

ZZZ/0380A

SEGMENT II TERATOLOGY EVALUATION OF
SC-19129 IN RABBITS S.A. NO. 2643

FIRST AMENDMENT TO PROTOCOL

Sponsor : G D Searle & Co
Study Director : F.W. Ross, B.Sc., M.I. Biol.
Date of amendment : 22 May 1985
Source of amendment : Staff Biologist
Purpose of amendment : To update and correct existing protocol

AMENDMENT

1.1 The LSR Schedule Number is : SEA/047/SC-19129

1.2 Section 2 SCHEDULED TIME PLAN

Amend to read as follows:-

2.1 Insemination commences : 22 April 1985
2.2 Draft report to Sponsor : September 1985

1.3 Section 3 DESIGN CONDITIONS

Amend first paragraph to read as follows:-

3.1 Animals

Sexually mature virgin female New Zealand White rabbits from an accredited closed colony (Ranch Rabbits, Crawley Down, Sussex, England) are used in the investigation.

Continues unchanged.

For LIFE SCIENCE RESEARCH LIMITED

Issued by :

Date: 3 June 1985

Approved by:

Date: 3 June 85

For G D SEARLE & CO

Accepted by:

Date: June 11, 1985



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4 DEC 1985
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LSR Schedule No. SEA/047/SC-19129
LSR Enquiry No. ZZZ/0380A

SEGMENT II TERATOLOGY EVALUATION OF
SC-19129 IN RABBITS S.A. No. 2643

SECOND AMENDMENT TO PROTOCOL

Sponsor : G D Searle & Co
Study Director : F.W. Ross, B.Sc., M.I.Biol.
Date of amendment : 4 December 1985
Source of amendment : Staff Biologist
Purpose of amendment : To correct existing protocol

AMENDMENT

1.1 Section 3.1 Animals

Amend first sentence of second paragraph to read as follows:

"The animals will be in the approximate weight range of 2.7-3.7 kg on arrival."

Continues unchanged.

1.2 Section 3.4 Basal diet

Amend first sentence to read as follows:

"A commercially available certified laboratory animal diet, S.Q.C. Standard Rabbit Diet (813 181W); Special Diet Services Limited, Witham, Essex, England) will be fed ad libitum throughout the study."

1.3 Section 5 TERMINAL STUDIES

Sub-section 5.1

Amend g) to read as follows:

g) External abnormalities of individual foetuses and placentae.

For LIFE SCIENCE RESEARCH LIMITED

Issued by : *T. Nijffinga* Date: *4 December 1985*
Approved by : *[Signature]* Date: *4 December 1985*

For G D SEARLE & CO

Accepted by : *[Signature]* Date: *Dec 4, 1985*

R&D PRODUCT DEVELOPMENT FUNCTION
REPORT REVIEW AND RELEASE

Page 1 of 11

DEPARTMENT: Product Development Analytical

DOCUMENT NUMBER: F-344-034-12A

TITLE OF REPORT: SC-19129

TYPE OF REPORT: Analytical Summary in Support of Product Safety
Assessment Study Number 2643

AUTHOR(S):	DATE	REVIEWER(S):	DATE
<u>Mary E. Napier</u>	<u>12/16/85</u>	<u>Charles Sumner</u>	<u>12/16/85</u>
_____	_____	_____	_____
_____	_____	_____	_____

TECHNICAL WRITER:

Michele Newcomb Michele Newcomb 12/17/85

APPROVAL:	DATE
<u>[Signature]</u>	<u>12-16-85</u>
_____	_____

Reason for Revision: Data for Lot 84K-052-101 was deleted from page two, because this lot was not used by the contract lab.

APPROVAL FOR RELEASE:

<u>[Signature]</u>	<u>12/20/85</u>	<u>Larry Hansen</u>	<u>12/23/85</u>
R. Baum, Director	Date	L. Hansen,	Date
Analytical Development		Senior Director	
		Product Development	

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Test Article Characterization and Stability

Lot 84K-047-101 of SC-19129 was analyzed using the release methods of testing, released against the current specifications (NS-S84-015-A), and given a re-evaluation period of one year prior to use in this study. The results of analysis are presented in Table 1.

Table 1

SC-19129, Lot 84K-047-101

	Pre-Study	Post-Study
Report of Analysis	84N1058	85N0682
Completion Date	10/16/84	08/20/85
Identity (HPLC)	Conforms to standard	Conforms to standard
Assay (HPLC) (on Dried Basis)	100.0% n = 3 s = 0.2	99.7% n = 3 s = 0.2
Water	9.8%	9.3%

These results and all other results, coupled with the use of lot 84K-047-101 within its re-evaluation period indicate that this lot of SC-19129 was suitable for use in this study.

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Stability of Test Article in Carrier

The stability of SC-19129 (Lot 84K-047-101) in 0.5% methyl cellulose - 0.1% polysorbate 80 at 1 and 300 mg/mL was determined using a stability indicating HPLC method (M85-001-A). Duplicate suspensions for each concentration were stored at ambient conditions and sampled at $t = 0, 1, 2, 4, 6, 8,$ and 16 hours. The results of the analysis are presented in Tables 2 and 3.

For the 1 mg/mL dosing concentration (Table 2), the results of the linear regression analysis (MINITAB II, Reference 1), gave $t = -0.77$, which is less than the table value of $t(0.95, 14) = 1.761$ (Reference 2, Table A-4), indicating no significant downward trend. The correlation between the observed recovery values and the predicted values exhibited a normal probability plot (Reference 1 and 3). The results indicate SC-19129 in suspension vehicle at 1 mg/mL, stored at ambient conditions, does not undergo significant degradation for at least 16 hours.

For the 300 mg/mL dosing concentration (Table 3), the results of the linear regression analysis (MINITAB II, Reference 1), gave $t = 0.98$, which is less than the table value of $t(0.95, 14) = 1.761$ (Reference 2, Table A-4), indicating no significant downward trend. The correlation between the observed recovery values and the predicted values exhibited a normal probability plot (Reference 1 and 3). The results indicate SC-19129 in suspension vehicle at 300 mg/mL, stored at ambient conditions, does not undergo significant degradation for at least 16 hours.

Since SC-19129 in suspension vehicle at 1 and 300 mg/mL is stable for at least 16 hours at ambient conditions, all dosing concentrations between 1 and 300 mg/mL are considered to be stable for at least 16 hours when stored under equivalent conditions.

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Table 2

Stability of SC-19129
in Suspension Vehicle, 1 mg/mL

Report of Analysis 85-0208

Time (Hours)	Sample	% SC-19129 Recovered
0	1	100.3
	2	100.7
	3	100.4
	4	100.6
1	1	99.9
	2	100.2
2	1	101.3
	2	100.7
4	1	101.0
	2	100.8
6	1	100.1
	2	98.6
8	1	100.3
	2	101.3
16	1	98.8
	2	101.2
Intercept		100.5
Slope		- 0.03
t-Ratio		- 0.77
t(0.95, 14)		1.761
Correlation: Predicted vs Observed		0.975

Notebook Reference: S. Lai, PDAD-0085, pp. 70-91

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Table 3

Stability of SC-19129
in Suspension Vehicle, 300 mg/mL

Report of Analysis 85-0209

Time (Hours)	Sample	% SC-19129 Recovered
0	1	101.7
	2	101.4
	3	100.1
	4	99.9
1	1	100.2
	2	99.7
2	1	99.8
	2	99.7
4	1	100.1
	2	100.8
6	1	100.8
	2	99.9
8	1	100.1
	2	101.4
16	1	100.8
	2	101.0
Intercept		100.3
Slope		0.03
t-Ratio		0.98
t(0.95, 14)		1.761
Correlation: Predicted vs Observed		0.949

Notebook Reference: S. Lai, PDAD-0085, pp. 70-91

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Homogeneity of Test Article in Carrier

The homogeneity of SC-19129 (Lot 84K-047-101) in 0.5% methyl cellulose - 0.1% polysorbate 80 at dosing concentrations of 62.5 and 187.5 mg/mL was determined during the study at the month 2 treatment phase time point. (Upfront homogeneity samples were sent for analysis, but due to problems with the shipping of the samples the results will not be reported.) The analysis was conducted using a stability indicating HPLC method (M85-001-A). Nine samples were taken for each concentration: three each from the top, middle, and bottom strata of the suspension contained in a beaker. Contract lab personnel delivered the samples into small glass vials using a syringe and gavage needle to parallel the techniques used in dosing. Each vial was capped with teflon lined crimp top caps. The results of the analysis are presented in Table 4.

The results for the 62.5 mg/mL suspension were shown to be normally distributed ($\alpha = 0.05$), having a normality correlation coefficient of 0.922, which is greater than the table value of $r = 0.905$ (Reference 1 and 3). The calculated tolerance interval, (Reference 2, Table A-6), indicates that with 95% confidence, at least 95% of future samples should be between 58.2 and 81.4 mg/mL for the 62.5 mg/mL dosing concentration. This is equivalent to $\pm 16.6\%$ of the mean sample concentration. The SC-19129 in suspension vehicle is considered homogeneous for the 62.5 mg/mL dosing concentration.

The results for the 187.5 mg/mL suspension were shown to be normally distributed ($\alpha = 0.05$), having a normality correlation coefficient of 0.971, which is greater than the table value of $r = 0.912$ (Reference 1 and 3). The calculated tolerance interval, (Reference 2, Table A-6), indicates that with 95% confidence, at least 95% of future samples should be between 169 and 225 mg/mL for the 187.5 mg/mL dosing concentration. This is equivalent to $\pm 14.2\%$ of the mean sample concentration. The SC-19129 in suspension vehicle is considered homogeneous for the 187.5 mg/mL dosing concentration.

Since the results indicate the SC-19129 in suspension vehicle is homogeneously distributed at 62.5 and 187.5 mg/mL, all the suspensions of SC-19129 made between 62.5 and 187.5 mg/mL are also homogeneously distributed.

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Table 4

Homogeneity of SC-19129 in Suspension Vehicle

Report of Analysis	85-1169	85-1171		
Dosing Concentration	62.5 mg/mL	187.5 mg/mL		
	Sample	mg/mL	Sample	mg/mL
	G00204	72.2	G00216	194
	G00205	*	G00217	182
	G00206	73.6	G00218	208
	G00207	70.6	G00219	198
	G00208	63.1	G00220	193
	G00209	69.7	G00221	200
	G00210	70.8	G00222	207
	G00211	70.0	G00223	192
	G00212	68.6	G00224	195
\bar{X}		69.8		197
s		3.1		8
Normality Correlation Coefficient		0.922		0.971
Tolerance Interval		± 11.6		± 28

* Sample spilled during preparation

Notebook Reference: M. Napier, PDAD-0103, pp. 42-56

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Concentration of Test Article in Carrier

The concentration of SC-19129 (Lot 84K-047-101) in 0.5% methyl cellulose - 0.1% polysorbate 80 was determined at the month 2 treatment phase time point. The target concentrations were 0 (control), 62.5, 125, and 187.5 mg/mL. The analysis was conducted using a stability indicating HPLC method (M85-001-A). The results of the analysis are presented in Tables 5 and 6.

Table 5

In-Study Concentration for
SC-19129 in Suspension Vehicle

Dosing Control Samples

Report of Analysis 85-1168

Dosing Concentration 0 mg/mL

Sample

G00201	Less than 0.00195
G00202	Less than 0.00195
G00203	Less than 0.00195

Notebook Reference: M. Napier, PDAD-0103, pp. 42-60

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

Table 6

In-Study Concentration for
SC-19129 in Suspension Vehicle

Month 2 Treatment Phase

85-1169		Report of Analysis 85-1170		85-1171	
62.5 mg/mL		Dosing Concentration 125 mg/mL		187.5 mg/mL	
Sample		Sample		Sample	
G00204	72.2	G00213	139	G00216	194
G00205	*	G00214	139	G00217	182
G00206	73.6	G00215	141	G00218	208
G00207	70.6			G00219	198
G00208	63.1			G00220	193
G00209	69.7			G00221	200
G00210	70.8			G00222	207
G00211	70.0			G00223	192
G00212	68.6			G00224	195
\bar{X}	69.8		140		197
s	3.1		1		8

* Sample spilled during preparation

Notebook Reference: M. Napier, PDAD-0103, pp. 42-60

ANALYTICAL SUMMARY
Product Development Analytical Department

Page 10 of 11

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

References:

1. Ryan, Jr., T. A., Joiner, B. L., and Ryan, B. F., "MINITAB Student Handbook", 1976, Wadsworth Publishing Co., Inc.
2. Natrella, M. G., "Experimental Statistics, National Bureau of Standards Handbook 91", 1963, US Government Printing Office
3. Filliben, J., Technometrics, 17 (1), 111 (1975)

ANALYTICAL SUMMARY
Product Development Analytical Department

Page 11 of 11

Subject: SC-19129

Summary Number: F-344-034-12A

Applicable to SA Study Number: 2643

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.

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

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 2642

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

Authors:

Gary Chmielewski 2/5/86
Gary Chmielewski, M.A. Date
Research Associate
Product Safety Assessment

James W. Noveroske Feb. 5, 1986
James W. Noveroske, Ph.D. Date
Study Director
Product Safety Assessment

Approvals:

Martin A. Sidor 2/5/86
Martin A. Sidor, D.V.M., M.S. Date
Director
Laboratory Animal Resources
Product Safety Assessment

Frederick M. Radzialowski 2/5/86
Frederick M. Radzialowski, Ph.D. Date
Acting Director, Toxicology
Product Safety Assessment

Frederick M. Radzialowski 2/5/86
Frederick M. Radzialowski, Ph.D. Date
Senior Director
Product Safety Assessment

February 5, 1986

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

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DEPARTMENT OF PRODUCT SAFETY ASSESSMENT

G. D. Searle & Co., Skokie, IL

Title: A Range-Finding Study of SC-19200 in Pregnant Rabbits

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.

Author: James W. Noveroske and Gary Chmielewski

Study No.: S.A. 2642

Date: February 5, 1986

Type of Report: Final

Summary:

SC-19200 was administered once daily by oral intubation to six rabbits per group from days 6 through 18 of gestation at dosage levels of 125, 250, 500, 750, and 1000 mg/kg/day. A control group received the vehicle, 0.5% methylcellulose and 0.1% polysorbate 80, in the same dosing regimen as the drug-treated groups.

Clinical signs of decreased food intake or not eating occurred in all groups, but with greater frequency at the higher dosage levels.

Although several deaths occurred due to intubation errors, no compound-related deaths were attributable to SC-19200 at dosage levels of 750 mg/kg/day or less. At 1000 mg/kg/day, some of the deaths were considered compound related.

Average maternal body weights were unaffected at dosage levels of 125, 250, and 500 mg/kg/day. At 750 and 1000 mg/kg/day average maternal body weights decreased during dosing but were significant only on day 16 of gestation in the 1000 mg/kg/day group.

Examination of the reproductive status of pregnant rabbits at day 28 of gestation revealed no embryotoxic or fetotoxic effects of SC-19200.

S.A. 2642

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

INTRODUCTION

The study was conducted 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 a teratology study.

MATERIALS AND METHODS

Thirty-six female rabbits (New Zealand White strain, Hazleton-Dutchland, Denver, PA) approximately 4 months of age and weighing 3.12 to 4.59 kg, were divided into 6 groups of 6 rabbits each for this study.

Each rabbit was artificially inseminated (day 0 of gestation) with approximately six million motile spermatozoa contained in 0.25 ml of 0.9% physiological saline, and then injected intravenously via the marginal ear vein with 50 USP units of chorionic gonadotropin to help induce ovulation. The females were then assigned to treatment groups using a block design of random permutations, and given unique identification numbers using an ear tag. The rabbits were individually housed in stainless steel cages, and given approximately 150 g of Certified Purina Rabbit Chow #5322 per day and had free access to municipally supplied tap water throughout the study.

The animal room was maintained at $65^{\circ}\pm 5^{\circ}\text{F}$ temperature and 25% or greater relative humidity with a 12-hour light and 12-hour dark cycle. The study was started on September 10, 1985 and terminated on October 9, 1985.

SC-19200, N-L- β -aspartyl-L-phenylalanine was given to 5 groups of 6 rabbits each for 13 consecutive days (days 6 through 18 of gestation) at dosage levels of 125, 250, 500, 750, and 1000 mg/kg/day. SC-19200 was administered orally by gavage as a suspension of 0.5% methylcellulose (w/v) and 0.1% polysorbate 80 (v/v) in distilled water. The doses of SC-19200 Lot #N41-30, were prepared as fresh suspensions daily and the initial quantity (4 ml/kg) administered was based on the most recent body weight. The quantity was then increased to 6 ml/day (See Protocol Amendment #2) in an attempt to decrease the difficulty of dosing due to viscous consistency of the suspension. The sixth group of rabbits served as controls and received the vehicle, 0.5% methylcellulose and 0.1% polysorbate 80 in distilled water, in the same volume as the drug-treated rabbits.

The identity, strength, purity, and composition of the test article were determined before use and the test article was analyzed for a minimum of identity after use. The results of the test article analyses are shown in Appendix B.

Body weights of the rabbits were recorded on days 0, 6, 8, 10, 13, 16, 19, and 28 of gestation. The rabbits were examined daily for adverse clinical signs throughout the study, sacrificed by an overdose of an euthanizing agent injected via the marginal ear vein on day 28 of gestation, and the reproductive organs exposed to obtain the numbers of corpora lutea, implantations, resorptions, and live or dead fetuses.

Maternal body weights and body weight changes 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 Levene's test for homogeneity of variance was done. The Kruskal-Wallis test was used to analyze the following variables: numbers of corpora lutea, implantations, resorptions, and live and dead 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. All t-tests were two-tailed and significance levels achieved have been reported for 5% for t-tests, Levene's tests and Mann-Whitney U tests.

The study was conducted at G. D. Searle & Co. and the final report, protocol, amendments, 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	A. Mackenthun
Teratology	G. Chmielewski
Product Development	
Analytical Department	J. Jiu

RESULTS AND DISCUSSION

Although clinical signs of decreased food intake or not eating occurred in all groups, they were more severe at the higher dosage levels. At 750 and 1000 mg/kg/day, there was a decrease in food consumption which started on days 7 or 8 of gestation, and continued throughout dosing or until the animals died. An additional clinical sign of lethargy was noted preceeding death in some animals.

During the study 2, 0, 3, 2, 5, and 6 of 6 animals from the control, 125, 250, 500, 750, and 1000 mg/kg/day groups, respectively, died. However, ten of these deaths (2, 0, 1, 1, 4, and 2 from the control, 125, 250, 500, 750, and 1000 mg/kg/day groups, respectively) were confirmed intubation errors and one additional death at 250 mg/kg/day was probably due to an intubation error. Thus, 2 of 2, 0 of 0, 2 of 3, 1 of 2, 4 of 5, and 2 of 6 deaths from the control, 125, 250, 500, 750, and 1000 mg/kg/day groups, respectively, were confirmed or probable intuabtion errors. Therefore, the deaths that occurred at dosage levels of 750 mg/kg/day or less were not considered compound related but were the result of intubation errors attributable to excessive struggling by animals being held for dosing and some resistance that made it extremely difficult to introduce the gavage needle into the esophagus. The remaining three deaths, one each at 250, 500, and 750 mg/kg/day were not considered compound related, but probably undetected intubation errors. At 1000 mg/kg/day, 4 of 6 deaths were considered compound related.

Two animals from the 250 mg/kg/day and 1 animal from the 750 mg/kg/day group aborted prior to day 28. These

abortions were not considered compound related, because there was no dose response, no abortions occurred at 500 mg/kg/day, and furthermore, spontaneous abortions are not uncommon in rabbits.

At dosage levels of 125, 250, and 500 mg/kg/day, average maternal body weight gain was unaffected compared to that of the control group (Tables 1 and 3). Average maternal body weights for the 750 and 1000 mg/kg/day groups decreased throughout dosing, but were statistically significant ($p < 0.05$) only on day 16 of gestation in the 1000 mg/kg/day group when compared to that of the control group.

Examination of the reproductive status of pregnant rabbits at day 28 of gestation revealed no adverse effects on average numbers of corpora lutea, implantations, resorptions, and live or dead fetuses per litter at 125, 250, and 500 mg/kg/day of SC-19200 (Tables 2 and 4). No surviving pregnant animals were available for evaluation in the 750 or 1000 mg/kg/day groups.

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. One known deviation occurred as follows:

1. Animal room temperature levels rose above protocol specified limits for approximately 2 hours on September 14, 1985, and for approximately 4 hours on October 5, 1985.

However, this deviation 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 RABBITS
Maternal Body Weights

		(mg/kg/day)				
	Control	125	250	500	750	1000
Average Body Weight (Kg)						
Day 0	3.53	3.67	3.81	3.70	3.78	3.66
Day 6	3.47	3.66	3.77	3.70	3.75	3.66
Day 8	3.48	3.70	3.75	3.71	3.68	3.61
Day 10	3.48	3.68	3.75	3.65	3.65	3.41
Day 13	3.66	3.74	3.79	3.68	3.41	3.25
Day 16	3.66	3.84	3.84	3.69	3.17	2.96*
Day 19	3.63	3.82	3.80	3.71	3.38	- ^a
Day 28	3.78	4.04	3.75	3.77	-	-
Change						
Days 0-6	-0.06	-0.01	-0.04	0.00	-0.03	0.00
Days 6-19	+0.08	+0.16	-0.05	+0.05	-0.59 ^b	-
Days 19-28	+0.14	+0.22	+0.13	+0.06	-	-
Days 0-28	+0.09	+0.37	+0.14	+0.11	-	-

* Significantly different ($p < 0.05$) from control

-^a No surviving pregnant animals

^b Represents only 1 animal

TABLE 2

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

Reproductive Status Of Females At Sacrifice

	(mg/kg/day)					
	Control	125	250	500	750	1000
Females						
Total No.	6	6	6	6	6	6
No. Live Pregnant	4	5	2	4	0	0
No. Live Not Pregnant	0	1	0	0	0	0
No. aborted	0	0	1 ^a	0	1	0
No. Died Pregnant	2	0	3	2	4	6
No. Died not Pregnant	0	0	0	0	1	0
Corpora Lutea						
Total No.	39	54	22	44	- ^b	-
No./Pregnant Female	9.8	10.8	11.0	11.0	-	-
Implantations						
Total No.	31	44	21	33	-	-
No./Pregnant Female	7.8	8.8	10.5	8.3	-	-
Resorptions						
Total No.	8	1	3	2	-	-
No./Pregnant Female	2.0	0.2	1.5	0.5	-	-
Fetuses						
Total No.	23	43	18	31	-	-
No. Live	23	43	17	31	-	-
No. Dead	0	0	1	0	-	-
No. Live/Pregnant Female	5.8	8.6	8.5	7.8	-	-
No. Dead/Pregnant Female	0	0	0.5	0	-	-

^aExcludes one animal which aborted and later died^bAll animals aborted or died prior to Day 28 sacrifice

TABLE 3

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

Individual Female Body Weights

0 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		0	6	8	10	13	16	19	28
85-1551	Pregnant	4.10	3.80	3.81	3.83	3.91	3.97	3.99	4.25
85-1552	Pregnant	3.87	3.70	3.73	3.74	3.91	3.72	3.64	3.64
85-1553	Pregnant	3.32	3.33	3.32	3.30	3.28	3.40	3.35	3.45
85-1554	Pregnant	3.44	3.40	3.39	3.41	3.53	3.54	3.56	3.77
85-1555	Pregnant	3.19	3.23	3.28	3.11	— ^a	—	—	—
85-1556	Pregnant	3.22	3.32	3.34	—	—	—	—	—

—^a Animal died

TABLE 3 (Cont.)

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

Individual Female Body Weights

125 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		0	6	8	10	13	16	19	28
85-1557	Pregnant	3.71	3.77	3.81	3.82	3.92	4.01	4.05	4.24
85-1558	Pregnant	3.77	3.76	3.81	3.78	3.91	3.99	3.98	4.23
85-1559	Pregnant	4.21	4.04	4.07	4.10	4.17	4.32	4.25	4.46
85-1560	Not Pregnant	3.51	3.55	3.57	3.60	3.58	3.54	3.45	3.68
85-1561	Pregnant	3.25	3.34	3.38	3.27	3.30	3.40	3.38	3.63
85-1562	Pregnant	3.41	3.40	3.44	3.44	3.40	3.50	3.45	3.65

TABLE 3 (Cont.)

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

Individual Female Body Weights

250 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		0	6	8	10	13	16	19	28
85-1563	Pregnant	3.92	3.89	3.83	3.86	3.83	3.79	3.66	3.81
85-1564	Pregnant	3.75	3.74	3.69	3.73	3.67	— ^a	—	—
85-1565	Pregnant	4.59	4.50	4.39	4.42	4.43	4.38	4.19	— ^b
85-1566	Pregnant	3.57	3.49	3.55	3.41	—	—	—	—
85-1567	Pregnant	3.32	3.33	3.35	3.39	3.46	3.57	3.58	3.70
85-1568	Pregnant	3.72	3.68	3.68	3.71	3.58	3.65	3.77	x ^c

—^a Animal died—^b Animal abortedx^c Animal aborted and subsequently died

TABLE 3 (Cont.)

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

Individual Female Body Weights

500 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		0	6	8	10	13	16	19	28
85-1569	Pregnant	3.69	3.74	3.69	3.69	3.70	3.81	3.79	3.98
85-1570	Pregnant	3.47	3.48	3.54	3.42	3.51	3.60	3.59	3.37
85-1571	Pregnant	3.64	3.63	3.59	3.63	3.68	3.55	3.61	3.67
85-1572	Pregnant	3.73	3.75	3.76	3.72	— ^a	—	—	—
85-1573	Pregnant	3.84	3.81	3.83	3.63	—	—	—	—
85-1574	Pregnant	3.85	3.81	3.83	3.81	3.82	3.81	3.87	4.07

—^a Animal died

TABLE 3 (Cont.)

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

Individual Female Body Weights

750 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		0	6	8	10	13	16	19	28
85-1575	Pregnant	3.88	3.83	3.74	3.53	— ^a	—	—	—
85-1576	Pregnant	4.41	4.31	4.22	3.87	3.67	—	—	—
85-1577	Pregnant	3.57	3.54	3.44	3.33	3.00	2.82	—	—
85-1578	Pregnant	3.12	3.11	3.03	—	—	—	—	—
85-1579	Not Pregnant	3.40	3.41	3.40	3.22	2.97	2.88	—	—
85-1580	Pregnant	3.94	3.97	3.96	3.85	3.57	3.52	3.38	— ^b

—^a Animal died—^b Animal aborted

TABLE 3 (Cont.)

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

Individual Female Body Weights

1000 mg/kg/day Group

Female	Reproductive Status	Gestation Day							
		0	6	8	10	13	16	19	28
85-1581	Pregnant	3.37	3.44	3.28	2.96	-- ^a	—	—	—
85-1582	Pregnant	3.48	3.48	3.46	3.19	3.02	2.69	—	—
85-1583	Pregnant	3.57	3.54	3.58	3.33	3.04	2.97	—	—
85-1584	Pregnant	3.90	3.92	3.94	3.72	3.50	—	—	—
85-1585	Pregnant	4.01	3.89	3.84	3.84	3.45	3.21	—	—
85-1586	Pregnant	3.63	3.67	3.56	—	—	—	—	—

--^a Animal died

TABLE 4

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

Individual Fetal Data

0 mg/kg/day Group

Animal Number	Reproductive Status	Number of				
		Corpora Lutea	Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-1551	Pregnant	14	12	0	12	0
85-1552	Pregnant	11	6	6	0	0
85-1553	Pregnant	5	5	0	5	0
85-1554	Pregnant	9	8	2	6	0
85-1555	Pregnant	ANIMAL DIED				
85-1556	Pregnant	ANIMAL DIED				

TABLE 4 (Cont.)

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

Individual Fetal Data

125 mg/kg/day Group

Animal Number	Reproductive Status	Number of				
		Corpora Lutea	Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-1557	Pregnant	14	8	1	7	0
85-1558	Pregnant	8	8	0	8	0
85-1559	Pregnant	13	12	0	12	0
85-1560	Not Pregnant	0	0	0	0	0
85-1561	Pregnant	10	8	0	8	0
85-1562	Pregnant	9	8	0	8	0

TABLE 4 (Cont.)

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

Individual Fetal Data

250 mg/kg/day Group

Animal Number	Reproductive Status	Number of				
		Corpora Lutea	Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-1563	Pregnant	12	12	2	9	1
85-1564	Pregnant			ANIMAL DIED		
85-1565	Pregnant			ANIMAL ABORTED		
85-1566	Pregnant			ANIMAL DIED		
85-1567	Pregnant	10	9	1	8	0
85-1568	Pregnant			ANIMAL ABORTED AND DIED		

TABLE 4 (Cont.)

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

Individual Fetal Data

500 mg/kg/day Group

Animal Number	Reproductive Status	Number of				
		Corpora Lutea	Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-1569	Pregnant	12	6	0	6	0
85-1570	Pregnant	11	7	0	7	0
85-1571	Pregnant	12	11	1	10	0
85-1572	Pregnant	ANIMAL DIED				
85-1573	Pregnant	ANIMAL DIED				
85-1574	Pregnant	9	9	1	8	0

TABLE 4 (Cont.)

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

Individual Fetal Data

750 mg/kg/day Group

Animal Number	Reproductive Status	Number of				
		Corpora Lutea	Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-1575	Pregnant			ANIMAL DIED		
85-1576	Pregnant			ANIMAL DIED		
85-1577	Pregnant			ANIMAL DIED		
85-1578	Pregnant			ANIMAL DIED		
85-1579	Not Pregnant			ANIMAL DIED		
85-1580	Pregnant			ANIMAL ABORTED		

TABLE 4 (Cont.)

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

Individual Fetal Data

1000 mg/kg/day Group

Animal Number	Reproductive Status	Number of				
		Corpora Lutea	Implant- ations	Resorp- tions	Live Fetuses	Dead Fetuses
85-1581	Pregnant			ANIMAL DIED		
85-1582	Pregnant			ANIMAL DIED		
85-1583	Pregnant			ANIMAL DIED		
85-1584	Pregnant			ANIMAL DIED		
85-1585	Pregnant			ANIMAL DIED		
85-1586	Pregnant			ANIMAL DIED		

PROTOCOL

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

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: March 26, 1985

B. Initiate Dosing: April 1, 1985

C. Initiate Day 28 Sacrifice: April 23, 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 a Teratology study.

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	6
2	SC-19200	125	6
3	SC-19200	250	6
4	SC-19200	500	6
5	SC-19200	750	6
6	SC-19200	1000	6

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 suspended in 0.5% methylcellulose (w/v), 0.1% polysorbate 80 (v/v) in distilled water.
- C. Administration:
 - 1. Route: Orally by gavage.
 - 2. Frequency: Once daily.
 - 3. Duration: The females will be dosed from day 6 through day 18 of gestation. The quantity of control vehicle or test article suspensions will be based on the most recent body weight.
 - 4. Volume: Both the control vehicle and test article suspensions will be given at 4 ml/kg.
- 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 prepared fresh daily.
- F. Estimated Test Article Requirements: 1200g

10. Study Design Conditions:

- A. Animals: 36 virgin female rabbits of the New Zealand White strain (H.A.R.E., Hewitt, N.J.) will be used in this study. The rabbit is widely used as the non-rodent species for teratogenic studies, and a vast amount of historical control data is available. The rabbits will be approximately 4 months of age and weigh approximately 3 to 5 kg at the start of the study. The rabbits will be allowed approximately 3 weeks acclimatization prior to the start of the study.
- B. Husbandry and Diet: Rabbits will be individually housed in stainless steel cages during the study. The rabbits will be given approximately 150 g of Certified Purina Rabbit Chow #5322 per day and have free access to municipally supplied tap water throughout the study. No special analyses of feed and water will be performed since no contaminants known to be capable of interfering with the study are reasonably expected to be present. Animal room temperature will be $65^{\circ} + 5^{\circ}\text{F}$ 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 rabbits will be artificially inseminated (day 0 of gestation) with semen from breeder colony males of the same strain and source. Each female will then receive 50 USP units of a chorionic gonadotropin intravenously via the marginal ear vein to help induce ovulation. The females will then be assigned to treatment groups by using a block design of random permutations 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 rabbits that die will be examined internally to verify reproductive status and to possibly determine cause of death.
- C. Body Weight: Females will be weighed on gestation days 0, 6, 8, 10, 13, 16, 19, and 28.

D. Food Consumption: Estimated for all females throughout the study.

12. Caesarean Section:

On day 28 of gestation, all females will be sacrificed with an overdose of a euthanizing agent. 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. 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: numbers 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 compound-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 3/5/85

Date

B. F. N. Kotsonis, Ph.D.

Diplomate, A.B.T.

Director, Toxicology

Product Safety Assessment: F. N. Kotsonis 3/5/85

Date

C. F. E. Kohn, Ph.D.

Senior Director,

Product Safety Assessment: F. E. Kohn 3/5/85

Date

PROTOCOL AMENDMENT
August 16, 1985

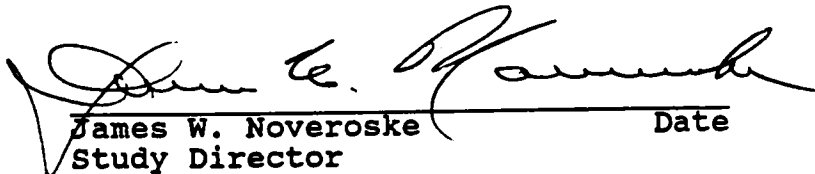
Protocol Amendment #1
SA 2642
A Range-Finding Study of SC-19200 in Pregnant Rabbits

The following is a change to the approved protocol:

1. Page 1, Sect. 4. A. - change "March 26, 1985" to "September 10, 1985".
2. Page 1, Sect. 4. B. - change "April 1, 1985" to "September 16, 1985".
3. Page 1, Sect. 4. C. - change "April 23, 1985" to "October 8, 1985".
4. Page 3, Sect. 10. A. - change "(H.A.R.E., Hewitt, N.J.)" to "(Hazelton-Dutchland, Denver, P.A.)".
5. Page 4, Sect. 13. - fourth sentence add "corpora lutea" to "the following variables: numbers of . . .".
6. Page 4, Sect. 13. - change "Bartlett-Box" to "Levene's".
7. Page 4, Sect. 15. - Senior Director, Product Safety Assessment is now "F. Radzialowski".

Reason for change - original study postponed due to unavailability of test article, intended animals no longer exist, and protocol was lacking current procedures and information.

Approval:


James W. Noveroske
Study Director

Date

Aug. 16, 1985

PROTOCOL AMENDMENT
September 17, 1985

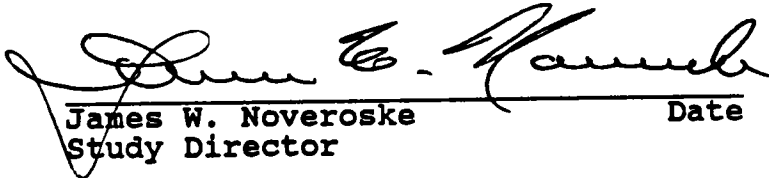
Protocol Amendment #1
SA 2642
A Range-Finding Study of SC-19200 in Pregnant Rabbits

The following is a change to the approved protocol:

1. Page 2, Sect. 9.C.4. - change "given at 4 ml/kg" to "given at 6 ml/kg".

Reason for change - prepared suspensions easier to administer
at 6 ml/kg.

Approval:

 James W. Noveroske Sept. 17, 1985
Study Director Date

S.A. 2642

PROTOCOL AMENDMENT
FEBRUARY 5, 1986


Protocol Amendment #3
SA 2642
A Range-Finding Study of SC-19200 in Pregnant Rabbits

The following is a change to the approved protocol amendment dated September 17, 1985:

1. Subheading - change "Protocol Amendment #1" to "Protocol Amendment #2".

Reason for change - protocol amendment dated September 17, 1985 was incorrectly labeled as amendment #1.

Approval:


James W. Noveroske Date
Study Director

Feb. 5, 1986

S.A. 2642

APPENDIX B

R&D PRODUCT DEVELOPMENT FUNCTION
REPORT REVIEW AND RELEASE

Page 1 of 3

DEPARTMENT: Product Development Analytical

DOCUMENT NUMBER: F-362-035-02

TITLE OF REPORT: SC-19200

TYPE OF REPORT: Analytical Summary in Support of Product Safety
Assessment Study Number 2642

AUTHOR(S):

DATE

REVIEWER(S):

DATE

James J.

26-Nov-85

Michael J. Swenson

11-26-85

APPROVAL:

DATE

James J.

27-Nov-85

TECHNICAL WRITER: Michele Newcomb

Michele Newcomb 11/27/85

APPROVAL FOR RELEASE:

James J. for R. Baum
R. Baum, Director
Analytical Development

02-Dec-85
Date

L. Hansen FOR L. HANSEN
L. Hansen,
Senior Director
Product Development

12/2/85
Date

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

S.A. 2642

B-1

Subject: SC-19200

Summary Number: F-362-035-02

Applicable to SA Study Number: 2642

Test Article Characterization and Stability

Lot N41-30 was analyzed using the release method of testing, released against the current specifications (NS-S85-001-B), 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	N41-30	N41-30
Analysis Report #	85N0418/ 85N0419	85N0863
Completion Date	04/24/85	11/04/85
Identity (IR)	Conforms to standard spectrum	Conforms to standard spectrum
Assay (titration)	100.3% n = 3 s = 0.3	99.3% n = 3 s = 0.6

These results and all other results, coupled with the use of lot N41-30 within its re-evaluation period indicate that lot N41-30 of SC-19200 was suitable for use in this study.

Subject: SC-19200

Summary Number: F-362-035-02

Applicable to SA Study Number: 2642

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.

Plasma and Urine Concentrations of [^{14}C]-SC-19129
and Major Metabolites Following Administration
of [^{14}C]-SC-19129 in the Diet to Pregnant Female Rats

Department of Drug Metabolism
Research and Development Division - G.D. Searle & Co.

MRC-851-0005


Plasma and Urine Concentrations of [^{14}C]-SC-19129
and Major Metabolites Following Administration
of [^{14}C]-SC-19129 in the Diet to Pregnant Female Rats

Study Initiated: January 21, 1985

Study Completed: February 12, 1986

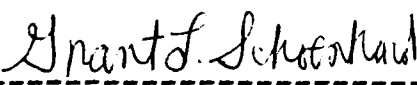
Document Number: MRC-851-0005

Author:

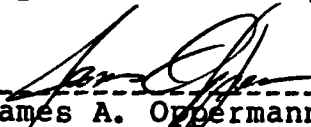
 2/12/86

Earl Burton, Ph.D. Date
Research Scientist
Department of Drug Metabolism

Reviewed and Approved by:

 2/12/86

Grant L. Schoenhard, Ph.D. Date
Section Head, Metabolism
Department of Drug Metabolism

 2/13/86

James A. Oppermann, Ph.D. Date
Director
Department of Drug Metabolism

MRC-851-0005

List of Contributors

Department of Drug Metabolism

Dr. E. Burton, Research Scientist
Mr. K. Hoglund, Biochemist
Ms. I. Dressler, Research Biochemist
Mr. B. Belonio, Biologist
Ms. E. Duarte, Document Preparation Specialist

Physical Methodology Department

Dr. J. Hribar, Senior Research Scientist
Mr. W. Aksamit, Research Associate
Mr. N. Liu, Research Analytical Chemist

Reference Citation

E. Burton, K. Hoglund, I. Dressler, J. Hribar. Plasma and Urine Concentrations of [^{14}C]-SC-19129 and Major Metabolites Following Administration of [^{14}C]-SC-19129 in the Diet to Pregnant Female Rats. Department of Drug Metabolism, Research and Development Division, G.D. Searle & Co. MRC-851-0005, February, 1986.

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**Plasma and Urine Concentrations of [¹⁴C]-SC-19129
and Major Metabolites Following Administration
of [¹⁴C]-SC-19129 in the Diet to Pregnant Female Rats**

I. Abstract

Plasma and urine concentrations of total ¹⁴C and plasma and urine metabolite profiles were determined following administration of [¹⁴C]-SC-19129 in the diet to pregnant rats. The low, medium and high doses administered were 5.8 ± 0.3 mg/kg (mean \pm standard error), 180 ± 10 mg/kg and 950 ± 90 mg/kg respectively.

Mean plasma concentrations of total ¹⁴C at 6 a.m. (beginning of light cycle) were approximately proportional to dose and were 0.86 ± 0.17 mcg/ml (expressed as mcg SC-19129 equivalents/ml), 25 ± 2 mcg/ml and 110 ± 30 mcg/ml for the low, medium and high doses, respectively. [¹⁴C]-SC-19129 was not detected in plasma. The concentrations of [¹⁴C]-SC-19200, the free acid metabolite, in pooled plasma samples at 6 a.m. were 0.29 mcg/ml (expressed as SC-19129 mcg equivalents/ml), 1.9 mcg/ml and 10 mcg/ml for the low, medium and high dose group respectively. The concentrations of [¹⁴C]-SC-19200 in the medium and high dose group plasma at 6 a.m. were approximately proportional to dose. [¹⁴C]-Phenylalanine, [¹⁴C]-phenylacetic and [¹⁴C]-phenylacetyl glycine were also present in plasma profiles. The concentration of [¹⁴C]-phenylacetic acid was disproportionately high in the high dose group plasma (2.0 mcg/ml) compared to the concentration in the medium dose group plasma (0.18 mcg/ml).

The percentages of dose excreted in the 0 to 24 hour urine as total ^{14}C were $9.6 \pm 0.4\%$, $39 \pm 3\%$ and $33 \pm 12\%$ for the low, medium and high dose groups, respectively. Approximately 85%, 95% and 95% of the radiolabel in the urine samples from the low, medium and high dose animals respectively, was present in the form of [^{14}C]-phenylacetylglycine.

The results indicate dose proportional absorption from the dietary admix dosage form but suggest dose dependence in the route of elimination.

**Plasma and Urine Concentrations of [¹⁴C]-SC-19129
and Major Metabolites Following Administration
of [¹⁴C]-SC-19129 in the Diet to Pregnant Female Rats**

II. Introduction

SC-19129 (N-L- β -aspartyl-L-phenylalanine, 1-methyl ester, β -APM) and its free acid SC-19200 (N-L- β -aspartyl-L-phenylalanine, β -AP), have been identified as conversion products of aspartame (SC-18862, N-L- α -aspartyl-L-phenylalanine methyl ester, APM) in sweetened soft drinks (1).

The purpose of this study was to determine the concentrations of SC-19129, if present, and its major metabolites in plasma and urine following administration of [¹⁴C]-SC-19129 in the diet to pregnant rats. The study was primarily designed to allow examination of the relationship of plasma concentrations to dosage over a range of doses likely to be used in toxicity studies.

III. Materials and Methods

A. Overview of Study Design:

Pregnant female rats were fed diets containing [¹⁴C]-SC-19129 at 3 dose levels. Plasma and urine samples were collected. Total radioactivity was determined for all samples. Pooled plasma and urine samples were examined by high performance liquid radiochromatography (HPLC) for SC-19129, SC-19200 (β -AP) the free acid of SC-19129, and other major metabolites.

B. Test Article and Dosage Forms:

[U-¹⁴C-Phe]-SC-19129 was prepared by the Radiochemistry Group, G.D. Searle & Co. The radiolabeled test article was supplied at two specific activities to facilitate the preparation of diet mixtures at 3 dosage levels as described in the protocol (Section X.9.C). Lot MRC-532-118-1 had a specific activity of 32.5 mCi/mg (9.55 mCi/mmol) and was used to prepare the low dose diet mixture. Lot MRC-532-155-1 had a specific activity of 0.730 mCi/mg (0.228 mCi/mmol) and was used to prepare the medium and high dose diet mixtures. The actual concentration of each test article-diet mixture, prepared with Purina Certified Rodent Chow 5002 as described in the protocol (Section X.9.C.), is given in Table A-1 (Appendix 1; Section IX). The actual specific activities of the radiolabeled constituents measured in plasma and urine (Section IV) may have been somewhat lower than the specific activities of the [¹⁴C]-SC-19129 administered in the diets due to the

administration of unlabelled SC-19129 diet mixtures (similar respective concentrations) for 24 hours prior to administration of the [^{14}C]-SC-19129 diets (Section X.9.D.2). This was done to acclimatize the animals to the dosage form.

C. Animals, Animal Treatment and Test Article Administration:

Female CD rats (Charles River Breeding Laboratory, Portage, MI) weighing 240-290 g at the time of dosing were used. Pregnant animals were provided by the Reproductive Toxicology Group, G.D. Searle & Co. The animals were housed and fed as delineated in the protocol (Section X.10). The [^{14}C]-SC-19129 diet mixture was made available ad libitum for a 24 hour period from approximately 5 p.m. on day 7 of gestation until approximately 5 p.m. on the following day. The actual doses received were calculated as described in the protocol (Section X.9.D.3).

D. Sample Collection:

1. Plasma:

Three blood samples were collected from each animal, two via a tail vein and the third by cardiac puncture, as described in the protocol (Section X.11.B). Diethyl-p-nitrophenyl phosphate (esterase inhibitor) was added to each blood collection tube to give a final concentration of approximately 1×10^{-4} molar. Plasma was prepared and an aliquot taken for total radioactivity determination. The remainder of each plasma sample was stored frozen at approximately -20°C until analysis. [^{14}C]-SC-19129 was added to control rat plasma (approximately 10 mcg/ml plasma) which had been pre-treated with 1×10^{-4} molar diethyl p-nitrophenyl

phosphate. Aliquots of the control plasma containing [^{14}C]-SC-19129 were analyzed prior to, and following, frozen storage.

2. Urine:

Urine was collected as described in the protocol (Section X.11.C) and stored frozen at approximately -20°C until analysis. Control urine was spiked with [^{14}C]-SC-19129 (50 mcg/ml) and stored frozen until analysis.

E. Sample Analysis:

1. [^{14}C]-SC-19129-Diet-Mixtures:

Five weighed samples from each test article diet mixture were oxidized using a sample oxidizer (Packard Model 306, Packard Instruments Co., Downers Grove, IL). Total ^{14}C in the combustion products was determined by liquid scintillation counting (LSC, Section III.H). A 1g sample of each test article diet mixture was extracted (2) and the extract was analyzed by HPLC (Section III.F) to determine the radiochemical purity of the [^{14}C]-SC-19129 in the diet mixture.

2. Plasma:

Total ^{14}C was determined by LSC (Section III.H) using duplicate aliquots. Extracts of pooled plasma or spiked control plasma were prepared for HPLRC analysis (Section III.f) by mixing 0.5 ml of plasma with 0.5 ml of distilled water and then adding 0.025 ml of formic acid (88%) while mixing on a vortex mixer. The sample was applied to a 500 mg C18 Bond Elut[®] column which had been preconditioned by washing sequentially with one column volume each of methanol and 0.1 molar phosphate buffer, pH 7. Labelled compounds were eluted from the

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column with 1 ml of water followed by 1 ml of water:acetonitrile (65:35, v/v). The eluants were combined and lyophilized (Freeze-Dryer-8, Labconco Corp., Kansas City, MO). The dried samples were dissolved in 0.4 ml of mobile phase (Section III.F) and filtered through a 0.45 micron filter (Gelman Acrodisc[®]; Gelman Sciences, Inc., Ann Arbor, MI).

3. Urine:

Total ¹⁴C in duplicate aliquots of each urine sample was determined by LSC (Section III.H). One ml aliquots of pooled urine or spiked control urine were applied to 500 mg Cl8 Bond Elut[®] columns which had been preconditioned as described above (Section III.E.2). Labelled compounds were eluted from the column with 1 ml of water followed by 1 ml of methanol. The water and methanol eluants were combined, lyophilized and the residue dissolved in mobile phase (Section III.F).

F. High-Performance Liquid Radiochromatography (HPLRC-System for Profiles of Radioactivity in Plasma and Urine:

Extracts of diet mixtures, plasma and urine were profiled by HPLRC (3) on a Supelcosil LC-8-DB column (15 cm x 4.6 mm; Supelco, Inc., Bellefonte, Pa) using a mobile phase of methanol:0.18 M monobasic sodium phosphate, pH 2.0 containing 0.2 molar heptane sulfonic acid 28:72, v/v) and flow rate of 1.0 ml/min. The extracts were dissolved in the mobile phase prior to injection onto the column. Unlabelled standards of SC-19129, β -AP, phenylalanine and tyrosine, used to calibrate the system, were detected by absorbance at 210 nm using a model 480 variable wavelength detector (Waters Associated, Medford, MA). Radiolabelled compounds were detected using a radioactive flow detector Flow-One[®]

Model CU, RadioAnalytic, Inc., Tampa, FL). The effluent from the HPLC column and Flo-Scint® III (RadioAnalytic, Inc., Tampa, FL) were mixed at a ratio of 1.0 ml/min to 5.5 ml/min in the Flo-One® mixing chamber. Counting efficiency was determined by mixing HPLRC mobile phase containing a known amount of radioactivity (Oxi-Test® CO, RadioAnalytic, Inc., Tampa, FL) and Flo-Scint® III in the above ratio and counting the mixture in the Flo-One in the stopped-flow mode.

G. Isolation and Identification of Metabolites from Urine:

Pooled urine from the high dose group (5 ml) was adjusted to pH 1 and extracted with 2 volumes of toluene. The toluene extract was evaporated to dryness and reconstituted in water. Aliquots of the reconstituted extract were applied to a C18 column (3.9 mm x 15 cm; μ Bondapak C18, Waters Associates, Milford, MA) equilibrated with a mobile phase consisting of 0.028 M sodium acetate in water, pH 4.95:methanol (95:5, v/v) (4).

The column was eluted with the same mobile phase at a flow rate of 1 ml/min. Fractions were collected for consecutive 0.4 min intervals with a fraction collector and radioactive compounds were detected by LSC (Section III.H) of an aliquot of each fraction. Metabolite peaks were observed with retention times of 12 minutes (Metabolite A) and 16 minutes (Metabolite B). Fractions containing the two metabolites were evaporated to dryness. Derivatives of Metabolite A and Metabolite B were prepared by treatment with thionylchloride in methanol at 60° C for 15 minutes and by treatment with bis(trimethylsilyl) trifluoroacetamide (BSTFA) in acetonitrile at 60° C for 15 minutes, respectively.

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Mass spectra were obtained with a Finnegan 4023 GCMS (Finnegan MAT, San Jose, CA) with electron impact ionization (EI) at 0.3 torr source pressure and 200° C source temperature. The samples were chromatographed on a 15 meter X 25 mm DB-1 fused silica capillary column (J & W Scientific Inc., Rancho Cordova, CA) coupled directly to the mass spectrometer. The column was kept at 10 psi helium and the sample was injected with the column temperature at 100° C. The temperature was increased to 250° C at a programmed rate of 15° C/min beginning one minute after sample injection.

H. Radioactivity Measurements:

Samples of 0.050 ml plasma were mixed with 10 ml of PCS° (Amersham Corp., Arlington Heights, IL). Samples of 0.10 ml urine were mixed with 4 ml water and then mixed with 5 ml of PCS° to form a stable gel. The combustion products from oxidized test article diet mixtures were mixed with 9 ml of Carbosorb° and 12 ml of Permafluor V (both from Packard Instruments Co., Downers Grove, IL). Radioactivity was measured with scintillation spectrometers (Mark II or Mark III, Tracor Analytic, Elk Grove Village, IL). Counting efficiency was determined by the automatic external standard channels ratio method.

I. Protocol Deviations:

1. Dosage:

[¹⁴C]-SC-19129 diet mixtures were prepared, to give intended doses of 10, 250 and 1000 mg/kg, based on body weights and projected food consumptions. However the low and medium doses were lower than intended (Table 1) due to consumption of less than the projected amounts of

the test article-diet mixtures. The intended high dose was achieved (Table 1) by increasing the [¹⁴C]-SC-19129 content of the diet to compensate for the observed food consumption (the high dose group was the last of three groups to be dosed). Since the intent of the study design was to allow examination of the relationship of plasma concentrations to dosage over a range of doses likely to be used in toxicity studies, and since the intended high dose was attained, the lower than intended low and medium doses are not considered to have adversely affected the study.

2. Body-Weight:

Animals weighing 250-350 g at the time of dosing were specified. The actual weight range was 240-290 g (Table A-1). Since the actual weight range was narrower than the specified range, the inclusion of one animal smaller than 250 g is not considered to have adversely affected the study.

3. Number-of-Animals:

Five animals were included initially in each dosage group. One medium dose animal (rat 10) died during blood sample collection at 9 a.m. and one high dose animal (rat 13) died during blood sample collection at 6 a.m. The 5 p.m. plasma samples from two medium dose animals (rat 11 and rat 12) were lost due to breakage of the sample tubes during centrifugation. One additional animal (rat 7) was administered the medium dose test article-diet mixture (on the day following treatment of the first 5 animals) and samples were collected as specified by the protocol in order to provide a minimum of three plasma samples for the medium dose group 5 p.m. time point. Samples from at least 4 animals were available for the analyses of urine and of plasma taken

at 6 a.m. and 9 a.m. This number of samples is considered to be adequate for evaluation of the relationship of plasma concentrations, and percentages of dose excreted in the urine, to dose.

IV. Results and Discussion

A. Doses Received and Radiochemical Purity of Dosage Forms:

The doses received by the low, medium and high dose groups were 5.8 ± 0.3 (mean \pm standard error [SEM]) mg/kg, 180 ± 10 mg/kg and 950 ± 90 mg/kg respectively (Table 1). Doses received by individual animals are given in Table A-1 (Section IX). The radiochemical purity of [^{14}C]-SC-19129 extracted from the diet mixtures (Section III.E.1) was $99.6 \pm 0.2\%$ (mean \pm SEM of the three dose groups).

B. Recovery of [^{14}C]-SC-19129 from Control Plasma and Urine:

The recovery of [^{14}C]-SC-19129 from control urine with the Bond Elut[®] procedure (Section III.E.3) was 88.6%. The percentage of the radioactivity extracted from spiked control urine which was present at the retention time of [^{14}C]-SC-19129 in HPLRC profiles was 96.3% when the urine was extracted immediately and 95.6% after 6 days storage at -20°C .

The recovery of [^{14}C]-SC-19129 from control plasma was 66.3% with the Bond Elut[®] procedure (Section III.E.2). HPLRC analysis showed that 96.3% and 95.2% of the extracted radioactivity had the HPLRC retention time appropriate for SC-19129 when the extraction was performed immediately or after 5 days storage at -20°C , respectively.

C. Total Radioactivity in Plasma:

Mean plasma concentrations of total ^{14}C are given in Table 1 and shown in Figure 1. The ratios of total ^{14}C concentrations to dose were very similar (dose

Proportional) for the low and medium dose groups but the mean plasma total ^{14}C concentration in the high dose group was somewhat lower than expected (based on the low and medium dose values) for dose proportionality. This departure from dose proportionality by the high dose group can be attributed to very low concentrations in one animal (rat 14; Table A-2, Section IX) which is considered to be an outlier. The remaining high dose animals had plasma total ^{14}C concentrations consistent with dose proportionality (Table A-2). These results are consistent with absorption of approximately the same percentage of the radiochemical dose from the range of doses administered (5.8 mg/kg to 950 mg/kg) in the diet in this study.

D. Distribution of Plasma Radioactivity:

HPLRC profiles of plasma extracts revealed very little, if any, intact [^{14}C]-SC-19129 (<2% of total radiolabel). However SC-19200 (β -AP) and phenylalanine (identified by comparison of HPLRC retention times with authentic standards; 8.5 minutes and 7.5 minutes respectively) and at least two other major metabolite peaks (retention times of approximately 5.5 minutes and 9.5 minutes) were present in HPLRC profiles (Figures 2 and 3). The metabolites were identified as phenylacetyl glycine (5.5 minute peak) and phenylacetic acid (9.5 minute peak) by isolation and identification of the same metabolite peaks from urine (Section IV.G). Plasma concentrations of [^{14}C]-SC-19200 were approximately proportional to dose at 6 a.m. (Table 1; Figure 4). HPLRC profiles of the 5 p.m. plasma samples showed no detectable [^{14}C]-SC-19200 in the low and medium dose groups, while [^{14}C]-SC-19200 was still present

in the high dose group. The fact that [^{14}C]-SC-19200 was present in plasma from high dose animals 24 hours (5 p.m.) after initiation of dosing with the diet admixture indicates that SC-19200 plasma concentrations may increase somewhat during chronic administration of high doses of SC-19129.

The average plasma concentration of [^{14}C]-phenylacetic acid at 9 a.m. was disproportionately high in the high dose group (Figure 3) compared to the medium dose group (Figure 2). The [^{14}C]-phenylacetic acid concentrations (adjusted for molecular weight) in the medium dose (180 mg/kg) and high dose (950 mg/kg) groups were calculated to be 0.18 mcg/ml and 2.0 mcg/ml respectively.

E. Urinary Excretion of Total Radioactivity:

The percentages of the administered radiolabeled doses excreted in the 24 hour urine samples were $9.6 \pm 0.4\%$ (mean \pm standard error), $39 \pm 3\%$ and $33 \pm 12\%$ for the low, medium and high dosage groups respectively (Table 1). The somewhat lower excretion of total radioactivity (as a percentage of dose) by the high dose group compared to the medium dose group can be attributed to very low excretion by one animal (rat 14; Table A-2, Section IX) which is considered to be an outlier. The remaining animals in the high dose group had urinary excretion values consistent with dose proportionality of the high dose compared to the medium dose group. The percentage of dose excreted in the urine by the low dose animals was clearly lower than the percentages excreted by the medium and high dose animals.

The urinary excretion results for the medium and high doses, taken together with the plasma total ^{14}C results (Section IV.C), indicate absorption of a constant percentage

of the dose from the diet admix dosage form. However the route of elimination is dose dependent, as indicated by the lower percentage of dose excreted in the urine by the low dose group.

F. Distribution of Urinary Radioactivity:

HPLRC profiles of pooled urine samples from the medium and high dose groups are shown in Figure 5. Approximately 95% of the radiolabel was present in a peak eluting at 6 minutes, corresponding to metabolite A in plasma profiles (Figures 2,3). This metabolite was isolated and identified as phenylacetyl glycine (Section IV.G). The small and relatively broad peak eluting between approximately 9 and 11 minutes (Figure 5) contained SC-19200 and a metabolite which corresponded to metabolite B in plasma profiles (Figures 2,3). This peak contained 3% to 4% of the radiolabel present in the urine and represented 1% to 1.5% of the administered dose. Metabolite B was isolated and identified as phenylacetic acid (Section IV.G). HPLRC analysis of pooled urine from the low dose group (not shown in Figure 5) showed that 85% of the radioactivity in the sample was present in peak A (phenylacetyl glycine) and 10% was in the broad peak containing SC-19200 and phenylacetic acid.

G. Metabolite Identification and Metabolic Pathway:

Identification of SC-19200 (β -AP), the free acid of SC-19129, and phenylalanine on chromatograms was made by comparison to the chromatographic properties of authentic standards. Metabolites A and B (Figures 2,3) were isolated from pooled urine and identified by gas chromatography-mass spectrometry (GCMS) as described in Section III.G.

Metabolite A was identified as phenylacetylglutamine by comparison of its HPLC retention time with that of a standard and by comparison of the GC retention time and mass spectrum of the methylated metabolite to that of the methyl ester of the standard. The spectra of both the standard and metabolite (Figure 6) contain a molecular ion of 207 m/z, a base peak of 91 m/z and peaks at 88 m/z, 118 m/z, 148 m/z and 176 m/z containing representative fragments as indicated.

Metabolite B was identified as phenylacetic acid by comparison of the GC retention time and mass spectrum of the trimethylsilyl (TMS) derivative with an authentic standard (Figure 7).

The proposed metabolic pathway of SC-19129 is shown in Figure 8. The methyl ester bond appears to be completely metabolized pre-systemically by enzymes in the intestine and/or liver since no intact SC-19129 was observed in plasma. A minor portion of the dose reaches the systemic circulation as SC-19200, the free acid of SC-19129. A portion of the administered [^{14}C]-SC-19129 is also hydrolyzed at the β -aspartyl bond to release free [^{14}C]-phenylalanine (present in plasma) and presumably free aspartic acid (not labeled). The majority of the radiochemical dose is metabolized to phenylacetylglutamine and excreted as such in the urine. In a study of [^{14}C]-SC-19129 in the rhesus monkey, the major metabolite was found to be phenylacetylglutamine (5). The finding of phenylacetylglutamine as the major metabolite in rat is consistent with known species differences in the conjugation of phenylacetic acid (6). Phenylacetic acid and its amino acid conjugates are normal mammalian metabolites of

phenylalanine (7). However, this is usually a minor pathway in mammals, with hydroxylation to tyrosine being the major route of metabolism (7).

The high proportion of the phenylalanyl moiety of SC-19129 metabolized via phenylacetic acid is hypothesized to be the result of bacterial metabolism in the lower gastrointestinal (GI) tract. This would occur if the absorption of SC-19129 and SC-19200 is sufficiently slow so that a large percentage reaches the lower GI tract where it can be metabolized by bacteria to phenylalanine and phenylacetic acid. The phenylacetic acid would be conjugated with glycine by the rat after absorption from the GI tract.

The N-acetyl derivative of SC-19200 (Figure 8) is also a metabolite in the rat, as shown following intravenous administration of [^{14}C]-SC-19200 (8). This metabolite was not detected in the present study, probably as a consequence of the relatively small amounts of SC-19200 which were absorbed orally.

V. Conclusions

Plasma concentrations of total ^{14}C , and peak plasma concentrations of $[\text{}^{14}\text{C}]\text{-SC-19200}$, the free acid metabolite, were approximately proportional to dose following administration of 5.8 mg/kg, 180 mg/kg and 950 mg/kg doses of $[\text{}^{14}\text{C}]\text{-SC-19129}$ in the diet to pregnant rats. The percentages of dose excreted in the urine as total ^{14}C were approximately equal for the medium and high dose animals, but the percentage for the low dose group was lower. The results are interpreted as indicating dose proportional absorption from the diet admix dosage form, with dose dependence in the route of excretion.

The major metabolites of $[\text{U-}^{14}\text{C-Phe}]\text{-SC-19129}$, in addition to the free acid $[\text{}^{14}\text{C}]\text{-SC-19200}$ and $[\text{}^{14}\text{C}]\text{-phenylalanine}$, were found to be $[\text{}^{14}\text{C}]\text{-phenylacetic acid}$ and its conjugate, $[\text{}^{14}\text{C}]\text{-phenylacetylglycine}$. The $[\text{}^{14}\text{C}]\text{-phenylacetic acid}$ concentration in the pooled plasma of high dose animals was disproportionately high compared to the concentration in pooled plasma from medium dose animals.

VI. References

1. Igyarto, M. High Performance Liquid Chromatography Method for Mock Beverage Stability Studies. Nutrasweet™ Research and Development Department, G.D. Searle & Co. NS-M84-019-A, December, 1984.
2. Napier, M. and Boettcher, J. HPLC Method for SC-19129 in Rodent Diet Admixture. Research and Development Division, G.D. Searle & Co. M84-045-A, November, 1984.
3. Wynne, B.J., Schmidt, R.E., Hill, J. and Schoenhard, G.L. Method Development and Validation of the High Performance Liquid Chromatographic Assay for N-L- β -Aspartyl-L-Phenylalanine (β -AP) in Rat Plasma. Department of Drug Metabolism, Research and Development Division, G.D. Searle & Co., MRC-851-0013, June, 1985.
4. Gusovsky, F., Fawcett, J., Javaid, J.I., Jeffries, H. and Sabelli, H. A High-Pressure Liquid Chromatography Method for Plasma Phenylacetic Acid, A Putative Marker for Depressive Disorders. *Analytical Biochem.* 145, 101-105 (1985).
5. Burton, E., Dressler, I., Hoglund, K. and Hribar, J. Pharmacokinetics and Metabolism of [14 C]-SC-19129 in the Rhesus Monkey. Department of Drug Metabolism, Research and Development Division, G.D. Searle & Co. MRC-842-0056, July, 1985.
6. James, M.O., Smith, R.L., Williams, R.T. and Reidenbert, M. The Conjugation of Phenylacetic Acid in Man, Sub-Human Primates and Some Non-Primate Species. *Proc. R. Soc. Lond. B.* 182, 25-35 (1972).
7. Harper, A.E. Phenylalanine Metabolism. In: Stegink, L.D. and Filer, L.J. (Editors) *Aspartame: Physiology and Biochemistry*. Marcel Dekker, Inc., New York, 1984, pp. 141-159.
8. Burton, E., Hoglund, K., and Dressler, I. Pharmacokinetics and Metabolism of [14 C]-SC-19129 and [14 C]-SC-19200 its Free Acid, in the Rat. Department of Drug Metabolism, Research and Development Division, G.D. Searle & Co. MRC-851-0042, February, 1986.

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VII. Table

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Table 1
Plasma Concentrations of Total ^{14}C and [^{14}C]-SC-19200
and Urinary Excretion of Total ^{14}C
Following Administration of [^{14}C]-SC-19129
by Dietary Admix to Pregnant Rats

Dosage Group	Low	Medium	High
Intended Dose (mg/kg)	10	250	1000
Dose Received (mg/kg) ^a	5.8 ± 0.3 (5)	180 ± 10 (6)	950 ± 90 (5)
Plasma Total ^{14}C (mcg equivalents/ml)			
6 a.m.	0.86 ± 0.17 (5)	25 ± 2 (6)	110 ± 30 (5)
9 a.m.	1.2 ± 0.3 (5)	32 ± 2 (6)	120 ± 40 (4)
5 p.m.	1.6 ± 0.3 (5)	53 ± 1 (3)	130 ± 40 (4)
Plasma [^{14}C]-SC-19200 ^c (mcg equivalents/ml)			
6 a.m.	0.29 (5)	1.9 (5)	10 (5)
9 a.m.	0.14 (5)	1.9 (4)	5.4 (4)
5 p.m.	d (5)	d (3)	4.1 (4)
Urinary Excretion Total ^{14}C (% of Dose)	9.6 ± 0.4 (5)	39 ± 3 (5)	33 ± 12 (4)

- a Values are the mean ± standard error (SEM); the number of animals is indicated in parentheses. Individual animal values are given in Table A-1.
- b Values are the mean ± SEM; the number of animals is indicated in parentheses. Individual animal values are given in Table A-2.
- c Values obtained by HPLRC analysis of pooled plasma from the number of animals indicated in parentheses.
- d [^{14}C]-SC-19200 peak not detectable in HPLRC profile.
- e Values are the mean ± SEM; the number of animals is indicated in parentheses. Individual animal values are given in Table A-2.

VIII. Figures

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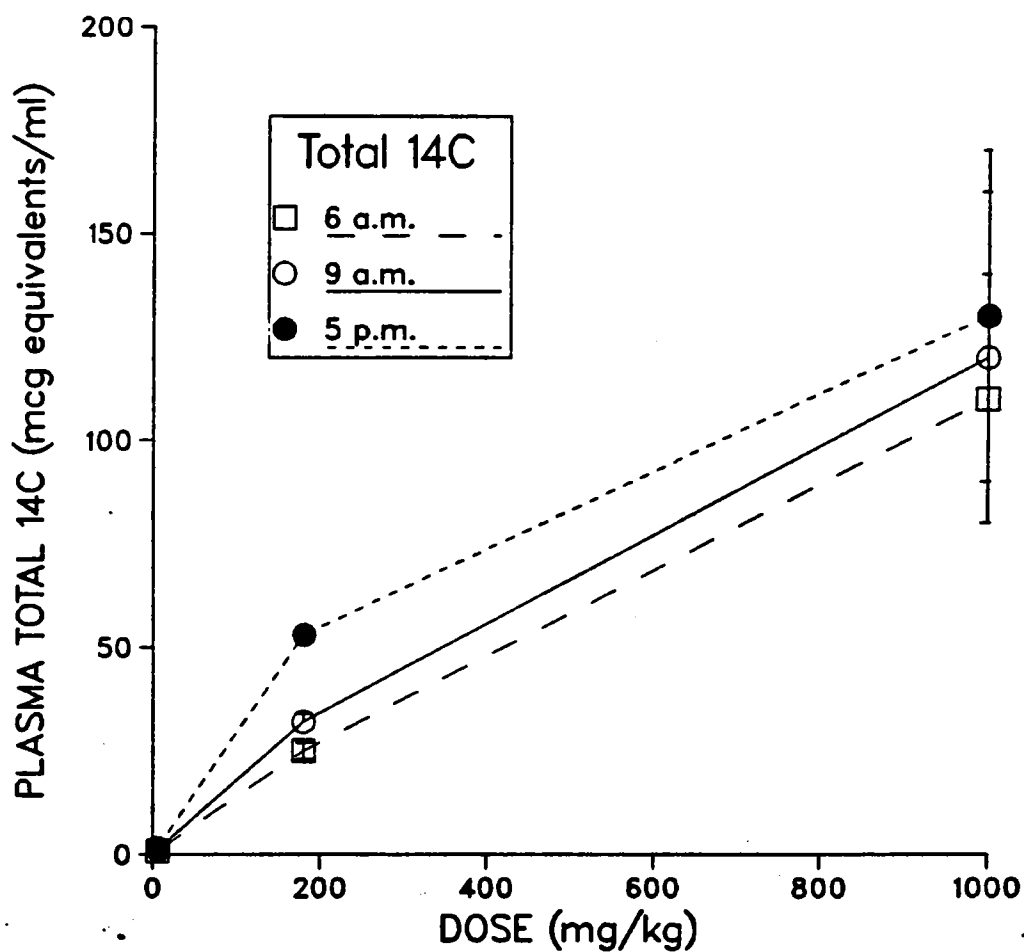


Figure 1. Plasma concentrations of total ^{14}C at approximately 6 a.m. (□), 9 a.m. (○) and 5 p.m. (●) on the day following administration of [^{14}C]-SC-19129 in the diet. Abscissa: average dose received in mg/kg body weight. Ordinate: mean concentrations of total ^{14}C in plasma in mcg equivalents of [^{14}C]-SC-19129/ml. The vertical bars indicate the standard errors of the means, in some cases the standard error bars are completely within the boundaries of the curve symbols.

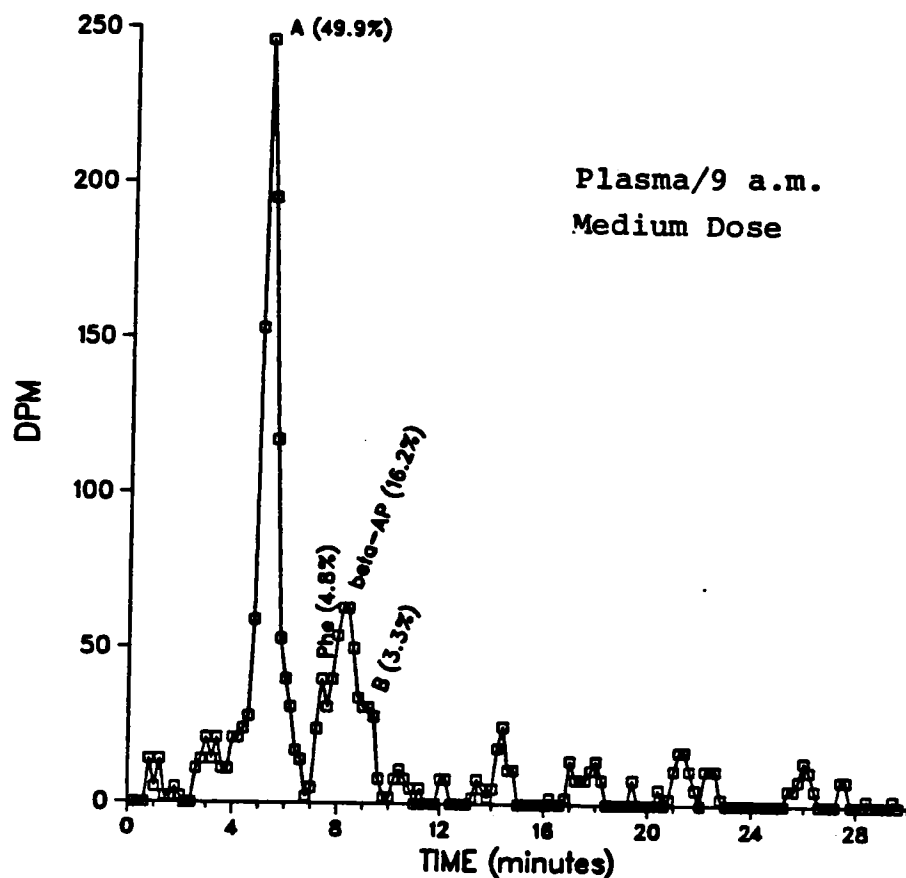


Figure 2. High performance liquid radiochromatogram of the Bond Elut[®] extract of the 9 a.m. plasma sample (pooled) from the medium dose treatment group. The locations of the reference standards of SC-19200 (β -AP) and phenylalanine (Phe) are marked on the chromatogram. Metabolite peaks A and B correspond to phenylacetyl glycine and phenylacetic acid respectively based on isolation and identification of metabolites with the same retention times from urine. The percentages of radioactivity eluted from the column that are associated with the above peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute.

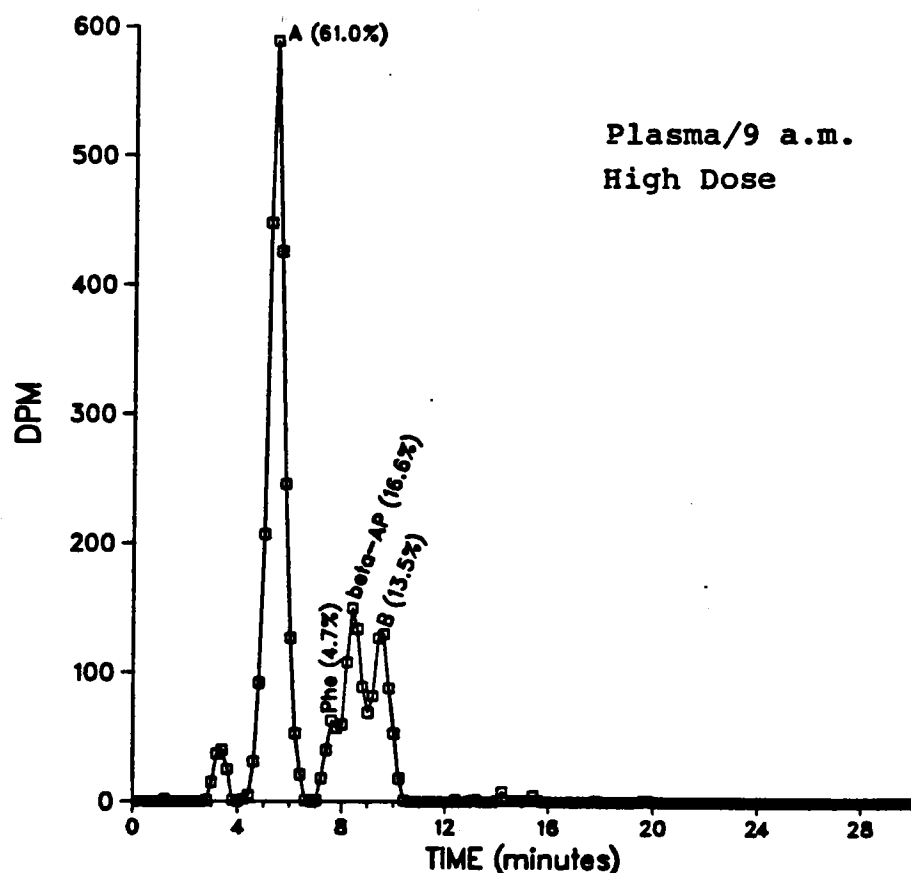


Figure 3. High performance liquid radiochromatogram of the Bond Elut[®] extract of the 9 a.m. plasma sample (pooled) from the high dose treatment group. The locations of the reference standards of SC-19200 (β -AP) and phenylalanine (Phe) are marked on the chromatogram. Metabolite peaks A and B correspond to phenylacetyl glycine and phenylacetic acid respectively based on isolation and identification of metabolites with the same retention times from urine. The percentages of radioactivity eluted from the column that are associated with the above peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute.

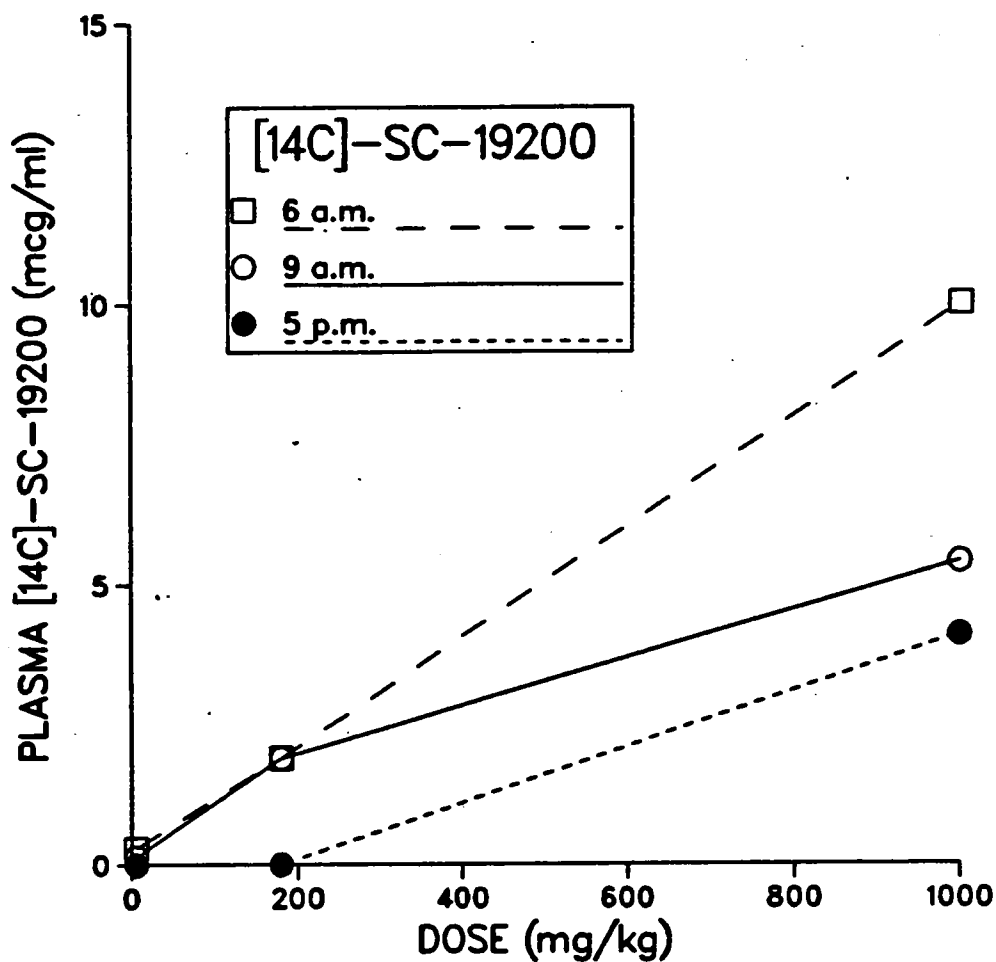


Figure 4. Plasma concentrations of [^{14}C]-SC-19200 at approximately 6 a.m. (\square), 9 a.m. (\circ) and 5 p.m. (\bullet) on the day following administration of [^{14}C]-SC-19129 in the diet. Abscissa: average dose received in mg/kg body weight. Ordinate: [^{14}C]-SC-19200 in pooled plasma samples in mcg equivalents of [^{14}C]-SC-19129/ml.

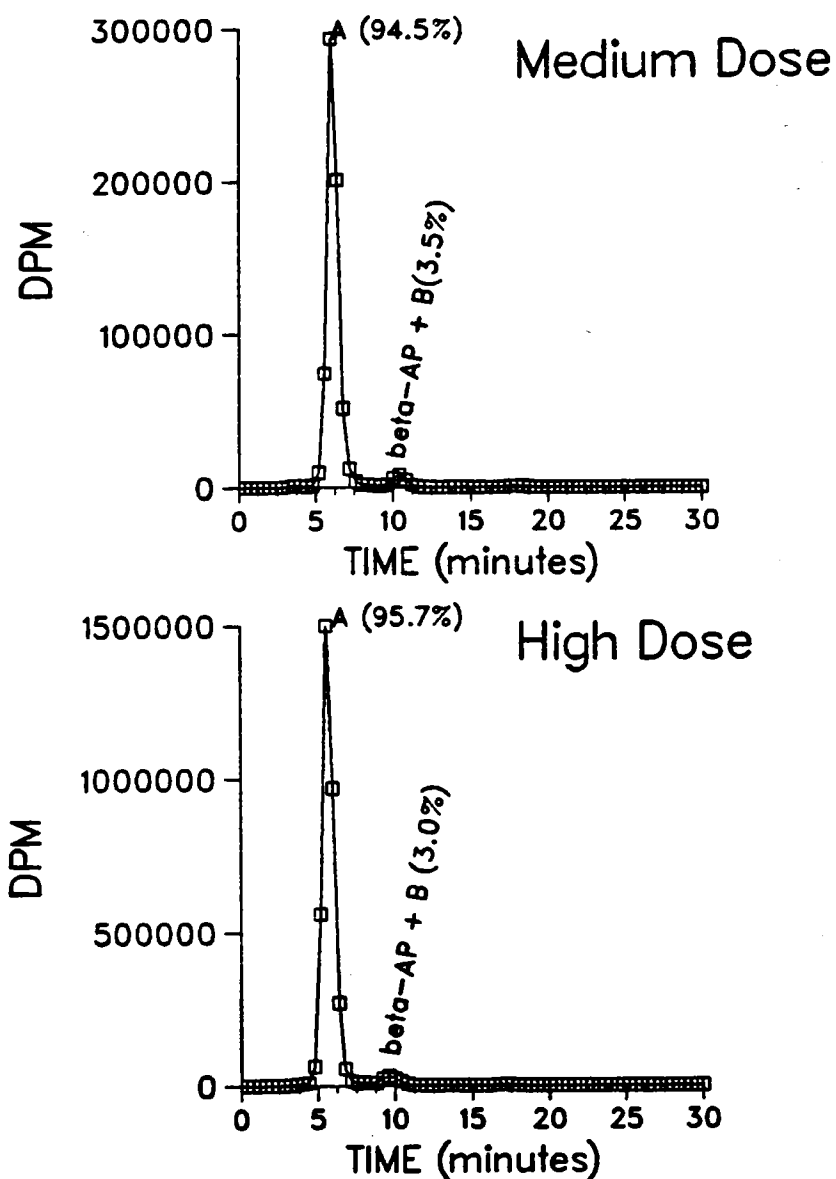


Figure 5. High performance liquid radiochromatogram of the 24 hour urine samples from the medium (A) and High (B) dose treatment groups. The locations of the reference standard of SC-19200 (β -AP) is marked on the chromatogram. Metabolite peaks A and B correspond to phenylacetylglutamine and phenylacetic acid respectively based on isolation and identification of metabolites with the same retention times from urine. The percentages of radioactivity eluted from the column that are associated with the above peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute.

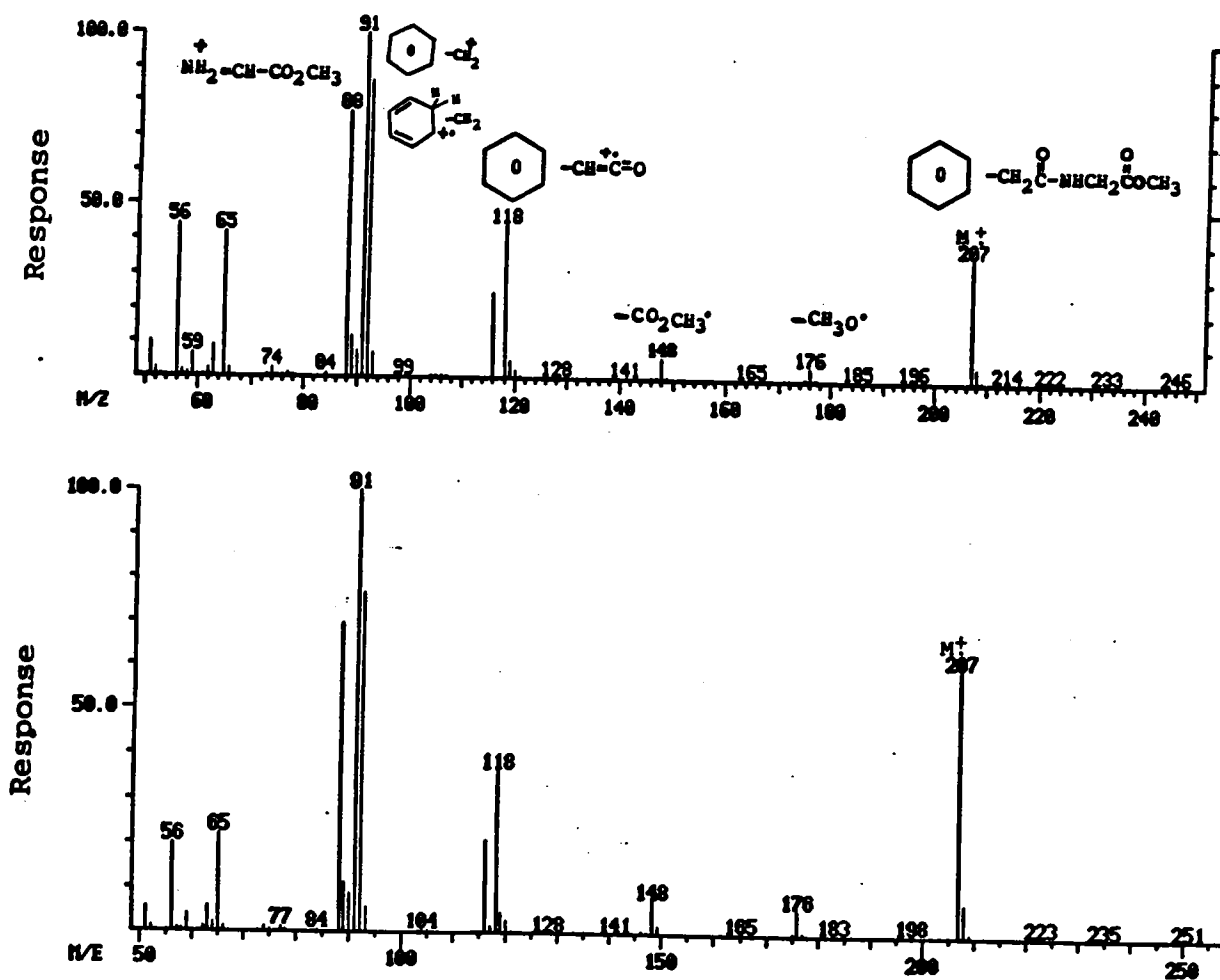


Figure 6. The electron impact mass spectrum of the methylester derivative of phenylacetylglycine (upper panel) and the methylated derivative of metabolite A (lower panel).

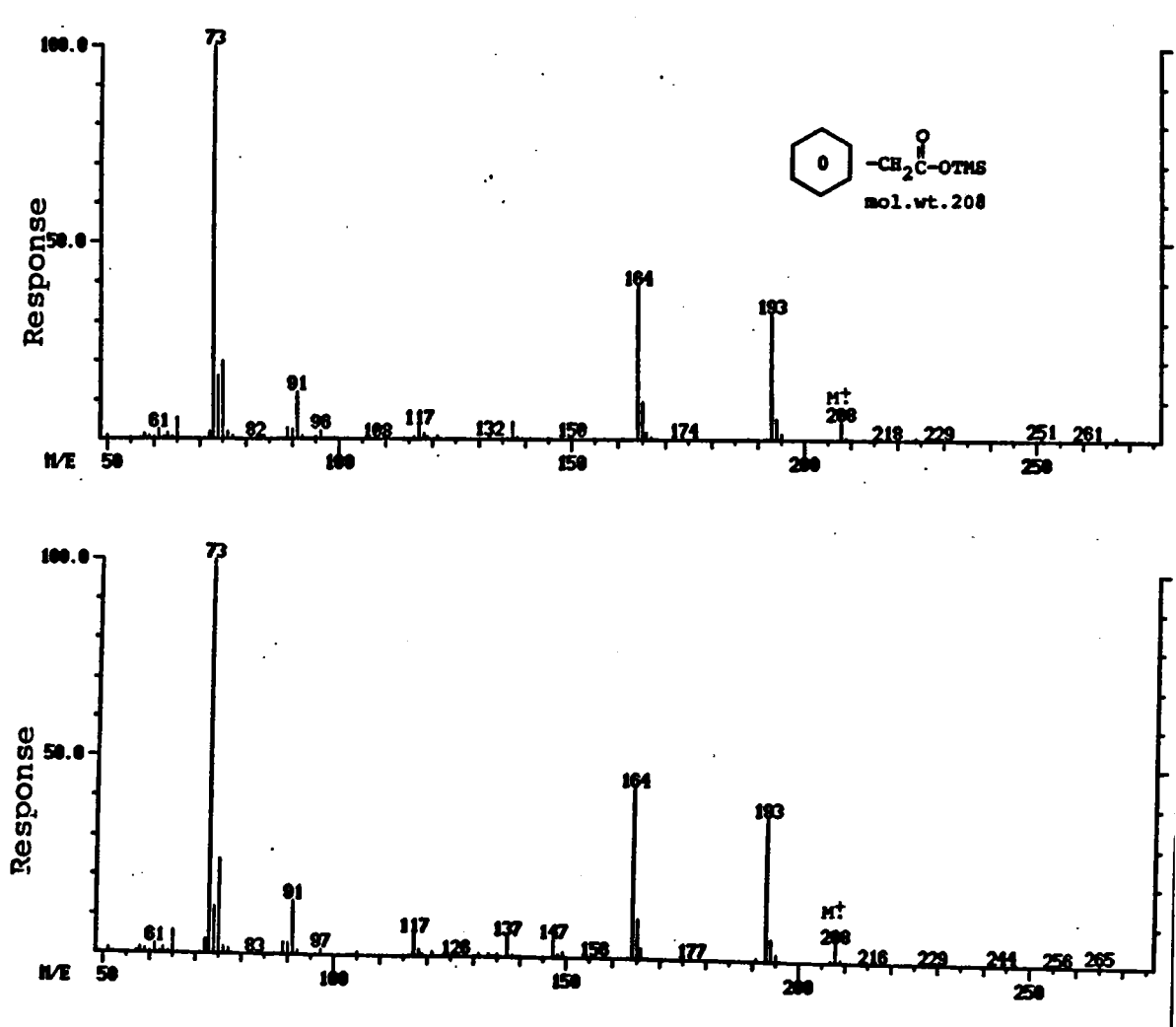


Figure 7. The electron impact mass spectrum of the methylester derivative of phenyl acetic acid (upper panel) and the trimethylsilyl derivative of metabolite B (lower panel).

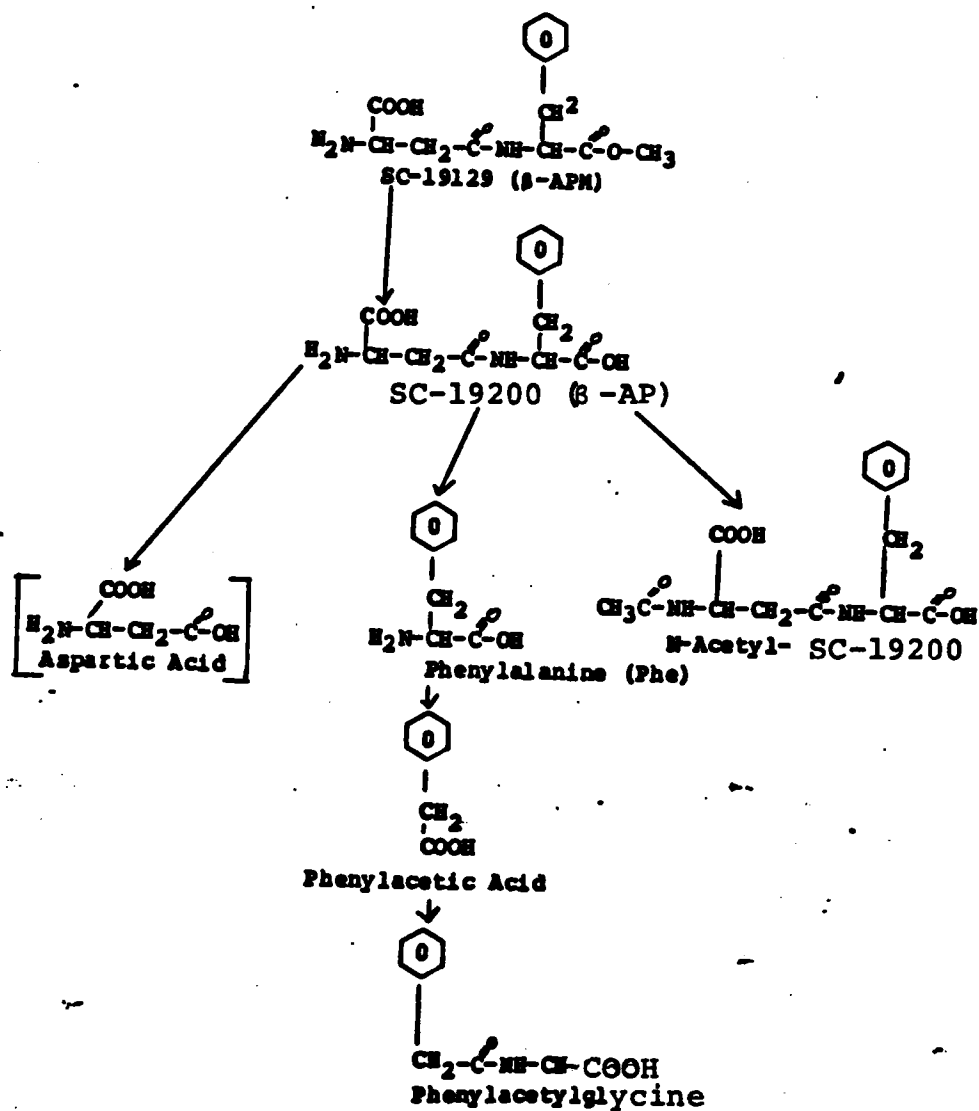


Figure 8. Structures and proposed metabolic pathway of SC-19129. Aspartic acid [in brackets] is a hypothetical metabolite which was not identified in this study. N-acetyl-SC-19200 was not identified in this study but was identified as a metabolite in the rat in a subsequent study (8).

IX. Appendix 1: Individual Animal Data

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Table A-1
Individual Body Weights and Doses Received

Dosage Group	Animal Number	Body Weight (g)	Intended Dose (mg/kg)	¹⁴ C]-SC-19129 (mg/g) in Diet ^a	Dose Received ^b (mg/kg)
Low	1	280	10	0.113	6.2
	2	280			6.6
	3	270			5.9
	4	270			5.0
	5	270			5.5
Medium	7	260	250	3.76	2.75 ± 0.09
	8	260			
	9	280			
	10	270			
	11	290			
	12	290			
					170
High	13	250	1000	18.2	13.3 ± 1.0
	14	270			
	15	270			
	16	240			
	17	260			
					950
					1000
					670
					1200
					860

^a The specific activity of the [¹⁴C]-SC-19129 in the low, medium and high dose diet mixtures was 32.5 mCi/mg, 0.730 mCi/mg and 0.730 mCi/mg respectively.

^b Dose received (mg/kg) = $\frac{\text{diet mixture consumed (g)} \times [\text{¹⁴C]-SC-19129 in diet (mg/g)}}{\text{body weight (kg)}}$

Table A-2

Individual Plasma Concentrations of Total ¹⁴C
and Percentages of Dose Excreted in the Urine

Dosage Group	Animal Number	Plasma Total ¹⁴ C _a (mcg equivalent/ml)			Urinary Excretion of Total ¹⁴ C (% of Dose)
		6 a.m.	9 a.m.	5 p.m.	
Low	1	0.54	0.73	1.1	9.8
	2	0.74	1.4	1.9	9.5
	3	1.5	2.2	2.5	8.1
	4	0.79	0.99	1.3	11
	5	0.71	0.88	1.2	9.8
Medium	7	29	42	54	35
	8	24	34	51	42
	9	19	26	53	49
	10	29	29 ^c	c	33 ^d
	11	20	27	e	39
	12	27	32	e	31
	13	140	f	f	169
	14 ^h	3.4 ^h	1.6 ^h	2.8 ^h	0.3 ^h
High	15	200	220	130	48
	16	110	130	170	30
	17	110	130	210	52

^a Samples were collected, at the time of day indicated, on the day following administration of the [¹⁴C]-SC-19129 diet mixtures.

^b Urine samples were collected from 0 to 24 hours, from approximately 5 p.m. on the day of [¹⁴C]-SC-19129 diet mixture administration until approximately 5 p.m. on the following day, unless otherwise indicated.

^c Animal died during 9 a.m. blood sample collection. The volume collected was sufficient for total ¹⁴C determination but not for inclusion in the pooled plasma samples (Table 1).

^d Urine sample collected from approximately 0 to 16 hours. This value was not included in the calculation of the 0-24 hour mean value for the group (Table 1).

^e Sample tube broke in centrifuge during plasma preparation.

Table A-2 (cont'd)

- f Animal died during 6 a.m. blood sample collection.
- g Urine sample collected from approximately 0 to 13 hours. This value was not included in the calculation of the 0-24 hour mean value for the group (Table 1).
- h Plasma and urine concentrations for this animal were very low. This animal was also observed to excrete the lowest volume of urine (9 ml versus 18 ± 7 ml [mean \pm standard deviation] for all animals), and to lose a marked amount of weight (31 g) during the 48 hour period ending at the time of sacrifice. Although diet (and thus [^{14}C]-SC-19129) consumption appeared normal (Table A-1) this animal is considered to be an outlier and the data from this animal is considered suspect.

X. Appendix 2: Protocol

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Protocol

1. **Study Title:**

Plasma and Urine Concentrations of [^{14}C]-SC-19129 and Major Metabolites Following Administration of [^{14}C]-SC-19129 in the Diet to Pregnant Female Rats.

2. **Study Sponsor:**

G.D. Searle & Co.

3. **Facility:**

G.D. Searle & Co., 4901 Searle Parkway,
Skokie, IL 60077.

4. **Proposed Initial Dosing Date:**

First Dosing: January 21, 1985

5. **Introduction:**

Toxicology studies, including teratology studies, are currently in progress to assess the safety of SC-19129. The study described in this protocol is designed to support dietary admix toxicology studies of SC-19129.

6. **Purpose:**

The purpose of this study is to determine the concentration of SC-19129, if present, and of its major metabolites in plasma and urine following administration in the diet to pregnant female rats.

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7. Overview of Study Design:

Pregnant female rats will be fed diets containing [¹⁴C]-SC-19129 at 3 dose levels. Five rats each will be dosed at 10 mg/kg, 250 mg/kg and 1000 mg/kg. Plasma and urine samples will be collected. Total radioactivity will be determined for all samples. Plasma and urine concentrations of [¹⁴C]-SC-19129 (if present), β-AP (the free acid of SC-19129) and other major metabolites will be determined in pooled samples using appropriate chromatographic procedures.

8. Laboratory Procedures:

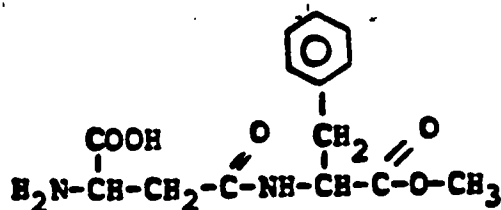
This study is not within the scope of Good Laboratory Practice Regulations.

9. Test Article:

A. Chemical Name:

SC-19129 (β-APM) is N-L-β-aspartyl-L-phenylalanine, 1-methyl ester.

B. Chemical Structure:



C. Dosage Forms:

1. [U-¹⁴C-Phe]-SC-19129 with a specific activity of approximately 32 mCi/mg (approximately

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9.6 mCi/mmol) will be supplied by the Radiochemistry Group, G.D. Searle & Co. This [¹⁴C]-SC-19129 will be used either undiluted (low dose) or diluted 1:40 (w/w) with unlabeled SC-19129 and recrystallized (medium and high dose) for dosage form preparation.

2. Test article-diet mixtures will be prepared by mixing the appropriate amount of [¹⁴C]-SC-19129 with powdered Purina Certified Rodent Chow 5002 to give intended doses of 10 mg/kg, 250 mg/kg and 1000 mg/kg. The actual test article concentration in each test article-diet mixture will be based on the body weight and projected food consumption of the animals for the day of dose administration.

D. Administration:

1. Route: The test article will be administered in the diet.
2. Frequency: The [¹⁴C]-SC-19129-diet mixtures will be available *ad libitum* for a 24 hour period from approximately 5:00 P.M. on day 1 of the experiment until approximately 5:00 P.M. on the following day. Unlabeled SC-19129-diet mixture, prepared to give the appropriate dose, will be made available to each animal *ad libitum* for approximately 24 hours prior to administering the [¹⁴C]-SC-19129-diet mixture.
3. Dosage: The intended doses are 10 mg/kg (low dose), 250 mg/kg (medium dose) and 1000 mg/kg (high dose). The [¹⁴C]-SC-19129 diet mixture for each animal will be weighed before dosing and at the end of the 24 hour dosing period. The actual dose received by each animal will be calculated

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from the weight of test article-diet mixture consumed, the concentration of test article in the diet and the animal's body weight at the time of dosing.

E. Analyses:

Homogeneity of the [^{14}C]-SC-19129-diet mixtures will be determined by sample oxidation and liquid scintillation counting of 5 weighed aliquots of each batch. The radiochemical purity of the test article in each diet mixture will be determined by extraction of an aliquot of each mixture and thin layer radiochromatography of a portion of the extract.

F. Storage:

[^{14}C]-SC-19129 will be stored at approximately 4°C. The [^{14}C]-SC-19129-diet mixtures will be prepared on the day of administration.

10. **Test System, Housing and Diet:**

A. Test System:

Pregnant female CD rats (Charles River Breeding Laboratory, Portage, MI) weighing 250-350 g at the time of dosing will be used. The animals will be dosed on day 7-8 of gestation. Five animals will be included in each dosage group.

B. Housing:

Animals will be housed individually in stainless steel cages prior to dose administration. They will be maintained on a 12 hour light, 12 hour dark cycle, with lights on at 6:00 A.M. and lights off at 6:00 P.M. Each rat will be uniquely identified with a cage card identifying the rat number. After [^{14}C]-SC-19129 administration, each rat will be

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housed in an individual metabolism cage for the remainder of the study.

C. Diet:

- i. Food: The rats will be maintained on Purina Certified Rodent Chow™ 5002 (Ralston Purina, St. Louis, MO) for a minimum of one week prior to dose administration. Food will be available throughout the study.
- ii. Water: Tap water from the municipal water supply will be available *ad libitum*.
- iii. Special analyses of food and water will not be performed since no contaminants known to be capable of interfering with the study are reasonably expected to be present.

11. Sample Collection, Times and Storage:

A. Animal Sacrifice:

Animals will be anesthetized with ether. Sacrifice will be by exsanguination through cardiac puncture at approximately 24 hours after initiation of dosing with the [^{14}C]-SC-19129 diet mixture.

B. Blood:

Each animal will be bled 3 times. Blood will be collected from each animal from a tail vein cannula between 6:00 A.M. and 7:00 A.M. and between 9:00 A.M. and 10:00 A.M. and by cardiac puncture between 5:00 P.M. and 6:00 P.M. on the day following initiation of dosing. Sample volumes will be approximately 1 ml. The blood will be placed in chilled tubes containing heparin and an esterase inhibitor and plasma will be prepared by centrifugation. It will be either stored frozen or

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further processed for analysis within three hours.

C. Urine:

The urine will be collected into containers surrounded by dry ice from 0-24 hours after dose administration. Care will be taken to collect any urine voided by the animals during blood sample collection and to combine it with the urine collected from the metabolism cages. The urine samples will be stored frozen until analyzed.

D. Control Urine and Plasma:

Plasma and urine will be collected from control rats which have not been treated with the test article. Aliquots of the urine and plasma from control rats will be spiked with [^{14}C]-SC-19129 prior to frozen storage. The spiked samples will be used to determine stability and efficiency of extraction with each matrix.

12. Sample Analysis:

Total ^{14}C will be measured by direct liquid scintillation counting (LSC). Pooled plasma and urine samples will be analyzed by sample extraction followed by analysis by high performance liquid radiochromatography (HPLRC) for [^{14}C]-SC-19129 (if present), [^{14}C]- β -AP (the free acid of β -APM) and related compounds.

13. Statistical Procedure:

Individual data for total radioactivity determinations will be tabulated and the means and standard errors of the means calculated. The recovery of ^{14}C in the urine samples will be calculated. Plasma and urine

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concentrations of [^{14}C]-SC-19129 (if present), [^{14}C]- β -AP and other major metabolite will be calculated from the data obtained from the metabolic profiles of pooled samples.

14. Archiving of Materials:

A report will be written and submitted to the R&D Central File. The raw data will be submitted to the R&D Central File after completion of the report.

15. Study Participants:

Study Director	E. Burton
Test article administration,	I. Dressler
specimen collection,	K. Hoglund
dosage form preparation and	
analysis and sample analysis	
Report	E. Burton


16. **Protocol Review:**

J. Oppermann
M. Crager
C. Tschanz
G. Schoenhard
J. Noveroske

17. **Protocol Approval:**



J. Oppermann 1/21/85
Date



E. Burton 1/21/85
Date
(Responsible Scientist)

