

Pharmacokinetics and Metabolism
of [^{14}C]-SC-19129 and [^{14}C]-SC-19200,
its Free Acid, in the Rabbit

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

MRC-852-0066

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Study Initiated: September 18, 1985

Study Completed: October 31, 1986

Document Number: MRC-852-0066

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Reference Citation

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Pharmacokinetics and Metabolism of [14 C]-SC-19129 and
[14 C]-SC-19200, its Free Acid, in the Rabbit. Department of
Drug Metabolism, Research and Development Division, G.D.
Searle & Co. MRC-852-0066, October, 1986.

Table of Contents

	Page
I. Abstract	I.1
II. Introduction	I.1
III. Materials and Methods	III.1
A. Overview of Study Design	III.1
B. Test Article and Dosage Forms	III.1
C. Animals, Animal Treatment and Test Article Administration	III.1
D. Sample Collection	III.2
1. Plasma	III.2
2. Urine and Feces	III.2
3. Breath	III.2
E. Sample Analysis	III.3
1. Dose Solution	III.3
2. Plasma	III.3
3. Urine	III.3
4. Feces	III.4
5. Breath	III.4
F. High Performance Liquid Radiochromatography (HPLRC)	III.4
G. Liquid Scintillation Counting (LSC)	III.5
H. Calculations	III.6
I. Pharmacokinetic Calculations	III.6
J. Protocol Deviations	III.7
IV. Results	IV.1
A. Radiochemical Purity of Dosage Forms	IV.1
B. Recovery of [¹⁴ C]-SC-19129 and [¹⁴ C]-SC-19200 from Control Plasma, Urine and Feces	IV.1
C. Total Radioactivity in Plasma	IV.2
D. Distribution of Plasma Radioactivity	IV.3
E. [¹⁴ C]-SC-9200 in Plasma	IV.4
F. Elimination of ¹⁴ CO ₂ in Breath	IV.5
G. Urinary and Fecal Excretion of Total Radioactivity	IV.6
H. Distribution of Urinary and Fecal Radioactivity	IV.7

Table of Contents (cont'd)

	<u>Page</u>
V. Discussion and Conclusions	V.1
A. Metabolic Formation of Free Phenylalanine (Phe)	V.1
B. Oral Bioavailability	V.2
C. Metabolic Pathway	V.4
D. Conclusions	V.5
VI. References	VI.1
VII. Tables	VII.1
VIII. Figures	VIII.1
IX. Appendix 1. Tables	IX.1
X. Appendix 2. Protocol	X.1

**Pharmacokinetics and Metabolism
of [^{14}C]-SC-19129 and [^{14}C]-SC-19200,
its Free Acid, in the Rabbit**

I. Abstract

The pharmacokinetics and metabolism of intravenously (IV) and orally administered [^{14}C]-SC-19200 (the free acid of [^{14}C]-SC-19129) and orally administered [^{14}C]-SC-19129 were studied in female rabbits. The dose in each case was approximately 10 mg/kg. The results obtained were as follows:

- 1) Absorption of total radioactivity from oral solution doses of [^{14}C]-SC-19200 and [^{14}C]-SC-19129 was prolonged. The mean times to peak plasma concentrations (C_{max}) and mean C_{max} were 5 hours and 6.06 mcg/ml following [^{14}C]-SC-19200 administration and 5 hours and 5.87 mcg/ml following [^{14}C]-SC-19129 administration.
- 2) The areas under the plasma concentration-time curves (AUC) for total radioactivity following oral doses of [^{14}C]-SC-19200 and [^{14}C]-SC-19129 were 465% and 409% respectively of the AUC following an IV dose of [^{14}C]-SC-19200. This was due to a marked quantitative difference in the various metabolites formed following oral compared to IV doses (see point #8 below).
- 3) [^{14}C]-SC-19129 was present in plasma at concentrations of 0.03 mcg/ml to 0.06 mcg/ml from 0.5 to 1 hour after oral administration of [^{14}C]-SC-19129. The AUC for SC-19129 was less than 2% of the AUC for SC-19200 in the same plasma samples.
- 4) Mean peak plasma concentrations of [^{14}C]-SC-19200 were 0.662 mcg/ml at 6 hours after oral

MRC-852-0066

[¹⁴C]-SC-19200 doses and 0.383 mcg/ml at 2 hours after [¹⁴C]-SC-19129 doses. The respective oral bioavailabilities were 23% and 8.5%. Since some [¹⁴C]-SC-19129 did reach the systemic circulation following its oral administration and since its volume of distribution may be larger than that of SC-19200, the oral bioavailability of [¹⁴C]-SC-19129 may be underestimated by plasma AUC comparisons.

- 5) The mean plasma elimination half-lives of total radioactivity were 77.3 hours, 184 hours and 148 hours following IV [¹⁴C]-SC-19200, oral [¹⁴C]-SC-19200 and oral [¹⁴C]-SC-19129 doses, respectively.
- 6) The mean plasma elimination half-life of [¹⁴C]-SC-19200 following IV administration was 0.58 hours. The plasma elimination phase of SC-19200 following oral doses contained too few data points to calculate half-lives.
- 7) The volumes of distribution of total radioactivity and of [¹⁴C]-SC-19200 following IV administration were 0.211 l/kg and 0.236 l/kg, respectively, indicating that [¹⁴C]-SC-19200 may be largely confined to the extracellular fluid volume.
- 8) The major metabolites of SC-19200 were phenylalanine (Phe), phenylacetylglycine (PAGly) and N-acetyl-SC-19200. Metabolite patterns differed quantitatively between IV and oral administration with N-acetyl-SC-19200 (and unchanged SC-19200) predominating after IV administration and PAGly predominating after oral administration.

- 9) The major route of excretion of total radioactivity was in the urine (90.7% of dose) following IV [^{14}C]-SC-19200, with excretion of small amounts in the feces (0.61%) and breath (0.59%). Recovery of the IV dose was 91.9% in urine, feces and breath. Following oral administration of [^{14}C]-SC-19200 or [^{14}C]-SC-19129, excretion in the urine (72.7% and 66.5% respectively) was lower than following IV administration of [^{14}C]-SC-19200. However excretion of total radioactivity in the breath (4.3% and 4.1% respectively) and in the feces (5.3% and 8.4% respectively) was higher following oral administration of [^{14}C]-SC-19200 and [^{14}C]-SC-19129 than that following the IV dose. Estimation of oral bioavailability of [^{14}C]-SC-19129 and [^{14}C]-SC-19200 from the urinary excretion data was not considered appropriate due to the quantitative differences in the metabolites excreted after oral and IV doses. Lower limits for the oral bioavailabilities were estimated from fecal excretion data and were 95% and 92% for [^{14}C]-SC-19200 and [^{14}C]-SC-19129 oral doses.
- 10) Urinary excretions of [^{14}C]-SC-19200 and N-acetyl-SC-19200 were 2.0% and 4.5% of dose, respectively, following oral doses of [^{14}C]-SC-19200 and 2.1% and 4.5%, respectively, following oral doses of [^{14}C]-SC-19129. Comparison with the excretion of SC-19200 and N-acetyl-SC-19200 following IV administration of [^{14}C]-SC-19200 (67.4% and 16.9% of dose, respectively) indicates oral absorption of very similar amounts of intact SC-19200 (7.7-7.8% of dose) from either SC-19200 or SC-19129.

**Pharmacokinetics and Metabolism
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its Free Acid, in the Rabbit**

II. Introduction

SC-19129 (N-L- β -aspartyl-L-phenylalanine, l-methyl ester, β -APM) and its free acid, SC-19200 (β -AP, N-L- β -aspartyl-L-phenylalanine), 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 pharmacokinetics and metabolism of orally administered [^{14}C]-SC-19129 have been examined in the rhesus monkey (2) and in the rat (3). In addition plasma and urine concentrations of total ^{14}C and major metabolites were determined in pregnant rats administered [^{14}C]-SC-19129 in dietary admix (4). The objectives of the present study were to determine the pharmacokinetics and metabolism of intravenously (IV) and orally administered [^{14}C]-SC-19200 and orally administered [^{14}C]-SC-19129 in the rabbit.

III. Materials and Methods

A. Overview of Study Design:

Eight female rabbits were dosed orally and eight female rabbits were dosed IV with [^{14}C]-SC-19200 at 10 mg/kg body weight. Eight female rabbits were dosed orally with [^{14}C]-SC-19129 at 10 mg/kg body weight. Plasma, urine and feces were collected from 4 of the 8 animals in each group and $^{14}\text{CO}_2$ in breath was collected from the remaining 4 animals in each group. Total radioactivity was determined for all samples. Pooled plasma and urine samples from selected time points were further analyzed by HPLRC.

B. Test Article and Dosage Forms:

[U- ^{14}C -Phe]-SC-19129 (lot MRC-532-118-1) and [U- ^{14}C -Phe]-SC-19200 (lot MRC-553-23-2) were supplied by the Radiochemistry Group, G.D. Searle & Co. The respective specific activities were 9.55 mCi/mmol (32.5 mCi/mg) and 26.9 mCi/mmol (96.0 mCi/mg). The dosage forms were prepared using the appropriate amounts of [^{14}C]-SC-19129 and unlabelled SC-19129 (lot 84K-047-101) or [^{14}C]-SC-19200 and unlabelled SC-19200 (lot CD-158-146A) as described in the protocol (Section IX.9.C.2.). The actual specific activities of the doses administered to each treatment group are given in Table 1.

C. Animals, Animal Treatment and Test Article Administration:

Female NZW rabbits (Hare Marland Laboratories, Hewitt, NJ) weighing 2.09-3.00 kg at the time of dosing were used. The animals were housed and fed (Purina Rabbit Chow #5322,

MRC-852-0066

Ralston Purina, St Louis, MO) as specified in the protocol (Section IX.10.B-C). Administration of the oral and intravenous (IV) dosage forms was as described in the protocol (Section IX.9.D). The mean dose administered to each treatment group is given in Table 1.

D. Sample Collection:

1. Plasma:

Blood samples were collected from the ear veins from four animals per treatment group as described in the protocol (Section IX.12.B). Plasma was prepared by centrifugation and an aliquot was taken for total radioactivity determination (Section III.E.2). The remainder of each plasma sample was stored frozen at approximately -70° until analysis (Section III.E.2). [^{14}C]-SC-19129 or [^{14}C]-SC-19200 was added to control rabbit plasma which was pretreated with 1×10^{-4} molar diethyl-p-nitrophenylphosphate (Section IX.11.B) to inhibit esterase activity. Aliquots of the control plasma containing [^{14}C]-SC-19129 or [^{14}C]-SC-19200 were analyzed immediately, or following frozen storage.

2. Urine and Feces:

Urine and fecal samples were collected as described in the protocol (Section IX.12.C-D) and stored frozen at approximately -70°C until analysis. Control urine was spiked with [^{14}C]-SC-19129 or [^{14}C]-SC-19200 and analyzed immediately or stored frozen until analysis.

3. Breath:

$^{14}\text{CO}_2$ eliminated in the breath was collected as described in the protocol (Section IX.12.E).

E. Sample Analysis:

1. Dose Solution:

Aliquots of the dosing solutions were taken for determination of total radioactivity by liquid scintillation spectrometry (LSC; Section III.H) and determination of radiochemical purity by HPLRC analysis (Section III.F).

2. Plasma:

Total ^{14}C was determined by LSC (Section III.H) using duplicate 0.01 ml or 0.05 ml aliquots, depending on the expected levels of radioactivity in the samples. Extracts of pooled plasma (1.0 ml containing 0.25 ml from each animal) or spiked control plasma (1.0 ml) were prepared for HPLRC analysis (Section III.F) by a C18 Bond Elut[™] (Analytichem International, Inc., Harbor City, CA) extraction procedure. This procedure was carried out as described for the assay of SC-19200 (β AP) in rat plasma (5) except 1) the solution used to elute radiolabelled compounds from the first Bond Elut[™] column was 30% (v/v) methanol in water instead of water and 2) the solution used to elute radiolabelled SC-19129 and metabolites from the second Bond Elut[™] column (1.5 ml of acetonitrile:0.18 M NaH_2PO_4 , pH 2.0, containing 0.02 M heptane sulfonic acid) contained 25% (v/v) acetonitrile rather than 20%. These changes were necessary to achieve acceptable recoveries of SC-19129.

3. Urine:

Total ^{14}C in duplicate aliquots of each urine sample was determined by LSC (Section III.G). Pooled

MRC-852-0066

urine samples or spiked control urine samples were filtered through a 0.45 micron filter (Gelman Acrodisc[®], AR; Gelman Sciences, Inc., Ann Arbor, MI) prior to HPLRC analysis (Section III.F).

4. Feces:

Fecal samples were suspended in a volume (ml) of water equal to 2.5 times the sample weight (g) using a blender (Stomacher Lab-Blender 80; Tekmar Co., Cincinnati, OH). Triplicate aliquots (0.2-1.2 ml) of suspension were oxidized with a Packard Tri-Carb Sample Oxidizer (Packard Model 306, Packard Instruments, Co., Downers Grove, IL). Total ^{14}C in the combustion products was determined by LSC (Section III.G).

5. Breath:

Total ^{14}C in 1 ml aliquots of CO_2 trapping solution (Appendix 1, Section II.C) was determined by LSC (Section III.G).

F. High-Performance Liquid Radiochromatography (HPLRC):

Plasma extracts, urine and dosing solutions were analyzed for the distribution (profile) of radioactivity by HPLRC on a Supelcosil LC-8-DB column (15 cm x 4.6 mm; Supelco, Inc., Bellefonte, PA) using a mobile phase of 0.18 M monobasic sodium phosphate, pH 2.0, containing 0.02 M heptane sulfonic acid:methanol (68:32, v/v) and a flow rate of 1.0 ml/min. Unlabelled standards of SC-19129, SC-19200 and phenylalanine, used to calibrate the system, were detected by absorbance at 210 nm using a model 480 variable wavelength detector (Waters Associates, Milford, MA). Radiolabeled compounds were detected using a radioactive

MRC-852-0066

flow detector (Flo-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. Liquid Scintillation Counting (LSC):

Samples of 0.010 ml or 0.050 ml plasma were mixed with 10 ml of PCS[®] (Amersham Corp., Arlington Heights, IL). Samples larger than 0.050 ml (plasma, urine, CO₂ trapping solution or HPLRC mobile phase) were mixed with sufficient water to give approximately 4 ml total aqueous volume and then mixed with 5 ml of PCS[®] to form a stable gel. The combustion products from oxidized fecal samples 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 liquid 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.

B. Calculations:

The concentrations of metabolites present in plasma were calculated as follows:

$$\left[\begin{array}{l} \text{Metabolite} \\ \text{Concentration} \\ \text{(mcg} \\ \text{equivalents/ml)} \end{array} \right] = \left[\begin{array}{l} \text{Plasma} \\ \text{Radioactivity} \\ \text{Concentration} \\ \text{(mcg} \\ \text{equivalents/ml)} \end{array} \right] \times \left[\begin{array}{l} \text{Fraction of} \\ \text{Radioactivity} \\ \text{Recovered in} \\ \text{Bond Elut} \end{array} \right] \times \left[\begin{array}{l} \text{Fraction of} \\ \text{Total Elute} \\ \text{from HPLRC} \\ \text{as metabolite} \end{array} \right]$$

All concentrations are expressed in terms of mcg equivalents of [^{14}C]-SC-19129, based on the specific activity of [^{14}C]-SC-19129 administered. The results were corrected for recovery of total radioactivity (but not for recovery of specific compounds) from the Bond Elut[™] extractions (Table 2).

The percentage of the dose excreted in the urine as metabolites was calculated as follows:

$$\left[\begin{array}{l} \text{Metabolite} \\ \text{Excreted} \\ \text{(\% of Dose)} \end{array} \right] = \left[\begin{array}{l} \text{Radioactivity} \\ \text{Excreted in} \\ \text{Urine} \\ \text{(\% of Dose)} \end{array} \right] \times \left[\begin{array}{l} \text{Fraction Eluted} \\ \text{from HPLRC} \\ \text{as Metabolite} \end{array} \right]$$

I. Pharmacokinetic Calculations:

Maximum plasma concentrations (C_{max}) and the times at which C_{max} values occurred (T_{max}) were determined by inspection of the plasma concentration-time curves. Areas under the plasma concentration-time curves were calculated using the trapezoidal rule (6).

Plasma concentration versus time curves of total ^{14}C and [^{14}C]-SC-19200 were analyzed using the CSTRIP computer program (7). The initial parameters estimated from CSTRIP were then used in the NONLIN computer program (8).

The percent of dose absorbed (bioavailability) using fecal excretion data was calculated according to the following equation:

$$\text{Bioavailability (\%)} = 100\% \times \frac{100\% - Z}{100\% - Y}$$

In the above equation Y is equal to the percent of dose recovered in the feces following IV administration and Z is the percent of dose recovered in the feces following oral administration.

J. Protocol Deviations:

1. The radiochemical purity of [^{14}C]-SC-19129 in dose solutions was determined by HPLRC rather than by thin layer radiochromatography (TLRC). HPLRC is judged to be equal to, or better than, TLRC for the above purpose and was chosen to increase efficiency of utilization of personnel.
2. The protocol specified that animals would weigh 3-4 kg at the time of dosing. The actual weight range was 2.09 to 3.00 kg (Table 1A, Section IX). The intent of the protocol was to place an upper limit on the variation in size (and age) between animals and also allow use of animals large enough to facilitate serial bleeding for plasma collection. Plasma sample collection was successfully accomplished with the animals used and the variation in size between animals was equivalent to that allowed by the protocol. The fact that the animals weighed 3 kg or less is not considered to have adversely affected the study.

3. Fecal extracts were not analyzed by HPLRC due to the relatively low percentages of dose excreted by this route. In the rat, fecal excretion of radiolabel following oral doses of [^{14}C]-SC-19200 or [^{14}C]-SC-19129 was due almost entirely to excretion of unabsorbed [^{14}C]-SC-19200 (3). This was assumed to also be the case for the rabbit. If fecal excretion following oral doses in the rabbit was due to excretion of metabolites rather than unabsorbed [^{14}C]-SC-19200, then bioavailability calculations using fecal excretion data (Section III.I. and IV.G.) would tend to underestimate oral bioavailability by as much as approximately 5%.

IV. Results

A. Radiochemical Purity of Dosage Forms:

Solutions of [^{14}C]-SC-19129 or [^{14}C]-SC-19200 were prepared each day that animals were dosed. The dosing solutions were analyzed within 4 hours of preparation, as specified in the protocol (Section X.9.E), by HPLRC (Section III.F). The radiochemical purities of dosing solutions of [^{14}C]-SC-19129 (N=3) and [^{14}C]-SC-19200 (N=6) were 93.7 ± 4.8 (mean \pm standard error) and $97.4 \pm 0.7\%$ respectively.

B. Recovery of [^{14}C]-SC-19129 and [^{14}C]-SC-19200 from Control Plasma, Urine and Feces:

The recoveries of radiolabel from [^{14}C]-SC-19129 and [^{14}C]-SC-19200 added to control plasma using the Bond Elut[™] procedure (Section III.E.2) were 83.9% and 69.2%, respectively (Table 2). The respective percentages of the extracted radiolabel present in the appropriate peak in HPLRC profiles were 98.4% and 97.8% when analyzed immediately and 96.6% and 98.8% when the extraction and HPLRC were performed after frozen storage of the spiked plasma (Table 3).

The recovery of radiolabel added to control urine samples was 98.8%-100% during the filtration procedure (Table 2). The percentages of the radiolabel from [^{14}C]-SC-19129 and [^{14}C]-SC-19200 present in the appropriate HPLRC peak were 95.6% and 95.3% when analyzed immediately and 92.2% and 96.0% after frozen storage for 9 days (Table 3). The difference in the amount of extracted radiolabel present at the appropriate HPLRC retention time for [^{14}C]-SC-19129 was accounted for by the formation of [^{14}C]-SC-19200 during storage.

MRC-852-0066

The recovery of radiolabel from [^{14}C]-SC-19129 and [^{14}C]-SC-19200 added to control feces was 100% (Table 2).

C. Total Radioactivity in Plasma:

Mean plasma concentrations of total radioactivity, expressed as mcg equivalents/ml, are given in Table 4 and are shown in Figure 1. Individual animal values are given in Tables 4A-6A. The pharmacokinetic parameters are summarized in Table 4.

Following intravenous administration of [^{14}C]-SC-19200, mean plasma total ^{14}C was 34.0 mcg/ml at 0.08 hours. Plasma total radioactivity declined in a triexponential manner as determined by CSTRIP and NONLIN analysis (Table 4 and Table 7A). The distribution half life (α) was 0.115 hours. The elimination half-lives were 0.562 hours and 77.3 hours for the first (β) and second () phases respectively. The mean volume of distribution of total radioactivity was calculated to be 0.211 l/kg. The mean AUC from time 0 to infinity was 165 (mcg/ml) hours.

Absorption of total radioactivity from oral doses of [^{14}C]-SC-19129 and [^{14}C]-SC-19200 appeared to occur over a period of several hours (Table 4, Figure 1). The mean peak plasma concentration (C_{max}) was 6.06 mcg/ml at 5.0 hours following [^{14}C]-SC-19200 administration and 5.87 mcg/ml at 5.0 hours following [^{14}C]-SC-19129 administration. The elimination half-lives of total radioactivity were 184 ± 15 hours and 148 ± 4 hours following oral doses of [^{14}C]-SC-19200 and [^{14}C]-SC-19129 respectively. The calculated elimination half-lives show good agreement between animals within treatment groups (Tables 8A, 9A) but are based on a sample collection period of only 120 hours. A sample collection period of approximately 3 half-lives

(approximately 500 hours) would provide a more reliable measure of the terminal half-lives.

The AUC values of total radioactivity from time 0 to infinity were 767 (mcg/ml) hours and 675 (mcg/ml) hours for orally administered [^{14}C]-SC-19200 and [^{14}C]-SC-19129 respectively. These AUC values were 465% and 409% respectively of the corresponding AUC for the IV dose (Table 4). The fact that the oral AUC values are much higher than the IV AUC value reflects a marked difference in the amounts of various metabolites formed following oral compared to IV doses (see Section V.A).

D. Distribution of Plasma Radioactivity:

The radioactive compounds in the plasma were separated chromatographically, using Bond Elut[™] columns (Section III.E.2), by a method developed for the assay of unlabeled SC-19200 in rat plasma (5). The recovery of SC-19200 and SC-19129 from rabbit plasma using this procedure is given in Table 2.

SC-19200 was the major component in HPLRC profiles of the Bond Elut[™] fraction following IV administration (Figure 2, Tables 5 and 6). Small amounts of phenylalanine (Phe) the N-acetyl derivative of SC-19200 and phenylacetylglycine (PAGly) were also present following IV administration.

Small amounts of intact SC-19129 were found in pooled plasma samples following oral administration of [^{14}C]-SC-19129. The concentrations at 0.5, 0.75 and 1.0 hours were estimated to be 0.030, 0.043 and 0.057 mcg/ml respectively. Measurable amounts of SC-19129 (> 0.03 mcg/ml) were not detected at other times, although the esterase inhibitor used to treat plasma samples (Section III.D.1) effectively protected SC-19129 added to plasma in

vitro from hydrolysis (Table 3). SC-19200 (see Section IV.E) and Phe were also present in plasma following oral doses of [^{14}C]-SC-19200 or [^{14}C]-SC-19129, (Figures 3-4, Tables 5-6) but the major plasma metabolites following oral administration were phenylacetic acid and its conjugate, PAGly (Table 6). The concentrations of metabolites given in Table 6 may be somewhat underestimated since the recoveries of these metabolites during the Bond Elut[™] extraction procedure has not been determined.

The Bond Elut[™] fraction containing SC-19200 (second Bond Elut[™] column; Section III.E.2) contained approximately 90% of the total radioactivity present in the 0.08 hour plasma sample following IV administration. However this percentage declined at later times and was less than 5% at 24 hours after IV administration. The majority of the radioactivity which was not recovered in the SC-19200 Bond Elut[™] fraction was recovered in an earlier eluting Bond Elut[™] fraction and was precipitable with 0.5N perchloric acid. This fraction presumably contains [^{14}C]-Phe incorporated into high molecular weight plasma protein (see reference 10 and 11).

E. [^{14}C]-SC-19200 in Plasma:

The concentrations of [^{14}C]-SC-19200 in pooled plasma after IV or oral administration are given in Table 5 and shown in Figure 5. The pharmacokinetic parameters for SC-19200 are listed in Table 5. The elimination of SC-19200 from plasma following IV administration was biexponential, with half-lives of 0.119 hours for the first (α) phase and 0.583 hours for the second (β) phase. Extrapolation of the SC-19200 plasma concentration-time curve, using the parameters calculated by the NONLIN program, indicated a maximum of 42.4 mcg/ml at zero time. The volume of

distribution of SC-19200 was calculated to be 0.236 l/kg. This volume is comparable to the estimated extracellular fluid volume (approximately 0.16-0.27 l/kg, reference 9), but smaller than the total body water volume (approximately 0.6-0.7 l/kg, reference 9), in the rabbit. This indicates that SC-19200 is largely excluded from tissues in the rabbit.

Peak concentrations of SC-19200 in pooled plasma were 0.662 mcg/ml at 6 hours following oral doses of [^{14}C]-SC-19200 and 0.383 mcg/ml at 2 hours following oral doses of [^{14}C]-SC-19129. The disappearance of SC-19200 from plasma following oral administration of [^{14}C]-SC-19129 occurred with a half-life of approximately 2.4 hours (Table 5). The elimination phase of SC-19200 from plasma following oral [^{14}C]-SC-19200 doses contained too few data points to calculate a half-life (Table 5).

The bioavailabilities of SC-19200, based on comparison of oral AUC values to the IV AUC values, were 22.6% for orally administered SC-19200 and 8.51% for orally administered SC-19129. Given the high oral bioavailability of total ^{14}C (see Section IV.G. and Section V.B), these values for the oral bioavailability of SC-19200 indicate extensive first pass metabolism of SC-19129 and SC-19200.

F. Elimination of $^{14}\text{CO}_2$ in Breath:

The cumulative excretion of $^{14}\text{CO}_2$ in the breath from 0 to 7 hours is given in Table 7 and shown in Figure 6. Excretion of $^{14}\text{CO}_2$ in the breath was similar following oral doses of [^{14}C]-SC-19200 and [^{14}C]-SC-19129. In both cases there was a lag time of about 2 hours followed by nearly linear rates of excretion of approximately 1% of dose per hour from 3 to 5 hours. The cumulative percentages of the

MRC-852-0066

oral dose of [^{14}C]-SC-19200 and the oral dose of [^{14}C]-SC-19129 excreted from 0 to 7 hours were 4.28 ± 0.51 and 4.14 ± 0.33 , respectively. The mean peak rate of excretion (Table 7) occurred somewhat earlier following the SC-19200 oral doses (2-4 hours) compared to the SC-19129 oral doses (3-5 hours).

The elimination of $^{14}\text{CO}_2$ in the breath following IV administration of [^{14}C]-SC-19200 was much slower (Figure 6) than that following the oral doses and the cumulative percentage of dose excreted from 0 to 7 hours was only 0.610 ± 0.056 (Table 7). Excretion of $^{14}\text{CO}_2$ in the breath during the period from 24 to 25 hours after administration was 0.014%, 0.080% and 0.062% of dose respectively for the SC-19200 IV dose, the SC-19200 oral dose and the SC-19129 oral dose.

G. Urinary and Fecal Excretion of Total Radioactivity:

The mean cumulative excretion of radioactivity in urine and feces is given in Table 7 and shown in Figures 7 and 8, respectively. Excretion in the urine and feces of individual animals is given in Tables 13A-15A. The mean percentages of dose excreted in the urine through 120 hours after the IV dose of SC-19200, the oral dose of SC-19200 and the oral dose of SC-19129 were $90.7 \pm 2.4\%$, $72.7 \pm 2.2\%$ and $66.5 \pm 3.7\%$, respectively. The mean percentages of dose recovered in feces after administration of IV and oral doses of [^{14}C]-SC-19200 were $0.592 \pm 0.078\%$ and $5.30 \pm 0.34\%$, respectively. The mean percentage recovered in feces after an oral dose of [^{14}C]-SC-19200 was $8.39 \pm 0.77\%$. The average total recoveries in breath, urine and feces after IV and oral doses of [^{14}C]-SC-19200 were 91.9% and 82.3%,

respectively. The average total recovery after an oral dose of [^{14}C]-SC-19129 was 79.0%.

The apparent bioavailabilities of total radioactivity from orally administered SC-19200 and SC-19129, estimated by comparison of urinary excretion data to data from the SC-19200 IV treatment group, were 80.1% and 73.3% respectively. The percentages of total radioactivity absorbed from oral doses of SC-19200 and SC-19129 based on fecal excretion data were estimated (see equation in Section III.I) to be 95.3% and 92.2% respectively.

H. Distribution of Urinary and Fecal Radioactivity:

The distribution of radioactivity in HPLRC profiles of pooled urine samples is shown in Figures 9-11. Approximately 74% of the radioactivity excreted in the urine following IV administration was found to be intact [^{14}C]-SC-19200 (Figure 9 and Table 8). Thus about 67% of the dose was excreted as SC-19200 and an additional 17% of the dose was excreted as the N-acetyl derivative of SC-19200 (Table 8). Only approximately 2% of the dose was excreted as phenylacetylglycine. However phenylacetylglycine was the major metabolite in urine (approximately 80% to 90% of the radioactivity present in urine samples) following oral administration of [^{14}C]-SC-19200 or [^{14}C]-SC-19129 (Figures 10, 11), and accounted for approximately 61% and 58% of the administered dose respectively. [^{14}C]-SC-19200 and its N-acetylated metabolite accounted for 2.0% and 4.5% of the dose respectively following oral administration of [^{14}C]-SC-19200 and 2.1% and 4.5% of the dose respectively following oral administration of [^{14}C]-SC-19129 (Table 8). Comparison of the combined amounts of [^{14}C]-SC-19200 and [^{14}C]-N-acetyl-SC-19200 excreted in the urine following

oral versus IV doses indicated absorption of 7.7% of the [¹⁴C]-SC-19200 oral dose and 7.8% of the [¹⁴C]-SC-19129 oral dose with the β-aspartyl peptide bond intact.

Due to the relatively low percentages of dose excreted in the feces, metabolic profiles were not obtained for this route of excretion (see Section III.J).

V. Discussion and Conclusions

A. Metabolic Formation of Free Phenylalanine (Phe):

The major radiolabeled constituents in plasma following IV administration of [^{14}C]-SC-19200 were unchanged [^{14}C]-SC-19200, [^{14}C]-Phe and [^{14}C]-N-acetyl-SC-19200. Unchanged [^{14}C]-SC-19200 was cleared rapidly from plasma (terminal half-life of 0.583 hour) but total radioactivity was cleared slowly (terminal half-life of 77.3 hours) and at 2 hours and later after IV administration was largely in a form not retained on Bond Elut[™] columns. This unretained radioactivity was precipitable by perchloric acid and is assumed to be present in the form of [^{14}C]-Phe incorporated biosynthetically into plasma proteins (10,11). Approximately 10% or less of [^{14}C]-SC-19200 given IV was estimated to be metabolized to free [^{14}C]-Phe in the rabbit based on the observed recovery of radiolabel from the IV dose in breath $^{14}\text{CO}_2$ (0.61%, Table 7) and in the urine and feces (91.3%, Table 7). The predicted excretion of radiolabel from [^{14}C]-Phe in the rabbit (given as [U- ^{14}C -Phe] aspartame which is metabolized via hydrolysis to its constituent subunits, Phe, aspartic acid and methanol; reference 10) would be 7.1% in the breath $^{14}\text{CO}_2$ (slightly more than 10 times the amount recovered from [^{14}C]-SC-19200). Likewise, the predicted excretion of radiolabel from [^{14}C]-Phe in the urine and feces in the rabbit would be approximately 9% (10) or approximately one tenth of the amount recovered from [^{14}C]-SC-19200.

The major metabolite formed following oral doses of [^{14}C]-SC-19200 or [^{14}C]-SC-19129 was the glycine conjugate of phenylacetic acid (PAGly). PAGly is a normal urinary metabolite of Phe in non-primate mammalian species (11).

However oxidation to phenylacetic acid followed by amino acid conjugation is normally a minor pathway of Phe elimination in mammals, with hydroxylation to tyrosine and subsequent oxidation to CO_2 being the major route of elimination from the body (11). Comparison of the percentages (Table 2) of radiolabel from orally administered [^{14}C]-SC-19200 and [^{14}C]-SC-19129 eliminated in breath and $^{14}\text{CO}_2$ (4.28% and 4.14% respectively), urine (72.7% and 66.5% respectively) and feces (5.30% and 8.39% respectively) to the amounts expected to be excreted from [^{14}C]-Phe suggests that approximately 25-40% of each dose was metabolized via free [^{14}C]-Phe. The high proportion of the phenylalanine subunits of SC-19200 and SC-19129 metabolized, via phenylacetic acid, to PAGly is probably the result of bacterial metabolism in the lower gastrointestinal tract. This is supported by the prolonged absorption phase and late T_{max} seen for plasma radioactivity (Table 4), the 2-3 hour lag phase in $^{14}\text{CO}_2$ excretion in breath (Table 7, Figure 6) and the late T_{max} of PAGly in plasma (Table 6). The absolute bioavailabilities of SC-19200 following oral administration of SC-19200 and SC-19129 were 7.7% and 7.8% respectively based on urinary excretion data (Table 7) and 22.6% and 8.5% respectively based on plasma concentration data (Table 5). Thus it appears that SC-19200 is slowly absorbed and that a large fraction reaches the lower gastrointestinal tract where it is metabolized by gut bacteria to Phe and phenylacetic acid.

B. Oral Bioavailability:

The absorption of total radioactivity from oral doses of [^{14}C]-SC-19200 and [^{14}C]-SC-19129 appeared to be very similar based on plasma concentration data (Table 4),

MRC-852-0066

urinary excretion data (Table 7) or fecal excretion data (Table 7). However, the absolute bioavailabilities of total radioactivity calculated from these three types of data differed from each other.

Fecal excretion appeared to provide a reasonable measure of oral bioavailability of total radioactivity since excretion by this route following the IV dose was very low (0.592%). Comparison of the fecal excretion data thus gives a lower limit of 95.3% and 92.2% absorption of the [^{14}C]-SC-19200 and [^{14}C]-SC-19129 oral doses respectively.

Bioavailability estimates based on urinary excretion were lower than those based on fecal excretion by approximately 15% and 19% for [^{14}C]-SC-19200 and [^{14}C]-SC-19129 respectively. This is probably due to the fact that Phe, which is a quantitatively more important metabolite following oral administration compared to IV administration, is eliminated to only a very small extent (5% of dose or less; see reference 10) in urine. Thus urinary excretion data will tend to underestimate oral bioavailability of total radioactivity.

Plasma AUC values cannot be used to determine oral bioavailability of total radioactivity from [^{14}C]-SC-19200 or [^{14}C]-SC-19129 since these AUC values appear to be primarily a measure of the amount of [^{14}C]-Phe produced by metabolism. The very long half-life of [^{14}C]-Phe incorporated into tissue and plasma proteins results in large AUC values and contributions from metabolites other than Phe are relatively small compared to the contribution of Phe. When the percentage of the dose metabolized to Phe is larger for oral doses than for IV doses, as in the rabbit (Section V.A), plasma data will overestimate total radioactivity bioavailability (greater than 400% for the rabbit, see Table 4). The major routes of Phe clearance are

MRC-852-0066

incorporation into tissue proteins and hydroxylation to tyrosine followed by oxidation to CO_2 (11). Excretion of $^{14}\text{CO}_2$ in breath was very similar for the oral doses of ^{14}C -SC-19200 and ^{14}C -SC-19129 and probably also reflects the amount of Phe produced by metabolism as discussed above for the plasma concentration data.

The absorption of intact SC-19200 from orally administered SC-19200 and SC-19129 was similar (7.7% and 7.8% respectively) based on urinary excretion data (Section IV.H). However AUC values for plasma SC-19200 indicated greater absorption of SC-19200 from SC-19200 doses (22.6%) than from SC-19129 doses (8.5%). Since some ^{14}C -SC-19129 did reach the systemic circulation following its oral administration and since its volume of distribution may be larger than that of SC-19200, the percent of SC-19129 doses absorbed with the β -aspartyl peptide bond intact may be underestimated by plasma AUC comparisons.

C. Metabolic Pathway:

The proposed metabolic pathway of SC-19129 and SC-19200 is shown in Figure 12. The methyl ester bond of orally administered SC-19129 is extensively metabolized presystemically by enzymes in the intestines and/or liver since only small amounts of SC-19129 were observed in plasma. A fraction (approximately 8.5-23% in the rabbit) of the SC-19200 formed by enzymatic hydrolysis of SC-19129, or of an oral dose of SC-19200 is absorbed and reaches the systemic circulation intact. SC-19200 in the systemic circulation (IV or oral) is extensively metabolized with the formation of free Phe and N-acetyl-SC-19200. Free aspartic acid may also be formed, but since it was not labeled it was not detected in the study. The majority of the orally

administered radiochemical dose is metabolized to phenylacetic acid which in turn is conjugated with glycine to form PAGly and excreted as such in the urine. The extensive formation of phenylacetic acid is presumed to be due to bacterial metabolism in the lower GI tract (as discussed in Section V.A), whereas its subsequent conjugation with glycine occurs following absorption into the body. In a study in the rhesus monkey the major metabolite of orally administered [^{14}C]-SC-19129 was phenylacetylglutamine (2). These results are consistent with known species differences in the conjugation of phenylacetic acid (10).

D. Conclusions:

1. Oral absorption of total radioactivity from [^{14}C]-SC-19200 or [^{14}C]-SC-19129 is prolonged with peak levels occurring at approximately 5 hours.
2. Plasma elimination half-lives for total radioactivity following IV and oral doses of SC-19200 (77.3 to 184 hours) were much longer than the elimination half-life for intact SC-19200 (0.583 hours following IV administration). The long plasma elimination half-life of total radioactivity appears to be due to metabolic formation of [^{14}C]-Phe and its subsequent incorporation into tissue and plasma proteins.
3. The absolute bioavailabilities of total radioactivity from orally administered SC-19200 and SC-19129 were estimated to be 95.3% and 92.2% based on fecal excretion data and 82.3% and 79.0% respectively based on urinary excretion data. The urinary excretion data were judged to underestimate

the oral bioavailability due to the relatively higher amounts of Phe produced following oral administration compared to IV administration and the low urinary excretion of Phe. Conversely plasma AUC comparisons markedly overestimated oral bioavailability of total radioactivity due to the long plasma half-life of [^{14}C]-Phe incorporated into plasma proteins.

4. SC-19200 was rapidly cleared from the systemic circulation with a terminal half-life of 0.583 hours following IV administration. The volume of distribution of SC-19200 was 0.236 l/kg indicating low uptake of intact SC-19200 into tissues.
5. [^{14}C]-SC-19129 was found in plasma at low concentrations following its oral administration. The AUC for SC-19129 was less than 2% of the SC-19200 AUC following the same dose.
6. The bioavailabilities of SC-19200 from orally administered SC-19200 and SC-19129 were 7.7% and 7.8% respectively based on urinary excretion data but were 23% and 8.5% respectively based on plasma concentration data. Since some SC-19129 did reach the systemic circulation following its oral administration, and since its volume of distribution may be larger than that of SC-19200, absorption prior to beta-aspartyl peptide bond hydrolysis may be underestimated by plasma data.
7. The major metabolites of SC-19200 were the N-acetyl derivative, Phe, phenylacetic acid and PAGly. Clearance of Phe appeared to occur by known routes including oxidation to $^{14}\text{CO}_2$ (expired in air) and incorporation into plasma proteins by peptide/protein synthesis.

MRC-852-0066

8. The major route of elimination of radioactivity absorbed after oral or IV administration was in the urine. Recovery from the IV dose was 90.7% in urine, 0.61% in breath and 0.59% in feces, for a total of 91.9%. Recoveries from oral doses of SC-19200 and SC-19129 were, respectively, 72.7% and 66.5% in urine, 5.3% and 8.4% in feces and 4.3% and 4.1% in breath. The total ^{14}C recoveries in breath are based on 0-7 hour samples (urine and fecal recoveries are based on 0-120 hour samples) and should be taken to be minimum estimates.

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VII. Tables

MRC-852-0066

-VII.1-

Table 1
Mean Body Weights and Dosages

Test Article	Route	Sample Type	Body Weight ^a (kg)	Specific ^b Activity (mCi/mg)	Dose (mg/kg) ^a	Dose (mCi/kg)
SC-19200	IV	Plasma, Urine & Feces	2.47 ± 0.05	5.05	10.0 ± 0.0	50.5
		Breath	2.53 ± 0.05	2.68	10.0 ± 0.0	26.8
SC-19200	Oral	Plasma, Urine & Feces	2.8 ± 0.1	5.09	10 ± 0	50.9
		Breath	2.29 ± 0.08	2.60	10.0 ± 0.0	26.0
SC-19129	Oral	Plasma, Urine & Feces	2.8 ± 0.1	4.74	10 ± 0	47.4
		Breath	2.41 ± 0.07	2.39	10.0 ± 0.0	23.9

^a Values are the mean ± standard error of 4 animals per group. Individual animal values are given in Tables 1A-3A, Section IX.

^b Doses were prepared by mixing appropriate amounts of [¹⁴C]-SC-19129 and unlabeled SC-19129 or [¹⁴C]-SC-19200 and unlabeled SC-19200 as described in Section III.B.

Table 2
Extractability of [¹⁴C]-SC-19129
and [¹⁴C]-SC-19200
from Rabbit Plasma, Urine and Feces

Sample Type	Mean Percent of Radiolabel Recovered	
	[¹⁴ C]-SC-19129 ^a	[¹⁴ C]-SC-19200 ^a
Plasma	83.9 ± 4.9 ^b	69.2 ± 2.2 ^b
Urine	98.8 ± 0.6 ^c	100 ^d
Feces	100 ^d	100 ^d

^a Compound added to indicated biological sample type.

^b Mean ± standard error of 6 samples.

^c Mean ± standard error of 3 samples.

^d Mean of duplicate samples.

Table 3
Stability of [¹⁴C]-SC-19129 and
[¹⁴C]-SC-19200 in Biological Samples

Biological Sample Type	Compound Added	Conditions	Percent Eluted from HPLRC as Compound Added
Plasma ^a	[¹⁴ C]-SC-19129	Immediate	98.4
		Stored at -70°C for 2 days	96.6
	[¹⁴ C]-SC-19200	Immediate	97.8
		Stored at -70°C for 14 days	98.8
Urine	[¹⁴ C]-SC-19129	Immediate	95.6
		Stored at -70°C for 9 days	92.2
	[¹⁴ C]-SC-19200	Immediate	95.3
		Stored at -70°C for 9 days	96.0

^a Control rabbit plasma was pretreated with 1×10^{-4} molar diethyl-p-nitrophenyl phosphate to inhibit esterase activity (Section III.D.1).

Table 4
Mean Plasma Concentrations and Pharmacokinetic Parameters
of ^{14}C -SC-19129 or ^{14}C -SC-19200 to Female Rabbits^a

Parameter	^{14}C -SC-19200 Intravenous	^{14}C -SC-19200 Oral	^{14}C -SC-19129 Oral
Plasma Concentration (mcg/ml) at Indicated Time (hours)	0.08 0.25 0.50 0.75 1 2 4 6 8 12 24 48 72 96 120	0.452 0.487 0.684 0.819 3.74 3.35 3.62 4.63 3.60 2.69 2.34 2.27 1.96 1.80	0.120 0.337 0.506 1.26 4.34 4.16 4.03 3.81 2.80 2.85 2.55 2.29 2.09 1.79
	34.0 20.5 13.5 8.71 6.82 2.95 1.40 1.32 1.31 1.06 0.852 0.706 0.613 0.541 0.505	b + + + + + + + + + + + + + +	b + + + + + + + + + + + + + +
	1.5 1.0 1.1 1.03 1.02 0.67 0.22 0.22 0.12 0.14 0.091 0.069 0.053 0.072 0.046	0.169 0.106 0.071 0.156 1.22 0.65 0.72 1.07 1.14 0.68 0.49 0.41 0.39 0.34	0.033 0.079 0.041 0.34 1.09 0.67 0.85 0.87 0.63 0.57 0.51 0.54 0.42 0.35
Cmax (mcg/ml)	48.6	6.06	5.87
Tmax (hour)	h	+	+
AUC ₀₋₂₀ (mcg/ml)hr	109	291	297
AUC ₀₋₁₂₀ (mcg/ml)hr	165	767d	675d
Bioavailability (%)	100	154	122
Half-lives (hours)	0.115 0.562 77.3	0.024 0.048 2.4	0.54 1 54
Volume of Distribution (l/kg)	0.211	0.019	0.49

Table 4 (cont'd)

- a Values are the mean + standard error of 4 animals unless otherwise indicated. Individual animal values are given in Tables 4A-6A for plasma radioactivity concentrations and in Tables 7A-9A for pharmacokinetic parameters. The plasma concentrations (Tables 4A-6A) have been rounded to 3 significant digits. The pharmacokinetic parameters (Tables 7A-9A) were calculated using unrounded (6 significant digits) values obtained in the calculation of the plasma concentrations.
- b Sampling time not applicable to this route of administration.
- c Obtained from the results of NONLIN analysis by extrapolation to zero time; see Table 7A, Czero.
- d Calculated using the mean AUC_0^∞ values.
- e Half-lives calculated by the NONLIN program (see Table 7A) by fitting the plasma concentration to the equation:
$$Cp = Ae^{-at} + Be^{-bt} + Ce^{-yt}$$
- f Pharmacokinetic constants could not be meaningfully calculated by CSTRIP or NONLIN for these groups.
- g Terminal elimination calculated by linear regression, see Tables 8A and 9A.
- h Not applicable.

Table 5
Plasma Concentrations and Pharmacokinetic Parameters
of [¹⁴C]-SC-19200 or [¹⁴C]-SC-19200 to Female Rabbits^a

Parameter	[¹⁴ C]-SC-19200 Intravenous	[¹⁴ C]-SC-19200 Oral	[¹⁴ C]-SC-19129 Oral
Plasma Concentration (mcg/ml) at Indicated Time (hours)	0.08 0.25 0.50 0.75 1 2 4 6 8	30.2 16.3 8.45 5.78 3.67 1.21 0.230 c c	b 0.226 0.245 0.206 0.320 0.285 0.603 0.662 0.441
Cmax (mcg/ml)	42.4 ^d	0.662	0.383
Tmax (hour)	1	6	2
AUC ₀ [∞] (lmcg/ml)hr)	16.8	3.80	1.43
Absolute Bioavailability (%) ^e	100	22.6	8.51
Half-lives (hours)	α 0.119 ^f β 0.583 ^f Volume of Distribution (l/kg)	g g g 1	g 2.38 ^{g/h} 1

- ^a Values obtained using pooled plasma samples from 4 rabbits per treatment group.
^b Sampling time not applicable to this route of administration.
^c Not detected in HPLC profile for this sample.

Table 5 (cont'd)

- d Obtained from the parameters calculated by the NONLIN program and extrapolation to zero time.
- e Calculated using the AUC values.
- f Half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$C_p = A e^{-\alpha t} + B e^{-\beta t}$$
- g Pharmacokinetic rate constants could not be meaningfully calculated by CSTRIP or NONLIN for these groups.
- h Terminal elimination half-life calculated by linear regression of the natural logarithm of the plasma concentrations versus time from 2 to 8 hours.
- i Not applicable.

Table 6
Plasma Concentrations of [14 C]-Phenylalanine, [14 C]-Phenylacetylglutamine,
[14 C]-Phenylacetic Acid and [14 C]-N-Acetyl-SC-19200
Following Administration of
[14 C]-SC-19200 or [14 C]-SC-19129 to Female Rabbits*

Dose	Time (hours)	Metabolite Concentration (mcg equivalents/ml)			
		Phenylalanine	Phenylacetylglutamine	Phenylacetic Acid	N-Acetyl-SC-19200
[14 C]-SC-19200 Intravenous	0.08	0.372	0.188	b	0.375
	0.25	0.348	0.230	b	0.272
	0.50	0.261	b	b	0.120
	0.75	b	b	b	0.236
	1	0.541	b	b	b
	2	0.110	b	b	b
	4	b	b	b	b
	6	b	b	b	b
	8	b	b	b	b
	12	b	b	b	b
[14 C]-SC-19200 Oral	0.25	b	0.174	b	b
	0.50	b	0.155	b	b
	0.75	0.069	0.110	b	b
	1	0.118	0.148	0.168	b
	2	b	1.40	1.33	b
	4	b	1.01	1.22	b
	6	0.472	1.01	0.910	b
	8	b	1.73	1.85	b
	12	0.457	0.555	0.788	b
[14 C]-SC-19129 Oral	0.25	0.004	0.024	b	b
	0.50	0.055	0.020	b	b
	0.75	0.121	0.050	b	b
	1	0.240	0.118	0.070	b
	2	b	0.930	1.03	b
	4	b	0.425	0.555	b
	6	0.108	0.567	0.330	b
	8	0.032	0.181	b	b
	12	b	0.108	b	b

Table 6 (cont'd)

- a Plasma concentrations were obtained by analysis of pooled plasma samples from 4 animals per treatment group. Concentrations are expressed as mcg equivalents of the administered compound without correction for the molecular weight of the metabolite.
- b Not detected in HPLRC profile for this sample.

Table 7
Cumulative Percentage of Radioactive Dose Excreted
in Breath, Urine and Feces of Female Rabbits
Following Administration of [^{14}C]-SC-19129 or [^{14}C]-SC-19200

Sample	Collection Time (hours)	Percent Recovery of Radioactivity ^a					
		[^{14}C]-SC-19200 Intravenous		[^{14}C]-SC-19200 Oral		[^{14}C]-SC-19129 Oral	
Breath ^b	0-0.5	0.039	+	0.003	+	0.008	+
	0-1	0.101	+	0.006	+	0.032	+
	0-2	0.175	+	0.013	+	0.187	+
	0-3	0.313	+	0.025	+	0.187	+
	0-4	0.424	+	0.035	+	0.829	+
	0-5	0.505	+	0.044	+	1.82	+
	0-6	0.564	+	0.051	+	2.84	+
Urine ^c	0-7	0.610	+	0.056	+	3.61	+
	0-12	86.0	+	3.7	+	4.14	+
	0-24	90.1	+	42.8	+	53.0	+
	0-48	90.4	+	60.6	+	62.4	+
	0-72	90.6	+	71.9	+	65.9	+
	0-96	90.6	+	72.5	+	66.2	+
	0-120	90.7	+	72.7	+	66.5	+
Feces ^c	0-24	0.253	+	1.44	+	2.42	+
	0-48	0.388	+	3.80	+	5.60	+
	0-72	0.470	+	4.48	+	7.42	+
	0-96	0.542	+	5.08	+	8.04	+
	0-120	0.592	+	5.30	+	8.39	+
Breath, Urine & Feces ^d		91.9		82.3		79.0	

Table 7 (cont'd)

- a Values are the mean \pm standard error (SEM) of 4 animals.
- b Individual animal values are given in Tables 10A-12A.
- c Individual animal values are given in Tables 13A-15A.
- d Values are the sums of the respective mean values.

Table 8

Urinary Excretion of [^{14}C]-SC-19200,
 [^{14}C]-N-Acetyl-SC-19200 and
 [^{14}C]-Phenylacetylglycine
 Following Administration of
 [^{14}C]-SC-19129 or [^{14}C]-SC-19200
 to Female Rabbits

Metabolite	Percent Recovery of Radioactivity ^a		
	[^{14}C]-SC-19200	[^{14}C]-SC-19200	[^{14}C]-SC-19129
	Intravenous	Oral	Oral
SC-19200	67.3	1.98	2.10
N-Acetyl-SC-19200	16.7	4.49	4.47
Phenylacetylglycine	1.87	60.7	58.3
Total ^{14}C ^b	90.7	72.7	66.5

^a Percentages of dose recovered as the metabolites were determined by analysis of pooled urine samples from 4 animals per treatment group.

^b Mean percentages of dose recovered, repeated here from Table 7, for reference.

VIII. Figures

MRC-852-0066

-VIII.1-

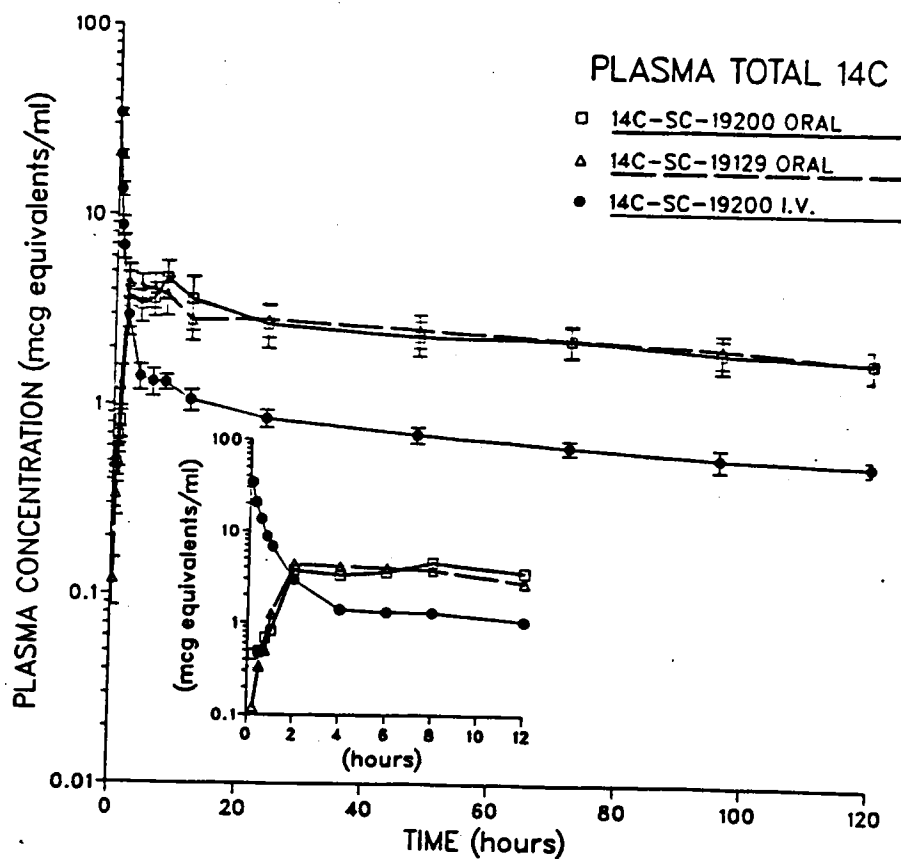


Figure 1. Mean plasma concentrations of total radioactive compounds following intravenous administration of [^{14}C]-SC-19200 (●) or oral administration of [^{14}C]-SC-19200 (□) or [^{14}C]-SC-19129 (△) to groups of 4 female rabbits. The vertical bars represent the standard errors of the means. The insert shows the mean plasma concentrations for the first 12 hours with the time axis expanded. Units: ordinate, concentration in plasma expressed as mcg equivalents of compound administered per ml; abscissa, time in hours after administration.

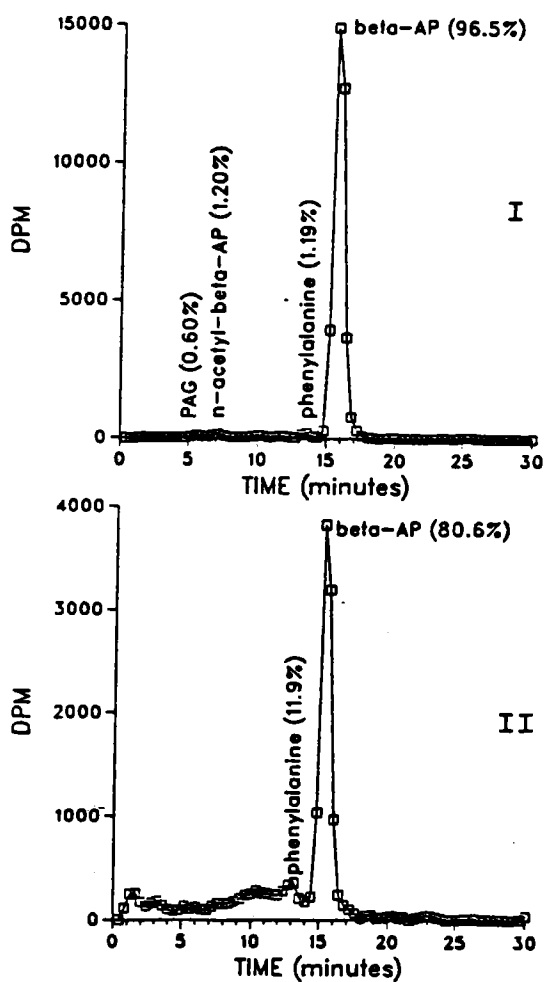


Figure 2. High performance liquid radiochromatograms of the acetonitrile eluent from Bond Elut[™] extraction of pooled plasma samples collected at 0.08 hour (I) and 1 hour (II) after IV administration of [¹⁴C]-SC-19200 to female rabbits. The locations of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

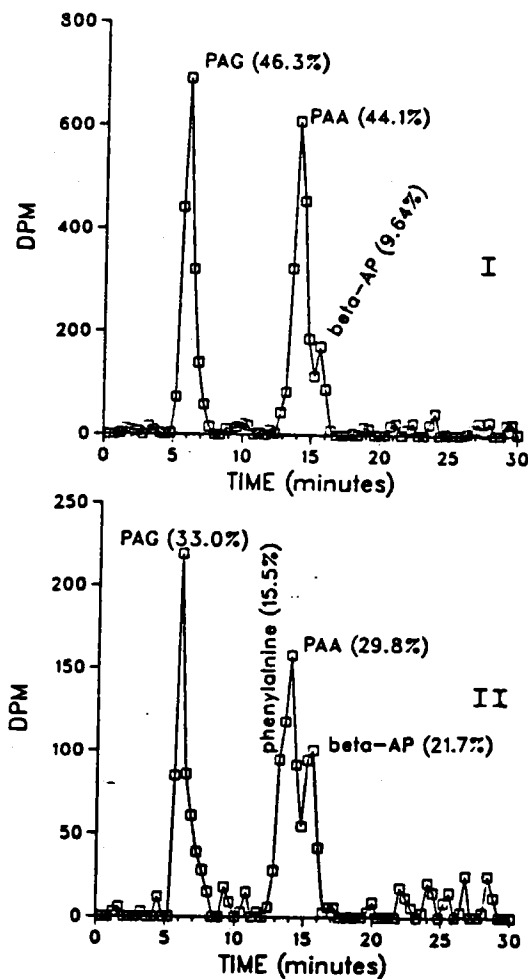


Figure 3. High performance liquid radiochromatograms of the acetonitrile eluent from Bond Elut™ extraction of pooled plasma samples collected at 2 hours (I) and 6 hour (II) after oral administration of [14 C]-SC-19200 to female rabbits. The locations of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

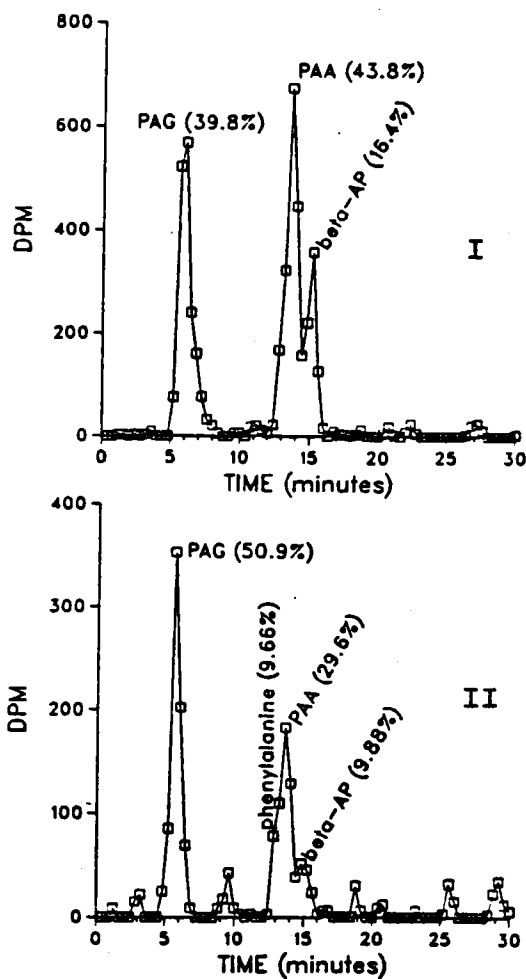


Figure 4. High performance liquid radiochromatograms of the acetonitrile eluent from Bond Elut[™] extraction of pooled plasma samples collected at 2 hours (I) and 6 hours (II) after oral administration of [¹⁴C]-SC-19129 to female rabbits. The location of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

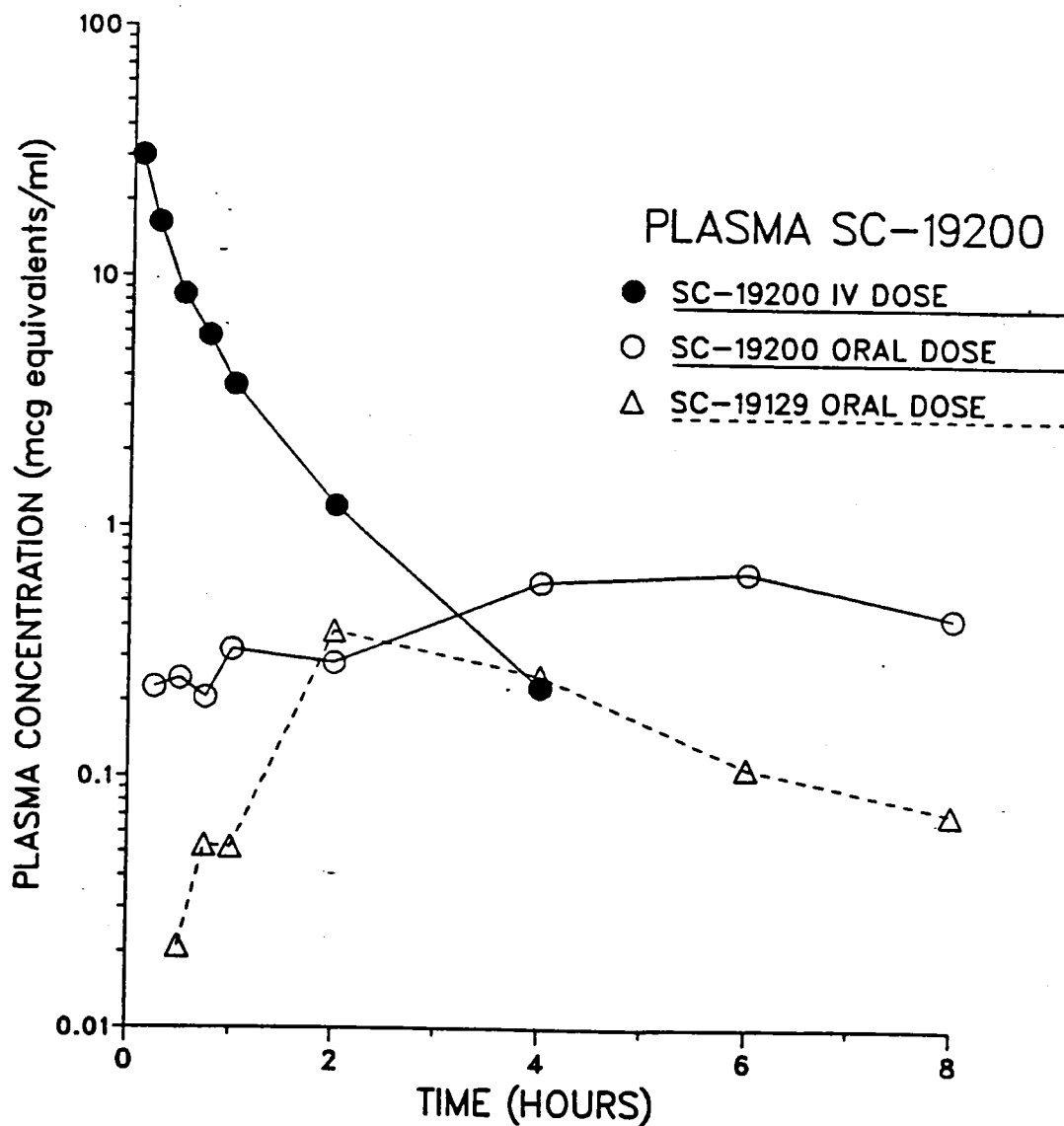


Figure 5. Mean plasma concentrations of [^{14}C]-SC-19200 in pooled plasma from rabbits following IV administration of [^{14}C]-SC-19200 (●) or oral administration of [^{14}C]-SC-19200 (○) or [^{14}C]-SC-19129 (△). Abscissa: time after dose administration in hours. Ordinate: concentrations in plasma expressed as mcg equivalents/ml.

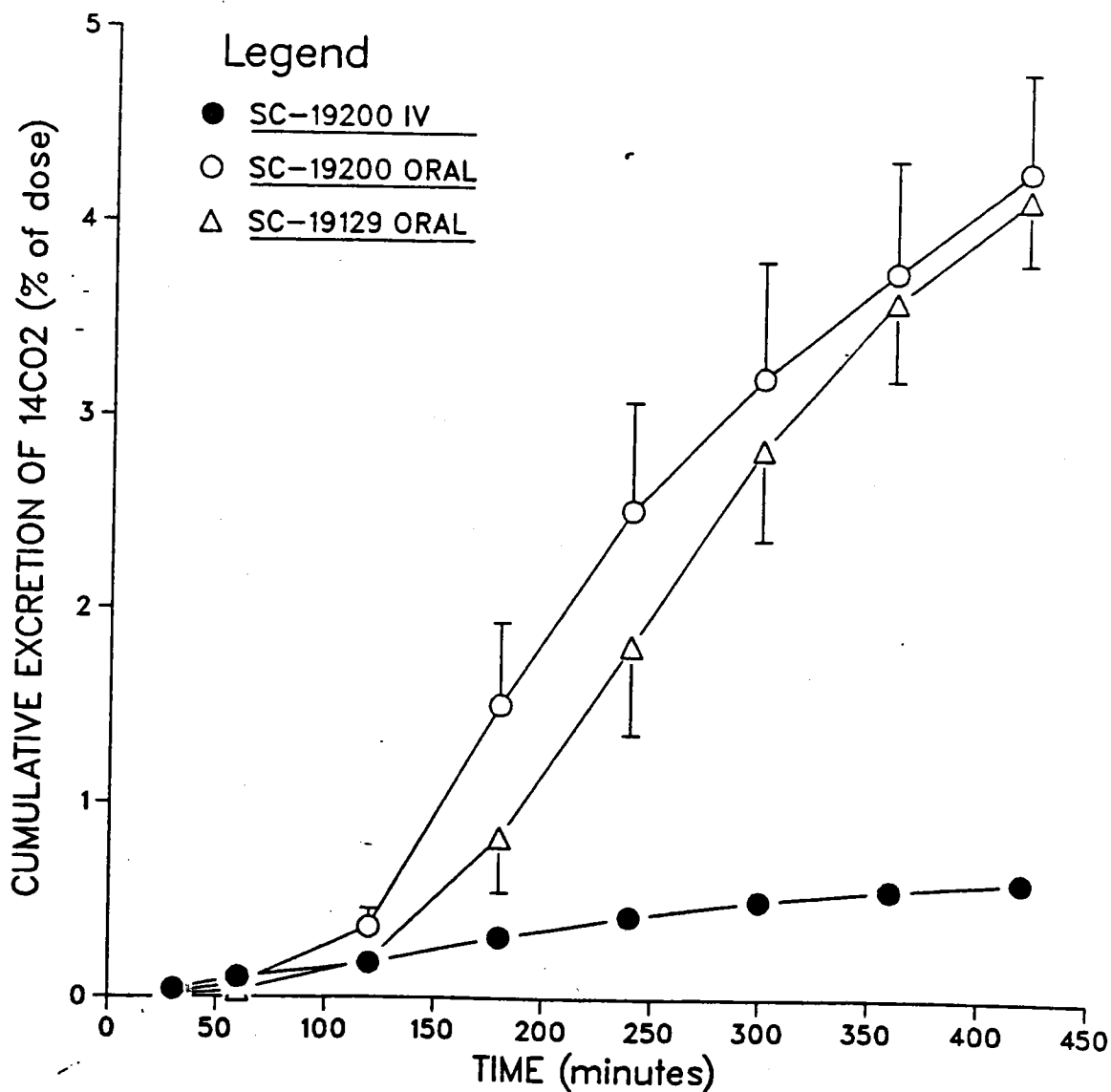


Figure 6. Mean cumulative excretion of $^{14}\text{CO}_2$ in the breath following IV administration of [^{14}C]-SC-19200 (●) or oral administration of [^{14}C]-SC-19200 (○) or [^{14}C]-SC-19129 (△) to female rabbits. The vertical bars indicate the standard errors of the means. Ordinate; cumulative excretion of radioactivity as a percentage of dose. Abscissa; time after dose administration in hours.

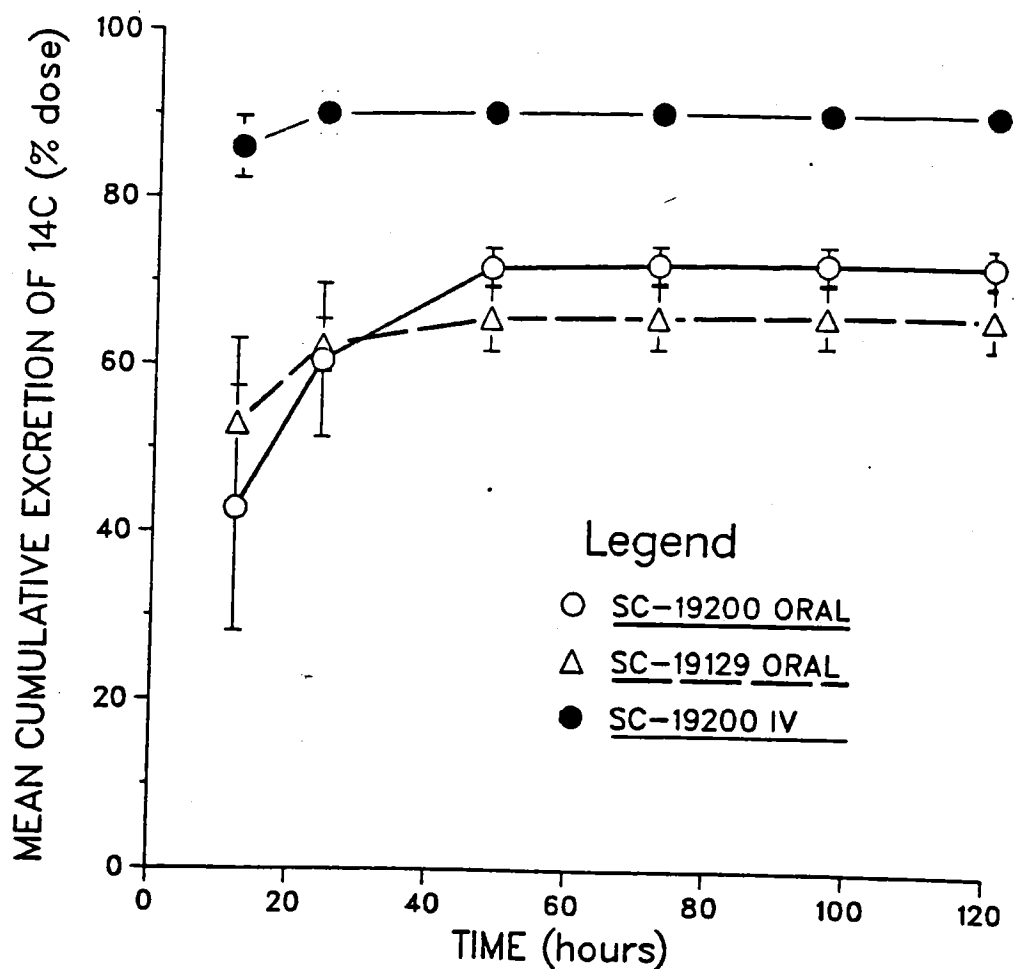


Figure 7. Mean cumulative excretion of radioactivity in urine following IV administration of [^{14}C]-SC-19200 (●) or oral administration of [^{14}C]-SC-19200 (○) or [^{14}C]-SC-19129 (Δ) to female rabbits. The vertical bars indicate the standard errors of the means. Abscissa; time after dose administration in hours. Ordinate: cumulative excretion of radioactivity as a percentage of dose.

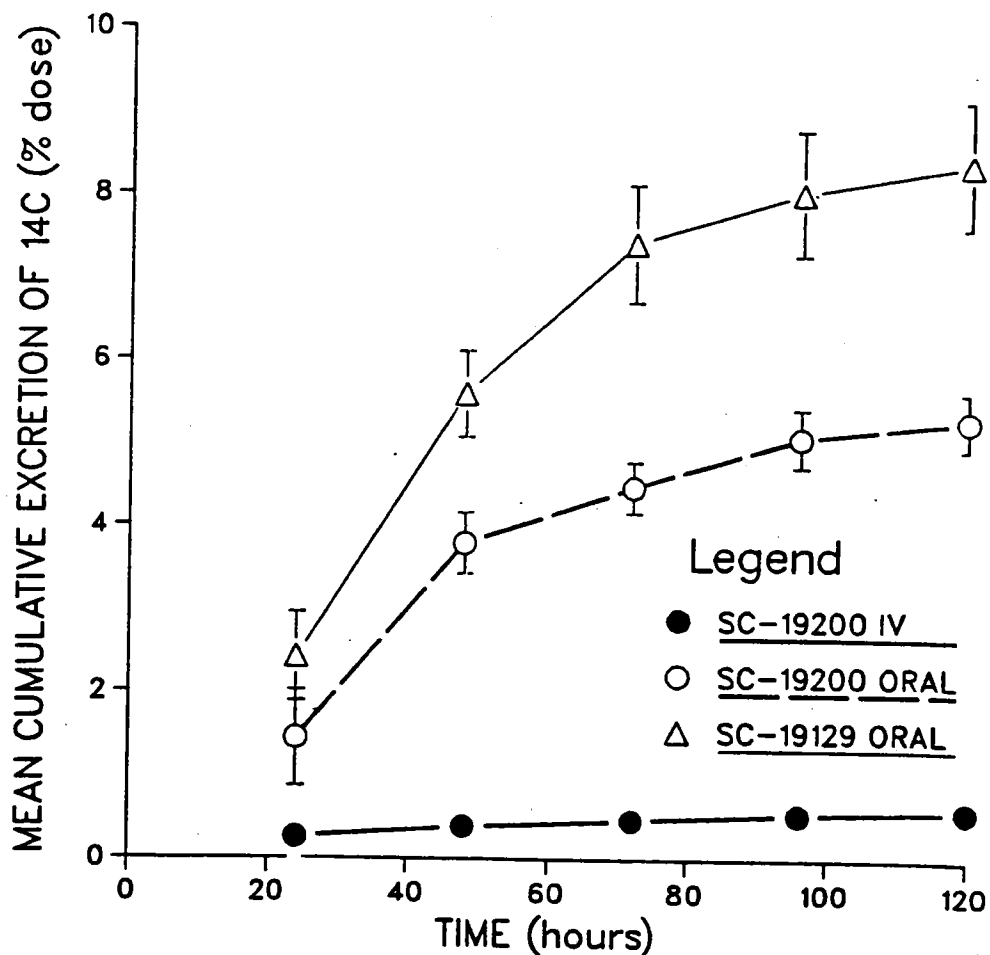


Figure 8. Mean cumulative excretion of radioactivity in feces following IV administration of [^{14}C]-SC-19200 (●) or oral administration of [^{14}C]-SC-19200 (○) or [^{14}C]-SC-19129 (△) to female rabbits. The vertical bars indicate the standard errors of the means. Abscissa; time after dose administration in hours. Ordinate; cumulative excretion of radioactivity as a percentage of dose.

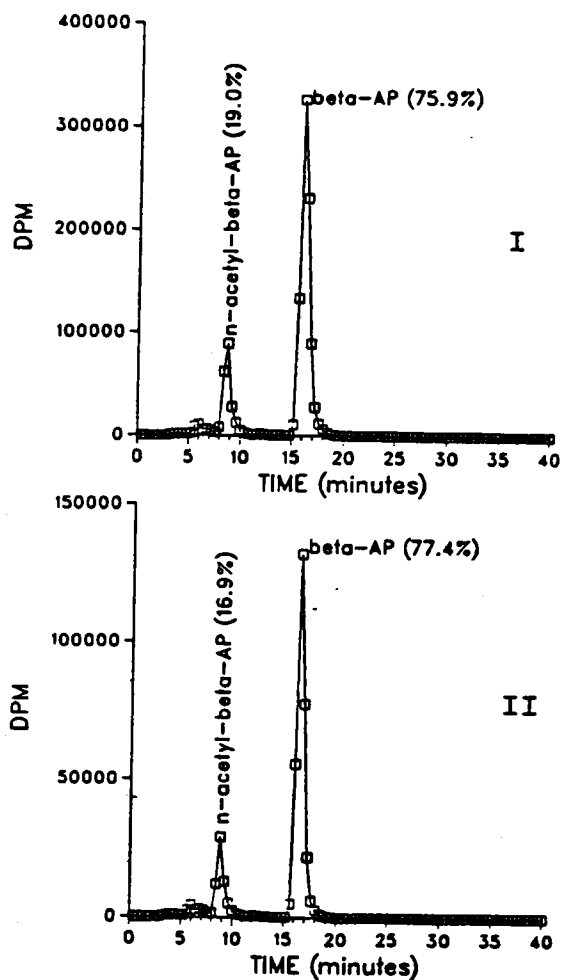


Figure 9. High performance liquid radiochromatograms of the pooled urine samples collected from 0-6 hours (I) and 6-12 hours (II) after IV administration of [14 C]-SC-19200 to female rabbits. The locations of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

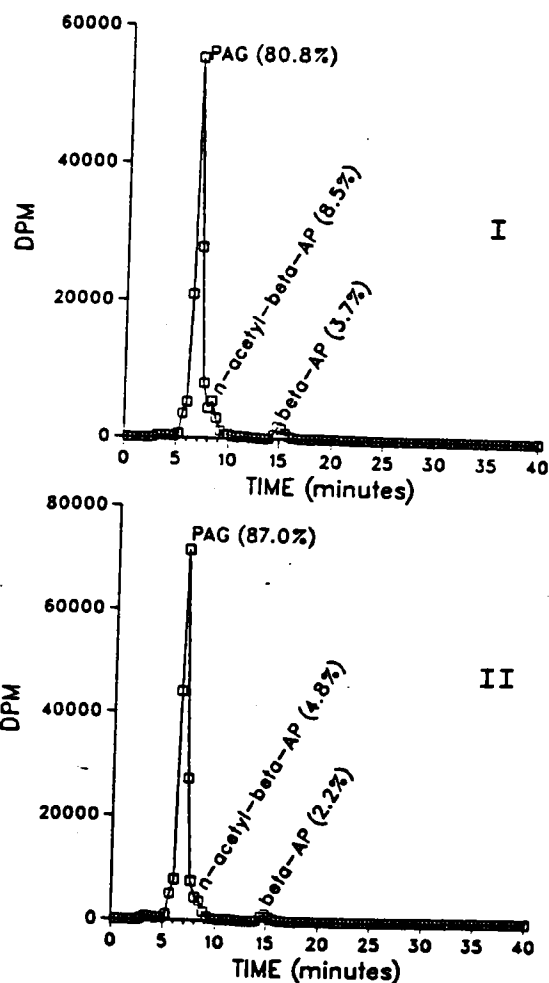


Figure 10. High performance liquid radiochromatograms of the pooled urine samples collected from 0-12 hours (I) and 12-24 hours (II) after oral administration of [^{14}C]-SC-19200 to female rabbits. The locations of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

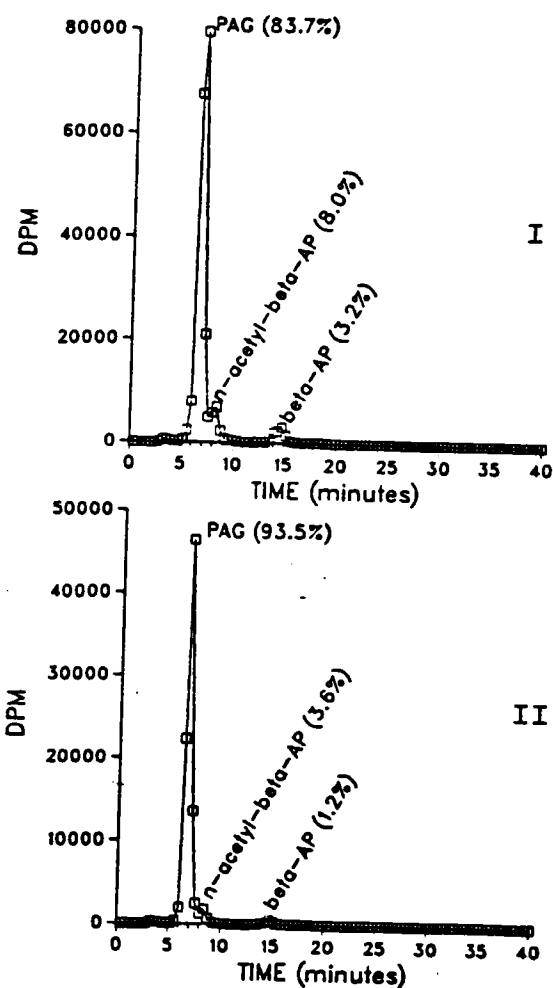


Figure 11. High performance liquid radiochromatograms of the pooled urine samples collected from 0-12 hours (I) and 12-24 hours (II) after oral administration of [14 C]-SC-19129 to female rabbits. The location of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

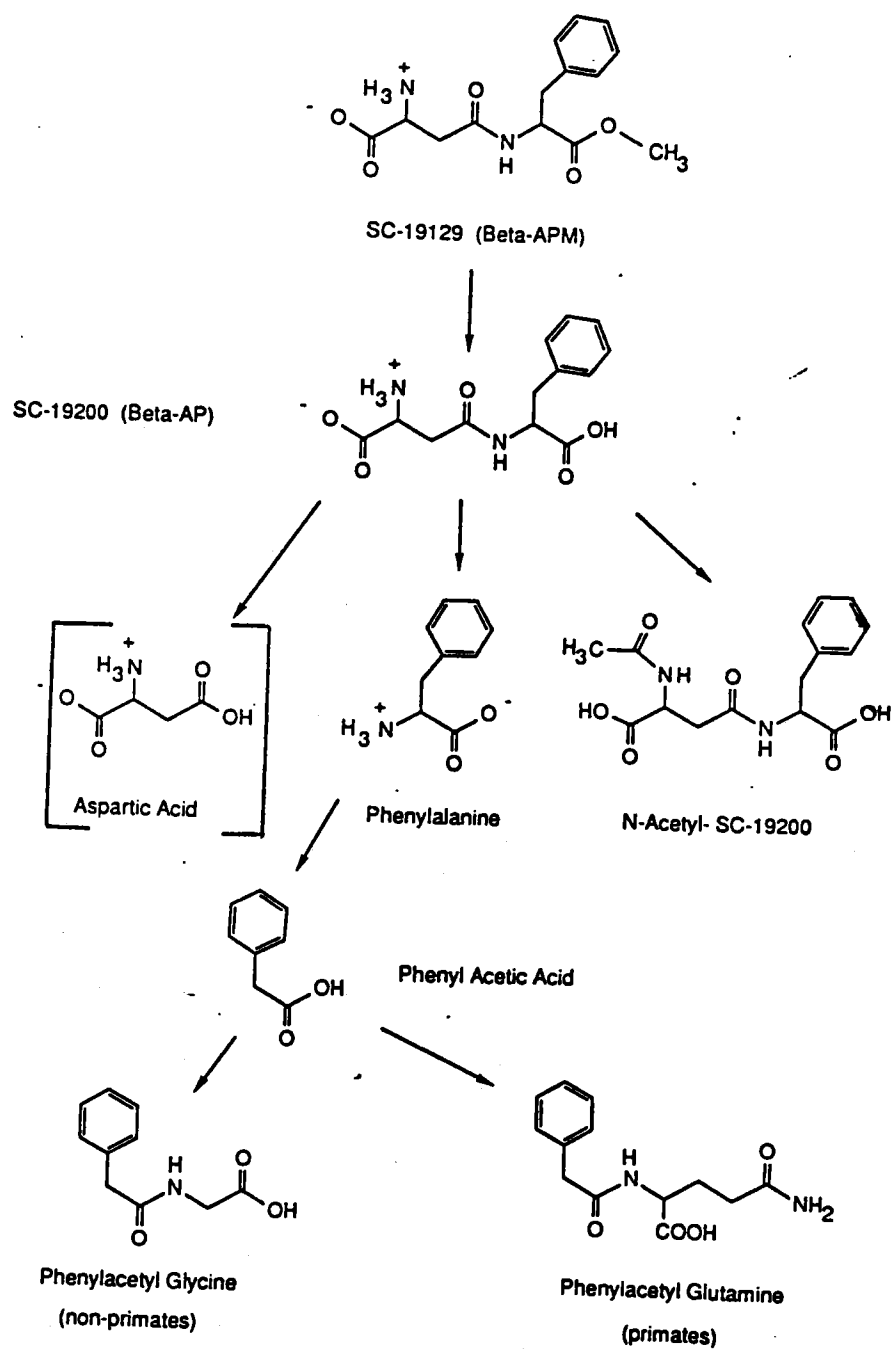


Figure 12. Structures and proposed metabolic pathway of SC-19129. Aspartic acid [in brackets] is a hypothetical metabolite which was not identified in this study.

IX. Appendix 1. Tables

MRC-852-0066

Table 1-A
Individual Rabbit Body Weights and Dosages
SC-19200 Intravenous Administration

Sample Type Collected	Rabbit #	Body Weight (kg)	Dose (mg/kg)
Plasma, Urine & Feces	1	2.42	10.0
	2	2.35	10.0
	3	2.56	10.0
	4	2.54	10.0
Breath	13	2.54	10.0
	14	2.45	10.0
	15	2.47	10.0
	16	2.66	10.0

Table 2A

Individual Rabbit Body Weights and Dosages
SC-19200 Oral Administration

Sample Type Collected	Rabbit #	Body Weight (kg)	Dose (mg/kg)
Plasma, Urine & Feces	17	2.9	10
	18	2.9	10
	19	2.9	10
	20	2.6	10
Breath	5	2.48	10.0
	6	2.09	10.0
	7	2.24	10.0
	8	2.35	10.0

Table 3A

Individual Rabbit Body Weights and Dosages
SC-19129 Oral Administration

Sample Type Collected	Rabbit #	Body Weight (kg)	Dose (mg/kg)
Plasma, Urine & Feces	21	2.8	10
	22	3.0	10
	23	2.7	10
	24	2.7	10
Breath	9	2.42	10.0
	10	2.55	10.0
	11	2.22	10.0
	12	2.44	10.0

Table 4A

Plasma Concentrations of Total Radioactivity
[¹⁴C]-SC-19200 Intravenous Dose

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME ^a	Rabbit #1	Rabbit #2	Rabbit #3	Rabbit #4	MEAN	SEM ^b
0.08	32.8	37.9	34.5	30.9	34.0	1.48
0.25	19.3	23.2	18.7	20.9	20.5	1.01
0.5	16.0	13.4	10.6	13.8	13.5	1.11
0.75	10.9	7.84	6.26	9.79	8.71	1.03
1.0	9.31	6.09	4.49	7.39	6.82	1.02
2.	4.84	2.22	1.83	2.90	2.95	0.668
4.	2.04	1.16	1.17	1.22	1.40	0.216
6.	1.95	1.19	0.971	1.16	1.32	0.216
8.	1.64	1.30	1.08	1.23	1.31	0.120
12.	1.45	1.04	0.883	0.852	1.06	0.137
24.	1.11	0.790	0.679	0.828	0.852	0.0914
48.	0.898	0.658	0.575	0.693	0.706	0.0686
72.	0.760	0.585	0.506	0.599	0.613	0.0533
96.	0.744	0.545	0.435	0.440	0.541	0.0723
120.	0.635	0.476	0.419	0.491	0.505	0.0459

^a Time after dose administration in hours.

^b Standard error of the mean.

Table 5A

Plasma Concentrations of Total Radioactivity
[¹⁴C]-SC-19200 Oral Dose

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME ^a	Rabbit #17	Rabbit #18	Rabbit #19	Rabbit #20	MEAN	SEM ^b
0.25	0.235	0.235	0.945	0.391	0.452	0.169
0.5	0.382	0.346	0.803	0.417	0.487	0.106
0.75	0.670	0.581	0.888	0.595	0.684	0.0708
1.0	0.635	1.29	0.688	0.666	0.819	0.156
2.0	6.30	5.30	1.27	2.09	3.74	1.22
4.0	2.59	2.11	5.03	3.65	3.35	0.648
6.0	3.26	1.90	5.35	3.98	3.62	0.718
8.0	3.65	2.25	7.17	5.46	4.63	1.07
12.0	1.99	1.30	5.89	5.20	3.60	1.14
24.0	2.09	1.06	3.77	3.84	2.69	0.679
48.0	1.82	1.26	3.38	2.90	2.34	0.485
72.0	1.90	1.34	3.15	2.69	2.27	0.405
96.0	1.56	1.06	2.72	2.51	1.96	0.393
120.0	1.45	1.00	2.43	2.31	1.80	0.343

a Time after dose administration in hours.

b Standard error of the mean.

Table 6A

Plasma Concentrations of Total Radioactivity
[¹⁴C]-SC-19129 Oral Dose

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME ^a	Rabbit #21	Rabbit #22	Rabbit #23	Rabbit #24	MEAN	SEM ^b
0.25	0.104	0.212	0.0518	0.113	0.120	0.0334
0.5	0.570	0.226	0.297	0.254	0.337	0.0790
0.75	0.621	0.490	0.485	0.428	0.506	0.0409
1.	0.607	1.48	2.11	0.828	1.26	0.339
2.	4.23	3.09	7.44	2.60	4.34	1.09
4.	5.30	5.32	3.06	2.94	4.16	0.667
6.	4.97	4.66	1.50	5.00	4.03	0.847
8.	5.71	3.76	1.50	4.27	3.81	0.874
12.	3.92	2.64	1.10	3.55	2.80	0.629
24.	3.93	3.08	1.23	3.16	2.85	0.573
48.	2.98	2.58	1.13	3.50	2.55	0.510
72.	2.63	2.29	1.05	3.19	2.29	0.454
96.	2.41	2.02	0.982	2.96	2.09	0.417
120.	2.12	1.84	0.810	2.41	1.79	0.348

a Time after dose administration in hours.

b Standard error of the mean.

Table 7A
Pharmacokinetic Parameters in Individual Rabbits
[14C]-SC-19200 Intravenous Dose

Parameter	Rabbit #1	Rabbit #2	Rabbit #3	Rabbit #4	Mean	SEM ^a
C _{max} (mcg/ml)	32.8	37.9	34.5	30.9	34.0	1.5
T _{max} (hour)	0.08	0.08	0.08	0.08	0.08	0.00
AUC ₀ [(mcg/ml)hr]	140	104	88	102	109	11
AUC ₀ [(mcg/ml)hr]	205	159	134	160	165	15
Rate Constant (k, hr ⁻¹) ^b						
α	14.7	4.38	5.57	5.29	7.49	2.42
β	1.00	1.35	1.46	1.22	1.26	0.10
γ	0.00971	0.00865	0.00918	0.00844	0.00900	0.00029
C zero (mcg/ml) ^c	59.7	48.8	47.3	38.4	48.6	4.4
Half-life (hours) ^b						
α	0.047	0.158	0.124	0.131	0.115	0.024
β	0.693	0.514	0.474	0.566	0.562	0.048
γ	71.4	80.1	75.5	82.1	77.3	2.4
Correlation ^d	0.999	1.00	1.00	1.00	1.00	0.00
Volume of Distribution (l/kg)	0.168	0.205	0.211	0.260	0.211	0.019

^a Standard error of the mean.

^b Absorption and/or elimination rate constants and the corresponding half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:
 $C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$

^c Concentration time zero obtained from results of NONLIN analysis by addition of the coefficient (A+B+C) in the above equation (footnote b).

^d Correlation between observed plasma concentrations and concentrations predicted by the NONLIN program.

Table 8A
Pharmacokinetic Parameters in Individual Rabbits
(¹⁴C)-SC-19200 Oral Dose

Parameter	Rabbit #17	Rabbit #18	Rabbit #19	Rabbit #20	Mean	SEM ^a
C _{max} (mcg/ml)	6.30	5.30	7.17	5.46	6.06	0.43
T _{max} (hour)	2	2	8	8	5	2
AUC ₀ [(mcg/ml)hr]	230	153	411	368	291	60
AUC ₀ [(mcg/ml)hr]	626	412	923	1107	767	154
Half-Life (hours) ^b	169	179	146	221	184	15

^a Standard error of the mean.

^b Calculated by linear regression of the natural logarithm of plasma concentrations versus time from 48 to 120 hours.

Table 9A
Pharmacokinetic Parameters in Individual Rabbits
[¹⁴C]-SC-19129 Oral Dose

Parameter	Rabbit #21	Rabbit #22	Rabbit #23	Rabbit #24	Mean	SEM ^a
C _{max} (mcg/ml)	5.71	5.32	7.44	5.00	5.87	0.54
T _{max} (hour)	8	4	2	6	5	1
AUC ₀ [(mcg/ml)hr]	365	301	143	379	297	54
AUC ₀ [(mcg/ml)hr]	823	688	325	863	675	122
Half-Life (hours) ^b	150	146	156	139	148	4

^a Standard error of the mean.

^b Calculated by linear regression of the natural logarithm of plasma concentrations versus time from 48 to 120 hours.

Table 10A

Mean Cumulative Excretion of $^{14}\text{CO}_2$ in Expired Air
 $[^{14}\text{C}]$ -SC-19200 Intravenous Dose

Individual Cumulative Percent Recoveries of Radioactivity

B R E A T H

Time (minutes)	Rabbit #14	Rabbit #15	Rabbit #13	Rabbit #16	MEAN	SEMA
0 - 30	0.0460	0.0419	0.0374	0.0315	0.0392	0.0031
0 - 60	0.114	0.115	0.0915	0.0832	0.101	0.0079
0 - 120	0.200	0.192	0.155	0.150	0.175	0.0127
0 - 180	0.361	0.347	0.255	0.290	0.313	0.0247
0 - 240	0.483	0.468	0.327	0.419	0.424	0.0353
0 - 300	0.578	0.554	0.379	0.509	0.505	0.0443
0 - 360	0.649	0.621	0.418	0.567	0.564	0.0514
0 - 420	0.699	0.673	0.448	0.620	0.610	0.0564

a Standard error of the mean.

Table 11A

Mean Cumulative Excretion of $^{14}\text{CO}_2$ in Expired Air
 ^{14}C -SC-19200 Oral Dose

Individual Cumulative Percent Recoveries of Radioactivity

B R E A T H

Time (minutes)	Rabbit #5	Rabbit #6	Rabbit #7	Rabbit #8	MEAN	SEM ^a
0 - 30	0.0367	0.0359	0.00618	0.0167	0.0239	0.0075
0 - 60	0.0617	0.0926	0.0352	0.0605	0.0625	0.0117
0 - 120	0.609	0.314	0.153	0.364	0.360	0.0944
0 - 180	2.48	1.27	0.474	1.82	1.51	0.425
0 - 240	3.29	2.53	0.937	3.33	2.52	0.559
0 - 300	3.57	3.58	1.47	4.22	3.21	0.600
0 - 360	3.87	4.29	2.12	4.76	3.76	0.577
0 - 420	4.20	4.94	2.87	5.12	4.28	0.511

a Standard error of the mean.

Table 12A
Mean Cumulative Excretion of $^{14}\text{CO}_2$ in Expired Air
[^{14}C]-SC-19129 Oral Dose

Individual Cumulative Percent Recoveries of Radioactivity

B R E A T H

Time (minutes)	Rabbit #10	Rabbit #11	Rabbit #12	Rabbit #9	MEAN	SEM ^a
0 - 30	0.0117	0.0141	0.00230	0.00239	0.00764	0.00309
0 - 60	0.0397	0.0578	0.00642	0.0238	0.0319	0.0110
0 - 120	0.201	0.358	0.0592	0.128	0.187	0.0642
0 - 180	1.44	1.16	0.182	0.539	0.829	0.285
0 - 240	2.69	2.34	0.617	1.63	1.82	0.457
0 - 300	3.67	3.46	1.60	2.63	2.84	0.470
0 - 360	4.36	4.22	2.69	3.16	3.61	0.406
0 - 420	4.79	4.63	2.51	3.64	4.14	0.331

a Standard error of the mean.

Table 13A

Urinary and Fecal Excretion of Total Radioactivity
 $[^{14}\text{C}]$ -SC-19200 Intravenous Dose

Individual Cumulative Percent Recoveries of Radioactivity

U R I N E						
Time (hours)	Rabbit #1	Rabbit #2	Rabbit #3	Rabbit #4	MEAN	SEM ^a
0 - 6	75.3	b	b	b	c	c
0 - 12	b	87.1	92.1	89.4	86.0	3.70
0 - 24	84.1	87.9	93.4	95.1	90.1	2.52
0 - 48	84.5	88.3	93.6	95.4	90.4	2.47
0 - 72	84.8	88.4	93.7	95.4	90.6	2.45
0 - 96	84.8	88.4	93.7	95.5	90.6	2.44
0 - 120	84.9	88.5	93.8	95.5	90.7	2.43

F E C E S						
Time (hours)	Rabbit #1	Rabbit #2	Rabbit #3	Rabbit #4	MEAN	SEM ^a
0 - 24	0.273	0.375	0.215	0.150	0.253	0.0478
0 - 48	0.446	0.534	0.313	0.259	0.388	0.0626
0 - 72	0.558	0.607	0.407	0.306	0.470	0.0692
0 - 96	0.640	0.693	0.456	0.380	0.542	0.0742
0 - 120	0.711	0.735	0.513	0.411	0.592	0.0783

U R I N E & F E C E S						
Time (hours)	Rabbit #1	Rabbit #2	Rabbit #3	Rabbit #4	MEAN	SEM ^a
0 - 24	84.4	88.3	93.6	95.3	90.4	2.49
0 - 48	85.0	88.8	93.9	95.6	90.8	2.42
0 - 72	85.3	89.0	94.1	95.7	91.0	2.39
0 - 96	85.5	89.1	94.2	95.8	91.2	2.38
0 - 120	85.6	89.2	94.3	95.9	91.3	2.35

a Standard error of the mean.

b Animal did not urinate during the indicated time interval.

c Mean and SEM not calculated for this time interval due to limited number of samples.

Table 14A
Urinary and Fecal Excretion of Total Radioactivity
[¹⁴C]-SC-19200 Oral Dose

Individual Cumulative Percent Recoveries of Radioactivity

U R I N E

Time (hours)	Rabbit #17	Rabbit #18	Rabbit #19	Rabbit #20	MEAN	SEM ^a
0 - 6	b	0.121	b	b	c	c
0 - 12	5.09	67.0	34.1	64.9	42.8	14.6
0 - 24	76.2	67.2	34.1	64.9	60.6	9.16
0 - 48	76.7	75.0	66.7	69.1	71.9	2.37
0 - 72	76.9	75.2	67.1	70.8	72.5	2.20
0 - 96	77.1	75.3	67.3	70.9	72.7	2.20
0 - 120	77.2	75.3	67.4	71.0	72.7	2.19

F E C E S

Time (hours)	Rabbit #17	Rabbit #18	Rabbit #19	Rabbit #20	MEAN	SEM ^a
0 - 24	1.44	2.86	1.45	0.0305	1.44	0.577
0 - 48	3.92	4.25	4.30	2.72	3.80	0.370
0 - 72	4.44	4.63	5.17	3.68	4.48	0.308
0 - 96	4.99	5.28	5.86	4.18	5.08	0.350
0 - 120	5.18	5.45	6.11	4.46	5.30	0.343

U R I N E & F E C E S

Time (hours)	Rabbit #17	Rabbit #18	Rabbit #19	Rabbit #20	MEAN	SEM ^a
0 - 24	77.6	70.1	35.6	64.9	62.1	9.19
0 - 48	80.7	79.3	71.0	71.9	75.7	2.48
0 - 72	81.4	79.8	72.3	74.5	77.0	2.14
0 - 96	82.1	80.5	73.2	75.1	77.7	2.13
0 - 120	82.4	80.8	73.6	75.5	78.0	2.11

^a Standard error of the mean.

^b Animal did not urinate during the indicated time interval.

^c Mean and SEM not calculated for this time interval due to limited number of samples.

Table 15A

Urinary and Fecal Excretion of Total Radioactivity
[¹⁴C]-SC-19129 Oral Dose

Individual Cumulative Percent Recoveries of Radioactivity

U R I N E						
Time (hours)	Rabbit #21	Rabbit #22	Rabbit #23	Rabbit #24	MEAN	SEM ^a
0 - 6	36.9	b	b	b	c	c
0 - 12	51.4	67.6	67.9	24.9	53.0	10.1
0 - 24	57.8	67.6	67.9	56.1	62.4	3.16
0 - 48	59.3	68.8	75.6	59.7	65.9	3.92
0 - 72	59.4	68.9	75.7	60.8	66.2	3.80
0 - 96	59.7	69.0	75.8	61.3	66.5	3.72
0 - 120	59.9	69.1	75.8	61.3	66.5	3.70

F E C E S						
Time (hours)	Rabbit #21	Rabbit #22	Rabbit #23	Rabbit #24	MEAN	SEM
0 - 24	1.08	3.69	2.57	2.34	2.42	0.535
0 - 48	6.84	6.08	4.81	4.67	5.60	0.520
0 - 72	8.16	8.69	5.46	7.37	7.42	0.708
0 - 96	8.99	9.20	5.91	8.05	8.04	0.752
0 - 120	9.36	9.50	6.19	8.52	8.39	0.765

U R I N E & F E C E S						
Time (hours)	Rabbit #21	Rabbit #22	Rabbit #23	Rabbit #24	MEAN	SEM
0 - 24	58.9	71.3	70.5	58.4	64.8	3.54
0 - 48	66.2	74.9	80.4	64.4	71.5	3.77
0 - 72	67.6	77.6	81.2	68.2	73.6	3.40
0 - 96	68.7	78.2	81.7	69.3	74.5	3.24
0 - 120	69.2	78.6	82.0	69.9	74.9	3.19

^a Standard error of the mean.

^b Animal did not urinate during the indicated time interval.

^c Mean and SEM not calculated for this time interval due to limited number of samples.

X. Appendix 2. Protocol

MRC-852-0066

-X.1-

Protocol

1. Study Title:

Pharmacokinetics and Metabolism of [14C]-SC-19129 and [14C]-SC-19200 its Free Acid, in the Rabbit.

2. Study Sponsor:

G. D. Searle and Co.

3. Facility:

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

4. Proposed Date:

First Dosing: September, 1985

5. Introduction:

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

6. Purpose:

The purpose of this study is to determine the pharmacokinetics and metabolism of intravenously (I.V.) and orally (p.o.) administered [14]-SC-19200 and orally (p.o.) administered [14C]-SC-19129 in the rabbit.

7. Overview of Study Design:

Eight female rabbits will be dosed p.o. and eight female rabbits I.V. with [¹⁴C]-SC-19200 at 10 mg/kg. Eight female rabbits will be dosed p.o. with [¹⁴]-SC-19129 at 10 mg/kg. Plasma, urine and feces will be collected from four of the animals in each group and breath will be collected from the additional 4 animals in each group. Total radioactivity will be determined for all samples. Plasma and urine concentrations of SC-19129 (if present), SC-19200 and other major metabolites will be determined using pooled samples and appropriate chromatographic procedures. A schematic representation of the study design is given below.

Schematic Representation of Study Design

Test Dose Article	(mg/kg)	Approximate Radiochemical Dose (mCi/kg)	Route	Number of Female Sample Rabbits	Type
SC-19129	10	50	p.o.	4	Plasma, Urine & Feces
		25		4	Breath
SC-19200	10	50	I.V.	4	Plasma, Urine & Feces
		25		4	Breath
SC-19200	10	50	p.o.	4	Plasma, Urine & Feces
		25		4	Breath

8. Laboratory Procedure:

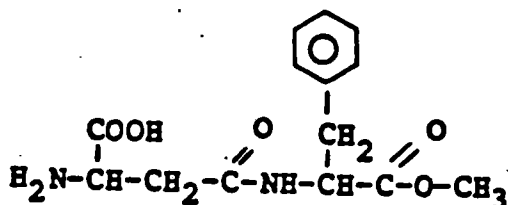
This study is not within the scope of Good Laboratory Practice Regulations.

9. Test Article:

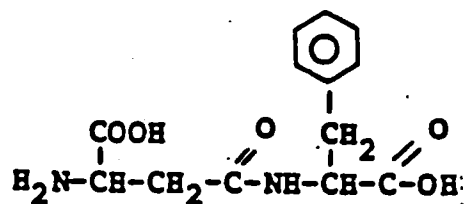
A. Chemical Name:

SC-19129 (β -APM) is N-L- β -aspartyl-L-phenylalanine, 1-methyl ester. SC-19200 (β -AP) is the free acid of SC-19129.

B. Chemical Structure:



SC-19129



SC-19200

C. Dosage Forms:

1. [U-14C-Phe]-SC-19129 with a specific activity of approximately 32 mCi/mg (approximately 9.6 mCi/mmol) and [U-14C-Phe]-SC-19200 with a specific activity of approximately 96 mCi/mg (approximately 27 mCi/mmol) will be supplied by the Radiochemistry Group, G. D. Searle & Co. Unlabelled SC-19129 (Lot 84K-047-101) and SC-19200 (Lot #CD-158-146A) will be

obtained from the Searle Test Article File.

2. The oral (p.o.) dosage forms will be prepared by dissolving the appropriate amounts of [^{14}C]-SC-19129 and unlabelled SC-19129, or [^{14}C]-SC-19200 and unlabelled SC-19200, in distilled water to give a final concentration of 5.0 mg/ml. The I.V. dosage form will be prepared by dissolving appropriate amounts of [^{14}C]-SC-19200 and unlabelled SC-19200 in 0.9% NaCl to give a final concentration of 5.0 mg/ml. The proportions of labelled and unlabelled test article will be adjusted to give approximately the specific activity (e.g. 5 mCi/mg for 50 mCi/kg) indicated for each treatment subgroup in Section 7 (Schematic Representation of Study Design).

D. Administration:

- i Route: Test article will be given p.o. by gavage or I.V. via a vein.
- ii Frequency: Each animal will receive the test article once.
- iii Volume and Dosage:
 - a. The [^{14}C]-SC-19200 I.V. dose will consist of 2.0 ml/kg administered via a vein cannula and followed by an equivalent volume rinse with 0.9% (w/v) NaCl. This is intended to provide a dose of 10 mg/kg.
 - b. Oral Dose: [^{14}C]-SC-19129 and [^{14}C]-SC-19200 oral doses will consist of 2.0 ml/kg administered intragastrically via a stomach tube attached to a syringe. This is intended to provide a dose of 10 mg/kg.

E. Analyses:

The radiochemical purity of the test article in the dose solution will be determined by high performance liquid radiochromatography (HPLRC) within 4 hours of dose administration.

F. Storage:

[14C]-SC-19129, [14C]-SC-19200 and the corresponding unlabelled compounds will be stored at room temperature in well closed containers and protected from light. The test article dosing solutions will be prepared fresh on the day of administration.

10. Test System, Housing and Diet:

A. Test System:

Twenty-four female New Zealand rabbits of less than 1 year in age will be obtained from Hare Marland Laboratories.

Rabbits will weigh between 2 and 3 kg at the start of the study and will be identified by a unique animal number.

B. Housing:

The rabbits will be housed in individual metabolism cages located in J-224 or J-226 during dosing and sample collections, except where otherwise indicated.

C. Diet:

1. Food:

The rabbits, maintained on Purina Rabbit Chow (Ralston Purina, St. Louis, MO), will be fasted for 18-24 hours prior to the administration of the compound.

Food will be available ad libitum from 6 hours

(plasma urine and feces group) or 7 hours (breath collection group) after dose administration and throughout the remainder of the study.

2. **Water:**

Tap water from the municipal water supply will be available ad libitum from 6 hours after dose administration and throughout the remainder of the study.

3. **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. Animal Observation

Animals will be observed for any visible changes such as emesis or diarrhea which might have impact on the interpretation of results.

12. Sample Collection, Times and Storage:

A. **Animal Sacrifice:** Animals will be anesthetized with ether. Sacrifice will be by exsanguination through cardiac puncture at 120 hours after dosing or by ether anesthesia, depending on whether animals were used for plasma, urine and feces collection or for breath collection respectively.

B. **Blood:** Blood will be collected from four animals per treatment group by venipuncture,, indwelling catheter or arterial puncture (if the veins collapse) at or near 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after dosing and by cardiac puncture at 120 hours after dose administration. A sample will also be

collected at or near 0.08 hour from animals dosed with [14C]-SC-19200 intravenously. Sample volumes will be approximately 1 ml during the first 12 hours and 2 ml at later time points. The blood will be placed in chilled tubes containing heparin. The tubes will also contain diethyl p-nitrophenyl phosphate for blood from the animals dosed with [14C]-SC-19129. Plasma will be prepared by centrifugation. It will be stored frozen if not further processed for analysis within 3 hours.

- C. Urine: The urine will be collected from four animals (same as in 12B above), into containers surrounded by dry ice from 0-6, 6-12, 12-24, 24-48, 48-72, 72-96 and 96-120 hours after dose administration. The urine samples will be stored frozen until analyzed.
- D. Feces: Feces from four animals (same as in 11.B above) will be removed from the metabolism cages at approximately 24, 48, 72, 96 and 120 hours after dose administration and stored frozen until analyzed.
- E. Breath: $^{14}\text{CO}_2$ eliminated in the breath will be collected from 4 animals per treatment group. Each rabbit will be placed in an individual restraining cage, enclosed in a plastic bag after dosing and expired air will be drawn, by means of a vacuum, through gas washers containing ethanolamine: 2-methoxyethanol (1:2, v/v). Samples will be collected from approximately 0-0.5, 0.5-1, 1-2, 2-3, 3-4, 4-5, 5-6 and 6-7 hours. Samples will also be collected on the following day from approximately 24-25 hours.
- F. Control Urine, Feces and Plasma: Plasma, urine and feces will be collected from control rabbits which have not been treated with the test article. Aliquots of the urine, feces and plasma from control animals will be spiked with [14C]-SC-19129 and [14C]-SC-19200 prior to

frozen storage. The spiked samples will be used to determine stability and efficiency of extraction with each matrix.

- G. Fluid Replacement: Following each blood sample taken up to 6 hours an equivalent volume of 0.9% (w/v) NaCl will be injected via the vein.

13. Sample Analysis:

- A. Plasma and Urine: Total ^{14}C will be measured by direct liquid scintillation counting (LSC). Based on the results from the total ^{14}C analysis, pooled plasma and urine samples from appropriate time points will be selected for sample extraction followed by analysis by high performance liquid radiochromatography (HPLRC) for [^{14}C]-SC-19129 (if present), [^{14}C]-SC-19200 and related compounds.
- B. Feces: Total ^{14}C will be measured by sample combustion and LSC of the trapped products. Based on the results of the total ^{14}C analysis, selected samples will be extracted and analyzed by TLRC or HPLRC.
- C. Breath: Total ^{14}C in each sample will be measured by LSC of aliquots of the ethanolamine: 2-methoxyethanol solutions used to trap CO_2 .

14. Statistical Procedure:

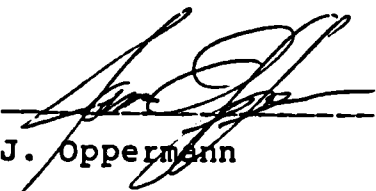
Individual data for total radioactivity determinations will be tabulated. Plasma total ^{14}C concentration-time curves will be prepared from the mean ^{14}C plasma concentrations of the animals at each time point and the related pharmacokinetic parameters determined. The recovery of ^{14}C will be calculated from the urinary, fecal, and breath data.

18. **Protocol Approval:**

Grant Schoenhard 9-18-85

G. Schoenhard

Date

 9/12/85

J. Oppermann

Date

Carl H. Burton 9/17/85

E. Burton

Date

(Responsible Scientist)