

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

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

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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 of orally administered [^{14}C]-SC-19129 were studied in four female dogs at doses of approximately 10 mg/kg. The following results were obtained:

1. Approximately 90% of the orally administered radioactive doses of SC-19200 and SC-19129 were absorbed by the dogs based upon their fecal excretion of radioactivity (10.2% and 11.2%, respectively). Urinary excretions of intact SC-19200 following oral SC-19200 and SC-19129 indicated that 16.4% and 14.0%, respectively, of the radiolabelled doses of SC-19200 and SC-19129 were absorbed by the dog with the peptide bond intact. SC-19129 could not be detected in plasma, indicating complete hydrolysis of the methyl ester prior to or during absorption.
2. Maximal plasma concentrations (C_{max}) of total radioactivity (11.8 and 10.4 mcg equivalents of test article per ml for [^{14}C]-SC-19200 and [^{14}C]-SC-19129, respectively) occurred 8.5 hours (T_{max}) after oral dosing for both groups. In contrast, C_{max} s of [^{14}C]-SC-19200 which were 2.34 and 1.56 mcg/ml, respectively, for oral SC-19200 and SC-19129, were attained 2 and 1.5 hours,

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respectively, after oral dosing. The differences in the Cmaxs for total radioactivity and [^{14}C]-SC-19200 probably are a consequence of the incorporation of ^{14}C -phenylalanine, released during the metabolism of SC-19200 and SC-19129, into plasma proteins.

3. The oral bioavailability of SC-19200 (based on a comparison of oral and IV AUCs of SC-19200 after the administration of SC-19200 and SC-19129) were 15.7% and 8.54%, respectively. By contrast, the bioavailabilities for total radioactivity following oral [^{14}C]-SC-19200 and [^{14}C]-SC-19129 (also based on $\text{AUC}_{\text{oral}}/\text{AUC}_{\text{IV}}$) were 287% and 250%, respectively, again indicating the incorporation of ^{14}C -phenylalanine into plasma proteins.
4. The mean plasma elimination half-lives of SC-19200 observed following intravenous SC-19200, oral SC-19200 and oral SC-19129 were 0.87, 1.13, and 1.57 hours, respectively. However, as would be expected with the incorporation of ^{14}C -phenylalanine into plasma proteins, the mean plasma elimination half-lives of total radioactivity following intravenous SC-19200, oral SC-19200 and oral SC-19129 were 399, 317 and 338 hours, respectively.
5. The volumes of distribution of total radioactivity and of SC-19200 following the intravenous administration of [^{14}C]-SC-19200 were 0.12 and 0.13 l/kg, respectively, indicating that SC-19200 is

largely confined to extracellular fluid.

6. The major route of excretion of total radioactivity following [^{14}C]-SC-19200 and [^{14}C]-SC-19129 was the urine. Following IV [^{14}C]-SC-19200, 72.2% of the administered radioactivity was recovered in the urine. Approximately 3% of the intravenous total radioactivity was recovered in the feces. Subsequent to the oral administration of [^{14}C]-SC-19200 and [^{14}C]-SC-19129, 27.4% and 21.1%, respectively, of the radioactive doses were recovered in the urine with 10.2% and 11.2%, respectively, being recovered in the feces. The lower recoveries of total radioactivity in urine and feces following oral SC-19200 and SC-19129 (which were approximately 50% of the intravenous recovery) also substantiate the incorporation of ^{14}C -phenylalanine into plasma proteins.
7. The urinary excretions of intact [^{14}C]-SC-19200 were 14.0, 16.4 and 70.1%, respectively, following the administration of oral [^{14}C]-SC-19129, oral [^{14}C]-SC-19200 and intravenous [^{14}C]-SC-19200, indicating that approximately 15% of the doses of the beta-aspartyl dipeptides were absorbed with the peptide bond intact.
8. SC-19200 and phenylalanine were the major constituents found in the plasma metabolic profiles of dogs receiving oral and intravenous SC-19200 and SC-19129. Phenylalanine was the sole metabolite present in the feces. SC-19200 was the major

constituent observed in the urinary metabolic profiles of dogs receiving oral and intravenous SC-19200 and SC-19129. Phenylacetylglycine also was present in urine, accounting for less than 1% of the dose. Finally, an unidentified metabolite "metabolite A" (which accounted for approximately 5% of the dose of radioactivity) was observed in the urine of dogs receiving oral SC-19200 and oral SC-19129. This metabolite, which has not been observed in the rat, rabbit or monkey, appears to be unique to the dog.

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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 pharmacokinetics and metabolism of orally administered [^{14}C]-SC-19129 have been examined in the rhesus monkey (2) the rat (3) and the rabbit (4). In addition, plasma and urinary concentrations of total ^{14}C and major metabolites were determined in pregnant rats administered [^{14}C]-SC-19129 in dietary admix (5). The objective of the present study was 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 dog.

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III. Materials and Methods

A. Overview of Study Design:

Four female beagle dogs were dosed intravenously (IV) with [^{14}C]-SC-19200. Following the IV dose, [^{14}C]-SC-19129 and [^{14}C]-SC-19200 were administered orally, in separate doses, to each of the same animals in a randomized crossover manner. Each dose consisted of approximately 10 mg of the indicated compound per kg body weight. A wash-out period of approximately 4 weeks occurred between doses for each animal.

Plasma, urine and feces were collected. Total radioactivity was determined in all samples. Pooled plasma, urine and fecal samples from selected time points were analyzed by high performance liquid radiochromatography (HPLRC). SC-19200 was analyzed in individual plasma samples, following IV administration, by a specific, validated HPLC method (6).

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-332-115-1) were supplied by the Radiochemistry Group, G.D. Searle & Co. The respective specific activities were 9.55 mCi/mmol (32.5 mCi/mg) and 1.33 mCi/mmol (4.73 mCi/mg). The dosage forms were prepared using the appropriate amounts of [^{14}C]-SC-19129 and unlabeled SC-19129 (lot 84K-047-101) or [^{14}C]-SC-19200 and unlabeled SC-19200 (lot CD-158-146A) as described in the protocol (Section X.9.C.2.). The specific activity of [^{14}C]-SC-19200 in the IV dose solution was 1.01 mCi/mg

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(Table 1). The average specific activities of [^{14}C]-SC-19200 and [^{14}C]-SC-19129 in the oral dose solutions (two solutions for each compound due to the crossover design for the oral doses; Section III.A and Table 1A) were 2.44 mCi/mg and 2.48 mCi/mg, respectively (Table 1).

C. Animals, Animal Treatment and Test Article Administration:

Female beagle dogs weighing 8.3-10.7 kg at the time of the first dosing were used. The animals were housed and fed as specified in the protocol (Section X.10.B-C). Administration of the dosage forms was as described in the protocol (Section X.9.D). The actual doses administered are given in Table 1 and Table 1A.

D. Sample Collection:

1. Plasma:

Blood samples were collected by venipuncture from four animals per treatment group as described in the protocol (Section X.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°C until analysis (Section III.E.2). [^{14}C]-SC-19129 or [^{14}C]-SC-19200 was added to control dog plasma which was pretreated with 1×10^{-4} molar diethyl-p-nitrophenylphosphate (Section X.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 X.12.C-D) and stored frozen at approximately -70°C until analysis. Control urine and fecal samples were spiked with [^{14}C]-SC-19129 or [^{14}C]-SC-19200 and analyzed immediately or stored frozen until analysis.

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.G) and determination of radiochemical purity by HPLRC analysis (Section III.F).

2. Plasma:

Total ^{14}C was determined by LSC (Section III.G) using duplicate 0.05 ml aliquots. Individual plasma samples taken following IV administration of SC-19200 were analyzed for unchanged SC-19200 by a validated method (6). Extracts of pooled plasma from selected sampling times following the IV or oral doses were prepared for HPLRC analysis (Section III.F) by a C18 Bond ElutTM (Analytichem International, Inc., Harbor City, CA) procedure. Pooled plasma (1 ml) was applied to a 100 mg C18 Bond ElutTM column which had been conditioned by washing sequentially with 1 column volume of methanol and 2 volumes of 0.01 M sodium phosphate, pH 7.4. The column was eluted with 0.9 ml of water and the plasma and water eluates were combined and saved for

further extraction (see below). The column was eluted with 1 ml of 0.05 M heptafluorobutyric acid (HFBA) in 0.20 M sodium phosphate, pH 2.0, and the eluate was discarded. The column was then eluted with 1 ml of acetonitrile:water (80:20 v/v) and the eluate was saved. The combined plasma and water eluates from the first column were mixed with 0.20 ml of 1N HCl and 0.30 ml of 0.10 M HFBA in 0.01 M phosphoric acid and the mixture was applied to a second, 500 mg C18 Bond ElutTM column (preconditioned as above for the first column). The column was then washed sequentially with 2 ml of 0.05 M HFBA in 0.20 M sodium phosphate, pH 2.0, and 0.10 ml of acetonitrile:water (80:20, v/v) and the eluates were discarded. Finally, the column was eluted with 1.5 ml of acetonitrile:water (80:20, v/v). The acetonitrile:water eluates from the first and second Bond ElutTM columns were combined and mixed with 0.050 ml of 1N HCl. The mixture was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 3 ml of acetonitrile:water (80:20) containing 0.050 ml of 1N HCl, and the solution was evaporated under a stream of nitrogen to remove residual HFBA. The residue was dissolved in 0.35 ml of methanol:0.020 M sodium phosphate, pH 3.0 (20:80, v/v) and an aliquot (0.20 ml) was analyzed by HPLRC (Section III.F).

3. Urine:

Total ¹⁴C in duplicate aliquots of each urine sample was determined by LSC (Section III.G). Pooled urine samples or spiked control urine samples were filtered through a 0.45 micron filter (Gelman Acrodisc^R, AR; Gelman Sciences, Inc., Ann Arbor, MI) prior to HPLRC analysis (Section III.F).

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4. Feces:

Fecal samples were suspended in a volume (ml) of water equal to 1.0 times the sample weight (g) using a blender (Stomacher Lab-Blender 80; Tekmar Co., Cincinnati, OH). Triplicate aliquots (0.5-1.5 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). Aliquots of the above fecal suspensions from the 0-24 hour samples were pooled within treatment groups in proportion to the individual animal sample weights. A 10 ml aliquot of pooled fecal suspension and 25 ml of water were combined and mixed for 15 minutes (Rugged RotatorTM, Kraft Apparatus, Inc., Mineola, NY). The mixture was centrifuged at 3000 x g for 5 minutes and an aliquot of the supernatant was further centrifuged at approximately 15,000 x g for 15 minutes. The supernatant from the second centrifugation was filtered as described above for urine (Section III.E.3) prior to HPLRC analysis (Section III.F).

F. High Performance Liquid Radiochromatography (HPLRC):

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.

Extracts of plasma, urine and feces (Section III.E) were profiled by HPLRC (standard system) on a Zorbax ODS column

(15 cm x 4.6 mm; Du Pont Instruments, Wilmington, DE) using a mobile phase of 0.020 M sodium phosphate, pH 3.0:methanol (80:20, v/v) and a flow rate of 1 ml/min. The column temperature was maintained at 40°C (Model CH-1445 Column Heater, Sys-Tec, Inc., New Brighton, MN).

Unlabelled standards of SC-19129, SC-19200, and phenylalanine, used to calibrate the HPLRC system, were detected by absorbance at 222 nm (Spectroflow 783 detector, Kratos Analytical, Ramsey, NJ). Radiolabeled compounds in profiles of dosing solutions or urine, or extracts of the plasma obtained following the IV dose, were measured using a radioactive flow detector (Flo-One^R, model β /IC, RadioAnalytic, Inc., Tampa, FL). The effluent from the HPLC column and Flo-Scint^R III (RadioAnalytic, Inc., Tampa, FL) were mixed at a ratio of 1.0 ml/min to 5.5 ml/min in the Flo-One^R mixing chamber. Counting efficiency was determined by mixing HPLRC mobile phase containing a known amount of radioactivity (Oxi-Test^R CO., RadioAnalytic, Inc., Tampa, FL) and Flo-Scint^R III in the above ratio and counting the mixture in the Flo-One^R in the stopped-flow mode. Radiolabeled compounds in profiles of extracts of feces, or of extracts of plasma obtained following oral doses, were measured by collecting the eluate from the column as 0.4 min aliquots (FoxyTM fraction collector, ISCO Inc., Lincoln, NE) followed by analysis of the aliquots by LSC (Section III.G). Plasma from untreated animals was carried through the extraction and HPLRC procedures, with collection of fractions of the column eluate, to provide appropriate samples for determination of background radioactivity by LSC (Section III.G).

The specificity of the HPLRC method used for plasma, urine and feces profiles was examined by further HPLRC (ion-pair system) of an aliquot of the Bond Elut extract of pooled plasma, obtained at 5 hours following oral administration of [^{14}C]-SC-19129, on a Nova PakTM C18 column (15 cm x 3.9 mm). The column was eluted with a mobile phase consisting of 0.020 M heptane sulfonic acid (containing 0.001 M dimethyloctylamine and 0.040 M phosphoric acid): acetonitrile (90:10, v/v) at a flow rate of 1 ml/min. Fractions (0.4 min) of the eluate were analyzed by LSC (Section III.G).

G. Liquid Scintillation Counting) (LSC):

Samples of plasma (0.050 ml) were mixed with 12 ml of PCS^R (Amersham Corp., Arlington Heights, IL). Samples of urine 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^R to form a stable gel. The combustion products from oxidized fecal samples were mixed with 9 ml of CarbosorbTM 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.

Values for radioactivity disintegrations per minute (DPM) for individual plasma, urine, red blood cell and fecal samples were corrected by subtracting DPM values obtained for appropriate control samples treated in the same manner. DPM in HPLRC fractions were corrected by subtracting 2 times the background DPM determined in control HPLRC fractions.

H. Calculations:

The crossover design of the study and the terminal elimination half-life of plasma radioactivity resulted in the presence of residual radioactivity in plasma at the time of administration of each of the oral doses. The results (DPM) of radioactivity determinations (Section III.G) of plasma samples collected following oral administration of test articles were adjusted for residual radioactivity, prior to calculation of the concentration (mcg equivalents/ml) of total radiolabeled compounds (Total ^{14}C), as follows: 1) The terminal elimination rate constant of total radioactivity was calculated (NONLIN program, Section III.I) for the dose (IV or oral, depending on the crossover schedule; Table 1A) immediately preceding the oral dose for which plasma DPM/ml were to be corrected. 2) The predicted residual DPM/ml was calculated for each plasma sample time (MULDOS 1 program on VAX computer) following the oral dose from the residual DPM/ml present at zero time and the elimination rate constant associated with the residual radiolabel. 3) The predicted residual DPM/ml was subtracted from the experimental value.

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

$$\begin{bmatrix} \text{Metabolite} \\ \text{Concentration} \\ \text{(mcg} \\ \text{equivalents/} \\ \text{ml)} \end{bmatrix} = \begin{bmatrix} \text{Plasma} \\ \text{Radioactivity} \\ \text{Concentration} \\ \text{(mcg} \\ \text{equivalents/} \\ \text{ml)} \end{bmatrix} \times \begin{bmatrix} \text{Fraction} \\ \text{of Radio-} \\ \text{activity} \\ \text{in Plasma} \\ \text{Recovered} \\ \text{from Bond} \\ \text{Elut} \end{bmatrix} \times$$

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$$\left[\begin{array}{l} \text{Fraction of Radio-} \\ \text{activity in Bond Elut} \\ \text{Fraction Recovered} \\ \text{from HPLRC} \end{array} \right] \times \left[\begin{array}{l} \text{Fraction of Radio-} \\ \text{activity Recovered} \\ \text{from HPLRC Present} \\ \text{as Metabolite} \end{array} \right]$$

All concentrations are expressed in terms of mcg equivalents of [¹⁴C]-SC-19129 or [¹⁴C]-SC-19200, based on the specific activity of the test article 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:

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

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 (AUC) were calculated using the trapezoidal rule (7).

Plasma concentration versus time curves of total ¹⁴C and [¹⁴C]-SC-19200 were analyzed using the CSTRIP computer program (8). The initial parameters estimated from CSTRIP were then used in the NONLIN computer program (9).

Whole body clearance (Q_b) of SC-19200 following IV administration was calculated according to the equation (reference 7, p. 249):

$$Q_b = \frac{\text{Dose}}{\text{SC-19200 Plasma AUC}}$$

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 Deviation:

The protocol specified that a wash-out period of at least four weeks would occur between doses for each animal. The actual wash-out period between the IV dose and the first oral dose for each animal was 27 days (see Table 1A). The difference of 1 day between the specified and actual wash-out periods is not considered to have adversely affected the study.

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 (Table 1A). 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 the dosing solutions of [^{14}C]-SC-19129 were 93.6% (3/31/86) and 97.8% (4/28/86). The radiochemical purities of the dose solutions of [^{14}C]-SC-19200 were 95.6% (3/4/86), 95.0% (3/31/86) and 97.5% (4/28/86).

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 ElutTM procedure (Section III.E.2) were 98.2% and 99.6%, respectively (Table 2). The respective percentages of the extracted radiolabel present in the appropriate peak in HPLRC profiles were 95.2% and 98.8% when analyzed immediately and 84.1% and 95.3% 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 approximately 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 93.0% and 92.8% when analyzed immediately and 93.1% and 92.0% after frozen storage for 95 days (Table 3).

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The recoveries of radiolabel from [^{14}C]-SC-19129 and [^{14}C]-SC-19200 added to control feces were 99.0% and 99.4% respectively. HPLRC analysis indicated that the extracted radioactivity was almost completely in the form of [^{14}C]phenylalanine, presumably resulting from bacterial metabolism.

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 2A - 4A. The pharmacokinetic parameters are summarized in Table 4.

Following intravenous administration of [^{14}C]-SC-19200, mean plasma total ^{14}C was 69.6 mcg/ml at 0.03 hours. Plasma total radioactivity declined in a manner best described as the sum of 4 exponentials, as determined by CSTRIP and NONLIN analysis (Table 4 and Table 5A). The distribution half life (α) was 0.0667 hours. The elimination half-lives were 0.615 hours and 55.9 hours for the first (β) and second (γ) phases respectively and 399 hours for the terminal (Δ) phase. The mean volume of distribution of total radioactivity was calculated to be 0.12 l/kg. The mean AUC from time 0 to infinity was 1240 (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 11.8 mcg/ml at 8.5 hours following [^{14}C]-SC-19200 administration and 10.4 mcg/ml at 8.5 hours following [^{14}C]-SC-19129 administration. The terminal elimination half-lives of total radioactivity were 317 ± 28 hours and 338 ± 49 hours following oral doses of

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[¹⁴C]-SC-19129 and [¹⁴C]-SC-19200, respectively. The calculated elimination half-lives are based on a sample collection period including a final sample taken at approximately 4 weeks after each dose and are thus based on a sampling period of approximately 1.5 - 2 half-lives.

The AUC values of total radioactivity from time 0 to infinity were 3560 (mcg/ml) hours and 3100 (mcg/ml) hours for orally administered [¹⁴C]-SC-19200 and [¹⁴C]-SC-19129 respectively. These AUC values were 287% and 250% respectively of the corresponding AUC for the IV dose (Table 4). The finding that oral AUC values are much higher than the IV AUC value reflects marked differences 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 extracted using Bond ElutTM columns (Section III.E.2). Recoveries of SC-19200 and SC-19129 from dog plasma using this procedure are given in Table 2.

SC-19200 was the major component in HPLRC profiles of the Bond ElutTM fraction following IV administration (Figure 2), with a minor amount of radiolabel present at the appropriate retention time for phenylalanine (Figure 2). The Bond ElutTM fraction containing SC-19200 also contained approximately 75-90% of the total radioactivity present in plasma samples taken up to 2 hours after IV administration. However this percentage declined markedly at later times. The radioactivity not recovered in the SC-19200 fraction was largely unretained by the Bond ElutTM columns. This fraction, as indicated in previous studies with

[¹⁴C]-SC-19200 and [¹⁴C]-SC-19129 (2-4) may contain [¹⁴C]Phe incorporated into high molecular weight plasma proteins.

The major metabolites in HPLRC profiles of plasma obtained following oral doses of either [¹⁴C]-SC-19200 or [¹⁴C]-SC-19129 were [¹⁴C]-SC-19200 and [¹⁴C]Phe, based on comparison to the retention times of authentic standards (Figures 3 and 4). Co-injection of authentic [¹⁴C]-SC-19200 or [¹⁴C]Phe resulted in an increase in the respective plasma metabolite peak without apparent peak broadening. Further analysis of the pooled plasma obtained at 5 hours following oral administration of [¹⁴C]-SC-19129, using an alternative (ion-pair) HPLRC system (Section III.F), also indicated that [¹⁴C]-SC-19200 and [¹⁴C]Phe were the major metabolites (Figure 5). The percentages of radioactivity present in the Bond Elut fraction from the [¹⁴C]-SC-19129 oral dose 5 hour sample which were recovered in the HPLRC eluate as [¹⁴C]-SC-19200 were 25.2% for the standard system and 29.4% for the ion-pair system (Table 5). The respective percentages recovered as [¹⁴C]Phe were 22.9% and 40.9%. The standard HPLRC system and the ion-pair system are thus in reasonable agreement for [¹⁴C]SC-19200. The standard system provides a more conservative estimate for [¹⁴C]Phe than the ion-pair system. The latter system appears to overestimate [¹⁴C]Phe due primarily to an unidentified metabolite which has a retention time longer than that of SC-19200 in the standard system (peak at approximately 18.5 minutes in Figure 4II) but similar to that of Phe in the ion-pair system.

The theoretical limit of detection of [¹⁴C]SC-19200, using the 2-times background criterion of peak significance (Section III.G), was approximately 0.014 mcg/ml. This

amount, given the specific activity (2.4 - 2.5 mCi/mg; Table 1) of [^{14}C]SC-19200 or [^{14}C]SC-19129 in oral dose solutions and the fraction (20/35; Section III.E.2) of the Bond ElutTM extract of 1 ml of plasma analyzed, would increase the DPM in an HPLRC fraction to twice the background (approximately 40 DPM) DPM. The calculated [^{14}C]SC-19200 concentrations (see Section III.H for equation) given in Table 6 are presumably underestimated by an amount equal to, or greater than, the theoretical detection limit. The actual underestimation will depend on the number of adjacent 0.4 minute fractions of eluate from the HPLRC column in which [^{14}C]SC-19200 is recovered and the variations inherent in measuring low levels of radioactivity.

[^{14}C]Phe peaks (expressed as DPM, or mcg equivalents, per ml) were much smaller than [^{14}C]-SC-19200 peaks in plasma shortly after (e.g. 0.5 hour, Figures 3I and 4I) oral administration of [^{14}C]-SC-19200 or [^{14}C]-SC-19129. The ratio of [^{14}C]Phe to [^{14}C]-SC-19200 increased with time and was approximately one at 5 hours (Figures 3II and 4II) and greater than one at 7 and 9 hours. Peak concentrations of [^{14}C]Phe occurred at 5 hours and were 0.526 and 0.371, expressed as mcg equivalents of the administered test article, for [^{14}C]-SC-19200 and [^{14}C]-SC-19129, respectively. Occasional smaller metabolite peaks were also observed in plasma HPLRC profiles, the most prominent of which had the appropriate retention time (4.5 minutes) for tyrosine (Figures 3-4). Intact [^{14}C]-SC-19129 was not detected (< 0.05 mcg/ml) in HPLRC profiles following its oral administration, although the inhibitor used to treat plasma samples (Section III.D.1) effectively protected SC-19129 against hydrolysis in vitro (Table 3).

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E. [¹⁴C]-SC-19200 in Plasma:

The concentrations of SC-19200 in individual plasma samples after IV administration and of [¹⁴C]-SC-19200 in pooled plasma after oral administration are given in Table 6 and shown in Figure 6. The plasma concentrations following the IV dose were determined by a validated method (6), but plasma concentrations following oral doses were determined by a non-validated HPLRC procedure (Section III.F) which may underestimate SC-19200 (see Section IV.D). The pharmacokinetic parameters for SC-19200 are listed in Table 6. The elimination of SC-19200 from plasma following IV administration was biexponential, with half-lives of 0.208 hours for the first (α) phase and 0.870 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 predicted maximum of 83.8 mcg/ml at zero time (C_{zero} , Table 5A). The volume of distribution SC-19200 was calculated to be 0.13 l/kg. This volume is intermediate to the plasma volume (approximately 0.05-0.06 l/kg) and the extracellular fluid volume (approximately 0.2-0.3 l/kg) in the dog (10). This indicated that SC-19200 is largely excluded from tissues in the dog. The whole body clearance of SC-19200 following IV administration was calculated to be 3.5 ml/min per kg body weight (Table 6).

Peak concentrations of SC-19200 in pooled plasma were 2.34 mcg/ml at 2 hours following oral doses of [¹⁴C]-SC-19200 and 1.56 mcg/ml at 1.5 hour following doses of [¹⁴C]-SC-19129. The disappearance of SC-19200 from plasma following oral administration of [¹⁴C]-SC-19200 and [¹⁴C]-SC-19129 occurred with terminal half-lives of

approximately 1.13 and 1.57 hours respectively (Table 6).

The bioavailabilities of SC-19200, based on comparison of oral AUC values with the IV AUC value, were 15.7% for orally administered SC-19200 and 8.54% for orally administered SC-19129. These values may be regarded as lower limits due to the tendency of the HPLRC procedure used for the oral plasma analysis to underestimate low concentrations (Section IV.D). 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. 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 9A-11A. The mean percentages of dose excreted in the urine through 120 hours following the SC-19200 IV dose, SC-19200 oral dose and the SC-19129 oral dose were $72.2 \pm 2.7\%$, $27.4 \pm 2.2\%$, and $21.2 \pm 3.2\%$, respectively. The mean percentages of dose recovered in feces after administration of [^{14}C]SC-19200 IV and oral doses were $2.87 \pm 0.56\%$ and $10.2 \pm 1.5\%$, respectively. The mean percentage recovered in feces after an [^{14}C]-SC-19129 oral dose was $11.2 \pm 4.3\%$. The average total recoveries of radioactivity in urine and feces after IV and oral doses of [^{14}C]-SC-19200 were 75.1% and 37.6%, respectively. The average total recovery after an [^{14}C]-SC-19129 oral dose was 32.4%

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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 38.0% and 29.2% 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 92.5% and 91.4% respectively.

G. Distribution of Urinary and Fecal Radioactivity:

The distribution of radioactivity in HPLRC profiles of pooled urine samples is shown in Figures 9-11. Approximately 95% 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 70% of the dose was excreted as SC-19200. Approximately 0.7% of the IV dose was excreted as an unidentified metabolite (Metabolite A) with a retention time of approximately 11.5 minutes. SC-19200 was also the major metabolite in urine (approximately 60% to 80% of the radioactivity present in urine samples) following oral administration of [^{14}C]-SC-19200 or [^{14}C]-SC-19129 (Figures 10 and 11) and accounted for 16.4% and 14.0% of the oral doses, respectively. Comparison of the SC-19200 excreted in the urine following oral versus IV doses indicated absorption of 23.4% of the [^{14}C]-SC-19200 oral dose and 20.0% of the [^{14}C]-SC-19129 oral dose with the β -aspartyl peptide bond intact. These values for [^{14}C]-SC-19200 bioavailabilities are judged to be more accurate than the respective values found for plasma (Section IV.D-E) due to the higher

concentrations available for analysis in urine. The other major metabolite in urine following SC-19200 or SC-19129 oral administration was Metabolite A (Figures 10 and 11), which accounted for approximately 30% to 40% of the radioactivity present in urine samples. [^{14}C]Phenylacetyl-glycine was a minor constituent of urine following oral administration of [^{14}C]-SC-19200 and [^{14}C]-SC-19129, accounting for less than 1% of the administered dose.

The major radiolabeled constituent in fecal extracts following either IV or oral doses was [^{14}C]Phe (Figure 12), accounting for approximately 80% to 100% of the radioactivity in the extracts. [^{14}C]-SC-19200 or [^{14}C]-SC-19129 were not found in fecal extracts. When [^{14}C]-SC-19129 was added to a homogenate of control feces and carried through the extraction procedure it was found by HPLRC to be completely converted to [^{14}C]Phe, presumably as the result of bacterial metabolism.

V. Discussion and Conclusions

A. Metabolic Formation of Free Phenylalanine (Phe):

The major radiolabeled constituent in plasma following IV administration of [^{14}C]-SC-19200 was unchanged SC-19200. [^{14}C]Phe was also present in plasma following IV administration, but at much lower concentrations. Unchanged [^{14}C]-SC-19200 was cleared rapidly from plasma (terminal half-life of 0.871 hours). However, total radioactivity was cleared slowly (terminal half-life of 399 hours) and, at 2 or more hours after IV administration, was largely in a form not retained on Bond ElutTM columns. This unretained radioactivity has been observed in previous studies with other species (2-4). Evidence from those studies and others (11-13) indicate that the unretained radioactivity results from the biosynthetic incorporation of [^{14}C]Phe, released during the metabolism of [^{14}C]SC-19200 and [^{14}C]SC-19129, into plasma protein. The predicted excretion of radiolabel from [^{14}C]Phe given orally to the dog would be approximately 10% in the urine and feces (approximately 5% each) from 0-96 hours and approximately 24% in breath, as $^{14}\text{CO}_2$, from 0-8 hours (14). Intact [^{14}C]Phe could not be detected in the urine in this study but the 2.87% of the IV dose excreted in the feces appeared to be almost completely in the form of [^{14}C]Phe.

[^{14}C]Phe was present in larger amounts in plasma following oral doses compared to the IV dose and reached peak plasma concentrations of approximately 0.4 - 0.5 mcg equivalents (expressed as mcg equivalents of the test article) per ml. Plasma concentrations of total ^{14}C were higher following the oral doses, compared to the IV dose, at 3 hours and later times after dosing and plasma total ^{14}C

AUC⁰ values were 2.5 to 3 times greater for the oral doses compared to the IV dose. This apparently reflects a higher percentage of dose converted to [¹⁴C]Phe, which is subsequently incorporated into tissue and plasma protein, following oral administration.

B. Oral Bioavailability:

The absorption of total radioactivity from oral doses of [¹⁴C]-SC-19200 and [¹⁴C]-SC-19129 was similar based on comparisons of plasma concentrations (Table 4), 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 (2.87%). Comparison of the fecal excretion data thus gives a lower limit of 92.5% and 91.4% absorption of the [¹⁴C]-SC-19200 and [¹⁴C]-SC-19129 oral doses respectively.

Bioavailability estimates based on urinary excretion were markedly lower than those based on fecal excretion. 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 (approximately 5%; see reference 14) 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 [¹⁴C]-SC-19200 or [¹⁴C]-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 which, in turn, make contributions from metabolites other than Phe relatively small. Since the percentage of the dose metabolized to Phe is larger for oral doses than for IV doses, plasma data will overestimate the bioavailability of total radioactivity.

The absorption of intact SC-19200 from orally administered SC-19200 was higher than from orally administered SC-19129 based on plasma concentration data (15.7% and 8.54% respectively) but was similar for both oral doses based on urinary excretion data (16.4% and 14.0% of dose, respectively). The higher bioavailability values determined from urinary excretion data are judged to be more accurate than the values determined from plasma data due to the larger concentrations available for analysis in urine. The HPLRC procedure used for SC-19200 analysis appears to underestimate SC-19200 at the low concentrations present in plasma after oral administration (Section IV.D).

C. Metabolic Pathway:

The proposed metabolic pathways for SC-19129 and SC-19200, based on studies in several other species (2-4) in addition to the present study, is shown in Figure 13. Hydrolysis of the methyl ester bond of orally administered SC-19129 apparently occurs presystemically due to enzymes in the intestines and/or liver. [^{14}C]-SC-19129 was not detected in plasma in this study. A fraction (approximately 20% in the dog based on urinary excretion data) of the SC-19200 formed by deesterification 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 metabolized at least in part to free Phe. Free aspartic acid may also be formed but would not be labeled and therefore was not detected in this study. N-Acetyl-SC-19200 was found in studies in the monkey (2), rat (3) and rabbit (4) but not in the present study. This is consistent with other studies which have indicated a lower capacity or narrower specificity for N-acetylation in the dog compared to other species (15). [^{14}C]-Phenylacetyl-glycine (PAGly) was also formed following oral doses of [^{14}C]-SC-19200 or [^{14}C]-SC-19129, but the amounts (< 1% of dose) were much smaller than the amounts of PAGly found in the rat (3) and rabbit (4) and also much smaller than the amount of the analogous glutamine conjugate of phenylacetic acid (PAGln) produced in the monkey (2). Since evidence was presented in previous studies (2-4) indicating that PAGly or PAGln formation is largely due to formation of phenylacetic acid by bacterial metabolism, the low formation of PAGly in the dog may be a reflection of differences in gut flora between species.

An additional metabolite (Metabolite A) not observed in previous studies was found in dog urine and was present in larger amounts following oral doses compared to the IV dose. The structure of this metabolite was not elucidated in this study.

D. Conclusions:

1. Oral absorption of total radioactivity from [^{14}C]-SC-19200 or [^{14}C]-SC-19129 occurred over several hours, with peak concentrations occurring

- at approximately 8.5 hours.
2. Plasma elimination half-lives for total radioactivity following IV and oral doses of SC-19200 (317 to 399 hours) were much longer than the elimination half-life for intact SC-19200 (0.87 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 92.5% and 91.4%, respectively, based on fecal excretion data and 38.0% and 29.2%, 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 0.871 hours following IV administration. The volume of distribution of SC-19200 was 0.13 l/kg indicating that this compound distributes primarily into the extracellular fluid.
 5. The bioavailabilities of SC-19200 from orally administered SC-19200 and SC-19129 were 16.4% and

14.0%, respectively, based on urinary excretion data and were 15.7% and 8.54%, respectively, based on plasma concentration data. The values based on urinary excretion data are judged to be more accurate due to the higher concentrations available for assay in urine.

6. The major metabolites of SC-19200 were Phe and an additional metabolite, the structure of which was not identified. Phenylacetylglycine was a minor metabolite of SC-19200.
7. The major route of elimination of radioactivity absorbed after oral or IV administration was in the urine. Recovery from the IV dose was 72.2% in urine and 2.87% in feces, for a total of 75.1%. Recoveries from oral doses of SC-19200 and SC-19129 were, respectively, 27.4% and 21.1% in urine, and 10.2% and 11.2% in feces.

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VII. Tables

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Table 1

Mean Body Weights and Dosages

Test Article	Route	Body Weight (kg) ^a	Specific Activity ^b (mCi/mg)	(mg/kg)	Dose ^{a,d} (mCi/kg)
SC-19200	IV	9.3 ± 0.5	1.01	10 ± 0.2	10 ± 0.2
SC-19200	Oral	9.1 ± 0.3	2.44 ^c	9.9 ± 0.1	24 ± 0.4
SC-19129	Oral	8.8 ± 0.2	2.48 ^c	10 ± 0.1	25 ± 0.4

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

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

^c Each oral solution dosage form was prepared twice due to the crossover design of the study. The values given are the average of the two solutions prepared (see Table 1A).

^d The mean and standard errors were obtained using unrounded individual animal values. The means were then rounded to 2 significant digits and the standard errors were rounded to the nearest 0.1 mg/kg or mCi/kg.

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

Sample Type	Mean Percent of Radiolabel Recovered	
	[¹⁴ C]-SC-19129 ^a	[¹⁴ C]-SC-19200 ^a
Plasma	98.2 ± 0.6 ^b	99.6 ± 0.2 ^b
Urine	102 ^c	100 ^c
Feces	99.0 ^c	99.4 ^c

^a Compound added to indicated biological sample type.

^b Mean ± standard error of 5 samples.

^c Average of two samples.

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

<u>Biological Sample Type</u>	<u>Compound Added</u>	<u>Conditions</u>	<u>Percent Eluted from HPLRC as Compound Added</u>		
Plasma ^a	[^{14}C]-SC-19129	Immediate	95.2	+	0.1 ^b
		Stored at -70°C for 226 days	84.1	+	0.2 ^b
	[^{14}C]-SC-19200	Immediate	98.8	+	0.6 ^b
		Stored at -70°C for 226 days	95.3	+	1.1 ^b
Urine	[^{14}C]-SC-19129	Immediate	93.0		
		Stored at -70°C for 95 days	93.1		
	[^{14}C]-SC-19200	Immediate	92.8		
		Stored at -70°C for 95 days	92.0		

^a Control dog plasma was pretreated with 2.3×10^{-3} Molar diethyl-p-nitrophenyl phosphate to inhibit esterase activity.

^b Mean \pm Standard error of duplicate samples.

Mean Plasma Concentrations and Pharmacokinetics Parameters
of ^{14}C -Total Radioactivity Following Administration
of ^{14}C -SC-19129 or ^{14}C -SC-19200 to Female Dogs^a

Table 4

Parameter	$[^{14}\text{C}]\text{-SC-19200}$ Intravenous		$[^{14}\text{C}]\text{-SC-19200}$ Oral		$[^{14}\text{C}]\text{-SC-19129}$ Oral	
Plasma Concentration (mcg/ml) at Indicated Time (hours)						
0.03	69.6	+ 4.4				
0.08	55.2	+ 2.7	b			
0.25	32.5	+ 2.6		0.29	0.87	+ 0.19
0.5	27.0	+ 2.9		0.24	1.56	+ 0.32
1.0	14.3	+ 0.4		0.29	2.96	+ 0.83
1.5	9.54	+ 0.44		0.37	3.95	+ 1.01
2.0	6.93	+ 0.34		0.52	4.75	+ 1.31
3.0	4.54	+ 0.27		0.93	5.56	+ 1.19
4.0	3.81	+ 0.30		1.12	6.68	+ 1.51
5.0	3.13	+ 0.36		1.21	7.92	+ 1.49
7.0	2.94	+ 0.26		1.3	10.1	+ 1.3
9.0	2.87	+ 0.22		1.2	10.3	+ 1.2
12	3.12	+ 0.28		1.2	9.27	+ 1.11
24	2.64	+ 0.34		1.12	7.18	+ 0.83
48	2.45	+ 0.21		0.88	5.75	+ 0.97
72	1.91	+ 0.27		0.75	5.26	+ 0.71
96	1.82	+ 0.27		0.66	4.76	+ 0.82
120	1.64	+ 0.28		0.53	4.52	+ 0.69
Cmax (mcg/ml)	69.6	+ 4.4		1.4	10.4	+ 1.2
Tmax (hours)	0.03	+ 0.00		0.5	8.5	+ 0.5
AUC ₀ [(mcg/ml)hours]	309	+ 33	816	+ 98	710	+ 101
AUC ₀ [(mcg/ml)hours]	1240	+ 202	3560	+ 600	3100	+ 470

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Table 4 (cont'd)

Parameter	$[^{14}\text{C}]\text{-SC-19200}$ Intravenous		$[^{14}\text{C}]\text{-SC-19200}$ Oral		$[^{14}\text{C}]\text{-SC-19129}$ Oral	
Bioavailability (%)	100		287 ^c		250 ^c	
Half-Lives (hours)						
α	0.0667 \pm 0.0108 ^d		4.58 \pm 0.49 ^e		4.18 \pm 0.76 ^e	
β	0.615 \pm 0.040 ^d		5.82 \pm 0.64 ^e		7.33 \pm 0.94 ^e	
γ	55.9 \pm 17 ^d		317 \pm 28 ^e		338 \pm 49 ^e	
δ	399 \pm 23 ^d					
Volume of Distribution (l/kg)	0.12 \pm 0.01		f		f	

^a Values are the mean \pm standard error of 4 animals. Individual animals plasma concentrations are given in Tables 2A-4A and pharmacokinetic parameters are given in Tables 5A-7A. Plasma concentrations have been rounded to 3 significant digits or, if less than 1.0 mcg/ml, to the nearest 0.01 mcg/ml. Pharmacokinetic parameters (Tables 5A-7A) were calculated using unrounded values obtained in the calculation of plasma concentrations.

^b Sampling time not applicable.

^c Calculated using the mean AUC_0^∞ values.

^d Calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$\text{Cp} = \text{Ae}^{-\alpha t} + \text{Be}^{-\beta t} + \text{Ce}^{-\gamma t} + \text{De}^{-\delta t}$$

^e Calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$\text{Cp} = \text{Ae}^{-\alpha t} + \text{Be}^{-\beta t} + \text{Ce}^{-\gamma t}$$

^f Not applicable.

Table 5
Comparison of Percentages of [^{14}C]-SC-19200 and [^{14}C]Phe
in Plasma HPLRC Profiles Determined by Two HPLRC Systems

HPLRC System	Percent of Radioactivity in Bond Elut Fraction Recovered in HPLRC Eluate	Percent of Radioactivity Recovered in HPLRC Eluate Present as Indicated Metabolite [^{14}C]-SC-19200	[^{14}C]Phe	Percent of Radioactivity in Bond Elut Fraction Recovered from HPLRC as Indicated Metabolite [^{14}C]-SC-19200	[^{14}C]Phe
Standard	57.1	44.1	40.1	25.2	22.9
Ion-Pair	83.7	35.1	48.9	29.4	40.9

^a The comparison in this table is for the pooled 5 hour plasma sample obtained following [^{14}C]-SC-19129 administration. The HPLRC profiles are shown in Figure 4B and Figure 5.

^b The HPLRC systems are described in Section III.F.

^c [Percent of Radioactivity in Bond Elut Fraction Recovered in HPLRC Eluate] X
[Percent of Radioactivity Recovered in HPLRC Eluate Present as Indicated Metabolite].

Table 6

Plasma Concentrations and Pharmacokinetic Parameters
of [^{14}C]-SC-19200 Following Administration of [^{14}C]-SC-19129 or [^{14}C]-SC-19200 to Female Dogs

Parameter	[^{14}C]-SC-19200 Intravenous ^a		[^{14}C]-SC-19200 Oral ^b	[^{14}C]-SC-19129 Oral ^b
Plasma Concentration (mcg/ml) at Indicated Time (hours)				
0.03	63.3 ±	3.5	c	c
0.08	56.3 ±	5.8	c	c
0.25	38.7 ±	5.8	0.727	0.227
0.5	29.7 ±	4.7	1.58	0.765
1	13.5 ±	1.8	1.36	0.827
1.5	8.67 ±	0.57	2.21	1.56
2	5.26 ±	0.17	2.34	0.726
3	2.26 ±	0.12	1.22	0.728
4	0.841 ±	0.134	1.06	0.613
5	0.411 ±	0.140	0.596	0.371
7	d		0.052	0.057
9	d		0.006	0.014
C _{max} (mcg/ml)	65.0 ±	4.1	2.34	1.56
T _{max} (hour)	0.04 ±	0.01	2	1.5
AUC ₀ [(mcg/ml)hr]	48.5 ±	2.4	7.61	4.14
Abs. Bioavailability (%)	100		15.7 ^e	8.54 ^e
Vol of Dist (L/kg)	0.13 ±	0.02	9	9
Clearance (ml/min) kg	3.5 ±	0.2	9	9
Half-lives (hours) ^f alpha beta	0.208 ± 0.870 ±	0.118 0.121	0.901 1.13	0.505 1.57

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Table 6 (cont'd)

- a Values are the mean \pm standard error of 4 animals. Individual animal values are given in Table 8A.
- b Values obtained using pooled plasma samples from 4 dogs.
- c Sampling time not applicable.
- d Not detected; see Table 8A.
- 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 Not applicable.

Table 7

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

Sample	Sample Collection Time (hours)	Percent Recovery of Radioactivity ^a					
		[^{14}C]-SC-19200 Intravenous		[^{14}C]-SC-19200 Oral		[^{14}C]-SC-19129 Oral	
Urine	0-6						
	0-12	•	•	•	•	•	•
	0-24	52.2	17.7	23.1	2.3	8.04	4.09 ^f
	0-48	71.6	2.8	25.8	2.0	16.6	2.3
	0-72	72.0	2.7	26.7	2.2	19.4	2.5
Feces	0-96	72.1	2.7	27.0	2.2	20.6	3.1
	0-120	72.2	2.7	27.2	2.2	20.8	3.2
						21.0	3.2
						21.1	3.2
Urine and Feces	0-24	1.59	0.80	6.38	1.74	7.25	4.2
	0-48	2.19	0.51	8.30	1.76	9.70	4.4
	0-72	2.55	0.54	9.21	1.65	10.5	4.3
	0-96	2.73	0.55	9.77	1.54	10.9	4.4
	0-120	2.87	0.56	10.2	1.5	11.2	4.3
Urine and Feces	0-24	53.4	18.0	32.2	1.4	26.7	3.0
	0-48	73.8	2.3	35.0	1.1	30.3	3.2
	0-72	74.6	2.2	36.2	1.2	31.4	3.1
	0-96	74.8	2.3	37.0	1.2	31.9	3.1
	0-120	75.1	2.2	37.6	1.2	32.4	3.1

^a Values are the mean \pm SEM of 4 animals unless otherwise indicated.

^b Individual animal values are given in Table 9A.

^c Individual animal values are given in Table 10A.

^d Individual animal values are given in Table 11A.

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

^f Value is the mean \pm SEM of 3 animals.

Table 8
Urinary Excretion of [^{14}C]-Labeled Metabolites
Following Administration of [^{14}C]-SC-19129 or [^{14}C]-SC-19200 to Female Dogs

Metabolite	Percent Recovery of Radioactivity ^a		
	[^{14}C]-SC-19200 Intravenous	[^{14}C]-SC-19200 Oral	[^{14}C]-SC-19129 Oral
SC-19200	70.1	16.4	14.0
Phenylacetylglutamine	0.03	1.11	0.58
Metabolite A - Retention Time 11:5 min	0.65	5.47	5.08
Total $^{14}\text{C}^b$	71.6	26.7	20.6

^a Percentages of dose recovered from 0-48 hours as the indicated metabolites were determined by analysis of pooled urine samples.

^b Mean percentage of total radiolabel recovered from 0-48 hours repeated here from Table 7, for reference.

VIII. Figures

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-VIII.1-

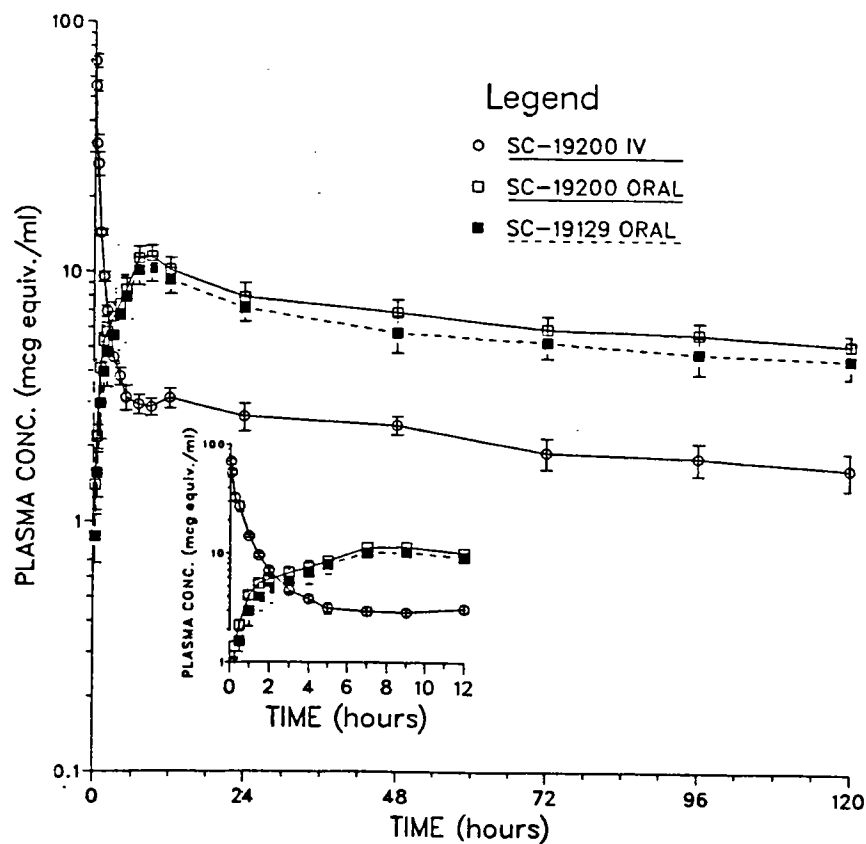


Figure 1. Mean plasma concentrations of total radioactivity following administration of intravenous [^{14}C]-SC-19200 (o), oral [^{14}C]-SC-19200 (\square) or oral [^{14}C]-SC-19129 (\blacksquare) to groups of 4 female dogs. The vertical bars represent the standard errors of the means. The insert shows 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.

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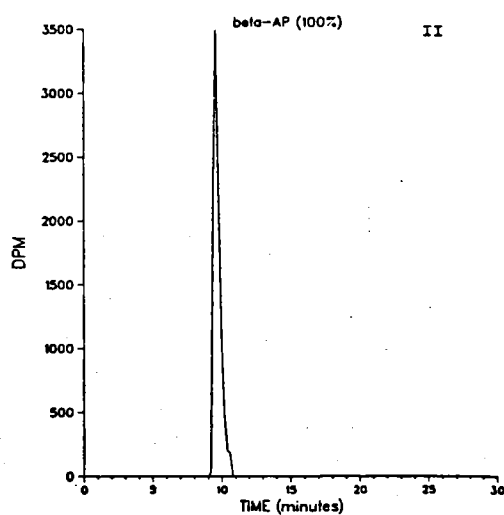
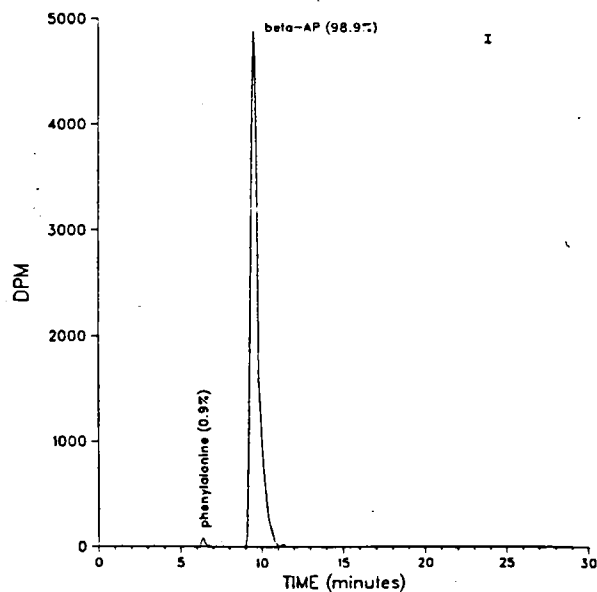


Figure 2. High performance liquid radiochromatograms of the acetonitrile eluate from Bond ElutTM extraction of pooled plasma samples collected at 0.5 hours (I) and 1 hour (II) after IV administration of [¹⁴C]-SC-19200 to female dogs. The locations of the reference standards are marked on the chromatograms. 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).

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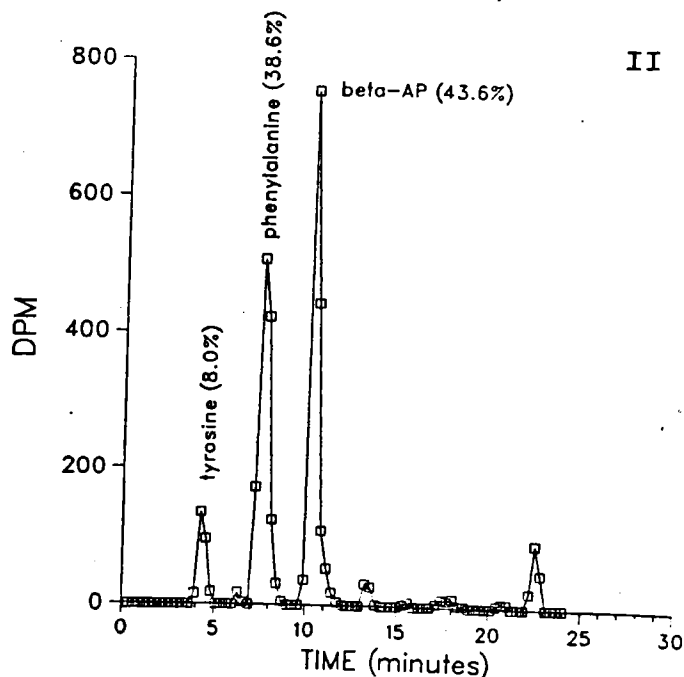
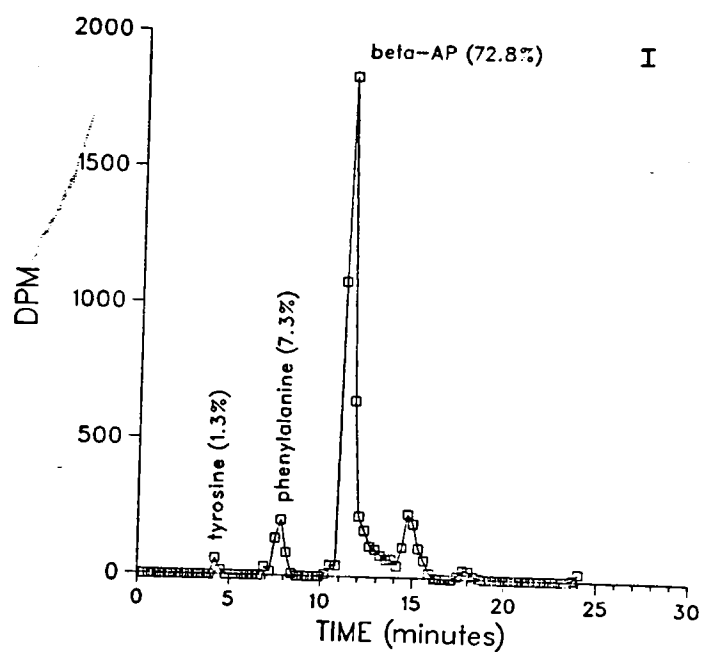


Figure 3. High performance liquid radiochromatograms of the acetonitrile eluate from Bond Elut™ extraction of pooled plasma samples collected at 0.5 hours (I) and 5 hours (II) after oral administration of [14 C]-SC-19200 to female dogs. The locations of the reference standards are marked on the chromatograms. 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).

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-VIII.4-

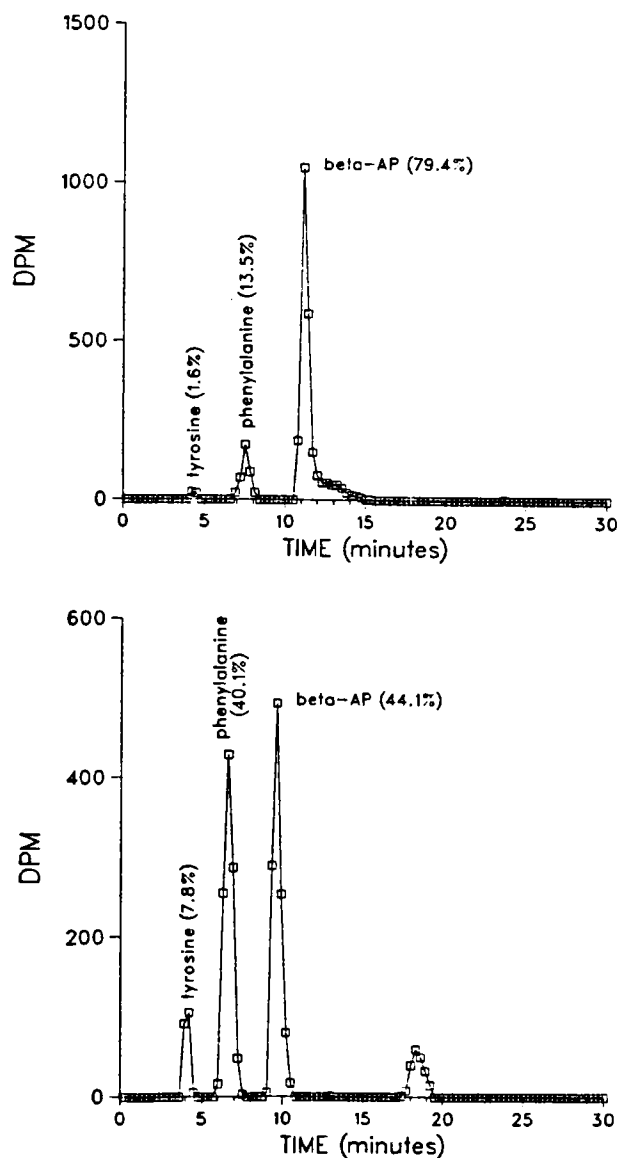


Figure 4. High performance liquid radiochromatograms of the acetonitrile eluate from Bond ElutTM extraction of pooled plasma samples collected at 0.5 hours (I) and 5 hours (II) after oral administration of [¹⁴C]-SC-19129 to female dogs. The locations of the reference standards are marked on the chromatograms. 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).

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-VIII.5-

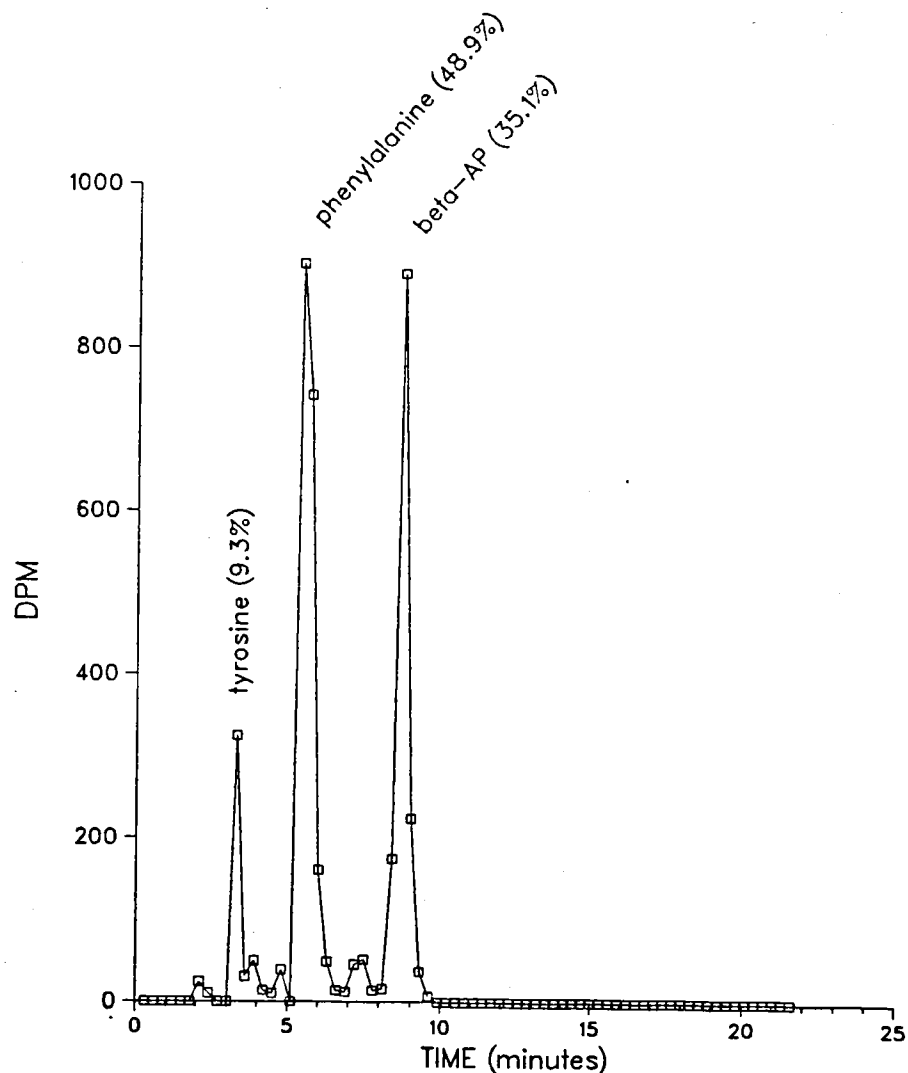


Figure 5. High performance liquid radiochromatogram, using the ion-pair HPLRC system (Section III.F), of the acetonitrile eluate from Bond Elut extraction of pooled plasma collected at 5 hours after oral administration of [14 C]-SC-19129 to female dogs. The location of reference standards are marked on the chromatogram. The percentages of radioactivity recovered from the column that are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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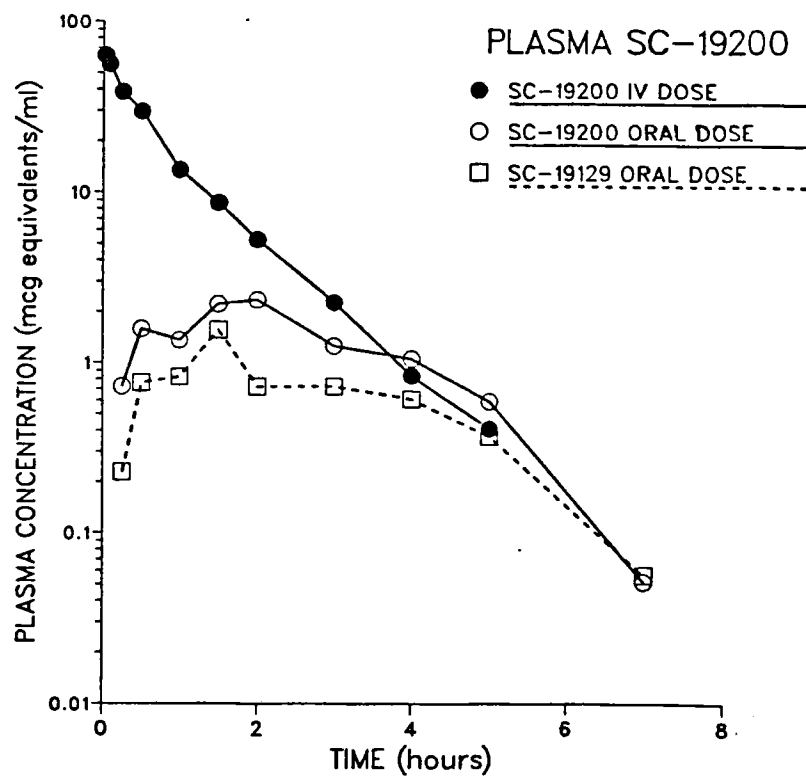


Figure 6. Mean plasma concentrations of SC-19200 in individual plasma samples following IV administration of [14 C]-SC-19200 (●) and in pooled plasma samples following oral administration of [14 C]-SC-19200 (○) or [14 C]-SC-19129 (□) to female dogs. Abscissa: time after dose administration in hours. Ordinate: concentrations in plasma expressed as mcg equivalents/ml.

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-VIII.7-

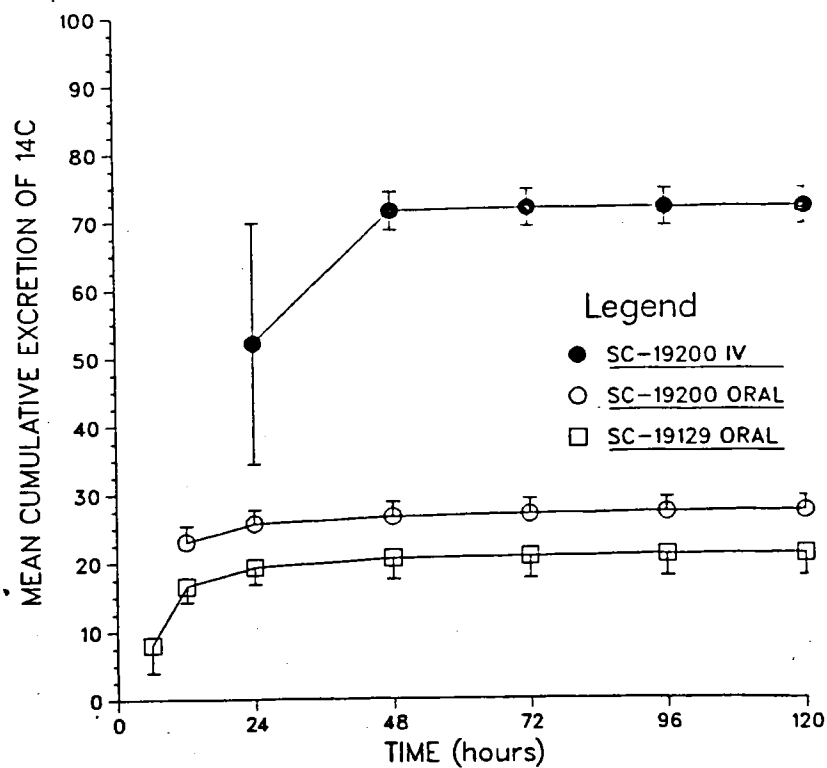


Figure 7. Mean cumulative excretion of radioactivity in urine following the administration of intravenous [^{14}C]-SC-19200 (●), oral [^{14}C]-SC-19200 (○) or oral [^{14}C]-SC-19129 (□) to female dogs. The vertical bars indicate the standard errors of the means. Abscissa: time after dose administration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

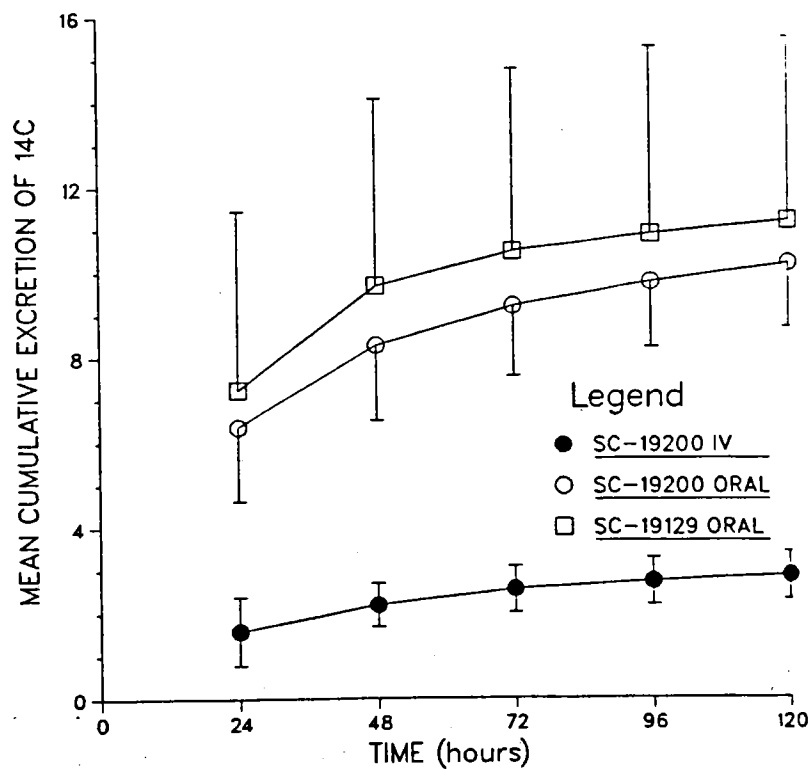


Figure 8. Mean cumulative excretion of radioactivity in feces following the administration of intravenous [^{14}C]-SC-19200 (●), oral [^{14}C]-SC-19200 (○) or oral [^{14}C]-SC-19129 (□) to female dogs. The vertical bars indicate the standard errors of the means. Abscissa: time after dose administration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

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POOLED DOG URINE
beta-AP I.V. DOSE, 0-6 HR.

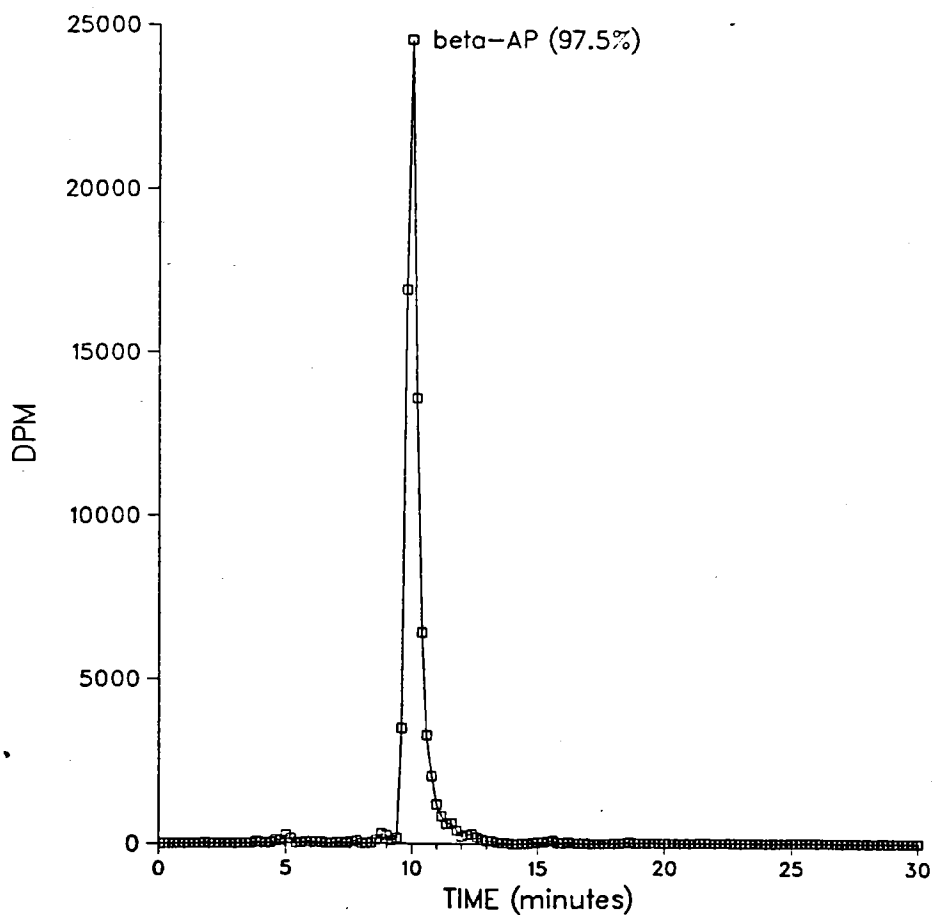


Figure 9. High performance liquid radiochromatograms of pooled urine sample collected from 0-6 hours after IV administration of [14 C]-SC-19200 to female dogs. The location of the reference standard is marked on the chromatograms. The percentages of radioactivity applied to the column associated with the standard is shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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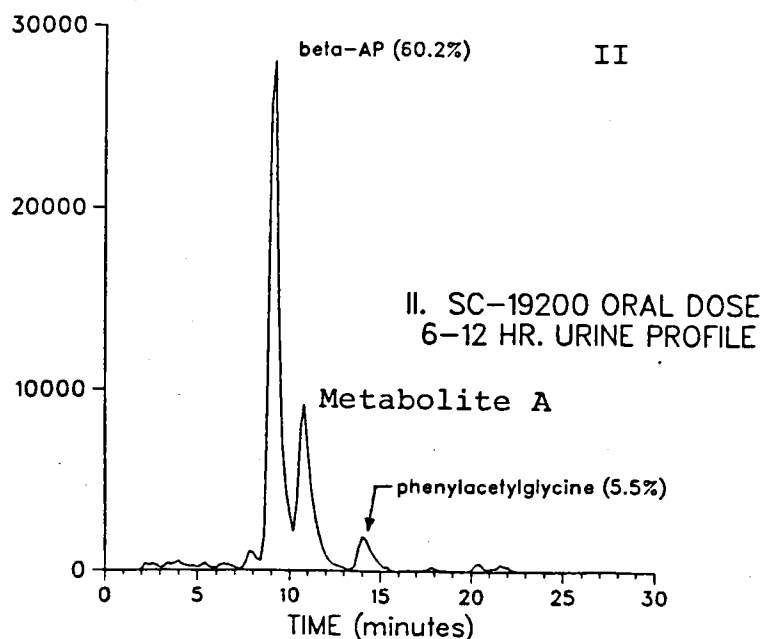
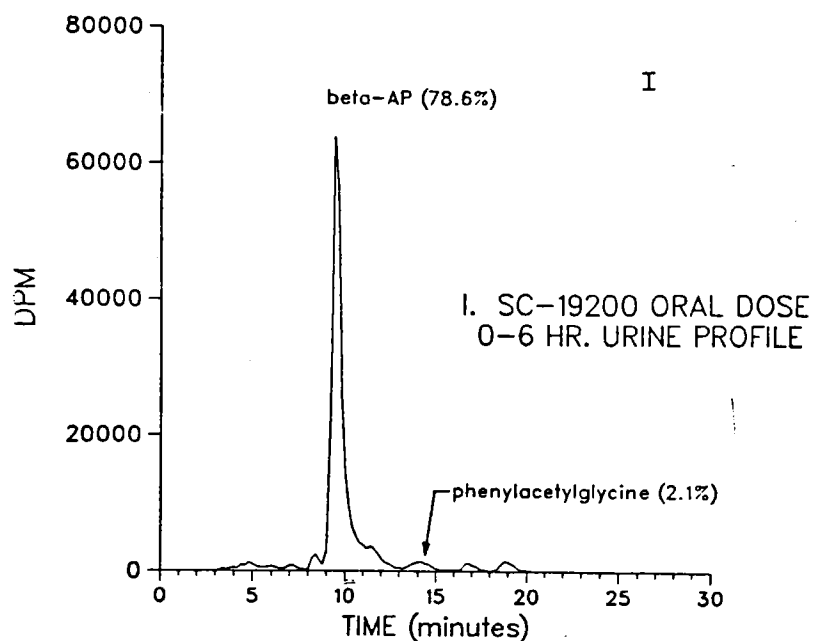


Figure 10. High performance liquid radiochromatograms of pooled urine collected from 0-6 hours (I) and 6-12 hours (II) after oral administration of [^{14}C]-SC-19200 to female dogs. The locations of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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-VIII.11-

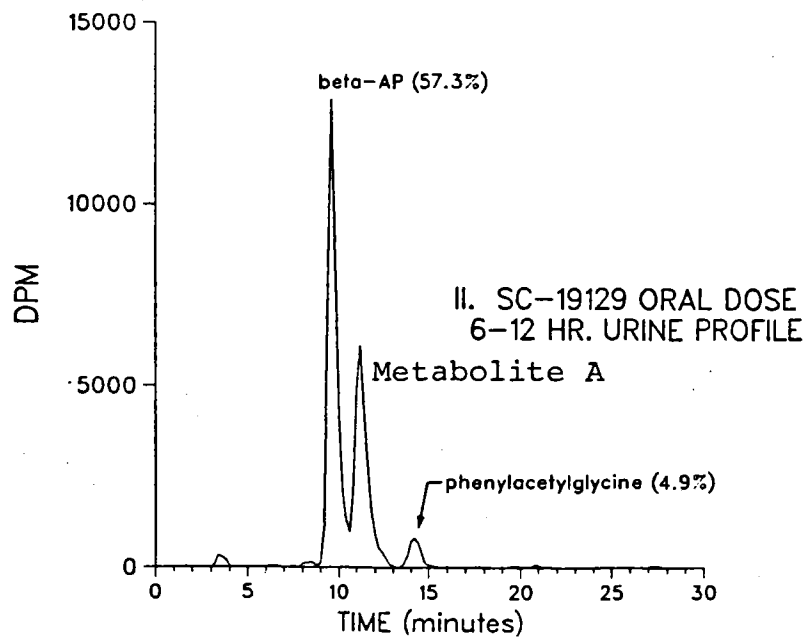
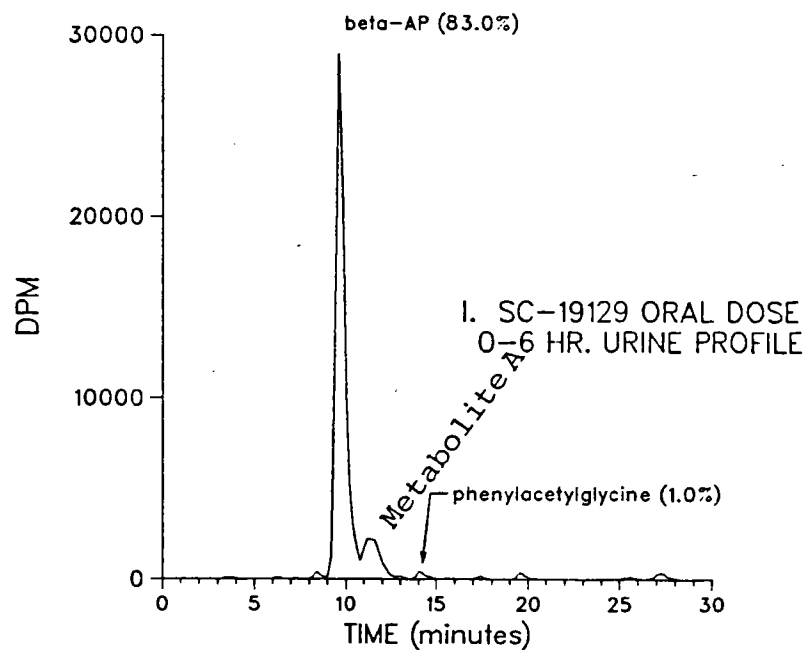


Figure 11. High performance liquid radiochromatograms of pooled urine collected from 0-6 hours (I) and 6-12 hours (II) after oral administration of [^{14}C]-SC-19129 to female dogs. The locations of the reference standards are marked on the chromatograms. The percentages of radioactivity applied to the column that are associated with the standards are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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-VIII.12-

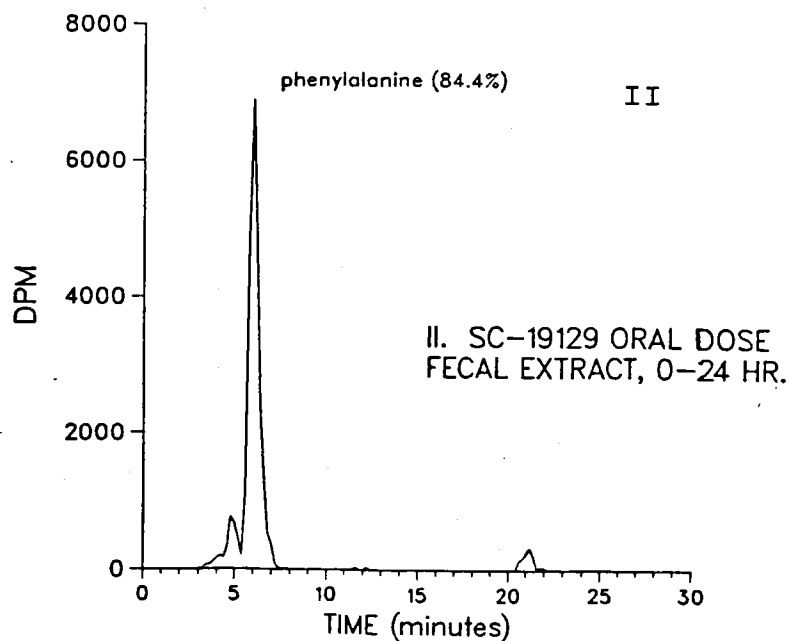
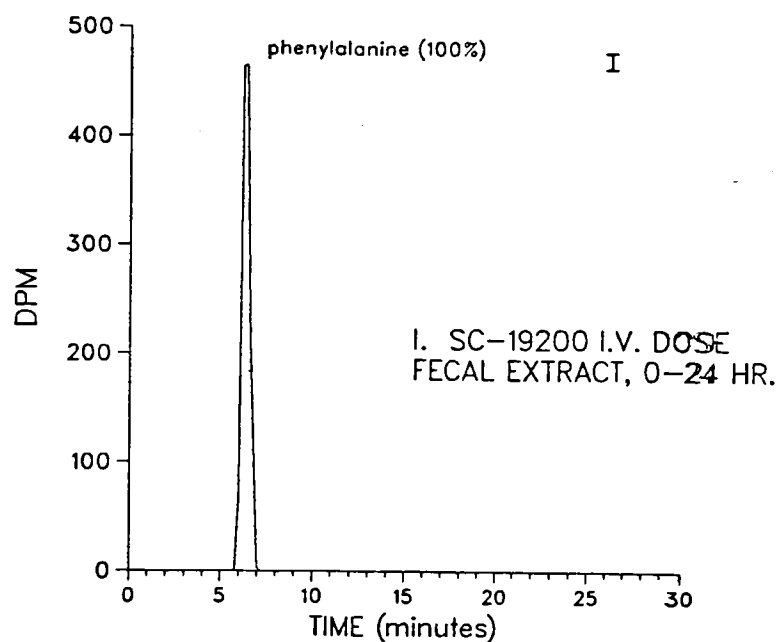


Figure 12. High performance liquid radiochromatograms of extracts of pooled feces collected from 0-24 hours following intravenous administration of [^{14}C]-SC-19200 (I) and oral administration of [^{14}C]-SC-19129 (II) to female dogs. The location of the reference standards are marked on the chromatograms. 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).

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-VIII.13-

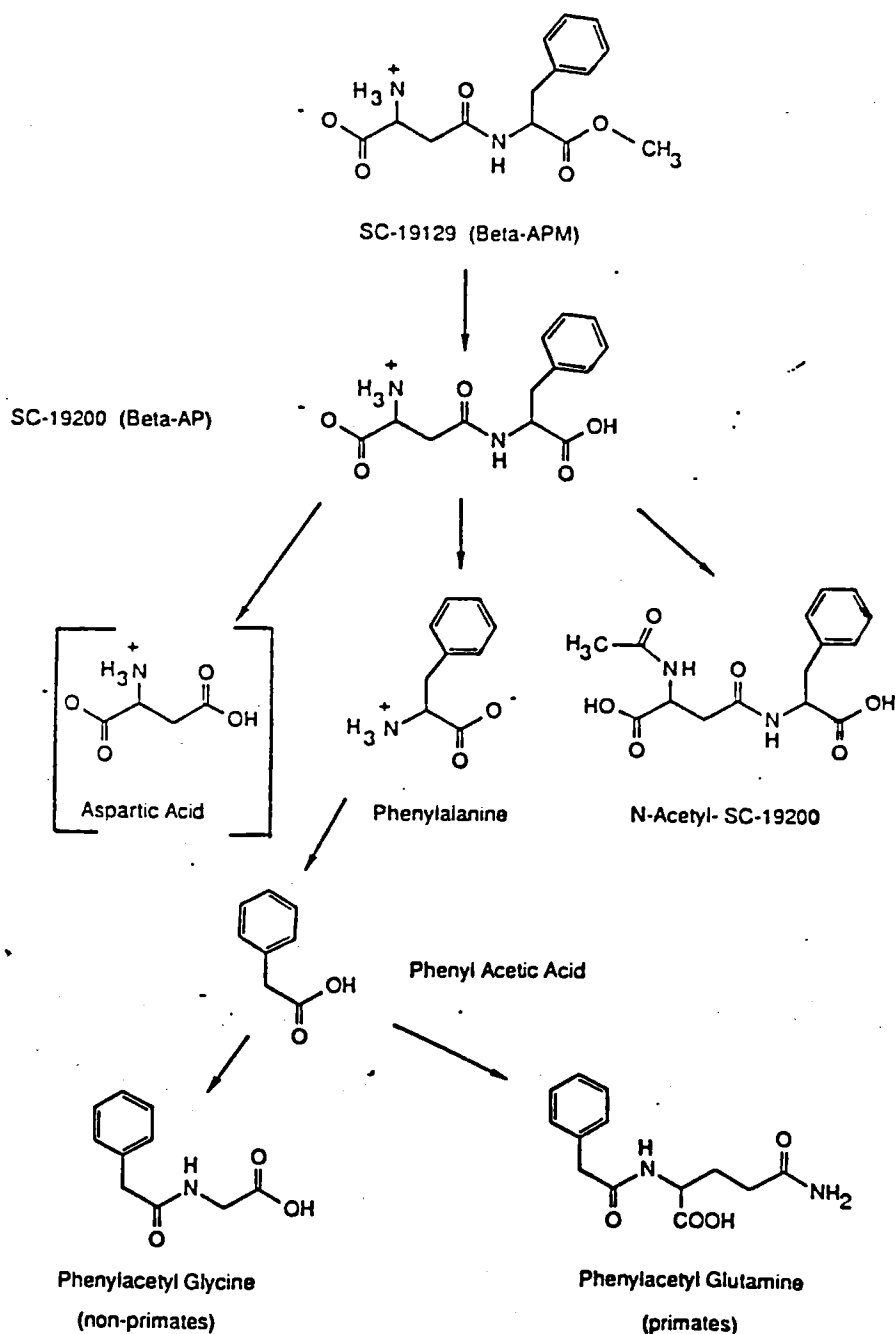


Figure 13. Structures and proposed metabolic pathway of SC-19129. Aspartic acid [in brackets] is a hypothetical metabolite which was not identified in this study.

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IX. Appendix 1. Tables

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Table 1A
Body Weights and Dosages

Test Article	Route	Dog ID #	Body Weight (kg) ^d	Dose (mg) ^e	Dose (mCi) ^e	Specific Activity (mCi/mg) ^f	Dose (mg/kg) ^g	Dose (mCi/kg) ^g
SC-19200	IV	AW35 ^a	10.7	107	109	1.01	10	10
		NNX4 ^a	9.0	89.7	91.1	1.01	10	10
		SLX4 ^a	9.0	90.0	91.3	1.01	10	10
		WN35 ^a	8.4	89.8	91.2	1.01	11	11
SC-19200	Oral	AW35 ^b	9.8	97.8	244	2.50	10	25
		NNX4 ^b	9.3	92.2	230	2.50	9.9	25
		SLX4 ^c	9.0	87.7	210	2.39	9.7	23
		WN35 ^c	8.4	82.4	197	2.39	9.8	23
SC-19129	Oral	AW35 ^c	8.9	93.0	234	2.52	10	26
		NNX4 ^c	9.0	89.9	226	2.52	10	25
		SLX4 ^d	8.9	90.1	220	2.45	10	25
		WN35 ^d	8.3	82.9	203	2.45	10	24

^a Doses were administered on March 4, 1986.

^b Doses were administered on March 31, 1986.

^c Doses were administered on April 28, 1986.

^d Body weights were measured to the nearest 0.1 kg.

^e Values are rounded to 3 significant digits.

^f Obtained by dividing dose in mCi by dose in mg, using unrounded values, and rounding the quotient to 3 significant digits.

^g Obtained by dividing dose (mg or mCi, as indicated) by body weight, using unrounded values, and rounding the quotient to 2 significant digits (for consistency with body weight significant digits).

Table 2A
Plasma Concentrations of Total Radioactivity
[¹⁴C]-SC-19200 Intravenous Dose

Plasma Concentration (mcg equivalents/ml) ^a						
Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM ^b
0.03	65.8	58.9	77.2	76.4	69.6	4.4
0.08	55.8	47.8	60.4	56.8	55.2	2.7
0.25	36.6	31.6	36.2	25.5	32.5	2.6
0.5	25.1	23.0	35.7	24.2	27.0	2.9
1.0	14.6	13.8	15.3	13.5	14.3	0.4
1.5	9.65	9.32	10.7	8.54	9.54	0.44
2.0	7.03	6.92	7.70	6.06	6.93	0.34
3.0	4.76	4.56	5.04	3.78	4.54	0.27
4.0	4.05	3.64	4.50	3.07	3.81	0.30
5.0	3.96	2.80	3.44	2.33	3.13	0.36
7.0	3.66	2.93	2.65	2.52	2.94	0.26
9.0	3.27	2.50	3.23	2.47	2.87	0.22
12.0	3.42	2.87	3.74	2.47	3.12	0.28
24.0	2.58	2.64	3.51	1.84	2.64	0.34
48.0	2.51	2.23	3.00	2.06	2.45	0.21
72.0	2.06	1.64	2.59	1.33	1.91	0.27
96.0	2.01	1.51	2.48	1.27	1.82	0.27
120.0	1.62	1.46	2.41	1.07	1.64	0.28
648.0 ^c	0.70	0.46	0.88	0.36	0.60	0.12

- ^a Values are rounded to 3 significant digits or, if less than 1 mcg/ml, to the nearest 0.01 mcg/ml.
- ^b Standard error of the mean, rounded to the same number of decimal places as the mean.
- ^c Sample taken just prior to administration of first oral dose (see Table 1A) for dosing schedule).

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Table 3A
Plasma Concentrations of Total Radioactivity
[¹⁴C]-SC-19200 Oral Dose

Time (hours)	Plasma Concentrations (mcg equivalents/ml) ^a					
	AW35	NNX4	SLX4	WN35	Mean	SEM ^b
0.25	0.55	1.45	1.79	1.81	1.40	0.29
0.5	1.51	2.56	2.49	2.15	2.18	0.24
1.0	3.24	4.45	4.45	4.25	4.10	0.29
1.5	4.26	5.39	6.00	5.44	5.27	0.37
2.0	4.16	6.07	6.41	6.22	5.72	0.52
3.0	3.82	7.17	7.47	7.89	6.59	0.93
4.0	4.15	8.31	7.90	9.23	7.40	1.12
5.0	5.25	8.88	8.66	11.1	8.47	1.21
7.0	8.79	9.72	12.3	14.4	11.3	1.3
9.0	8.93	9.88	13.9	13.4	11.5	1.2
12	8.08	8.13	13.0	11.5	10.2	1.2
24	6.31	5.74	9.49	10.2	7.93	1.12
48	5.58	5.25	8.51	8.42	6.94	0.88
72	4.86	4.47	7.21	7.28	5.95	0.75
96	4.67	4.43	6.95	6.72	5.69	0.66
120	4.48	4.08	6.27	5.86	5.17	0.53
672	1.02	1.10	c	c	d	d
768	c	c	2.15	1.72	d	d

^a Concentrations calculated after subtracting residual radioactivity remaining in plasma from previous dose(s), as described in Section III.H. See Table 1A for dosing schedule. Values are rounded to 3 significant digits or, if less than 1 mcg/ml, to the nearest 0.01 mcg/ml.

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Table 3A (cont'd)

- b Standard error of the mean, rounded to the same number of decimal places as the mean.
- c No sample taken at this time.
- d Mean and SEM not calculated for this time.

Table 4A
Plasma Concentration of Total Radioactivity
[¹⁴C]-SC-19129 Oral Dose

Time (hours)	Plasma Concentrations (mcg equivalents/ml) ^a					
	AW35	NNX4	SLX4	WN35	Mean	SEM ^b
0.25	0.893	0.494	1.38	0.716	0.87	0.19
0.5	1.65	1.11	2.43	1.06	1.56	0.32
1.0	2.10	2.36	5.44	1.93	2.96	0.83
1.5	2.49	4.14	6.74	2.44	3.95	1.01
2.0	2.41	5.01	8.31	3.25	4.75	1.31
3.0	2.79	6.40	8.38	4.68	5.56	1.19
4.0	3.41	7.08	10.6	5.65	6.68	1.51
5.0	4.51	8.59	11.6	6.99	7.92	1.49
7.0	7.25	11.9	12.8	8.60	10.1	1.3
9.0	7.86	11.6	13.0	8.71	10.3	1.2
12	7.38	11.2	11.2	7.32	9.27	1.11
24	5.29	8.09	8.97	6.39	7.18	0.83
48	4.32	5.03	8.61	5.03	5.75	0.97
72	3.90	5.53	7.15	4.46	5.26	0.71
96	3.04	5.07	6.89	4.04	4.76	0.82
120	3.06	4.88	6.26	3.88	4.52	0.69
672	c	c	1.80	0.81	d	d
768	1.00	1.64	c	c	d	d

^a Concentrations calculated after subtracting residual radioactivity remaining in plasma from previous dose(s), as described in Section III.H. See Table 1A for dosing schedule. Values are rounded to 3 significant digits or, if less than 1 mcg/ml, to the nearest 0.01 mcg/ml.

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Table 4A (cont'd)

- b Standard error of the mean, rounded to the same number of decimal places as the mean.
- c No sample taken at this time.
- d Mean and SEM not calculated for this time.

Table 5A
Plasma Total Radioactivity
Pharmacokinetic Parameters in Individual Dogs
[¹⁴C]-SC-19200 Intravenous Dose

Parameter	AM35	NNX4	SLX4	WN35	Mean	SEM ^a
C _{max} (mcg/ml)	65.8	58.9	77.2	76.4	69.6	4.4
T _{max} (hours)	0.03	0.03	0.03	0.03	0.03	0
AUC ₀₋₂₀ [(mcg/ml)hr]	324	281	393	239	309	33
AUC _{0-∞} [(mcg/ml)hr]	1290	1090	1770	810	1240	202
Rate Constant ^b (k, hr ⁻¹)						
α	7.60	9.15	16.6	12.0	11.3	2.0
β	1.29	1.08	1.23	0.970	1.14	0.07
γ	0.0570	0.0133	0.00714	0.0111	0.0221	0.0117
δ	0.00198	0.00150	0.00174	0.00181	0.00176	0.00010
C _{zero} (mcg/ml) ^c	73.7	67.1	96.5	97.8	83.8	7.8
Half-Life (hours) ^b						
α	0.0913	0.0757	0.0417	0.0580	0.0667	0.0108
β	0.536	0.644	0.564	0.715	0.615	0.040
γ	12.2	52.1	97.1	62.3	55.9	17.5
δ	350	462	398	384	399	23
Volume of Distribution (l/kg)	0.14	0.15	0.10	0.11	0.12	0.01

- ^a Standard error of the mean.
- ^b Elimination rate constants and the corresponding half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:
- $$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} + De^{-\delta t}$$
- ^c Concentration at time zero calculated by addition of the coefficients (A+B+C+D) obtained by NONLIN for the above equation.

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Table 6A
Plasma Total Radioactivity
Pharmacokinetic Parameters in Individual Dogs
[¹⁴C]-SC-19200 Oral Dose

Parameter	AM35	NNX4	SLX4	WN35	Mean	SEM ^a
C _{max} (mcg/ml)	8.93	9.88	13.9	14.4	11.8	1.4
T _{max} (hours)	9.0	9.0	9.0	7.0	8.5	0.5
AUC ₀ [(mcg/ml)hours]	654	637	987	986	816	98
AUC ₀ [(mcg/ml)hours]	2590	2510	4950	4200	3560	600
Rate Constant ^b (k, hr ⁻¹)						
α	0.129	0.209	0.129	0.162	0.157	0.019
β	0.110	0.174	0.100	0.114	0.125	0.017
γ	0.00247	0.00245	0.00174	0.00228	0.00224	0.00017
Half-Life (hr) ^b						
α	5.36	3.31	5.36	4.27	4.58	0.49
β	6.31	3.98	6.92	6.08	5.82	0.64
γ	281	283	398	304	317	28

^a Standard error of the mean.

^b Elimination rate constants and the corresponding half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$$

Table 7A
Plasma Total Radioactivity
Pharmacokinetic Parameters in Individual Dogs
[C]-SC-19129 Oral Dose

Parameter	AM35	NMX4	SLX4	WN35	Mean	SEM ^a
C _{max} (mcg/ml)	7.86	11.9	13.0	8.71	10.4	1.2
T _{max} (hours)	9.0	7.0	9.0	9.0	8.5	0.5
AUC ₀ [(mcg/ml)hr]	510	750	973	606	710	101
AUC ₀ [(mcg/ml)hr]	2390	3930	3910	2180	3100	470
Rate Constant ^b (k, hr ⁻¹)						
α	0.120	0.138	0.284	0.200	0.189	0.037
β	0.0697	0.107	0.123	0.0955	0.0988	0.0112
γ	0.00177	0.00154	0.00253	0.00291	0.00219	0.00032
Half-Life (hr) ^b						
α	5.79	5.03	2.44	3.46	4.18	0.76
β	9.95	6.49	5.62	7.26	7.33	0.94
γ	391	449	274	238	338	49

^a Standard error of the mean.

^b Elimination rate constants and the corresponding half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$C_p = A e^{-\alpha t} + B e^{-\beta t} + C e^{-\gamma t}$$

Table 8A
Plasma Concentrations and Pharmacokinetic Parameters of SC-19200
Following Intravenous Administration of [^{14}C]-SC-19200 to Female Dogs

Parameter	AW35	NNX4	SLX4	WN35	Mean ^b	SEM ^b
Plasma Conc. (mcg/ml at Indicated Time (hours)) ^a						
0.03	63.9	54.5	71.8	62.8	63.3	3.5
0.08	71.0	45.3	48.8	59.9	56.3	5.8
0.25	47.8	49.1	32.4	25.5	38.7	5.8
0.5	18.9	40.1	34.3	25.3	29.7	4.7
1	10.4	18.3	13.9	11.4	13.5	1.8
1.5	9.97	7.20	8.84	8.67	8.67	0.57
2	c	5.03	5.58	5.17	5.26 ^d	0.17 ^d
3	2.08	2.37	2.53	2.06	2.26	0.12
4	0.446	1.01	1.01	0.899	0.841	0.134
5	e	0.486	0.629	0.528	0.411	0.140
7	e	e	e	e	f	f
Cmax (mcg/ml)	71.0	54.5	71.8	62.8	65.0	4.1
Tmax (hours)	0.08	0.03	0.03	0.03	0.04	0.01
AUX ₀ [(mcg/ml)hr]	47.2	54.4	49.6	42.9	48.5	2.4
Rate Constant (k, hr ⁻¹) ^g						
α	3.80	1.27	27.0	8.90	10.3	5.8
β	0.879	0.565	1.02	0.884	0.836	0.096
Half-Life (hr) ^g						
α	0.182	0.547	0.026	0.078	0.208	0.118
β	0.789	1.23	0.682	0.784	0.870	0.121
C _{zero} (mcg/ml) ^h	78.3	59.3	109	78.5	81.2	10.2

Table 8A (cont'd)

Parameter	AM35	NNX4	SLX4	WN35	Mean ^b	SEM ^b
Volume of Distribution (l/kg)	0.13	0.17	0.092	0.14	0.13	0.02
Clearance (ml/min) / kg	3.5	3.1	3.4	4.2	3.5	0.2

- a The lowest concentration included in the standard curve was 1.0 (mcg/ml), the sensitivity limit of the validated assay (6). The detection limit of the assay is approximately 0.2 mcg/ml. Concentrations below 1.0 mcg/ml were obtained by extrapolation from the standard curve.
- b Values are the mean \pm standard error (SEM) of 4 animals unless otherwise indicated.
- c Sample extraction unsuccessful. Quantity not sufficient for repeat.
- d Values are the mean and SEM of 3 animals.
- e SC-19200 was not detected in the chromatogram. These samples were treated as having a concentration of zero for calculation of the mean and SEM.
- f Not calculated due to the number of samples having no detectable SC-19200.
- g Elimination rate constants and corresponding half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$C_p = A e^{-\alpha t} + B e^{-\beta t}$$
- h The theoretical concentration at zero time obtained by addition of the coefficients, A and B, calculated by the NONLIN program.

Table 9A
Urinary and Fecal Excretion of Total Radioactivity
[¹⁴C]-SC-19200 Intravenous Dose

Individual Cumulative Percent Recoveries of Radioactivity

U R I N E

Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM ^a
0 - 6	19.8	b	b	b	c	c
0 - 12	64.8	b	b	b	c	c
0 - 24	68.2	61.8	0.0818	78.9	52.2	17.7
0 - 48	69.3	70.3	67.1	79.6	71.6	2.8
0 - 72	69.5	71.0	67.8	79.8	72.0	2.7
0 - 96	69.6	71.1	67.9	79.9	72.1	2.7
0 - 120	69.7	71.2	68.1	80.0	72.2	2.7

F E C E S

Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM
0 - 24	1.82	2.84	b	0.101	1.59	0.80
0 - 48	2.04	3.17	2.72	0.819	2.19	0.51
0 - 72	2.27	3.50	3.28	1.13	2.55	0.54
0 - 96	2.45	3.72	3.44	1.29	2.73	0.55
0 - 120	2.62	3.91	3.56	1.39	2.87	0.56

U R I N E & F E C E S

Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM
0 - 24	70.0	64.6	0.08	79.0	53.4	18.0
0 - 48	71.3	73.5	69.8	80.4	73.8	2.3
0 - 72	71.8	74.5	71.1	80.9	74.6	2.2
0 - 96	72.0	74.8	71.3	81.2	74.8	2.3
0 - 120	72.3	75.1	71.7	81.4	75.1	2.2

a Standard error of the mean.

b No excretion during the indicated time interval.

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

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-IX.13-

Table 10A
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)	AW35	NNX4	SLX4	WN35	MEAN	SEM ^a
0 - 6	b	0.00171	18.9	b	c	c
0 - 12	19.2	27.2	19.2	26.9	23.1	2.3
0 - 24	20.9	30.0	24.3	28.1	25.8	2.0
0 - 48	21.4	30.4	24.9	29.9	26.7	2.2
0 - 72	21.6	30.7	25.2	30.6	27.0	2.2
0 - 96	21.8	30.8	25.4	30.9	27.2	2.2
0 - 120	21.9	31.0	25.5	31.2	27.4	2.2
F E C E S						
Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM
0 - 24	10.2	6.31	7.13	1.84	6.38	1.74
0 - 48	12.7	7.82	8.65	4.08	8.30	1.76
0 - 72	13.3	8.93	9.40	5.22	9.21	1.65
0 - 96	13.5	9.53	9.98	6.01	9.77	1.54
0 - 120	13.9	9.91	10.3	6.64	10.2	1.5
U R I N E & F E C E S						
Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM
0 - 24	31.1	36.3	31.4	29.9	32.2	1.4
0 - 48	34.1	38.2	33.6	34.0	35.0	1.1
0 - 72	34.9	39.6	34.6	35.8	36.2	1.2
0 - 96	35.3	40.3	35.4	36.9	37.0	1.2
0 - 120	35.8	40.9	35.8	37.8	37.6	1.2

a Standard error of the mean.

b No excretion during this time interval.

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

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Table 11A
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)	AW35	NNX4	SLX4	WN35	MEAN	SEM ^a
0 - 6	10.7	b	13.4	0.00422	8.04	4.09
0 - 12	14.3	15.3	13.4	23.4	16.6	2.3
0 - 24	15.0	16.6	19.6	26.4	19.4	2.5
0 - 48	15.3	17.3	20.1	29.5	20.6	3.1
0 - 72	15.5	17.6	20.3	29.9	20.8	3.2
0 - 96	15.7	17.8	20.4	30.0	21.0	3.2
0 - 120	15.8	18.0	20.5	30.2	21.1	3.2

F E C E S						
Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM
0 - 24	19.4	3.66	5.19	0.758	7.25	4.2
0 - 48	22.6	6.67	6.06	3.48	9.70	4.4
0 - 72	23.3	7.87	6.79	4.24	10.5	4.3
0 - 96	23.7	8.20	7.04	4.55	10.9	4.4
0 - 120	24.0	8.68	7.51	4.77	11.2	4.3

U R I N E & F E C E S						
Time (hours)	AW35	NNX4	SLX4	WN35	MEAN	SEM
0 - 24	34.4	20.3	24.8	27.2	26.7	3.0
0 - 48	37.9	24.0	26.2	33.0	30.3	3.2
0 - 72	38.8	25.5	27.1	34.1	31.4	3.1
0 - 96	39.4	26.0	27.5	34.6	31.9	3.1
0 - 120	39.8	26.7	28.0	35.0	32.4	3.1

a Standard error of the mean.

b No excretion during the indicated time interval.

MRC-861-0015

-IX.15-

X. Appendix 2. Protocol

MRC-861-0015

-X.1-

Protocol

1. Study Title:

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

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: March 4, 1986

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 and orally administered [^{14}C]-SC-19200 and orally administered [^{14}C]-SC-19129 in the dog.

7. Overview of Study Design:

The study design is a three way crossover in 4 female beagle dogs. Each animal will first be dosed intravenously (IV) with SC-19200. Following the IV dose, [^{14}C]-SC-19200 and ^{14}C -SC-19129 will be administered orally to each animal in a randomized crossover manner. Each dose will provide 10 mg of the indicated compound per kg body weight. A wash-out period of at least 4 weeks will occur between doses for each animal.

Plasma, urine and feces samples will be collected from each animal.

Total radioactivity will be determined for all samples. Concentrations of SC-19129 (if present), SC-19200 and other major metabolites will be determined in selected samples (based on total radioactivity results) by high performance liquid radiochromatography (HPLRC). A schematic representation of the study design is given below.

Schematic Representation of Study Design

Test Dose		Approximate Radiochemical Dose		Number of Female Dogs
<u>Article</u>	<u>(mg/kg)</u>	<u>(McCi/kg)</u>	<u>Route</u>	
SC-19200	10	10	I.V.	4
SC-19200	10	25	p.o.	4
SC-19129	10	25	p.o.	4

8. Laboratory Procedure:

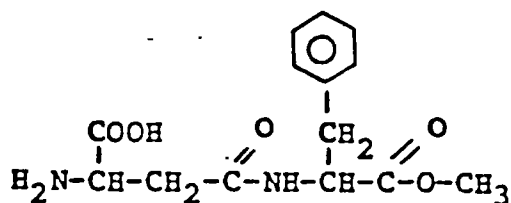
This study is not within the scope of Good Laboratory Practice Regulations.

9. Test Article:

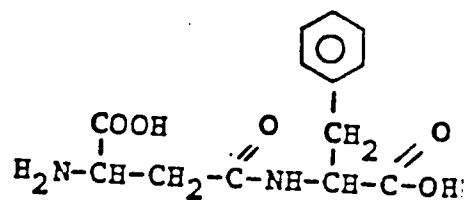
A. Chemical Name:

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/mole) and [U-¹⁴C-Phe]-SC-19200 with a specific activity of approximately 4.73 mCi/mg (approximately 1.33 mCi/mole) will be supplied by the Radiochemistry Group, G. D. Searle & Co. Unlabeled SC-19129 and unlabeled SC-19200 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 unlabeled SC-19129, or [^{14}C]-SC-19200 and unlabeled 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 unlabeled SC-19200 in 0.9% NaCl to give a final concentration of 10 mg/ml. The proportions of labeled and unlabeled test article will be adjusted to give approximately the specific activity (e.g. 2.5 mCi/mg for 25 mCi/kg) indicated for each treatment group in Section 7 (Schematic Representation of Study Design).

D. Administration:

1. Route:

- a. The oral solutions will be administered intragastrically through a gastric tube. The tube will be rinsed with water.
- b. The intravenous dose will be introduced via the saphenous vein.

2. Frequency:

Each animal will be dosed three times, as indicated in Section 7, with a 4 week interval between doses.

3. Volume and Dosage:

- a. The oral solution doses will consist of 2 ml per kg body weight to give doses of 10 mg per kg body weight. Each dose will be followed by approximately 20 ml of distilled water administered in such a way as to rinse the gastric tube.

- b. The [^{14}C]-SC-19200 I.V. dose will consist of 1.0 ml/kg; this is intended to provide a dose of 10 mg/kg. The catheter will be rinsed with 0.9% (w/v) NaCl immediately following administration of dose solution.

E. Analyses:

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

F. Storage:

[^{14}C]-SC-19129, [^{14}C]-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:

Four female beagle dogs weighing 8.0 to 11.0 kg at the time of the first dosing will be used. Each animal will be uniquely identified by a tattoo on the inner ear.

B. Housing:

The dogs will be housed in individual metabolism cages located in J-226 from 24 hours prior to dosing until the end of sample collection for each dose. They will be housed in the LAR dog colony at other times.

C. Diet:

1. Food: The dogs, maintained on Purina Dog Chow #5006 (Ralston Purina, St. Louis, MO), will be fasted for 18-24 hours prior to the administration of the compound. Food will be available from approximately 6 hours after dose administration and ad libitum throughout the remainder of the study.
2. Water: Tap water from the municipal water supply will be available ad libitum from 2 hours after dose administration.
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. Sample Collection, Times and Storage:

A. Blood:

Blood (approximately 5 ml) will be collected by venipuncture before dosing (0 hours) and at or near 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 12, 24, 48, 72, 96 and 120 hours after administration of dose solution. Additional blood samples will be obtained after intravenous dosing at 0.03 and 0.08 hours.

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.

B. Urine:

The urine will be collected into containers surrounded by dry ice, from -24-0, 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.

C. Feces:

Feces will be removed from the metabolism cages at approximately 0, 24, 48, 72, 96 and 120 hours after dose administration and stored frozen until analyzed.

D. Control Urine, Feces and Plasma: Plasma, urine and feces will be collected from the dogs prior to treatment with the test article. Aliquots of the control urine, feces and plasma will be spiked with [^{14}C]-SC-19129 and [^{14}C]-SC-19200 prior to frozen storage. The spiked samples will be used to determine stability and efficiency of extraction with each matrix.

12. 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, 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 HPLRC.

13. 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 and fecal data. Plasma concentration-time curves will be prepared from the [^{14}C]-SC-19129 (if present), [^{14}C]-SC-19200 or other major metabolite concentration data obtained from the metabolic profiles.

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, specimen collection	B. Belonio
Dosage form preparation and analysis and sample analysis	I. Dressler, K. Hoglund, D. Messing
Report	E. Burton

16. Protocol Review:

F. Kotsonis
A. Mackenthun
G. Schoenhard
J. Oppermann

17. Protocol Approval:

Grant Schoenhard 3-4-86

G. Schoenhard, Ph.D. Date

[Signature] 3/4/86

J. Oppermann, Ph.D. Date

Earl G. Burton 3/4/86

E. Burton Date
(Responsible Scientist)