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APPROVAL PACKAGE FOR:

APPLICATION NUMBER
NDA 21-437

Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

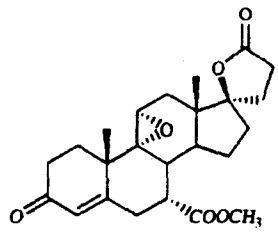
NDA number: 21437
Review number:
Sequence number/date/type of submission: N-000-BP, 1-17-02
Information to sponsor: Yes () No (x)

Sponsor and/or agent: Pharmacia
Manufacturer for drug substance:

Reviewer name: Elizabeth Hausner, DVM
Drug:
Trade name:
Generic name (list alphabetically): eplerenone
Code name: SC-66110, CGP 30 083
Chemical name: Draft

CAS registry number:
Mole file number:
Molecular formula/molecular weight: $C_{24}H_{30}O_6$
MW 414.50

Structure:



Eplerenone

Relevant INDs/NDAs/DMFs: IND [redacted]
IND [redacted]
Drug class: mineralocorticoid antagonist

Indication: hypertension

Clinical formulation: tablets for oral administration contain 25 mg, 50 mg or 100 mg of eplerenone and the following inactive ingredients: lactose, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl methylcellulose sodium lauryl sulfate, talc, magnesium stearate, titanium dioxide, polyethylene glycol, polysorbate 80 and synthetic iron oxides.

Route of administration: oral

Proposed use: alone or in combination with other anti-hypertensive agents

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

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Executive Summary

I. Recommendations

A. Recommendation on Approvability: The reviewer recommends approval.

B. Recommendation for Nonclinical Studies: No recommendations.

C. Recommendations on Labeling: Acceptable as written

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: Support for the proposed pharmacological mechanism was presented in the structural similarity to spironolactone, in vitro binding studies, animal models of hypertension and in the various toxicology studies where hormonal, urinalysis and clinical chemistry parameters were monitored.

Overall toxicology findings included adaptive induction of CYP450, found consistently in rats and mice (but not in dogs) with subsequent hepatocellular hypertrophy and predictable effects upon the thyroid and thyroid hormone metabolism. Adrenal zona glomerulosa hypertrophy was also consistently noted in rodents and dogs. The adrenal zona glomerulosa is the site of aldosterone synthesis, suggesting that the hypertrophy is an adaptive response to the pharmacological mechanism.

The main toxicological effect in the rat was an increase in the incidence and severity of chronic progressive nephropathy (CPN) primarily in female rats at dosages ≥ 250 mg/kg (approximately 15X the human exposure based on AUC for free eplerenone). It should be noted that females differ from male rats in their metabolism of eplerenone. The male rat metabolized 75% of a dose, excreting 25% unchanged. The female rat excretes 75% of the drug unchanged. The female rat shows 2-3X the body burden of a male rat given the same dosage. According to G. Robbie, Ph.D. (the biopharmaceutical reviewer), this sex-related difference in metabolism does not occur in humans.

The primary adverse effect in male dogs was prostate atrophy seen down to a NOEL of 5 mg/kg. Several possibilities exist to explain this. A striking dose-related increase in cortisol presents the possibility of cortisol-mediated atrophy. Subsequent to the cortisol changes, alterations in DHT and testosterone were reported. Mineralocorticoid-receptor antagonists may also bind to glucocorticoid receptors with subsequent effects on the hypothalamic-pituitary-adrenal axis. It is possible that a new hormonal equilibrium occurred secondary to the increased cortisol levels. Other possibilities include increased tissue aldosterone and/or local eplerenone competing for the androgen receptor. Spironolactone causes a similar effect in dogs (at a lower dosage) and is infrequently used in humans to deliberately produce such an effect.

Eplerenone did not show evidence of genotoxicity in the Ames assay, mouse lymphoma, chromosomal aberration (CHO cells), rat micronucleus and in vivo/in vitro unscheduled DNA synthesis in rat primary hepatocyte cultures.

There were no carcinogenicity findings deemed to be clinically relevant by the Exec CAC in a 26-week p53 mouse study, a standard 2-year rat carcinogenicity study and a 2-year restricted diet rat study.

Eplerenone given orally to rats appears to affect female fertility, embryo-fetal development and post-natal development only at dosages causing maternal toxicity. There are indications that eplerenone may alter male fertility at exposure levels approximately 23X the human exposure with no apparent paternal toxicity.

B. Pharmacological Activity: Anti-hypertensive activity through competitive binding to the mineralocorticoid (aldosterone) receptor.

C. Nonclinical Safety Issues Relevant to Clinical Use: Eplerenone appears to have the potential to cause the same secondary effects as does spironolactone. In those preclinical studies where spironolactone and eplerenone were examined concurrently, eplerenone seemed to be less potent on a mg/kg basis than spironolactone at causing secondary effects such as prostate atrophy. In vitro binding studies indicate that eplerenone has less binding affinity than spironolactone for steroid receptors other than the aldosterone receptor.

III. Administrative

A. Reviewer signature: Elizabeth Hausner, D.V.M. /S/

B. Supervisor signature: Concurrence - _____

Non-Concurrence - /S/ _____
(See memo attached)

C. cc: list:

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: decrease of blood pressure by decreasing sodium and fluid retention.

Mechanism of action: competitive inhibition of aldosterone binding to mineralocorticoid receptor.

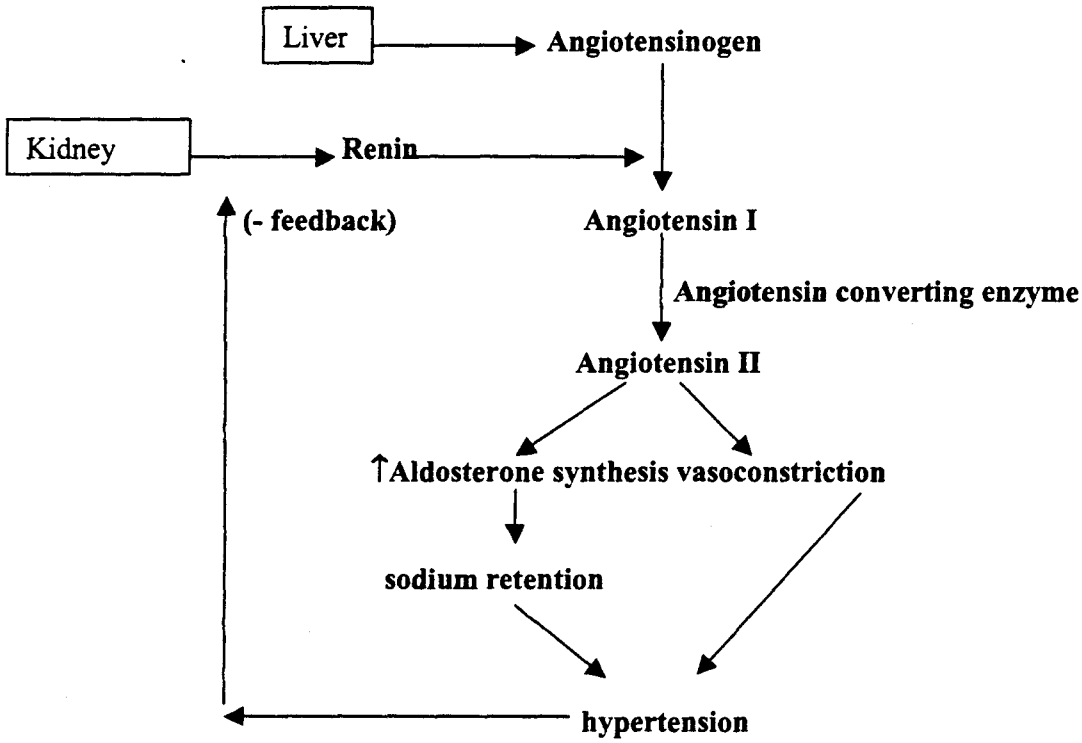
Drug activity related to proposed indication: decreases blood pressure through antagonism of receptor in kidney and possibly in non-classical tissues such as heart, brain and vasculature causing sodium excretion.

Secondary pharmacodynamics: Relative in vitro binding affinities for androgen, progesterone and glucocorticoid receptors are lower than those of spironolactone, an already marketed drug.

Pharmacology summary:

Maintenance of normal blood pressure and the ability to respond to transitory stimuli such as exercise, fear, emotion or pain depends upon a complex interaction of factors that ultimately regulate either mean cardiac output or the systemic vascular resistance. The kidney is thought to play a critical role in blood pressure regulation, especially long-term.

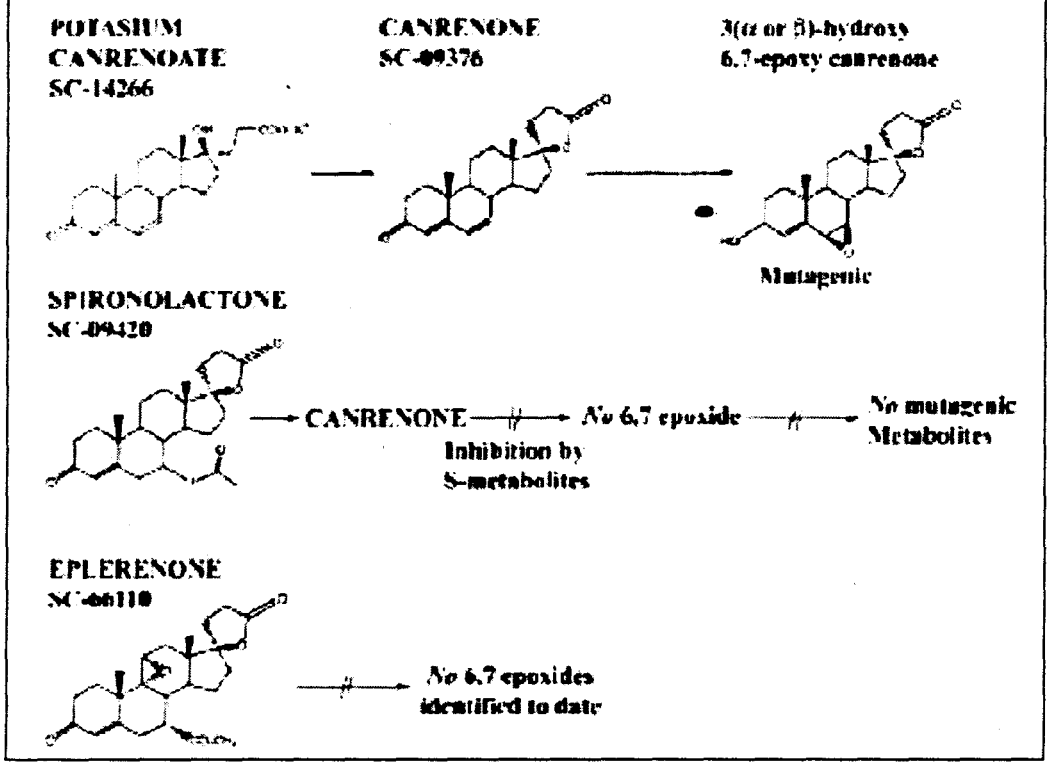
Renin is a proteolytic enzyme secreted by the juxtaglomerular cells of the kidney in response to stimuli such as decreased circulating volume or decreased sodium content of the macula densa of the renal distal tubules. Renin acts upon angiotensin, produced by the liver, to make Angiotensin I (AI). Angiotensin converting enzyme (ACE) acts in the lung to cleave AI to angiotensin II (AII), a pressor agent. AII effects the adrenal zona glomerulosa to increase the release of aldosterone. Aldosterone binds to the mineralocorticoid receptor (MR). The aldosterone-MR complex translocates to the nucleus and binds to hormone responsive elements upregulating expression of aldosterone induced proteins (AIP). In the renal tubules and collecting ducts this translates to increased reabsorption of sodium and water, expanding intravascular volume and raising blood pressure. In normal regulation, negative feedback is then exerted on renin secretion. This pathway is diagrammed below.



Eplerenone is a steroid-based anti-mineralocorticoid that competitively inhibits aldosterone at the receptor. Eplerenone is structurally similar to spironolactone, the only mineralocorticoid antagonist currently available in the US. Aldosterone antagonists are used as potassium sparing diuretics. Spironolactone produces adverse effects related to non-specific binding to other steroid receptors (progesterone and androgen). These effects appear clinically as gynecomastia, impotence, hirsutism and menstrual irregularities. Originally synthesized by Ciba-Geigy (CPG 30 083), the presence of the 9,11-epoxide group and/or the absence of the 7α thiol group on eplerenone decreases the binding affinity for the progesterone and androgen receptors while maintaining the mineralocorticoid blocking properties.

The sponsor's diagrammatic comparison of metabolism between spironolactone and eplerenone is shown below.

Figure 1. Relative Metabolism of Potassium Canrenoate, Spironolactone and Eplerenone



Evidence of pharmacological activity is apparent in the changes found in the urinary Na/K ratio and plasma aldosterone levels in the pre-clinical species in which these parameters have been measured. In vitro studies comparing spironolactone and eplerenone show that eplerenone has less affinity than spironolactone for the MR and for the other steroid receptors. Eplerenone inhibits human MR transcriptional activation by aldosterone in a concentration-dependent manner with a calculated IC50 of 291 nM.

Eplerenone was examined for pharmacological activity in numerous animal model systems. The interested reader is referred to the review of Amendment 275 to the IND.

Pharmacology conclusions: The sponsor presents both whole animal studies and in vitro binding data, serum aldosterone levels (in multiple toxicology studies) and altered urinary sodium excretion data to support the proposed mechanism of action.

II. SAFETY PHARMACOLOGY:

Neurological effects:
Four groups of rats received either vehicle or SC-66110 at 15, 45 or 150 mg/kg/day by oral gavage for 4 days. The highest dose achieved approximately 8X the expected human therapeutic plasma level. No deaths were reported. No effects of neurotoxicologic significance were reported.

Male rats, 6 per group, were given oral doses of 100, 300 or 1000 mg/kg of drug. Rectal temperature was measured before and 0.5, 1,2,3,4 and 6 hours after dosing. There was a significant ($p<0.05$) decrease in body temperature in the HD animals 1 hour after dosing. There was no difference from control at 2 hours.

Male mice, 6 per group were given oral doses of 100, 300 or 1000 mg/kg of drug. Locomotor activity, general behavior, thiopental sleep time, acetic acid induced writhing, convulsions, pentylenetetrazol-induced convulsions were assessed. At the HD there was a slight decrease in spontaneous motor activity and palpebral opening (sedation) from 0.5-2 hours after dosing. There was no effect on thiopental sleep time, acetic acid writhing, incidence or mortality associated with clonic seizures or pentylenetetrazol seizures.

Mice were orally dosed with suspensions of 25 or 50 mg/kg 1 hour prior to subcutaneous administration of 5 mg/kg apomorphine or 10 mg/kg apomorphine respectively. Rectal temperature was recorded from 30 minutes to 2 hours after apomorphine. Neither dose of eplerenone affected the apomorphine-induced hypothermia.
Another group of mice was given oral doses of 50 mg/kg eplerenone 1 hour before a lethal injection of physostigmine. Compared to the control, 50 mg/kg of eplerenone had no protective effect against physostigmine. Up to the oral dose of 50 mg/kg of eplerenone, there were no effects indicative of interaction with central noradrenergic, dopaminergic and serotonergic or cholinergic functions.

Cardiovascular effects: Conscious, instrumented Beagles were used for IV or oral drug administration. Four dogs received vehicle followed by 3 cumulative doses of SC-66110 (loading doses of 0.24, 0.52 and 1.88 mg/kg for 15 minutes plus maintenance doses for 15 minutes to sustain target plasma levels. 2 dogs received vehicle only using the same dosing scheme. For the oral studies, all 6 dogs received a single 22 mg/kg dose of SC-66110 by capsule. Dogs were monitored for 4 hours and at 24 hours. No deaths were reported. No cardiovascular or hemodynamic effects occurred at any dose. No gross pathologic findings were reported. Plasma levels were ≤2X the human therapeutic levels as summarized in the reviewer's table below.

Summary of plasma drug levels

Loading dose infusion mg/kg/15 min	Maintenance dose infusion Mg/kg/15 min	SC-66110 plasma conc (µg/ml) at end of maintenance	Multiple of human exposure (Cmax 1.12 µg/ml)
0.24	0.06	0.211±0.05	0.2X
0.52	0.17	0.638±0.126	0.6X
1.88	0.59	2.27±0.76	2X

Anesthetized Beagles (3 total) were given intravenous infusions of SC-66110 at 1,2 and 4 mg/kg at a rate of 2 ml/min. Cardiopulmonary parameters were monitored prior to dosing and for 30 minutes after administration of the negative control and test article. There were no reported effects on either systolic or diastolic blood pressure, heart rate and blood flow. At the lowest dose used, there was a statistically significant increase in QTc at 5 and 10 minutes after administration. This change was not dose-dependent. PR interval was increased slightly at 10 minutes after the infusion of the HD and at 15 minutes after the infusion of the MD. There is not apparent a pattern of PR increase consistent with a calcium channel effect and no apparent QRS effects. Total intravenous doses of 1,2 and 4 mg/kg had no significant effect on the respiratory or cardiovascular parameters measured.

Pulmonary effects: Anesthetized male guinea pigs were given either vehicle or a 15-minute loading dose of SC-66110 IV followed by a 45 minutes maintenance dose as follows:

Dosing Protocol in Guinea Pigs

Group	Loading dose (mg/kg)	Maintenance dose (mg/kg/)	Mean ±SD Plasma levels µg/ml (relative human exposure) Cmax 1.12 µg/ml
1	0	0	
2	0.4675	0.405	0.675±0.275 (0.6X)
3	1.4025	1.215	1.68±0.56 (1.5X)
4	4.675	4.05	5.32±1.09 (4.75X)

Pulmonary and cardiovascular parameters were evaluated for 60 minutes. No test article-related changes were observed in cardiopulmonary function.

There were no significant respiratory effects reported for the anesthetized Beagles.

Renal effects: Two oral doses of physiologic saline 30 minutes apart were given to 4 groups of 6 male rats per group. Thirty minutes after the second saline dose, the rats were orally dosed with 100, 300 or 1000 mg/kg of SC-66110 and placed immediately into metabolism cages. Urine was collected over 5 hours. Volume and electrolytes were measured. At all doses there was a statistically significant increase in total concentration of Na+ excreted.

Gastrointestinal effects: 6 male mice per group were orally dosed with 100, 300 or 1000 mg/kg of SC-66110. Three hours after dosing, a charcoal meal was orally administered. Thirty minutes later the mice were euthanized and charcoal powder transporting ratio was calculated. At the HD there was a statistically significant decrease in the ratio of charcoal meal transport.

Abuse liability: Not examined

Other: NA

Safety pharmacology summary: Did not appear to exhibit analgesic, pro-convulsive or anti-convulsive activity in mice. There did not appear to be significant neurotoxicological effects. There was a slight depression of intestinal motility and rectal temperature at very high doses in rats. At dosages achieving 2X (dogs) and 5X (guinea pigs) the human therapeutic exposure, there were no dose-related cardiopulmonary effects in these two species. The renal electrolyte effects were consistent with the expected pharmacology of the drug.

Safety pharmacology conclusions: The reported safety pharmacology studies did not identify any toxicologically significant cardiovascular, cardiopulmonary, gastrointestinal or CNS effects.

III. PHARMACOKINETICS/TOXICOKINETICS:

Note: unless otherwise specified, the vehicle for oral dosing is 0.5% methylcellulose and 0.1% polysorbate 80

Mice

1.DETERMINATION OF TOTAL RADIOACTIVITY AND PROFILING IN BIOLOGICAL SAMPLES OBTAINED AFTER ADMINISTRATION OF [^{14}C]SC-66110 IN THE MOUSE.M2098159

Urine, feces and rbc were collected after single oral doses of 0, 500, 1500 and 3000 mg/kg of [^{14}C]SC-66110 on Day 1 or after daily oral administration of 500 and 1500 mg/kg for 27 days and [^{14}C]SC-66110 administration on days 28, 29 and 30. Urine and feces were analyzed for metabolites with [redacted] There were no apparent metabolic differences between the sexes.
Urine: parent drug plus 19 metabolites containing SC-66110 derived radioactivity were found. Peaks designated MU-1-MU16, SC-71597 (6β-hydroxy SC-66110), MU-18 and MU19.
Feces: parent drug and 21 derivative peaks were found. MF-1- MF-17, SC-71597 and MF-19-MF-21

Excretion: Day 1 females given 500 mg/kg showed excretion of drug evenly divided between urine and feces. Feces then became major route of excretion for drug-derived radioactivity.

Nearly all radioactivity was excreted in the first 24 hours.

percentages of radioactivity excretion 168 hours post-dose

	urine	Feces
Males	53%	65%
Females	57%	56%

Percent of administered dose

Major components of the matrix after single oral dose		
Drug/metabolite	urine	feces
SC-66110	6.4-10.7%	13-28%
SC-71597	5.7%-11.1%	
MF12		7.3-15.2%
Major components of the matrix after 27 days of dosing		
Drug/metabolite	urine	feces
SC-66110	1.7-4.8%	9.1-35%
SC-71597	2.3-7.6%	
MF12		15.4-20.0%
MU19	2.7-5.3%	

2. *PHARMACOKINETICS AND EXCRETION OF [^{14}C]SC-66110 AFTER A SINGLE ORAL OR INTRAVENOUS ADMINISTRATION TO MALE CD-1, P53N5-W, AND P53N5-T KNOCKOUT MICE* (M2099198)

Single 15 mg/kg intravenous or oral doses of [^{14}C]-SC-66110 were given to CD-1 mice, p53N5-W and p53N5-T mice. Three mice per time point per strain were sampled at 0.033, 0.83, 0.25, 0.5, 1,2,3,4,5,8,24 and 48 hours after IV and 0.25, 0.5, 1,2,3,4,5,8,24 and 48 hours after oral dosing. Additional mice at the 1 and 3 hour timepoints were used to obtain samples for metabolic profiling.

The drug was rapidly absorbed with Tmax ~0.5 hours. The only notable strain difference was a secondary maximum peak of radioactivity in the p53 strains ~4-5 hours after dosing.

Summary of PK Parameters

	CD-1	P53N5-W	P53N5-T
Oral bioavailability	51%	13%	79%
(V_d)ml/k	7.46	15.9	8.21
Clearance ml/minute/g	0.761	0.97	0.653
$AUC_{0-\infty}$ for IV (µg equiv.hr/ml)	20.9	14.3	21.6
$AUC_{0-\infty}$ for oral (µg equiv.hr/ml)	11	21.7	19.3

The oral bioavailability of 135% may be due to serial euthanasias highlighting animal variability.

Excretion: feces was main route of excretion following oral and iv doses in all 3 strains. Most radioactivity recovered within 24 hours.

Metabolism: in plasma, parent drug and 19 derived metabolites were found.
Parent drug + 26 metabolites were found in the urine.

SC-66110 was found in all matrices of all strains at all sampling points.
Major plasma peaks: MP3, MP10, MP12, MP16, MP18 and SC-66110
Major urine peaks: MU17, MU24, MU 26 and SC66110
Major feces peaks: MF15, MF16 and SC-66110

There were no qualitative differences between the strains. There does seem to be greater plasma level exposure in the p53 strains.

3. *PLASMA CONCENTRATIONS OF SC-66110, SC-70303 FREE ACID AND TOTAL SC-66110 AFTER ORAL ADMINISTRATION OF [^{14}C]SC-66110 TO MALE MICE* M3098006

Male C57Bl/6TacfBR-[ko]P53WT mice were given oral gavage doses of 0, 250, 1000, 1500, 2000, 3000 and 5000 mg/kg. Blood samples were collected at 0.5, 1,2,3,4,5,8 and 24 hours after dosing.

Basically compared total SC-66110 to SC70303

The radiolabeled material was well absorbed at all doses. There were proportional increases in plasma levels between 250 and 1500 mg/kg. Greater than proportional increases were seen at dosages ≥2000 mg/kg. Plasma levels showed up to 2X more SC-66110 than SC-70303.

Summary of PK parameters

	Total radioactivity		Total SC-66110	
Dosage (mg/kg)	Cmax µg/ml	AUC µg.hr/ml	Cmax µg/ml	AUC µg.hr/ml
250	67.5	206	36.6	85.1
1000	111	721	73.5	329
1500	141	1579	92.0	689
2000	163	1577	99.0	651
3000	237	4104	171	2660
5000	241	4786	180	3500

4. *PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING A 4-WEEK RANGE-FINDING GAVAGE TOXICITY STUDY OF SC-66110 IN THE C57BL/6TacfBR MOUSE (EX4647)* M3098089
SC-66110 was given at oral dosages of 0, 100, 500 and 1000 mg/kg/day given to p53WT and heterozygotes (H) for 4 weeks during a range finding study. Blood samples from WT mice were collected day 1 and 31; blood was collected from H mice day 31 only.

Plasma levels of both SC-66110 and SC-70303 were found at all doses both days in all strains. Non-linear increases in plasma levels with increased dosage were apparent. Exposure also decreased from day 1 to day 31 suggesting induction of metabolism. No major sex-related differences were apparent in the measured parameters.

5. *PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING A 26-WEEK GAVAGE TOXICITY STUDY OF SC-66110 IN THE C57BL/6TacfBR-[KO]p53 HETEROZYGOUS MOUSE (SA4848)* M3000081
SC-66110 was given orally to p53 heterozygous mice at dosages of 0, 100, 300 and 1000 mg/kg/day. Blood samples were collected at specified times from individual animals on day 1, 94 and 177. Concentrations of SC-66110, SC-70303 and total SC-66110 were determined.

Drug was absorbed and available at all dosages with no apparent sex-related differences. The increases in plasma level exposure increased non-proportionally. There was a decrease in exposure over time at all doses, consistent with induction of metabolism, as determined by AUC and Cmax.

AUC and Cmax for SC-66110

Dosage (mg/kg/day)	Day1		Week 26	
	C_{max} (µg/ml)	AUC_{0-24}(µg.hr/ml)	C_{max} (µg/ml)	AUC_{0-24}(µg.hr/ml)
100	10	21	3.99	15.8
300	23.6	91.5	13.7	46.6
1000	83.1	359	26.1	95.6

There was less decline in plasma levels from day 94 to day 177 suggesting that steady state had been reached by Day 94.

6. *PHARMACOKINETIC SUPPORT TO A 2-WEEK FEASIBILITY STUDY OF SC-66110 DIETARY ADMIX AND ORAL GAVAGE IN MICE, EX4466* M3096131
Doses given in feed were equivalent to 0, 100, 1000 and 3000 mg/kg. Doses in the gavage group were 0, 100, 500 and 1000 mg/kg. Blood samples were collected after 16 days.

Summary of Pharmacokinetic Data

Dosage (mg/kg)	Cmax µg/ml		AUC $_{0-24}$ µg.hr/ml	
	Diet	gavage	diet	gavage
100	0.277±0.053	5.92±1.00	3.88	10.0
500		16.8±2.07		69.6
1000	0.976±0.21		10.6	
3000	6.10±0.95	21.1±3.23	84.5	173

As might be expected, the gavage administration produced higher Cmax and AUC values. The increase in exposure with increasing dosage was non-linear for both methods of administration.

7. *PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING A 13-WEEK RANGE-FINDING GAVAGE TOXICITY STUDY OF SC-66110 IN THE MOUSE, SA4513* M3097154
Male and female mice were gavaged with 0,100, 250, 500 and 1000 mg/kg/day for a 13-week range-finding study. Blood samples were collected days 1, 38 and 86. SC-66110 and SC-70303 were determined by [redacted] No sex-related differences were apparent in the pk data.

Non-linear kinetics were reported.

Summary of Mean Cmax for total SC-66110 (combined sexes)

Dosage mg/kg/day	Cmax µg/ml for total SC-66110		
	Day1	Day 38	Day 86
100	6.68	9.68	5.26
250	25.2	14.6	10.2
500	47.8	15.6	6.77
1000	71.8	27.9	13.4

Summary of Mean AUC_{0-24} for total SC-66110 (combined sexes)

Dosage mg/kg/day	AUC µg.hr/ml for total SC-66110 (SC-70303)		
	Day1	Day 38	Day 86
100	19.7 (10.5)	15.4 (6.24)	9.0 (3.90)
250	54.8 (28)	32.3 (13.2)	21.7 (9.41)
500	191 (89.4)	53.3 (21.4)	11.9 (5.20)
1000	406 (183)	118 (47.5)	107 (48.7)

8. *ABSORPTION AND EXCRETION OF SC-66110 AND SC-66110 RELATED MATERIALS DURING A 4-WEEK RANGE-FINDING GAVAGE TOXICITY STUDY OF SC-66110 IN THE C57BL/6TacfBR-[KO]P53 HETEROZYGOUS MOUSE (EX4751)* M3098205

During a 4-week range-finding study, p53heterozygous mice were orally dosed with [^{14}C]SC-66110 at doses of 500, 1500 and 3000 mg/kg on day 1. Mice were also given doses of the radiolabeled drug on days 28, 29 and 30 at dosages of 500 and 1500 mg/kg. Non-radiolabeled drug was given on the intervening days. Blood, urine and feces were collected at specified timepoints. Because of the many deaths at the highest dose, metabolic profiles were performed on urine and feces of the 500 and 1500 mg/kg groups only.

Exposure to SC-66110 and SC-70303 increased with increasing dose and decreased over time with no apparent sex-related differences in the data or in the urinary and fecal excretion of total radioactivity.
Total radioactivity was similar in plasma and RBC, suggesting that partitioning into RBC was not likely.
The percent of the dose excreted in urine did not differ significantly from dose to dose. Over time there was slight decrease in the amount excreted in urine (40-55% of total radioactivity on day 1 to 26-36% on Day 30) and an increase in the amount excreted via the feces (55-61% of total radioactivity Day 1 to 69-76% Day 30). A greater amount of the administered dose was excreted via the feces possibly due to induction of metabolism to compounds excreted in the bile. Major radioactive peaks in urine included SC-66110 and 6β-OH metabolite (SC71597). The major peak in the plasma was SC-66110 followed by the metabolite 6β-OH eplerenone. The major peak in the feces was SC-66110.

Rats

1. *PHARMACOKINETICS AND METABOLISM OF [^{14}C]SC-66110 AFTER IV AND ORAL ADMINISTRATION TO RATS (AN EXPLORATORY STUDY)*M3097046

Rats were given intravenous or oral doses of 15 mg/kg [^{14}C]SC-66110. Blood, urine and feces were collected at specified time points and analyzed for total radioactivity. Plasma samples were analyzed for SC-66110 and SC-70303. Metabolic profiles of selected plasma, urine or fecal samples were also obtained.

A sex-related difference in the elimination of drug was noted as shown in the table below.

Table 10. Mean Percentages of the Dose Excreted in Urine and Feces

Matrix	Sex	Total Radio-activity	SC-66110	Total SC-66110
IV				
Urine	M	22.6 ± 4.5	6.9 ± 1.6	7.1 ± 1.6
	F	33.2 ± 1.4	14.3 ± 1.4	19.8 ± 1.8
Feces	M	83.5 ± 0.9	6.7 ± 1.8	16.9 ± 0.8
	F	74.6 ± 3.6	9.2 ± 2.8	55.1 ± 3.3
Oral				
Urine	M	18.1 ± 0.5	<1	<1
	F	20.6 ± 0.6	1.8 ± 0.4	8.1 ± 1.4
Feces	M	79.2 ± 0.8	4.9 ± 0.6	14.5 ± 0.5
	F	77.8 ± 1.3	18.9 ± 4.6	52.3 ± 3.4

Females eliminated more of the total radioactivity as parent drug in the feces while the males excreted more of the total radioactivity in the urine, as metabolites rather than parent compound. Otherwise, only minor differences in PK parameters were apparent between the sexes.

Table 1. Pharmacokinetic Parameters of Total Radioactivity in Plasma

		Mean ± SEM	
Dose Route	Pharmacokinetic Parameter	Male	Female
IV	β (hr^{-1})	0.373 ± 0.031	0.473 ± 0.041
	$T_{1/2}$ (hr)	1.9	1.5
	AUC $_{0-\infty}$ (µg eq•hr/mL)	31.3 ± 3.5	33.7 ± 1.5
Oral	C_{max} (µg eq/mL)	7.6 ± 0.95	7.6 ± 0.53
	T_{max} (hr)	1.1 ± 0.3	0.8 ± 0.1
	$T_{1/2}$ (hr)	1.0	1.4
	AUC $_{0-\infty}$ (µg eq•hr/mL)	16.1 ± 2.3	21.3 ± 0.6
	Bioavailability (%)	59.6 ± 12.8	66.4 ± 3.5

2. THE PHARMACOKINETICS AND EXCRETION OF RADIOACTIVITY FOLLOWING INTRAVENOUS OR ORAL ADMINISTRATION OF [^{14}C]SC-66110 TO MALE AND FEMALE RATS M2095291

This report is the metabolite component from the previous study. [redacted] analysis of the radioactivity in the urine and feces of male rats under neutral or acidified conditions indicated that most of the radioactivity was in the form of metabolites following both IV and oral dosing. SC-66110 was the single largest radioactive peak in the urine of females receiving IV dosing whereas most of the radioactivity in feces was in the form of metabolites. Following acidification, SC-66110 represented the largest radioactive peak in urine and feces of female rats following both routes of administration.

Mean concentrations of radioactivity were higher in plasma than in red blood cells in both sexes following both IV and oral administration.

After oral dosing, most of the radioactivity at 1 hour post-dose was metabolites in both sexes. Five hours after dosing, plasma from males contained mostly metabolites while the plasma from females contained SC-66110 as the predominant peak, indicating a sex-related difference in metabolism.

Acidification of the plasma, urine and feces showed a decrease of polar metabolites in conjunction with an increase in parent drug and the more non-polar metabolites.

3. *PHARMACOKINETICS OF SC-66110 FOLLOWING A SINGLE ORAL ADMINISTRATION TO MALE AND FEMALE SPRAGUE DAWLEY RATS* M3000400

Male and female Sprague-Dawley rats were given single oral doses of 500, 1000 or 2000 mg/kg SC-66110. Blood samples were collected at specified timepoints out to 24 hours after dosing.

Table 7. Mean AUC_{0-24} and AUC_{0-24}/Dose of SC-66110, SC-70303 and Total SC-66110 in Male and Female Rats

Dose	Mean AUC_{0-24} (µg-h/mL)		Mean AUC_{0-24}/Dose	
(mg/kg)	Male	Female	Male	Female
SC-66110				
500	151	225	0.302	0.450
1000	327	364	0.327	0.364
2000	281	623	0.140	0.311
SC-70303				
500	62.6	111	0.125	0.222
1000	126	153	0.126	0.153
2000	117	259	0.0584	0.129
Total SC-66110				
500	211	332	0.423	0.664
1000	448	511	0.448	0.511
2000	393	871	0.196	0.436

Cmax and AUC for SC-66110, SC-70303 and total drug were greater in the females than males at all dosages as shown in the sponsor's table.

4. *ANALYSIS OF PLASMA, RBCS, URINE AND FECAL SAMPLES FROM RATS DOSED WITH SC–66110 DURING A 13-WEEK ORAL TOXICITY STUDY OF SC-66110 IN THE RAT* M3095302

Male and female rats received SC-66110 orally at doses of 20, 100 and 500 mg/kg for a period of 13 weeks. Day 1, Day 37 and Day 86 [^{14}C]SC-66110 was given to the rats in the 20 and 100 mg/kg groups. Blood, urine and feces were collected for determinations of total radioactivity and SC-66110.

There were no sex-related differences in total plasma radioactivity but there was a sex-related difference in the plasma levels of total SC-66110 with greater exposure in female rats due to less extensive metabolism than shown by the male rats. The increase in systemic exposure from 20 to 100 mg/kg was roughly proportional. Induction of metabolism effects were not obvious. The majority of total radioactivity was excreted in the feces regardless of dose and day of dosing. In unacidified samples the majority of plasma, urine and fecal radioactivity was due to metabolites, indicating extensive metabolism.

5. *PHARMACOKINETICS OF DIFFERENT DOSING REGIMENS OF SC-66110 IN THE RAT* M3000147

SC-66110 was given to male rats at 50 or 100 mg/kg once a day or 50 mg/kg twice a day for 7 days. Concentrations of SC-66110, SC-70303 and total SC-66110 were determined. Plasma levels were lowest with 50 mg/kg given sid. Plasma levels decreased over the 7 days dosing period at all doses, possibly due to induction of metabolism.

	Day 1				Day 7			
Dose (mg/kg)	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg•h/mL)	$AUC_{0-\infty}$ (µg•h/mL)	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg•h/mL)	$AUC_{0-\infty}$ (µg•h/mL)
50 q.d.	1	5.72	13.3	13.5	1	3.39	5.84	5.92
100 q.d.	1	6.85	16.5	16.7	1	4.45	8.98	9.06
50 b.i.d.	1	4.66	8.52	15.4	1*	4.35	3.68	9.44

* T$_{max}$ occurred after the second of the two daily doses

6. *THE PHARMACOKINETICS AND METABOLISM OF SC-66110 DURING AN 8 DAY RAT ORAL TOXICITY STUDY, EX4431 (AN EXPLORATORY STUDY)* M3097047

Doses of 100, 200, 500 and 1000 mg/kg were given to male rats for 8 days. Day 1 and 8, [^{14}C]-SC-66110 was given to the 100, 200 and 500 mg/kg groups. Blood, urine and fecal samples were collected at specified time points. Concentrations of radioactivity and SC-6110 (using a non-validated [redacted] procedure) were determined.

Drug was detectable in plasma at all doses. Plasma level exposure decreased over the 8 days of the study, probably due to induction of metabolism.

Table 8. AUC and AUC/Dose of SC-66110

			AUC (µg*mL)/hr		AUC/Dose	
Day	Dose mg/kg	Sex	0.00 to 8.00 hr	0.00 to 24.00 hr	0.00 to 8.00 hr	0.00 to 24.00 hr
1	0	M	0	0	*	*
	100	M	28.4	40.6	0.284	0.406
	200	M	72.3	76.7	0.362	0.384
	500	M	142	234	0.283	0.468
	1000	M	233	466	0.233	0.466
8	0	M	0	0	*	*
	100	M	11.9	12.9	0.119	0.129
	200	M	27.3	29.6	0.136	0.148
	500	M	76.4	102	0.153	0.204
	1000	M	114	177	0.114	0.177
* Not applicable to 0 mg/kg dose group						

The majority of radioactivity was excreted in the feces and was comprised of metabolites. Less than 31% of the fecal radioactivity was present as parent drug at any dosage or day. Results are consistent with other studies.

7. PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING THE TWO-YEAR ORAL GAVAGE COMBINATION CHRONIC TOXICITY AND CARCINOGENICITY STUDY OF SC-66110 WITH DIETARY CONTROL IN THE RAT (SA4664) M3000084

Male and female rats were given 250 mg/kg SC-66110 orally for 52 weeks. Blood samples were collected day 1 and day 360 of the study.

Day	Dose (mg/kg)	Sex	T_{max} (h)	C_{max} (µg/mL)	AUC_{24} (µg*h/mL)
SC-66110					
1	250	F	2.00	28.5	146
		M	3.00	20.6	84.1
360	250	F	2.00	14.8	81.2
		M	1.00	10.1	54.3
SC-70303					
1	250	F	2.00	7.54	37.4
		M	3.00	6.92	27.4
360	250	F	1.00	8.69	44.6
		M	1.00	3.99	26.6
Total SC-66110					
1	250	F	2.00	35.7	181
		M	3.00	27.2	110
360	250	F	1.00	23.0	124
		M	1.00	14.0	79.8

Results
Body burden as indicated by AUC decreased from day 1 to day 360. Plasma exposure was higher in females than in males at both timepoints. For total SC-66110 females had~1.5X the exposure of the males.

8. *PHARMACOKINETIC SUPPORT TO AN EMBRYO- FETAL DEVELOPMENTAL TOXICITY STUDY OF SC-66110 IN RATS (SEGMENT II), SA4468* M3096132

Pregnant rats received single daily doses of 20, 100 and 300 mg/kg/day from GD6 to GD17. Samples were collected both GD6 and GD17 at specified intervals for a period of 24 hours after Dosing.

Results

Parent drug was detectable in the plasma at all timepoints in all dose groups on both GD6 and GD17. Plasma concentrations decreased from GD6 to GD17, consistent with induction of metabolism. The sponsor reports no difference in plasma levels between the gravid rats and all rats combined. The number of non-gravid rats added in was very small and therefore from this study it cannot be said with confidence that there is no difference in the plasma level exposure between gravid and non-gravid female rats.

9. *PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING A RANGE-FINDING PRE- AND POSTNATAL TOXICITY STUDY OF SC-66110 IN RATS, EX4855* M3099162

Pregnant rats were given 500, 750 and 1000 mg/kg/day from GD6 to GD20 during a range-finding study. Blood samples were collected on days GD6 and GD20 at specified times for a period of 24 hours after dosing.

Results

Drug was detectable at all dosages at all time points. Plasma concentration increased from 500 mg/kg to 750 mg/kg then plateaued going to 1000 mg/kg. Concentrations at all 3 dosages decreased from Day 6 to Day 20.

Table 8. Pharmacokinetic Parameters of Total SC-66110 (AUC and AUC/Dose)

Day (mg/kg)	Dose	Sex	AUC (mcg/mL) x hr	AUC/Dose
6	500	F	532	1.06
	750	F	1010	1.34
	1000	F	949	0.949
20	500	F	235	0.470
	750	F	278	0.371
	1000	F	347	0.347

The results are consistent with saturation of absorption and induction of metabolism.

10. *PLASMA CONCENTRATIONS OF SC-66110 AND SC-70303 DURING A 4-WEEK ORAL GAVAGE RANGE FINDING TOXICITY STUDY OF SC-66110 WITH DIETARY RESTRICTION IN THE RAT, SA4566* M3097157

Table 9. Pharmacokinetic Parameters of Total SC-66110 (AUC and AUC /Dose)

Day	Dose mg/kg	Sex	AUC (a) (mcg-hr/mL) 0.00 to 24.00 hr	AUC/Dose 0.00 to 24.00 hr
1	250	F	214	0.857
		M	117	0.467
		F&M	165	0.662
	500	F	521	1.04
		M	239	0.479
		F&M	380	0.760
	750	F	615	0.820
		M	360	0.480
		F&M	501	0.668
	1000	F	595	0.595
		M	431	0.431
		F&M	513	0.513
28	250	F	139	0.557
		M	67.5	0.270
		F&M	96.7	0.387
	500	F	175	0.356
		M	112	0.224
		F&M	145	0.290
	750	F	311	0.415
		M	151	0.201
		F&M	231	0.308
	1000	F	272	0.272
		M	171	0.171
		F&M	222	0.222

(a) AUC of total SC-66110 was calculated by adding the mean AUC's of SC-66110 and SC-70303. SC-70303 values were normalized to SC-66110 equivalents to reflect conversion of SC-70303 to SC-66110 on the basis of SC-66110 assay.

Male and female rats were given SC-66110 at dosages of 0, 250, 500, 750 and 1000 mg/kg/day during a 4-week restricted diet toxicity study. Blood samples were collected at specified time intervals on days 1 and 28 for determination of SC-66110 and SC-70303 concentrations. On both days blood was collected for a period of 24 hours after dosing.

Results

Drug was detectable at all dosages at all time points on both days. Exposure decreased in both sexes from Day 1 to Day 28. On both days, plasma concentrations were higher in females than males.

Induction of metabolism and sex-related differences in body burden are apparent in this study consistent with other studies.

11. *PHARMACOKINETIC SUPPORT FOR A 13-WEEK GAVAGE TOXICITY STUDY OF SC-66110 IN THE RAT, SA4453* M3096081

Eplerenone was given orally to male and female rats for 13 weeks at dosages of 20, 100 and 500 mg/kg. Day 1,37 and 86, [^{14}C]SC-66110 was given to rats in the LD and MD groups. Blood, urine and feces were collected at specified timepoints after dosing on each of the three sampling days. The results of the radiolabel doses were discussed in a separate report. Concentrations of SC-66110, SC-70303 and total drug were described here.

Results

Drug was detectable at almost all sampling points of the three sampling days. Plasma levels increased with increasing dose in a non-linear fashion. Plasma concentrations of SC-66110 and

SC-70303 were higher in the females than in the males. Exposure in both sexes decreased over time.

The results are consistent with studies showing similar sex-related differences in pharmacokinetic parameters.

12. *Plasma concentrations of SC-66110, SC-70303 and total SC-66110 during a 26-week oral toxicity study of SC-66110 in the Rat (SA4516).* M3096284

Table 12 [illegible]

[illegible]				
1				
177				
[illegible]				

Male and female rats were given SC-66110 orally at dosages of 30, 100 and 500 mg/kg/day during a 26-week toxicity study. Blood samples were collected at specified times on days 1 and 177. Concentrations of SC-66110, SC-70303 and total drug were determined.

Results

AUC values for SC-66110 exceeded the AUC values for SC-70303 at all doses, both sampling days. The two forms appear to exist in equilibrium. As shown, females had higher AUC levels than males. Exposure was essentially dose proportional. However, at the high dose of 500 mg/kg/day, AUCs were much less on Day 177 than on Day 1. This was interpreted by the sponsor as autoinduction of metabolism.

13. *PHARMACOKINETICS OF SC-66110, SC-70303 AND TOTAL SC-66110 IN THE TWO YEAR ORAL GAVAGE COMBINATION CHRONIC TOXICITY AND CARCINOGENICITY STUDY OF SC-66110 IN THE RAT (SA4663)* M3000083

Male and female rats were given SC-66110 at dosages of 20, 75 and 250 mg/kg/day for 104 weeks by oral gavage. Blood samples were collected at specified time intervals on Day 1 and Day 358 (week 52) of the study. Concentrations of SC-66110 and SC-70303 were determined in the plasma.

Results

AUC values were consistently higher in females compared to males. Increases in exposure from 75 mg/kg to 250 mg/kg were non-proportional. Values are shown in the sponsor's table below

Summary table of PK parameters for total SC-66110.

Day	Dose (mg/kg)	Sex	T$_{max}$ (h)	C$_{max}$ (µg/mL)	AUC$_{0-24}$ (µg·h/mL)
Total SC-66110					
1	20	F	0.500	3.63	15.1
		M	0.500	3.04	5.20
1	75	F	0.500	18.0	54.2
		M	0.500	7.11	28.2
1	250	F	2.00	32.4	241
		M	3.00	20.7	152
358	20	F	0.500	8.69	25.8
		M	0.500	2.47	7.72
358	75	F	0.500	25.1	71.0
		M	0.500	10.5	29.3
358	250	F	0.500	32.9	156
		M	3.00	15.1	88.3

Compared to the AUC values obtained in healthy humans given multiple doses of 100 mg per day, total SC-66110 was found at levels 2-13X the human AUC in female rats and 0.6-7X the human AUC in male rats.

14. *EVALUATION OF PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING A STUDY OF THE EFFECTS OF SC-66110 ON EMBRYO/FETAL DEVELOPMENT IN RATS (SA4990)* M3000085

Pregnant rats were given SC-66110 as an oral suspension at doses of 100, 300 and 1000 mg/kg/day from gestation day 6 through gestation day 19/20. Blood samples were collected from maternal animals at specified times on Days 6 and 17 of gestation. Maternal and fetal blood samples were taken on day 19, 24 hours after the maternal dose for trough concentrations and day 20 at 1 hour post-dose for approximate peak concentrations. Concentrations of SC-66110 and SC-70303 were determined.

Results

Maternal plasma levels were found at Cmin (trough) at all three doses. Trough plasma levels were detectable in the fetuses only at 300 and 1000 mg/kg . Exposure determined at approximate Cmax showed detectable plasma levels in the fetuses at all 3 dosages. Maternal AUC for total SC-66110 increased approximately proportionally from 100 to 300 mg/kg and less than proportionally from 300 to 1000 mg/kg. Exposure at all dosages decreased from day 6 to day 17.

Table 3. Mean Plasma Concentrations of SC-66110, SC-70303 and Total SC-66110 in Dams and Fetuses on Day 19 and Day 20

Day 19	Dose (mg/kg)	Time (hr)	Female Mean (ug*hr/L)	Female SEM(a)	Female N(c)	Fetal Mean (ug*hr/L)	Fetal SEM(a)	Fetal N(c)
SC-66110	100	24	0.0256	0.00975	4	0	0	3
	300	24	0.0856	0.0149	4	0.0201	0.022	4
	1000	24	0.472	0.0972	4	0.479	(b)	2
SC-70303	100	24	0.00332	0.00332	4	0	0	3
	300	24	0.0813	0.0816	4	0.167	0.0395	4
	1000	24	0.124	0.0277	4	1.14	(b)	2
Total SC-66110	100	24	0.0330	0.0120	4	0	0	3
	300	24	0.0447	0.0226	4	0.182	0.0567	4
	1000	24	0.591	0.116	4	1.56	(b)	2
Day 20								
SC-66110	100	1	5.68	0.744	5	4.52	0.42	5
	300	1	8.86	1.43	5	6.77	1.24	5
	1000	1	23.3	4.21	5	16.4	3.1	5
SC-70303	100	1	1.28	0.121	5	1.33	0.165	5
	300	1	1.65	0.292	5	2.33	0.387	5
	1000	1	4.90	1.23	5	5.53	0.832	5
Total SC-66110	100	1	6.99	0.378	5	5.62	0.56	5
	300	1	10.4	1.7	5	9.00	1.59	5
	1000	1	28.2	5.57	5	21.7	3.74	5

(a) Standard Error of the Mean
(b) SEM is not reported for N=2
Female N = number of dams. Fetal N = number of litters with pooled samples

The results show fetal exposure at the dosages used for the study.

15. *TOXICOKINETIC SUPPORT FOR A STUDY OF THE EFFECTS OF ORAL ADMINISTRATION OF SC-66110 ON FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION IN MALE AND FEMALE RATS (SA4997).* M3000316

Male and female rats were given SC-66110 at oral dosages of 100, 300 or 1000 mg/kg. The TK were assessed only in the male rats. Blood samples were collected at specified times on Day 1 and Day 58 (week 9). Concentrations of SC-66110 and SC-70303 were determined.

Results

Plasma level exposure as measured by AUC_{0-24} increased less than proportionally with increasing dose. The plasma levels decreased over time, possibly due to induction of metabolism.

Table 8. **Toxicokinetic Parameters (AUC_{0-24} and AUC_{0-24}/Dose) of SC-66110, SC-70303 and Total SC-66110 for Male Rats on Day 1 and Day 58**

Day	Dose (mg/kg)	Sex	AUC_{0-24} (µg•hr/mL)	AUC_{0-24}/Dose
SC-66110				
1	100	M	46.5	0.465
	300	M	179	0.596
	1000	M	445	0.445
58	100	M	22.3	0.223
	300	M	65.7	0.219
	1000	M	149	0.149
SC-70303				
1	100	M	27.6	0.276
	300	M	110	0.368
	1000	M	221	0.221
58	100	M	14.1	0.141
	300	M	35.3	0.118
	1000	M	68.1	0.0681
Total SC-66110				
1	100	M	73.1	0.731
	300	M	277	0.924
	1000	M	668	0.668
58	100	M	35.8	0.358
	300	M	99.8	0.332
	1000	M	217	0.217

16. *TOXICOKINETIC SUPPORT FOR SC-66110: 13-WEEK ORAL TOXICITY AND IMPURITY QUALIFICATION STUDY IN THE RAT (. 2001-0160/SA5143)* M3001187

Male and female rats were given one of three different bulk drug lots of SC-66110 as oral suspensions once a day for 13 weeks at doses of 200 mg/kg/day (males) and 100 mg/kg/day (females). Blood samples were collected at specified intervals on day 79. Concentrations of SC-66110 and SC-70303 were determined.

Day	Dose (mg/kg)	Sex	Lot# 96K020-F1A AUC_{0-24} (µg•hr/mL)	Lot# 00K007-F5A AUC_{0-24} (µg•hr/mL)	Lot# SP12626 AUC_{0-24} (µg•hr/mL)
SC-66110					
79	100	F	34.1	32.7	45.1
	200	M	37.4	31.8	23.9
SC-70303 Free Acid					
79	100	F	34.5	29.4	35.9
	200	M	22.0	21.9	19.1
Total SC-66110					
79	100	F	67.2	60.3	76.6
	200	M	51.5	52.8	42.7

Results

Variability was expressed as standard error of the mean rather than standard deviation. However, the SEM values for a given parameter did not vary to a great extent between batches. There were no marked differences in exposure to the three lots of SC-66110 used in the study.

17. *EVALUATION OF PLASMA CONCENTRATIONS IN A STUDY OF EFFECTS ON PRE- AND POST-NATAL DEVELOPMENT IN CD RAT BY ORAL GAVAGE ADMINISTRATION OF SC-66110, SA4891* M3099360

Female rats were given eplerenone orally at dosages of 0, 100, 300 or 1000 mg/kg from GD6 to day 20 of lactation. Blood samples were collected from selected pups at 2 and 4 hours after maternal dosing on day 4 of age. Concentrations of SC-66110 and SC-70303 were determined.

Results

SC-66110 and SC-70303 were detectable in pups at both 2 and 4 hours at all dosages examined. There were no obvious sex-related differences in plasma concentrations. The concentrations of SC-66110 and SC-70303 were approximately equal. The increase in concentration from 100 to 300 mg/kg in male pups was more than proportional while the increase from 300 to 1000 mg/kg was less than proportional. The increases in exposure in the pups were non-linear.

Table 2. Mean Plasma Concentrations of SC-66110, SC-70303 and Total SC-66110 in Pups after Oral Administration of SC-66110 to the Rat

Dose (mg/kg)	Time (hr)	Male N	Male (µg/mL)	Female N	Female (µg/mL)	Male & Female N	Male & Female (µg/mL)
SC-66110							
100	2	8	0.0613	5	0.089*	13	0.0752
	4	10	0.214	6	0.186	16	0.200
300	2	7	0.230	8	0.227	15	0.217
	4	5	0.150	8	0.160	13	0.156
1000	2	4	0.265	7	0.624	11	0.434
	4	6	0.312	7	0.567	13	0.439
SC-70303							
100	2	8	0.0694	5	0.0466	13	0.0580
	4	10	0.343	6	0.457	16	0.400
300	2	7	0.421	8	0.280	15	0.350
	4	5	0.219	8	0.215	13	0.217
1000	2	4	0.250	7	0.489	11	0.375
	4	6	0.516	7	0.562	13	0.539
Total SC-66110							
100	2	8	0.128	5	0.134	13	0.131
	4	10	0.542	6	0.626	16	0.584
300	2	7	0.611	8	0.495	15	0.553
	4	5	0.360	8	0.387	13	0.364
1000	2	4	0.534	7	1.05	11	0.794
	4	6	0.806	7	1.11	13	0.957

Guinea Pigs

1. *SEARLE MEMO REPORT: PLASMA LEVELS OF SC-66110, SC-70303 AND TOTAL SC-66110 FOLLOWING A LOADING AND MAINTENANCE INFUSION TO MALE GUINEA PIGS* M3000403

In a cardiopulmonary function assay, guinea pigs received an intravenous loading infusion followed by a maintenance infusion. Plasma concentrations of SC-66110 and SC-70303 were determined from plasma samples collected at the end of the maintenance infusion at each dose level.

Results

Two dosage protocols were described. Plasma levels were increased with the higher loading and maintenance dosages. Concentrations of SC-66110 exceeded those of SC-70303 for each animal sampled.

2. *SEARLE MEMO REPORT: SC-66110 PLASMA CONCENTRATIONS IN THE GUINEA PIG CARDIOPULMONARY STUDY; EX4441* M3096082

The concentration of SC-66110 in male guinea pig plasma was determined from samples collected at the end of the maintenance dose infusion at each dose level. Results are summarized in the sponsor's table below.

Loading Dose (mg/kg/15 min)	Maintenance Dose (mg/kg/45 min)	Animal Number	SC-66110 Plasma Concentration (µg/mL)*	
0.4675	0.405	4 13 6	Mean (SD)	0.675 0.275
1.4025	1.22	7 8 9	Mean (SD)	1.68 0.56
4.675	4.05	10 11 12	Mean (SD)	5.32 1.09

* Means and standard deviations were calculated using unrounded individual animal values. Individual and mean values were then rounded to three significant digits. Standard deviations of the mean (SD) were rounded to the same decimal place as the mean.

The sponsor reports a projected therapeutic Cmax of 1.5 µg/ml in humans. Thus the reported concentrations in guinea pigs are approximately 0.6, 1.5 and 4.8X the human Cmax.

Rabbits

1. *PHARMACOKINETIC SUPPORT FOR AN EMBRYO-FETAL DEVELOPMENTAL TOXICITY STUDY OF SC-66110 IN RABBITS (SEGMENT II), SA4479* M3096255

In the above referenced reproductive toxicity study, NZW rabbits were given oral dosages of 0, 20, 100 and 300 mg/kg/day from GD7 to GD20. Blood samples were collected from the adults at specified intervals on GD7 and GD19. Pooled fetal blood samples were collected from each litter on GD20 approximately 2 hours after the final dose administration.

Results

Detectable plasma concentrations were reported for all doses, all time points on both days of determination in the adults.

Table 5. Mean (± SEM) Total SC-66110 AUC and AUC/Dose Values

			AUC (µg•hr/mL) 0.00 to 8.00 hr			AUC (µg•hr/mL) 0.00 to 24.00 hr			AUC/Dose 0.00 to 8.00 hr			AUC/Dose 0.00 to 24.00 hr		
Day	Dose (mg/kg)	Sex	Mean	SEM*	N	Mean	SEM*	N	Mean	SEM*	N	Mean	SEM*	N
7	0	F	0	(b)	2	0	(b)	2	(c)	(c)	0	(c)	(c)	0
	20.0	F	17.0	1.96	6	23.0	2.39	6	0.852	0.0989	6	1.16	0.119	6
	100	F	93.5	11.0	6	127	12.7	6	0.935	0.110	6	1.27	0.127	6
	300	F	270	16.4	6	457	56.6	6	0.900	0.0547	6	1.52	0.175	6
19	0	F	0	(b)	2	0	(b)	2	(c)	(c)	0	(c)	(c)	0
	20.0	F	14.3	1.55	5	17.6	2.28	5	0.714	0.0775	5	0.887	0.114	5
	100	F	98.0	10.3	5	119	14.4	5	0.980	0.103	5	1.19	0.144	5
	300	F	242	28.3	6	435	43.0	6	0.806	0.0944	6	1.45	0.144	6
20	0	F	(e)	(e)	0	(e)	(e)	0	(c)	(c)	0	(c)	(c)	0
	20.0	F	(e)	(e)	0	(e)	(e)	0	(e)	(e)	0	(e)	(e)	0
	100	F	(e)	(e)	0	(e)	(e)	0	(e)	(e)	0	(e)	(e)	0
	300	F	(e)	(e)	0	(e)	(e)	0	(e)	(e)	0	(e)	(e)	0

* Standard Error of the Mean

b SEM is not reported for N=2

c Could not be calculated for the 0 mpk dose group

e Not applicable, only fetal blood was taken on Day 20.

f Blood samples were collected at a single time point, therefore AUC could not be calculated

In adults, the increases in exposure with increasing dose were greater than proportional. The plasma levels as reported did not appear to change over the course of the study. In the fetuses, pooled samples showed detectable levels of drug at all dosages. The values were less than maternal values for concentration at 2 hours except at the lowest dose. At 20 mg/kg the fetal concentration exceeded maternal levels. The sponsor attributed this to one exceptionally high fetal value.

Table 3. Mean (± SEM) Plasma Concentrations of Total SC-66110 on Day 20 in Pooled Fetal Rabbits

Dose (mg/kg)	Time (hr)	Concentration (µg/mL)		
		Mean	SEM*	N
0	2.00	0	(b)	1
20.0	2.00	8.59	5.86	5
100	2.00	12.8	1.22	5
300	2.00	28.7	2.59	6

* Standard Error of the Mean

b SEM could not be calculated since N=1

2.*SEARLE MEMO REPORT: PHARMACOKINETICS OF SC-66110, SC-70303 FREE ACID AND TOTAL SC-66110 FOLLOWING A SINGLE ORAL ADMINISTRATION OF SC-66110 TO PREGNANT FEMALE RABBITS* M3000401

Female New Zealand White (NZW) rabbits were given single oral doses of 100 or 300 mg/kg on GD7. Blood samples were collected at 1,2,3,5,8 and 24 hours after dosing.

Results

Plasma concentrations of SC-66110 were greater than those of SC-70303. The increase in total SC-66110 with increased dose was approximately proportional.

Summary of AUC

Dose mg/kg	AUC μg.hr/ml		
	SC-66110	SC-70303	Total SC-66110
100	73.0±5.6	64.7±6.3	135±11
300	211±13	162±23	366±25

Values are mean±sem

3. *PHARMACOKINETICS,EXCRETION AND PLACENTAL TRANSFER OF [^{14}C]SC-66110 FOLLOWING ADMINISTRATION OF A SINGLE ORAL OR INTRAVENOUS DOSE TO PREGNANT RABBITS* M2099082

The PK, metabolism, excretion and placental transfer of radioactivity were examined following a single oral (15 mg/kg) or intravenous (15 mg/kg) dose of radiolabeled eplerenone given GD20. After oral dosing, blood was collected at 0.25, 0.5, 1,2,3,5,8,24, 48 and 72 hours. After intravenous dosing, blood was collected at 0.033, 0.083, 0.25, 0.5, 1,2,3,5,8,24,48 and 72 hours. For both routes of administration, urine and feces were collected at 24-hour intervals through 168 hours. From a separate group of orally dosed rabbits, tissues were collected at 1,8,24 and 72 hours. Included among tissues analyzed were amniotic fluid and fetal tissues such as brain, lungs, heart, liver and fetal kidneys.

Results

Absorption: Drug was rapidly absorbed with an oral bioavailability of ~73%.

Distribution: Highest concentrations of radioactivity in maternal tissues were found in kidneys (38.3 μg equivalents/g), liver (16.6), plasma (8.52), blood (6.10) and lungs (5.11). Lowest maternal tissue concentrations were in brain (0.781) and amniotic fluid (1.09).

Drug-derived radioactivity crossed the placenta and was found in the fetal blood and tissues. Fetal tissue levels were lower than maternal with two exceptions: fetal brain at all time points and fetal blood at 72 hours post-dose. Highest fetal concentrations were found in kidneys (2.82 μg equivalents/g), blood (2.47) and heart (2.11). Fetal brain had 1.68 μg equivalents/g.

Tissue:maternal plasma concentration ratios were greater than one for maternal liver and kidneys at all sampled time points, placenta at 24 hours post-dose and amniotic fluid at 72 hours post-dose. By 72 hours most of the radioactivity had cleared.

Metabolism
In all maternal matrices SC-66110 was found to be the main component followed by various metabolites. In the fetal plasma, parent drug plus six metabolites were reported with parent drug the predominant form. All except the RP1 metabolite corresponded to metabolites in the maternal plasma.

Excretion
Following oral dosing, the major route of elimination was by the urine. In the first 24 hours~51% was excreted in urine and ~11% in the feces. By 168 hours urine and feces accounted for ~69% and 30% respectively of the dosed radioactivity.
Urinary excretion was also the major form of elimination after intravenous dosing. By 24 hours ~61% of the radioactivity was found in the urine and ~13% in the feces. By 168 hours, urine and feces accounted for 74% and 25% respectively.

Summary
Radiolabeled SC-66110 was rapidly absorbed with good oral bioavailability. The radiolabel was widely distributed in tissues including maternal brain, amniotic fluid, and fetal tissues including the fetal brain.

4. *PHARMACOKINETICS, EXCRETION AND PLACENTAL TRANSFER OF [^{14}C]SC-66110 FOLLOWING ADMINISTRATION OF A SINGLE ORAL OR INTRAVENOUS DOSE TO PREGNANT RABBITS* M3000134

This is the in-house (sponsor's) summary of the preceding study. See original reviews of these two studies for comparisons.

Dogs

1. PRELIMINARY EVALUATION OF THE PHARMACOKINETIC PROPERTIES OF CGP30 083 IN THE CONSCIOUS DOG. BBBCVS 14/83. This very brief report informs us that plasma and urine were collected from dogs treated with 10 mg/kg po of the test article and processed on [redacted] Fractions thus isolated were further analyzed by [redacted]
Some fractions appear to have been monohydroxylated.

2. *TWO-WEEK ORAL TOLERABILITY STUDY OF CGP 30 083: PLASMA CONCENTRATIONS IN THE DOG; ESTRADIOL AND TESTOSTERONE LEVELS; TESTICULAR CYTOCHROME P-450 CONTENT AND 17α-HYDROXYLASE ACTIVITY.*
REP14M BBB CVS 2/85:TK This very brief, non-GLP report indicates that dogs received 100 mg/kg/po for 2 weeks. Blood samples were taken day 1 and day 12 at 1,2 and 3 hours after dosing. Note: the legend for the table where the study design was described said 3 hours for sampling, the table itself indicated 6 hours. A total of 3 dogs were used. Plasma levels were discernible at all sampling points. Estradiol and testosterone levels were measured and show some slight changes from day 1 to day 12. Given the lack of procedural detail, assay range data and a control group for comparison, little attention can be given to this study.

3. *SEARLE MEMO REPORT QF SC-66110 PLASMA CONCENTRATIONS IN THE DOG HEMODYNAMIC STUDY EX4465* M3096321

Dogs were first given SC-66110 as an intravenous infusion followed 48 hours later by a single oral (capsule) dose. Blood and plasma were collected at 2, 4 and 24 hours after the oral dose. Three different loading and maintenance infusion regimens were used.
Results: Plasma levels were discernible for each infusion regimen used and at almost each point of determination following oral dosing. The one exception was one animal with levels BLQ at 24 hours after dosing. The plasma level values were shown and discussed in the safety pharmacology section of this review.
Summary: The data supports plasma level exposure in the study.

4. *SYSTEMIC AVAILABILITIES OF SC-66110 AFTER ORAL ADMINISTRATION OF CONTROLLED AND IMMEDIATE RELEASE FORMULATIONS OF SC-66110 TO THE DOG (AN EXPLORATORY STUDY)* M3096292

Two groups of 4 female mongrels were given 15 different treatments of SC-66110 intra-gastrically. Animals were fasted overnight for each dose administration. A minimum washout period of 5 days between treatments was used. Blood samples were collected at specified intervals to 24 hours after dosing. Seven different formulations each of 200 mg were given to one group of dogs and 7 formulations of 100 mg were given to another group of dogs.
A food effect study was conducted with a separate group of animals receiving 100 mg IR tablets and was included in this report.

TABLES
Table 1. Dose, Lot Number and Descriptions of SC-66110 Formulations Administered to the Dog

Treatment*	Strength	Lot #	Formulation Description
1	200 mg	RCT10001	Immediate Release Bead Capsule
2	200 mg	GDS-3911-101B	Controlled Release Bead Capsule with a 10% Colonic Coating
3	200 mg	GDS-3911-104	Slow Release Coated Bead Capsule
4	200 mg	GDS-3911-106	Fast Release Coated Bead Capsule
5	200 mg	GDS-3911-108	Mixed Release Coated Bead Capsule (20% fast release and 80% colonic)
6	200 mg	GDS-3911-107	Colonic Coated Bead Capsule
7	200 mg	GDS-3911-109	Enteric Coated Bead Capsule
8	100 mg	GDS-7946-038D	6 hour Controlled Release Tablet
9	100 mg	GDS-7946-048	2 hour Controlled Release Tablet
10	100 mg	GDS-7946-060	4 hour Controlled Release Tablet
11	100 mg	RCT1000	Immediate Release Capsule
12	100 mg	GDS-7946-083A	Immediate Release Tablet
13	100 mg	GDS-7946-068B	Immediate Release Tablet containing Dicalcium Phosphate
14	100 mg	RCT10614	Immediate Release Tablet with Food
15	100 mg	RCT10614	Immediate Release Tablet without Food

*For treatments 1-13 one group of 4 dogs was used and for Treatments 14-15 a second group of 4 dogs was used.

Results
The highest AUC_{0-24} for SC-66110 and total SC-66110 was obtained from an immediate release formulation (36.8±2.2, 48.9±2.7 μg.hr/ml) and the lowest AUC_{0-24} with the controlled release with 10% colonic coating (6.70±0.48, 8.58±0.54 μg.hr/ml). Mean Cmax of SC-66110 was slightly greater under fed conditions versus fasted: 5.37 μg/ml and 4 μg/ml

Results
Systemic availability of SC-66110 was 90% after oral administration. The availability of SC-70303 was 17.5% after oral dosing.

The mean rate constant for the conversion of SC-70303 to SC-66110 was 4.09 hr^{-1}, approximately 6 times greater than the rate constant for the conversion of SC-66110 to SC-70303(0.646hr^{-1}). The mean percentage of dose converted to [^{13}CD3]SC-70303 acid after IV dosing was 55%. Mean percentage of dose converted to SC-66110 after IV dosing was 60%.

Both forms of SC-66110 were available after oral dosing, however, SC-70303 showed less bioavailability than SC-66110. There was rapid interconversion between the forms. SC-70303 was more rapidly converted to SC-66110 than vice versa.

7. *PHARMACOKINETICS AND EXCRETION OF [^{14}C]SC-66110 FOLLOWING SINGLE INTRAVENOUS OR ORAL ADMINISTRATION TO FAST AND SLOW METABOLIZER DOGS.* M2097117

Male and female dogs who had previously been phenotyped as "fast and slow metabolizers" without reference to what they are metabolizing were used. Phase I included a single IV dose of 15 mg/kg of [^{14}C]SC-66110. Phase II was a single oral dose of 15 mg/kg of [^{14}C]SC-66110. For both phases, blood was collected at specified times pre and post-dose. Urine and feces were collected at specified times to 168 hours post-dose.

Results

	Slow Metabolizer				Fast Metabolizer			
	Plasma		Red Blood Cells		Plasma		Red Blood Cells	
PK Parameter	IV	Oral	IV	Oral	IV	Oral	IV	Oral
[illegible]	4.98	4.98	4.21	5.40	5.06	5.17	4.22	4.29
[illegible]	0.033	1.25	0.033	2.00	0.033	1.38	0.033	1.25
[illegible]	36.4	13.7	14.9	9.75	33.2	12.6	13.9	9.06
[illegible]	140	119	85.6	76.8	130	116	79.3	78.3
[illegible]	140	119	85.7	79.0	130	117	79.4	78.3

equiv = equivalents
h = hours
Note: Red blood cell values are [illegible] equivalents

Urine and feces were the main routes of elimination for both groups of dogs. Pharmaco-kinetic parameters and elimination data are shown alongside.

	Slow Metabolizer		Fast Metabolizer	
	IV	Oral	IV	Oral
Urine	48.0%	35.8%	51.7%	32.1%
Feces	46.3%	55.1%	44.8%	59.7%
Cage Wash Wipes	2.00%	3.89%	3.36%	3.57%
Total Recovery	96.4%	94.8%	99.8%	95.4%

Summary
There are no apparent differences in PK parameters in the data as reported between the two metabolic profiles. The information as to what the dogs are metabolizing slowly or rapidly was not specified in the report.

8. *PHARMACOKINETICS OF SC-66110 IN SLOW AND FAST METABOLIZER DOGS AFTER IV AND ORAL ADMINISTRATION* M3098095

Dogs who had been previously phenotyped as slow or fast metabolizers using a dog CYP2D15 substrate, celecoxib, were given 15 mg/kg [^{14}C]SC-66110 IV and po in a cross-over design study. A washout period of two weeks was incorporated. This report is apparently the sponsor's version of the study described above in the report from the CRO.

The results presented by the sponsor are somewhat different from those presented above.

The interpretation that this particular enzyme contributes little to eplerenone metabolism in dogs does not change.

Table 7. Mean (± SEM) Pharmacokinetic Parameters of Total SC-66110 After IV Administration of SC-66110 to the Dog

Pharmacokinetic Parameter	Slow Metabolism Total SC-66110	Fast Metabolism Total SC-66110
A (µg/mL)	20.3 ± 2.2	17.2 ± 0.6
α (hr^{-1})	5.50 ± 0.57	3.44 ± 0.56
α t$_{1/2}$ (hr)	0.132 ± 0.034	0.172 ± 0.034
B (µg/mL)	15.0 ± 0.9	13.0 ± 0.6
β (hr^{-1})	0.227 ± 0.019	0.228 ± 0.021
β t$_{1/2}$ (hr)	2.51 ± 0.19	2.72 ± 0.50
V$_c$ (L/kg)	0.401 ± 0.041	0.467 ± 0.011
V$_{ss}$ (L/kg)	0.934 ± 0.085	1.01 ± 0.04
V$_{area}$ (L/kg)	0.817 ± 0.031	0.904 ± 0.044
Cl$_p$ (L/kg*hr)	0.238 ± 0.011	0.261 ± 0.026
AUC (µg*hr/mL)	74.8 ± 1.6	67.5 ± 8.1

Table 8. Mean (±SEM) Pharmacokinetic Parameters After Oral Administration of SC-66110 to Dogs

Pharmacokinetic Parameters	Slow Metabolism SC-66110	Slow Metabolism SC-70303	Slow Metabolism TOTAL SC-66110	Fast Metabolism SC-66110	Fast Metabolism SC-70303	Fast Metabolism TOTAL SC-66110
C_{max} (µg/mL)	8.50±0.12	2.39±0.78	10.6±0.6	7.82±0.44	1.71±0.20	9.42±0.66
T_{max} (hr)	1.13±0.24	1.13±0.13	1.00±0.20	1.25±0.14	1.13±0.13	1.25±0.14
AUC (µg*hr/mL)	51.9±1.6	11.3±1.4	62.7±2.7	50.1±9.2	9.97±1.29	58.7±10.4
Bioavailability (%)	84.8±4.7	80.5±5.1	84.0±4.6	87.2±7.4	89.4±5.5	87.5±7.0

9. *PHARMACOKINETICS AND METABOLISM OF [^{14}C]SC-66110 AFTER IV AND ORAL ADMINISTRATION TO DOGS (AN EXPLORATORY STUDY)* M3097041
Two male and 2 female dogs received 15 mg/kg of [^{14}C]SC-66110 IV and po in a cross-over manner with a 2-week washout period. Blood, urine and feces were collected at specified timepoints after dosing.

Results

Table 1. Pharmacokinetic Parameters of Total Radioactivity in Plasma

Dose Route	Pharmacokinetic Parameter	Mean	SEM
IV	β (hr^{-1})	0.173	0.005
	$T_{1/2}$ (hr)	4.0	N/A
	AUC (µg eq hr/mL)	130	8
Oral	C_{max} (µg eq/mL)	14.4	0.3
	T_{max} (hr)	1	0
	$T_{1/2}$ (hr)	4.0	N/A
	AUC (µg eq hr/mL)	123	11
	Bioavailability (%)	94.5	3.3
N/A = Not applicable			

Summary
Within the small sample size, the drug was rapidly and extensively absorbed after oral dosing. Plasma concentrations of SC-70303 were lower than SC-66110 regardless of route of administration. Urinary radioactivity associated with total SC-66110 was 36% for IV and 22% after oral dosing. Mean percentages of dose excreted as total radioactivity in urine and feces were ~54% and 41% respectively after IV dosing, and ~41% and 52% respectively after oral dosing. The differences in percent of dose excreted as total radioactivity and percent excreted as parent drug suggest extensive metabolism.

10. *REPORT AMENDMENT NO. 2: THE PHARMACOKINETICS AND EXCRETION OF RADIOACTIVITY FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION OF [^{14}C]SC-66110 TO MALE AND FEMALE DOGS* M2295241
This is the detailed protocol and amendments to the previous report.

11. *ANALYSIS OF PLASMA, RBCs, URINE AND FECAL SAMPLES FROM DOGS DOSED WITH SC-66110 DURING A 13-WEEK ORAL TOXICITY STUDY OF SC-66110 IN THE DOG, SA4451* M3095298
SC-66110 was given orally at dosages of 15, 100 and 300 mg/kg in gelatin capsules to male and female dogs for a 13-week toxicology study. Day 1, Day 38 and Day 85, [^{14}C] SC-66110 was given to dogs in satellite TK groups (15 and 100 mg/kg/day). Blood samples were collected at specified times. Urine and feces were also collected. Concentrations of total radioactivity in plasma, RBC, urine and feces were determined. Concentrations of SC-66110 and SC-70303 were determined after acidification of plasma samples. Urinary and fecal concentrations were determined without acidification. This report covers PK of total radioactivity and plasma concentrations of SC-66110 in the PK groups only. Plasma concentrations in the toxicology groups are reported separately.

Results

Plasma levels of drug were detectable at all doses. SC-66110 was the major component of plasma radioactivity without acidification. Percentages of parent drug increased with acidification. Drug-derived radioactivity was found associated with RBC also.

Urine
Without acidification less than 2% of the radioactivity was due to parent drug. The remainder was accounted for by metabolites. After acidification, the percentage of radioactivity associated with SC-66110 ranged from [redacted] regardless of day of sampling. Ten-13% of the total radioactivity of high dose was excreted in the urine compared to 25-29% of the low dose.

Feces
The main route of excretion appeared to be fecal. The majority of fecal radioactivity was associated with SC-66110 and did not change with sampling day. Sixty-one to 70% mean percentage of the low dose was excreted in the feces compared to 81-86% of the high dose.

Summary
There were no apparent sex-related differences in pharmacokinetics. Exposure increased with increasing dose but less than dose proportionally. Combined with the excretion data this suggests decreased or limited absorption at the higher dose. Induction of metabolism was not apparent in the data as presented in that plasma levels did not decrease with repeated dosing. Metabolism was more extensive at the lower dosage.

12. *SYSTEMIC AVAILABILITES FROM FORMULATIONS OF DIFFERENT RECRYSTALLIZATIONS AND PARTICLE SIZES OF SC-66110 AFTER ORAL ADMINISTRATION OF SC-66110 TO THE DOG (AN EXPLORATORY STUDY).* M3097151

Female dogs were given 200-mg dosages of one of 5 different treatments of SC-66110 to determine the systemic availabilities of different particle sizes and recrystallizations. Blood samples were collected at specified times out to 24 hours after dosing and concentrations of SC-66110 and SC-70303 were determined.

Summary of treatments

Treatment	Lot #	Formulation description
1	GDS-7945-125	Immediate release(IR) + 200 mg micronized recrystallized SC-66110
2	GDS-7945-129	IR capsules + 200 mg methanol recrystallized drug
3	GDS-7945-132	IR capsules with recrystallized drug
4	GDS-7945-133	IR capsules + methanol recrystallized drug
5	GDS-7243-069	IR capsules + recrystallized 20 mesh drug

Results
Treatments 1,2 and 3 produced comparable AUC values. Treatments 4 and 5 produced the lowest plasma exposures.

Table 7. Mean Pharmacokinetic Parameters of Total SC-66110 after Administration of SC-66110 to the Dog

Treatment*	Mean (±SEM) Pharmacokinetic Parameters		
	C_{max} (ug/mL)	T_{max} (hr)	AUC_{0-24hr} (ng•hr/mL)
1	7.90 ± 1.17	1.8 ± 0.3	56.4 ± 11.9
2	7.54 ± 2.35	1.8 ± 0.3	57.5 ± 15.6
3	8.76 ± 1.04	2.0 ± 0.4	62.3 ± 8.3
4	6.69 ± 1.44	1.8 ± 0.5	45.4 ± 10.7
5	4.54 ± 1.19	1.5 ± 0.3	30.9 ± 8.3

*For Treatment see Table 1.

13. *EVALUATION OF THE SYSTEMIC AVAILABILITY OF SC-66110 AFTER INTRAGASTRIC INTRADUODENUM, INTRAJEJUNUM AND INTRACOLON ADMINISTRATION TO THE CIAP DOG (AN EXPLORATORY STUDY)* M3097218

The report amendment at the beginning of this report refers to a study in humans and appears to be unconnected to this study.
Female dogs had chronic intestinal access ports implanted in the duodenum, jejunum and colon.

Table 4. Mean Pharmacokinetic Parameters of SC-66110 after IG, Duodenum, Jejunum and Colon Administration of SC-66110

Dose Route	Analyte	Mean (±SEM) Pharmacokinetic Parameters		
		C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
IG	SC-66110	4.91 ± 0.40	0.56 ± 0.16	13.6 ± 0.6
	SC-70303	0.491 ± 0.017	0.75 ± 0.14	1.67 ± 0.08
	Total SC-66110	5.31 ± 0.41	0.56 ± 0.16	15.2 ± 0.6
Duodenum	SC-66110	4.66 ± 0.39	0.63 ± 0.13	14.4 ± 0.9
	SC-70303	0.472 ± 0.040	0.75 ± 0.14	1.94 ± 0.18
	Total SC-66110	5.33 ± 0.41	0.63 ± 0.13	16.3 ± 1.0
Jejunum	SC-66110	6.46 ± 0.56	0.25 ± 0	15.4 ± 1.1
	SC-70303	0.811 ± 0.070	0.38 ± 0.07	2.15 ± 0.20
	Total SC-66110	7.22 ± 0.65	0.25 ± 0	17.4 ± 1.3
Colon	SC-66110	1.70 ± 0.12	1.8 ± 0.4	11.6 ± 2.2
	SC-70303	0.235 ± 0.017	1.8 ± 0.3	1.85 ± 0.36
	Total SC-66110	1.91 ± 0.13	1.5 ± 0.7	13.4 ± 2.5

Solutions of SC-66110 were given at 7.5 mg/kg IG, ID, IJ and IC. Plasma concentrations of SC-66110 and SC-70303 were then determined.

Results

Gastric and duodenal PK were comparable. Drug given intrajejunally had a shorter Tmax and reached a higher Cmax than when given via the other routes. This suggests greater absorption in the jejunum. Colonic administration produced the longest Tmax with lowest Cmax of the analyzed routes. Overall, AUC values were similar, regardless of site of absorption with the exception of the colon. AUC was slightly lower following colonic administration compared to the other routes of administration. The data indicates absorption throughout the GI tract.

14. *SITE ABSORPTION STUDIES OF SC-66110 IN BEAGLES USING A REMOTE CONTROLLED CAPSULE AND GAMMA SCINTIGRAPHY* M2096382

Four female beagles were used in a crossover design of 3 dosing periods. In each dosing period, 100 mg of SC-66110 was delivered to a section of the gastrointestinal tract using the [redacted] This is a large non-digestible capsule that will release its contents upon receiving the appropriate signal from an external transmitting device. Passage of the capsules was monitored by gamma scintigraphy and drug was released directly into stomach, early small bowel and colon. Serial blood samples were taken to characterize absorption from a given region.

Results

Efficiency and rate of release of the drug in any part of the GI tract appeared to depend upon water available and thus location in the GI tract. The release of drug was incomplete in the colon but apparently complete in the stomach and upper GI tract. The results presented do not raise new safety issues for the drug nor do they contribute new knowledge of the pharmacokinetics. The sponsor's reference to obtaining blood from the "sephanous [sic] vein of the front leg"(page 3) raises some question as to the reliability of the observations. The study authors concluded that a controlled release formulation was feasible considering that significant absorption in the colon was achieved even though no effort was made to improve drug release and permeability across the colon wall.

15. *THE COMPARATIVE BIOAVAILABILITY OF DIFFERENT DOSAGE FORMS OF SC-66110 IN THE DOG AFTER ORAL ADMINISTRATION (AN EXPLORATORY STUDY)* M3095276

SC-66110 was given to 4 female Beagles at a dose of 20 mg/kg in one of 3 dosage forms: micronized chemical, nonmicronized chemical and micronized chemical plus the excipient Avicel. Each dog received each dosage form with at least a one week washout period between dosings. Blood samples were collected to 24 hours after each dose administration.

Table 2. **Mean (± SEM) C_{max}, T_{max} and AUC Values for the Three Dosage Forms**

	Nonmicronized		Micronized		Micronized Plus Avicel	
	Mean	SEM	Mean	SEM	Mean	SEM
C_{max} µg/mL	5.16	0.53	14.8	2.4	16.3	2.2
T_{max} hours	1.0	0.1	1.9	0.1	1.5	0.2
AUC (µg•hr/mL)	47.9	7.5	109	18	122	20
AUC normalized to nonmicronized	1	0	2.30	0.19	2.60	0.34

Standard Error of the Mean

Results

The micronized and micronized plus excipient forms produced greater plasma levels than did the non-micronized form.

16. *PLASMA CONCENTRATIONS OF TOTAL SC-66110 DURING SINGLE ORAL DOSE OF SC-66110 IN THE DOG (AN EXPLORATORY STUDY)* M3095258

Two groups of 3 female dogs were given SC-66110 as neat chemical in a gelatin capsule at dosages of 100 and 500 mg/kg. Blood samples were collected at specified times to 48 hours after dosing.

Table 2. **Pharmacokinetic Parameters of Total SC-66110 (T_{max}, C_{max} and C_{max}/Dose) in the Female Dog**

Day	Dose (mg/kg)	Sex	N	T_{max} (hr)	C_{max} (µg/mL)	C_{max} SEM (µg/mL)	C_{max}/Dose
1	100	F	3	2.0	29.1	6.9	0.291
	500	F	3	10.0	64.6	7.0	0.129

Standard error of the mean of the concentrations at T_{max}

Results

Tmax at the LD was 2 hours and 10 hours at the HD. Cmax showed a less than proportional increase. AUC $_{0\text{-}24}$ increased with increasing dose but less than proportionally.

Table 3. **Pharmacokinetic Parameters (AUC and AUC/Dose) of Total SC-66110 in the Female Dog**

Day	Dose (mg/kg)	Sex	AUC (µg•hr/mL)	AUC/Dose
			0.00 to 24.00 hr	0.00 to 24.00 hr
1	100	F	397	3.97
	500	F	1227	2.45

17. *PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING A 13-WEEK STUDY OF THE EFFECTS OF SC-66110 ON REPRODUCTIVE FUNCTION AND ONSET OF PROSTATE SIZE CHANGE IN THE MALE BEAGLE DOG, EX4541* M3097389

Male dogs were given SC-66110 at 0, 5 and 25 mg/kg/day during a 13-week study to assess reproductive function and onset of prostate size change. Blood samples were collected from each dog on days 1, 3 and 85 at 1, 2 and 3 hours after dosing. Concentrations of SC-66110 and SC-70303 were determined.

Results
Limited samples were collected over a very brief period of time. Plasma levels were found at both doses, all time points. The mean concentrations increased with increasing dose but not proportionally.

18.*PLASMA CONCENTRATIONS OF SC-66110 DURING A 13-WEEK ORAL TOXICITY STUDY OF SC-66110 IN THE DOG, SA4451.* M3096238

In the course of a 13-week toxicology study, SC-66110 was orally dosed to male and female dogs at dosages of 15, 100 and 300 mg/kg as neat chemical in a gelatin capsule. On days 1,38 and 85 [^{14}C]SC-66110 was given to dogs in the satellite groups receiving 15 and 100 mg/kg/day. Blood, urine and feces were collected as previously described (see above). The concentrations of radioactivity in RBC, plasma, urine and feces were described elsewhere. The pharmacokinetic determinations were described in this report.

Table 6. AUC and AUC/Dose for SC-66110 in Male and Female Dogs (combined)

Day	Dose Group (mg/kg)	AUC (µg/mL) X hr (0 to 24 hr)	AUC/Dose (0 to 24 hr)
1	15	39.2 ± 4.68	2.61 ± 0.311
	100	200 ± 28.8	2.00 ± 0.288
	300	523 ± 30.7	1.74 ± 0.102
16	15	41.6 ± 2.26	2.78 ± 0.150
	100	165 ± 12.1	1.65 ± 0.121
	300	629 ± 151	2.10 ± 0.503
38	15	43.5 ± 2.71	2.90 ± 0.181
	100	117 ± 15.7	1.17 ± 0.157
	300	496 ± 173	1.66 ± 0.577
85	15	46.1 ± 2.26	3.08 ± 0.151
	100	152 ± 16.0	1.52 ± 0.160
	300	317 ± 54.9	1.06 ± 0.183

Results
Plasma levels were detectable at both dosages on all three sampling days. Exposure increased with increasing dose, less than proportionally from 15 to 100 mg/kg and approximately proportionally from 100 to 300 mg/kg. There were no consistent sex-related differences in PK. Exposure decreased with repeated dosing of the MD and HD.

19. *PLASMA CONCENTRATIONS OF SC-66110, SC-70303 AND TOTAL SC-66110 DURING A 13-WEEK ORAL TOXICITY STUDY OF SC-66110 IN THE MALE DOG, SA4512.* M3097030

Male dogs were given oral dosages of 0, 1.5, 5 and 25 mg/kg/day during a 13-week toxicity study. An additional group of dogs was given spironolactone at 5 mg/kg/day. Blood samples were collected at specified time intervals on days 1 and 85. Concentrations of SC-66110 and SC-70303 were determined. The major active metabolites of spironolactone, 7α-thiomethyl spironolactone, 6β-OH-7αthiomethyl spironolactone and canrenone were also determined.

Table 9. Pharmacokinetic Parameters of Total SC-66110 (T_{max}, C_{max}, and C_{max}/Dose)

Day	Dose (mg/kg)	Sex	N	T_{max} (hr)		C_{max} (µg/mL)		C_{max}/Dose	
				Mean	SEM*	Mean	SEM*	Mean	SEM*
1	0	M	12	0	0	0	0	0	0
	1.5	M	6	1.83	0.307	0.855	0.0777	0.592	0.0516
	5.0	M	6	1.50	0.224	2.75	0.167	0.714	0.0334
	25.0	M	12	1.92	0.236	6.27	0.745	0.321	0.0274
85	0	M	12	2.00	0	0	0	0	0
	1.5	M	6	1.17	0.167	0.940	0.0917	0.629	0.0346
	5.0	M	6	1.33	0.211	2.71	0.276	0.551	0.0459
	25.0	M	12	2.00	0.236	7.36	0.876	0.298	0.0342

* Standard Error of the Mean

exposure was apparent.

Results

Measurable plasma concentrations were found at all dosages at almost all timepoints. From Day 1 to Day 83 there was a slight decrease in both Cmax and AUC of SC-66110 and total SC-66110 at the high dose.

The decrease in exposure at the highest dose may have been due to decreased absorption or induction of metabolism. Non-linear

Table 12. Pharmacokinetic Parameters of Total SC-66110 (AUC and AUC (Time))

Day	Dose (mg/kg)	AUC (µg·mL⁻¹·hr) 0 to 24 (0) hr			AUC/Dose 0 to 24 (0) hr		
		Mean	SEM*	N	Mean	SEM*	N
1	0	0	0	0	0	0	0
	1.50	4.21	0.445	6	3.14	0.290	6
	5.00	20.5	1.15	6	4.74	0.239	6
	25.0	70.1	5.17	12	2.80	0.207	12
85	0	0	0	0	0	0	0
	1.50	5.48	0.455	6	3.66	0.303	6
	5.00	19.6	1.78	6	3.90	0.355	6
	25.0	57.1	3.92	12	2.29	0.156	12

* Standard Error of the Mean

AUC and SEM are calculated by Sampling time group

20. *PLASMA CONCENTRATIONS OF SC-66110 AND SC-70303 DURING A 52-WEEK ORAL TOXICITY STUDY OF SC-66110 IN THE DOG, SA4507: INTERIM REPORT* M3097188

Male dogs were given SC-66110 at dosages of 1.5, 5 and 100 mg/kg/day during the 52 week oral toxicity study. The present report is an interim report based on the results of the blood samples collected days 1,91 and 180. Concentrations of SC-66110 and SC-70303 were determined and PK parameters calculated.

Results

Exposure increased with increasing dose although the changes were non-proportional. Concentrations of SC-66110 exceeded SC-70303 at all dosages and on all sampling days. There was a tendency for the female dogs to have lower plasma exposure than the male dogs as determined by $AUC_{0\text{-}24}$. At 100 mg/kg/day, there was a decrease in exposure from day 1 to day 91. Induction of metabolism was thus apparent to a limited extent.

Table 12. Pharmacokinetic Parameters of Total SC-66110 (AUC and AUC/Dose)

			AUC (mcg*hr/mL) after 0.00 to 24.00 hr			AUC/Dose (mcg*hr/mL/mg/kg) after 0.00 to 24.00 hr		
Day	Dose (mg/kg)	Sex	Mean	SD	N	Mean	SD	N
1	1.50	F	3.75	0.51	8	3.50	0.34	8
		M	4.88	0.55	8	3.25	0.36	8
		F&M	4.31	0.39	16	3.88	0.26	16
	5.00	F	11.4	0.7	12	2.28	0.13	12
		M	16.5	1.1	12	3.31	0.22	12
		F&M	14.0	0.8	24	2.79	0.16	24
	100	F	224	35	12	2.26	0.35	12
		M	320	42	12	3.20	0.42	12
		F&M	274	28	24	2.74	0.28	24
91	1.50	F	4.86	0.91	8	3.24	0.61	8
		M	4.85	0.91	8	3.23	0.61	8
		F&M	4.85	0.50	16	3.24	0.34	16
	5.00	F	14.2	1.0	12	2.83	0.20	12
		M	17.6	0.9	12	3.53	0.17	12
		F&M	15.9	0.7	24	3.18	0.15	24
	100	F	163	14	12	1.63	0.14	12
		M	102	12	12	1.02	0.12	12
		F&M	133	11	24	1.33	0.11	24
180	1.50	F	3.92	0.35	8	2.61	0.23	8
		M	5.25	0.50	8	3.50	0.33	8
		F&M	4.59	0.54	16	3.06	0.23	16
	5.00	F	13.7	0.7	12	2.74	0.14	12
		M	18.2	1.0	12	3.65	0.21	12
		F&M	16.0	0.8	24	3.19	0.15	24
	100	F	138	16	12	1.38	0.16	12

21. *PLASMA CONCENTRATIONS OF SC-66110 AND SC-70303 DURING A 52-WEEK ORAL TOXICITY STUDY OF SC-66110 IN THE DOG, SA4507: FINAL REPORT.* M3098072

Male and female dogs were given oral dosages of 1.5, 5 and 100 mg/kg of SC-66110 during a 52-week oral toxicity study. Blood samples were collected at specified intervals out to 24 hours after dosing on days 1,91, 180 and 363. Concentrations of SC-66110 and SC-70303 were determined.

Results

Drug was detectable at all dosages. SC-66110 exceeded SC-70303 at all determinations. There were no consistent sex-related differences but there was a tendency to decreased exposure (AUC_{0-24}) in the females. There was a decrease in AUC from Day 1 to Day 91 in the 100 mg/kg/day group.

Table X **Pharmacokinetic Parameters of SC-66110 (AUC and AUC/Dose)**

Day	Dose mg/kg	Sex	AUC (µg/mL x hr)			AUC/Dose		
			Mean	SEM	N	Mean	SEM	N
1	0	F	0	0	0	0	0	0
		M	0	0	0	0	0	0
		F&M	0	0	0	0	0	0
	1.5	F	3.03	0.435	8	2.02	0.290	8
		M	3.49	0.416	8	2.33	0.278	8
		F&M	3.26	0.301	16	2.17	0.200	16
	5	F	11.9	0.917	12	2.38	0.183	12
		M	12.0	0.636	12	2.39	0.127	12
		F&M	12.0	0.622	24	2.38	0.125	24
	100	F	232	20.5	12	2.32	0.205	12
		M	264	30.4	12	2.64	0.304	12
		F&M	248	24.1	24	2.48	0.241	24
91	0	F	0	0	0	0	0	0
		M	0.0435	0.0435	12	0	0	0
		F&M	0.0217	0.0217	24	0	0	0
	1.5	F	3.66	0.435	8	2.44	0.290	8
		M	4.12	0.729	8	2.72	0.532	8
		F&M	4.07	0.431	16	2.71	0.299	16
	5	F	11.2	0.812	12	2.24	0.174	12
		M	14.5	1.21	12	2.91	0.245	12
		F&M	12.8	0.875	24	2.57	0.181	24
	100	F	235	11.9	12	2.35	0.119	12
		M	304	10.4	12	3.04	0.104	12
		F&M	270	9.33	24	2.70	0.0933	24
180	0	F	0	0	0	0	0	0
		M	0	0	0	0	0	0
		F&M	0	0	0	0	0	0
	1.5	F	3.35	0.297	8	2.24	0.198	8
		M	4.62	0.417	8	3.07	0.278	8
		F&M	3.99	0.297	16	2.65	0.198	16
	5	F	11.9	0.979	12	2.37	0.196	12
		M	15.2	0.904	12	3.07	0.181	12
		F&M	13.5	0.916	24	2.70	0.183	24
	100	F	219	13.7	12	2.19	0.137	12
		M	278	8.70	12	2.78	0.870	12
		F&M	248	30.7	24	2.48	0.307	24
363	0	F	0	0	0	0	0	0
		M	0	0	0	0	0	0
		F&M	0	0	0	0	0	0
	1.5	F	5.45	1.67	4	3.63	1.11	4
		M	4.84	0.778	4	3.23	0.519	4

Day	Dose mg/kg	Sex	AUC...a (µg/ml) x hr)			AUC.../Dose		
			Mean	SE Mean	N	Mean	SE Mean	N
		F&M	5.12	0.760	8	3.41	0.507	8
	5	F	12.8	0.960	8	2.56	0.192	8
		M	16.7	1.86	8	3.33	0.372	8
		F&M	14.7	1.10	16	2.94	0.220	16
	100	F	189	22.1	8	1.89	0.221	8
		M	156	31.4	8	1.56	0.314	8
		F&M	172	19.6	16	1.72	0.196	16

Standard error of the mean
Not calculated for the 0 mg/ml dose group

22. *TWO-WAY CROSSOVER STUDY WITH [^{14}C]-SC-66110 FOLLOWING A SINGLE ORAL OR RECTAL ADMINISTRATION TO MALE DOGS (M2097263)* M2097263

Four male dogs were dosed in a cross-over design study. In phase I each animal received a single oral dose and in phase II the same four dogs received a single rectal dose of aqueous solutions of [^{14}C]SC-66110 at 15 mg/kg with a target radioactive dose of 10µCi/kg. Blood samples were collected to 24 hours after dosing. Plasma samples were analyzed for total radioactivity, SC-66110 and SC-70303. Urine and fecal samples were collected at 24 hour intervals through 168 hours after dosing and analyzed for total radioactivity.

Results

Tmax was achieved at 0.5- 2 hours after oral dosing and at 4 hours following rectal administration. Mean Cmax after oral dosing was 12.8 µg equiv/ml vs 7.32 µg equiv/ml after rectal dosing. Mean AUC was likewise slightly greater with oral dosing but total systemic availability after oral and rectal administration were equivalent. Plasma concentrations of

Table 9. Pharmacokinetic Parameters in Plasma Following a Single Oral (Phase 1) or Rectal (Phase 2) Dose of [^{14}C]SC-66110 (15 mg/kg)

Phase	Animal Number	T... (hours)	C... (µg equiv/ml)	t... (hours)	AUC... µg equiv•hour/ml	AUC... µg equiv•hour/ml
1						
1						
1						
1						
Mean		1.25	12.8	4.22	102.5	106.2
SEM		0.323	0.626	0.382	6.30	6.92
2						
2						
2						
2						
Mean		4	7.32	6.12	97.8	99.4
SEM		0.00	0.347	1.13	6.91	7.50

equiv Equivalents.
SEM Standard error of the mean

SC-66110, SC-70303 and total SC-66110 showed that absorption was slower after rectal administration compared to oral administration. Plasma drug concentrations accounted for 63% of the dose administered regardless of the route.

23. *PHARMACOKINETICS OF SC-66110 ATFER ORAL AND RECTAL ADMINISTRATION TO THE DOG.* M3098277
This is the Pharmacia report for the study described above.

The only substantive additional information from this report is that the mean percentages of dose excreted as total radioactivity in urine were 33.5% and 32.3% following oral and rectal administration of [^{14}C]SC-66110 respectively. This further indicates that extent of absorption of SC-66110 was approximately the same after oral and rectal administration although the rate was slower following the latter.

24. *EVALUATION OF THE EFFECT OF SC-66110 FORM I CONTENT ON THE SYSTEMIC EXPOSURE TO SC-66110 IN THE DOG* M3001109

Four female dogs were given a single dose of SC-66110 (100 mg) as one of three different tablet formulations in a crossover design study. The purpose was to evaluate the effect of the content of Form I polymorph on systemic exposure following oral administration. Two polymorphic forms (I and II) of SC-66110 have been detected in GMP batches of drug substance. Form II is the principle polymorph and is the thermodynamically more stable form at ambient temperature. The three tablet lots were identical in composition and contained the following SC-66110 chemical: a) micronized (polymorph II), b) low polymorph I (~4.5%) or c) containing high polymorph I (~9%). After dosing, blood samples were collected to 24 hours.

Table 6. Mean Pharmacokinetic Parameters of Total SC-66110

Parameter	Mean	S.D.	SEM	N
Formulation A - SC-66110 Micronized (Polymorph II)				
T_{max} (h)	1.75	0.50	0.25	4
C_{max} (µg/mL)	5.99	1.36	0.68	4
AUC (µg•h/mL)	43.6	10.6	5.3	4
Formulation B - SC-66110 (Low Polymorph I)				
T_{max} (h)	1.75	0.50	0.25	4
C_{max} (µg/mL)	6.04	0.92	0.46	4
AUC (µg•h/mL)	39.9	12.3	6.2	4
Formulation C - SC-66110 (High Polymorph I)				
T_{max} (h)	1.25	0.50	0.25	4
C_{max} (µg/mL)	4.93	1.63	0.82	4
AUC (µg•h/mL)	36.4	12.7	6.3	4
S.D = Standard Deviation				
SEM = Standard Error of the Mean				
NA = Not Applicable				

Results
In all three formulations, plasma SC-66110 exceeded SC-70303. As summarized in the sponsor's table, Tmax was slightly shorter, Cmax slightly lower and AUC slightly less with the high polymorph I formulation.

25. *CALCULATION OF EXPOSURE MARGINS TO UNBOUND EPLERENONE IN NONCLINICAL TOXICOLOGY STUDIES* M3001079

This report is actually a compilation across species and studies in which the "exposure to eplerenone" was calculated. At the beginning of the developmental process bioanalytical methods were developed to quantitate eplerenone + SC-70303 free acid. As SC-70303 is relatively inactive, the sponsor felt that it would be more appropriate to express safety margins in non-clinical species in terms of eplerenone alone. The exposure margins are thus expressed in terms of unbound or free eplerenone, the pharmacologically active molecule. The calculations were expressed thusly:

Exposure to eplerenone = fractional contribution of eplerenone to the total eplerenone x AUC of total eplerenone

Fractional contributions of eplerenone to the total eplerenone were estimated from subsequent studies, in which both eplerenone and SC-70303 free acid (a lactone ring opened eplerenone derivative) were measured

Exposure to unbound eplerenone was also calculated using the percent binding to the plasma protein of a given species. For mouse, rat, rabbit and dog, binding of eplerenone was reported as concentration-independent and calculated as follows

[C_{max} (or AUC) of eplerenone/C_{max} (AUC) of total eplerenone] x 100

AUC of eplerenone x (100 - mean plasma protein binding)/100

Results

The report provides a summary of the pharmacokinetics for mice, rabbits, rats and dogs.

Table 1: Plasma Protein binding of eplerenone in animals

	P53 KO mouse	P53 WT Mouse	CD-1 Mouse	Rabbit	Dog	Rat
Mean	22.9	25.6	16.4	19.1	15.7	24.3
SEM	1.5	2.4	1.3	0.4	1.5	1.75

From the sponsor's summary tables there appears to be little variation in eplerenone as a percent of total from dose to dose within any given pre-clinical species. Within a species, sex also had little effect upon the ratio of eplerenone: total eplerenone. Duration of dosing also did not seem to effect the ratio.

Table 3. Contribution of fractional eplerenone to total eplerenone (C_{max}) by gender, species and study.

	Contribution (%) of Eplerenone to Total Eplerenone (C_{max})						
Species	Male		Female		M & F		Study Number
	Mean	SEM	Mean	SEM	Mean	SEM	
Mouse	65.0	1.6	64.6	1.5	64.8	1.2	EX4647
Mouse	61.0	2.3	64.4	1.5	62.7	1.4	EX4751
Mouse	57.0	1.7	56.4	1.9	56.7	1.3	SA4513
Mouse	61.0	2.8	65.2	2.4	63.1	1.9	SA4648
Rat	72.5	0.9	70.7	1.1	71.4	0.8	M3000401
Rat	72.7	0.9	A	A	B	B	M3000147
Rat	58.5	0.7	50.8	3.1	54.6	2.2	SA5143
Rat	75.3	1.8	A	A	B	B	EX4897
Rat	C	C	C	C	C	C	SA4955
Rat	68.0	1.8	72.2	1.9	70.1	1.4	SA4963
Rat	73.4	D	72.1	D	73.0	3.3	SA4964
Rat	67.9	0.7	A	A	B	B	SA4997
Rat	A	A	69.3	1.3	B	B	SA4990
Rabbit	A	A	57.6	D	B	B	M3004811
Dog	65.6	2.8	84.5	0.7	75.2	0.6	SA4957
Human	58.1	1.1	A	A	B	B	EE3-58-06-004

A = Not Applicable - Gender not dosed
B = Not Applicable, only one gender dosed
C = Not Applicable C_{max} of total eplerenone not calculated for this study
D = Not Applicable N=2
SEM = standard error of the Mean

Ratios also appeared unaffected by pregnancy (rats), administration by gavage or dietary admix (mice), strain of mouse or dietary restriction (rat). There was a difference across species of the fractional contribution of eplerenone to total eplerenone. From highest to lowest free: total eplerenone was human>dog>rat>mouse>rabbit.

Protein Binding

1. *PLASMA PROTEIN BINDING OF [^{14}C]SC-66110 AND [^{14}C]SC-70303 IN CD1 MICE, P53 KNOCKOUT MICE, P53 WILDTYPE MICE, RATS, RABBITS AND HUMANS* M3098056

Plasma protein binding of [^{14}C] SC-66110 and [^{14}C]SC-70303 was determined in samples from CD-1 mice, P53 knockout mice, P53 wildtype mice, rats, rabbits and humans. Plasma protein binding is summarized in the reviewer's table below.

Summary of Plasma Protein Binding

Concentration (µg/ml)	P53 ko mouse	P53 WT mouse	CD-1 mouse	rabbit	rat	human
			% bound			
0.02-60.0	28 - 16	35-19	19-14	20-18	31-21	59-34

The greatest degree of protein binding appears to occur in human plasma.

2. *PLASMA PROTEIN BINDING OF EPLERENONE (SC-66110) IN THE RAT, DOG AND MAN (AN EXPLORATORY STUDY)* M3096395

The plasma protein binding of [^{14} C]eplerenone was studied in the rat, dog and man at specified concentrations using a filtration method. The results obtained for rats and humans were similar to the previous study. Plasma protein binding was concentration dependent. Plasma protein binding in the dog and rat was lower than that in humans in the drug concentration range of 0.02-5.0 µg/ml.

Table 1. The Mean Percentages of Plasma Protein Bound Eplerenone in Rat, Dog and Human

Concentration (µg/mL)	% Bound		
	Rat	Dog	Human
0.02	25.1 ± 0.7	21.6 ± 0.7	60.6 ± 2.6
0.2	25.2 ± 0.4	15.8 ± 0.4	59.0 ± 3.1
1	18.9 ± 0.1	13.3 ± 0.7	36.2 ± 2.4
5	16.7 ± 0.3	13.4 ± 0.4	33.3 ± 2.1
6.0	13.1 ± 0.7	14.2 ± 0.9	16.8 ± 1.2

3. *BINDING OF [^{14}C]SC-66110 AND [^{14}C]SC-70303 TO HUMAN SERUM ALBUMIN AND ALPHA-1 ACID GLYCOPROTEIN* M3098041

The binding of [^{14}C]SC-66110 and [^{14}C]SC-70303 was determined in human serum albumin and alpha1-acid glycoprotein at specified concentrations using an ultrafiltration method. Binding of

Table 4. Contribution of eplerenone to total eplerenone (AUC) by gender, species and study.

	Contribution (%) of Eplerenone to Total Eplerenone (AUC)						
Species	Male		Female		M & F		Study Number
	Mean	SEM	Mean	SEM	Mean	SEM	
Mouse	69.2	1.3	63.3	1.4	69.2	0.9	EX4647
Mouse	62.6	1.7	64.8	1.2	63.7	1.0	EX4751
Mouse	57.7	1.2	57.0	1.2	57.3	0.8	SA4513
Mouse	66.5	3.8	69.9	3.0	68.1	2.4	SA4846
Rat	72.0	0.5	70.2	1.2	71.1	0.7	M3000400
Rat	71.9	0.6	A	A	B	B	M3000147
Rat	44.0	4.5	43.0	1.2	43.5	2.1	SA5143
Rat	A	A	76.3	1.6	B	B	EX4655
Rat	69.3	0.7	68.2	0.9	69.3	0.6	SA4566
Rat	67.4	2.1	68.1	2.4	67.8	1.5	SA4663
Rat	72.2	C	73.1	C	72.7	3.6	SA4664
Rat	65.5	1.0	A	A	B	B	SA4987
Rat	A	A	68.5	1.2	B	B	SA4980
Rabbit	A	A	55.9	C	B	B	M3000401
Dog	83.7	0.8	83.2	0.8	83.5	0.6	SA4527
Human	92.9	1.0	A	A	B	B	EE3-98-06-004

A = Not Applicable – Gender not dosed
B = Not Applicable only one gender dosed
C = Not Applicable N=2
SEM = standard error of the Mean

[^{14}C]SC-66110 to α 1-acid glycoprotein was about 50% and concentration independent in the

range of 0.02-1.00µg/ml range and appeared to saturate in the 5.00-6.00 µg/ml range. Binding to HSA was ~10% and concentration independent. Binding of [^{14}C]SC-70303 to α1-acid glycoprotein was ~2% and concentration independent from 0.02-600 µg/ml. The binding to HSA was ~30% and concentration independent.

Distribution/Accumulation

1.*INTERACTION OF SC-66110 TRANSPORT THROUGH CACO-2 CELL MONOLAYER WITH P-GLYCOPROTEIN SUBSTRATES AND ANTIBODY AND AMENDMENT*M3099193
To determine whether eplerenone is a substrate for p-glycoprotein transport, [^{14}C]SC-66110 was evaluated in a CACO-2 monolayer system and compared to [^{3}H] vinblastine, a known p-gly substrate. [^{3}H]mannitol was used as a negative control. The reported net secretion values of approximately 1.0 do not support eplerenone as a p-glycoprotein substrate.

2. *INHIBITION OF P-GLYCOPROTEIN BY EPLERENONE IN CaCo-2 CELLS AND CaCo-2 CELLS TRANSFECTED WITH PGY-1 cDNA.*
3 known substrates (digoxin, vinblastine and doxorubicin) of P-gly were measured in the presence of 2 concentrations of eplerenone or ketaconazole or verapamil. Permeabilities were also measured ±P-gly antibodies.

The transfected cells did not seem to produce results any different from the non-transfected cells. Eplerenone at 200 µM in this test system seemed to have a detectable inhibitory effect upon P-gp for digoxin and vinblastine. The purity of 95% seemed somewhat low, although this was not a GLP study. It is not clear how these study results will translate in vivo in the clinic.

3. *IN VITRO BINDING OF SC-66110 TO CHARCOAL* M3000396
Binding of [^{14}C]SC-66110 to powdered or granular activated charcoal was conducted over a range of pHs (1.5, 4.5, 7.5 and 9.0) and two ratios of test compound to adsorbent.

Binding of total radioactivity to powdered activated charcoal was >98% and concentration (100-500µg/ml) and pH independent. Binding of total radioactivity to granular activated charcoal was >81% and independent of pH and concentration.

4. *MILK SECRETION OF [^{14}C]SC-66110 FOLLOWING A SINGLE ORAL DOSE TO LACTATING RATS* M2098074

[^{14}C]SC-66110 was given as a single oral dose of 2 mg/kg to lactating rats, concentration of radioactivity and metabolite profiles in plasma and milk were determined at 0.5, 1, 2, 3, 5, 8, 24 and 48 hours.
PK Summary

	Cmax (µg equiv/ml)	Tmax (hours)	AUC_{0-t} (µg equiv.hr/ml)	$AUC_{0-\infty}$ (µg equiv.hr/ml)	$T_{1/2}$ (hrs)
Plasma	0.675	0.5	2.28	2.28	5.03
Milk	0.650	1.0	1.94	1.94	4.78

In both milk and plasma, the bulk of radioactivity was associated with SC-66110.