5. *THE TISSUE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [^{14}C]SC-66110 TO MALE PIGMENTED RATS* CHW6127-261

Tissue distribution and excretion of radioactivity were determined following single oral doses [^{14}C]SC-66110 to male Long-Evans rats at 20 mg/kg. Three animals per time point were euthanized to 504 hours (21 days) post-dose. The tissues with the highest mean Cmax values were liver, pancreas and kidneys. Tissues with lowest Cmax were eye (without lens), brain and spinal cord. By 96 hours post-dose, the radioactivity was below the limits of detection in all tissues except kidneys, liver and GI tract where the values were —— µg equivalents/g. Half-life in pigmented skin was 16.1 hours versus 1.21 hours for non-pigmented skin, suggesting binding to melanin.

Induction/Inhibition

1. *THE EFFECT OF SC-66110 ON LIVER MICROSOMAL CYTOCHROME P450 ACTIVITIES IN RATS FOLLOWING DAILY ORAL DOSING FOR 13-WEEKS* M2097313

Performed at —— Liver samples were received from a study in which the rats had received daily oral dosages from 20 to 500 mg/kg/day for a 13-week toxicity study. Postmitochondrial supernatant and microsomal fractions were prepared by differential centrifugation. Postmitochondrial supernatants were analyzed for total protein content. Microsomal fractions were analyzed for total protein content, total cytochrome P450 content, ethoxyresorufin O-deethylase activity, pentoxyresorufin O-dealkylase activity, testosterone 6β- and 16β-hydroxylase activities, erythromycin N-demethylase activity, p-nitrophenol hydroxylase activity, and lauric acid 11- and 12-hydroxylase activities.

Results

Dose dependent induction of CYP3A was seen in both male and female rats. CYP1A1 activity increased 2X in male rats. One marker for CYP2E activity was increased in both sexes while a second marker for this isozyme was not. Overall, while there were slight effects on CYP2B, CYP1A1 and CYP2E1, the effects were not consistent between the sexes nor were they consistent between the different test substrates for the same enzyme.

2. *THE EFFECT OF SC-66110 ON LIVER MICROSOMAL CYTOCHROME P450 ACTIVITIES IN RATS FOLLOWING DAILY ORAL DOSING FOR 36 DAYS.* M2096277

Sprague-Dawley rats received 0, 20, 100 or 500 mg/kg/day of SC-66110 or 100 mg/kg/day of spironolactone. Days 7, 24 and 36, 5 rats/sex/group were euthanized and the liver collected for preparation of post-mitochondrial supernatant and microsomes and enzyme analysis (CYP1A1, CYP2B, CYP3A, CYP2E1, CYP4A) and total CYP450 content.

Increases in absolute and normalized liver weight were apparent at 7 days in both sexes receiving 500 mg/kg/day eplerenone and spironolactone. Both SC-66110 and spironolactone possessed similar activity profiles for increasing liver weights and inducing CYP3A levels in rats, including slightly greater induction of CYP3A in males compared to females. Spironolactone possessed greater activity on a mg/kg basis than eplerenone. Since prostate development is dependent on the presence of testosterone, the results from this study of increased testosterone metabolism through induction of CYP3A (measured by increases in 6β-hydroxytestosterone levels) may be related to the decreased prostate weights observed in several rat toxicology studies.

3.

The effect of [redacted] on rat testicular cytochrome P-450, testicular 17 α-hydroxylase activity and liver cytochrome P-450

This 3-page report did not provide enough information to allow an independent interpretation of the data.

4. *THE EFFECT OF SC-66110 ON LIVER MICROSOMAL CYTOCHROME P450 ACTIVITIES IN DOGS FOLLOWING DAILY ORAL DOSING FOR 13 WEEKS*
M2098050
Dogs were treated with daily oral dosages of SC-66110 at 1.5, 5 and 25 mg/kg/day for 13 weeks (SA4512, males only) and 15, 100 and 300 mg/kg/day (SA4451, males and females). Spironolactone was used for comparison at a dose of 5 mg/kg/day in SA4512. After euthanasia, microsomal fractions were prepared and analyzed for total protein content, CYP450 content and selected activities. There were very modest effects upon enzymatic activity seen only at dosages of 100 and 300 mg/kg/day that may have been within the realm of normal variability. The data do not support SC-66110 as an inducer of hepatic CYP450 in the dog.

5.*DOG INTERACTION WITH CYP3A INHIBITORS.* M3098398
Female mongrel dogs were implanted with a chronic portal vein cannula and given an oral dose of 15 mg/kg/day SC-66110 for 7 days ±CYP3A inhibitors ketaconazole, bergmottin diol (1 mg/kg), bergmottin diol (5 mg/kg), fluconazole and grapefruit juice. Blood samples were collected at specified timepoints days 1 and 7 after dosing.

Mean plasma concentrations of both SC-66110 and SC-70303 were somewhat higher in the portal versus jugular blood samples, particularly at the early sampling points.
Day 1 and day 7 the AUC for SC-66110 was increased over control values with ketaconazole, bergamottin (5 mg/kg only day 1), fluconazole and grapefruit juice. The greatest increase in AUC for SC-66110 was in conjunction with ketaconazole administration, resulting in ~2.5X increase over control both day 1 and day 7. A similar increase was also reported for day 7 with portal sampling. The results are summarized in the sponsor's table below. A small n increased the variability.

Table 23. Mean Pharmacokinetic Parameters of Total SC-66110 from Jugular Plasma after Oral Administration of SC-66110

			PK Parameters		
Treatments	Dose (mg/kg)	Day	C_{max} (µg/mL)	T_{max} (h)	AUC (h•µg/mL)
Control	0	1	13.5 ± 1.5	1.25 ± 0.60	70.9 ± 10.8
Ketoconazole	10	1	15.5 ± 0.8	1.75 ± 0.45	192 ± 7
Fluconazole	5	1	12.9 ± 1.0	1.50 ± 0.76	103 ± 15
Bergamottin diol	1	1	11.4 ± 0.9	1.56 ± 0.60	74.5 ± 13.1
	5	1	10.3 ± 4.6	1.67 ± 0.33	101 ± 49
Grapefruit juice	20[a]	1	11.9 ± 0.6	2.25 ± 0.48	92.9 ± 15.0
Control	0	7	16.7 ± 1.0	0.75 ± 0.14	98.6 ± 11.4
Ketoconazole	10	7	21.0 ± 0.9	1.00 ± 0	248 ± 19
Fluconazole	5	7	13.1 ± 0.4	1.33 ± 0.33	105 ± 16
Bergamottin diol	5	7	18.1 ± 0.9	1.67 ± 0.33	203 ± 33
Grapefruit juice	20[a]	7	10.6 ± 0.6	2.00 ± 0.41	95.0 ± 10.6

a. Units are in mL/kg

Metabolism

1. *ISOLATION AND IDENTIFICATION OF [^{14}C]SC-66110 METABOLITES IN DOG AND RAT URINE* M3097045

After the 13-week oral toxicity study (SA4451), 0-48 hour urine samples from 1 male and 1 female dog in the 15 mg/kg group from Day 1 were analyzed for metabolites. Zero-48 hour urine samples from 3 female rats given 20 mg/kg on Day 1 of a 13-week oral toxicity study were combined and analyzed for major metabolites.

The major metabolites identified in the dog urine were C-6 β hydroxy, C-16 α hydroxy, C-21 hydroxy and C-6βC-21 hydroxy metabolites and the glucuronide of C-6βhydroxy metabolite.

The major metabolites identified in the rat urine were C-6βhydroxy and C-6β,C-3α hydroxy metabolites.

2. *ISOLATION AND IDENTIFICATION OF [^{14}C]SC-66110 METABOLITES IN RAT AND DOG FECES* M3001063

[^{14}C]eplerenone was given to male and female rats at 20 mg/kg and to a male and female dog at 15 mg/kg in both cases as part of 13-week oral toxicity studies. In this report, the number of animals from whom samples were collected was not detailed. Fecal samples for 0-24 hours were examined for the dogs and 0-48 hours for the rats.

Eplerenone was extensively metabolized (~80% of the dose) in the male rat and to a lesser extent (~25% of the dose) in female rats. All nine of the currently known human metabolites were identified in male rat feces and included: 6β-hydroxy, 21-hydroxy, 3β,6β-dihydroxy, 3α,6β-dihydroxy, 6β15α-dihydroxy, 6β,21-dihydroxy, 2α,3β,6β-trihydroxy, 3α,6β,21-trihydroxy and 3β,6β,21-trihydroxy. Five other rat metabolites were also reported including combinations of oxygen additions (by hydroxylation pathways) and hydrogens (by reductions). Major components of radioactivity excreted in male and female rat urine and feces were the 6β-hydroxy metabolite and parent eplerenone.

One of the human metabolites was identified in the dog feces: 21-hydroxy. One additional dog metabolite was identified, however, the majority of radioactivity excreted in dog urine and feces was parent drug and the 21-hydroxy metabolite.

3. *METABOLISM OF SC-66110 AFTER ORAL ADMINISTRATION OF [^{14}C]SC-66110 IN THE MOUSE* M3097227

[^{14}C]eplerenone was given to male and female CD-1, P53+/- and P53 WT mice at a dose of 100 mg/kg. Blood, urine and feces were collected from all 3 strains of mice and metabolic profiles generated from the different matrices.

There were many peaks in the ——— profiles that were reported to contain unresolved components. Therefore the percentage of dose excreted as each metabolite in the urine and feces was not calculated. By visual assessment it was determined that several metabolites stood out as major components of the radioactivity excreted in urine and feces. No apparent sex-related differences were noted in any of the strains.

For all strains and both sexes, the major plasma component was SC-66110 and the 6β-hydroxyeplerenone (MM12). Across strains and sexes, major components in the urine and feces were reported MM6 and MM7 (unidentified dihydroxymetabolites), MM12, 21-hydroxyeplerenone(MM14) and eplerenone. There were a variety of other metabolites reported to be present as minor components that could not be structurally identified by . ———

4. *THE IN VITRO METABOLISM OF [^{14}C]EPLERENONE, [^{14}C]SPIRONOLACTONE AND [^{14}C] CANRENONE BY HEPATOCYTES, LIVER S9 AND LIVER MICROSOMES OF THE DOG AND HUMAN,AND RECOMBINANT HUMAN CYP450 ISOZYMES* M3096144

In vitro CYP450 metabolic profiles for [^{14}C]eplerenone, [^{14}C]spironolactone and [^{14}C] canrenone were determined for human and dog hepatocytes, liver S9 and liver microsomes. Metabolism was also compared for selected cDNA expressed CYP450 from both species. Comparative reaction phenotyping was conducted for dogs and humans. The major in vitro metabolites of [^{14}C]eplerenone were identified by comparing their ——— and — retention times to those of the metabolites found previously in human and canine urine and feces.

Extensive metabolism was reported for human hepatocytes, liver S9 and liver microsomes. Less metabolism was observed in canine hepatocytes, liver S9 and liver microsomes. [^{14}C]eplerenone and [^{14}C]canrenone were minimally metabolized by hepatocytes and substantially metabolized by liver S9 and liver microsomes obtained from either humans or dogs.

Substantial metabolism of [^{14}C]spironolactone was observed with human liver S9 and microsomes. With human hepatocytes, canine hepatocytes, canine liver S9 and canine microsomes there were no metabolites observed other than canrenone and an occasional polar metabolite at the solvent front of the —— profile.

The major in vitro metabolites of eplerenone were 6β-hydroxy eplerenone and 21-hydroxy eplerenone in both species. Formation was mediated in humans by CYP3A4/5 and in dogs by CYP3A12. Metabolism of spironolactone and canrenone also appeared to be mediated by the same isozymes in the respective species.

The 3 tested compounds, [^{14}C]eplerenone, [^{14}C]spironolactone and [^{14}C]canrenone were all metabolized by cells expressing human CYP3A4 but not CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP2E1. Incubation of the same compounds with microsomes from cDNA-expressed dog enzymes showed the compounds metabolized by CYP3A12 and not CYP2D15. Metabolism of the 3 radiolabeled compounds correlated best with CYP3A activity as shown by reaction phenotyping.

5. *IN VITRO METABOLISM OF [^{14}C]SC-66110 TO 6β-OH SC-66110 BY RAT HEPATIC ENZYMES AFTER REPEATED TREATMENT OF SC-66110 OR SPIRONOLACTONE*
M3000411

Rat hepatic S9 fractions were prepared from 2 separate studies: the 36 day oral toxicity study where dosages of 20, 100 or 500 mg/kg/day of SC-66110 were used and the 13 week oral toxicity study in which dosages of 20, 100 and 500 mg/kg/day were used. Spironolactone at 100 mg/kg/day was given in the 36 day oral toxicity study. Rats were euthanized in the two studies days 7, 24,36 or week 13 and livers collected. [^{14}C]SC-66110 was incubated in vitro for 30 minutes at concentrations of 25 and 100 µg/ml using the hepatic S9 fractions. Concentrations of [^{14}C]SC-66110 and metabolites were determined by ——.

In vitro metabolism of SC-66110 to the 6β-OH metabolite was induced in a dose-dependent manner in the liver fractions from rats who received repeated dosages of SC-66110. Induction of SC-66110 metabolism was greater with spironolactone than with SC-66110 when both were dosed at 100 mg/kg/day.

Bioanalytical

1. *SC-66110 and SC-70303, addendum to the validation of an —— assay in mouse plasma at ——* M2197163

The report describes the extraction of plasma and analysis by —— Long-term storage (3 months) of samples at -70°C was assessed. The sensitivity limit for both SC-66110 and SC-70303 was — µg/ml plasma. Control mouse plasma did not show endogenous peaks that interfered with analysis. Hemolyzed plasma and plasma samples that had undergone 3 freeze-thaw cycles were analyzed with acceptable levels of precision and accuracy. Analytical recovery of one concentration level, — µg/ml SC-66110 was not acceptable. Stability of the analytes was to be re-assessed at 6 and 9 months.

2. [redacted] *test method TM-201: determination of SC-66110 in mouse plasma by* [redacted]
M2001209
The procedure details a [redacted] method for determination of SC-66110 in mouse plasma. The range of quantitation is [redacted] µg/ml based on analysis of [redacted] ml of plasma.

3. *Validation of an assay for SC-66110 and SC-70303 in rat plasma at* [redacted]
M2097068
The report describes the extraction of SC-66110 from rat plasma and the subsequent [redacted] analysis. The limit of sensitivity for SC-66110 and SC-70303 was [redacted] ug/ml plasma.

4. *Validation of an assay for eplerenone (SC-66110) in rat plasma at* [redacted]
M2097066
This report also details the extraction of rat plasma and [redacted] analysis. The standard curve range was [redacted] µg SC-66110/ml of plasma.

5. [redacted] *method for the determination of SC-66110 in rat plasma specific to Searle* M2001214

This report also describes the extraction and processing of rat plasma for the [redacted] determination of SC-66110.

6. *Validation of an assay for eplerenone (SC-66110) in rabbit plasma at* [redacted]
M2097344
The report describes the extraction and processing of rabbit plasma for [redacted] determination of SC-66110. The limit of sensitivity was [redacted] µg SC-66110/ml. Acceptable precision and accuracy were obtained over the standard curve range of [redacted] µg SC-66110/ml plasma.

7. *The method development and validation of* [redacted] *for SC-66110 and SC-70303 in rabbit plasma for Searle by* [redacted] M2000264

[redacted] methods using for quantitation of SC-66110 and SC-70303 using stable isotopes [D3,^{13}C1]SC-66110 and [D3,^{13}C1]SC-70303 as internal standards were described. The sensitivity of the rabbit plasma assay was [redacted] ng SC-66110/ml rabbit plasma with a standard curve range of [redacted] ng SC-66110/ml plasma. The same sensitivity and standard curve range were reported for SC-70303.

8. *Epoxymexrenone (SC-66110) validation of an* [redacted] *assay in dog plasma at* [redacted] M2097035
This report describes the processing and extraction of dog plasma for the [redacted] determination of SC-66110. The sensitivity of the assay was [redacted] ug SC-66110/ml plasma with a standard curve range of [redacted] µg SC-66110/ml plasma.

9. Validation of an assay for SC-66110 and SC-70303 in dog plasma at —— M2097062

The assay described here has a sensitivity limit of — µg/ml for both SC-66110 and SC-70303. Standard curves were linear over a range of — µg SC-66110/ml dog plasma.

10. Evaluation of the analytical interference of SC-66110 in the clinical chemistry analyses of dog serum and urine M3095297

Potential analytical interference of SC-66110 on the precision and accuracy of clinical chemistry assays in dog serum and urine was investigated. Analytical interference in 16 analytes in serum and urine was investigated with SC-66110 added at either — µg/ml or — µg/ml. No analytical interference was reported for either serum or urine parameters.

11. —— *method SOP: AL-S-1784-00, —— method for the determination of SC-66110 in dog plasma specific to Searle* M2001208

This report describes the SOP used for —— analysis of dog plasma samples.

PK parameters:

Absorption: Eplerenone appears to be well absorbed after oral administration to mice, rats,

Table 2. Single-dose Pharmacokinetic Parameters after Oral Administration

Species	Sex	Dose (mg/kg)	Formulation	T_{max} (h)	C_{max} (µg/ml)	AUC (µg·h/ml)	BA (%)	Ref
Mouse	M	15	β-CD	0.5	12.8	11	51	(6)
Rat	M	15	β-CD	0.5	1.7	2.38	25.6	(6)
Rat	F	15	β-CD	1	3.54	4.30	46.1	(6)
Rabbit	F	15	β-CD	0.75	2.09	10.9	31.7	(13)
Dog	M & F	15	β-CD	0.75	7.98	38.3	79.2	(19)
Human	M	~1.4	capsule	1.6	1.75	11.4	NA	(24)

[a] Data listed is total eplerenone
[b] total C14 concentrations
[c] estimated based on total C14 data
[d] suspension made in SBE7-β-CD phosphate buffer

rabbits and dogs with a Tmax occurring within 3 hours of dosing. Good oral bioavailability was reported for all the pre-clinical species. A substantial difference in oral bioavailability between male and female rats is apparent in the above table. Studies in the dog showed that eplerenone was absorbed throughout the gastrointestinal tract with greatest rate of absorption in the jejunum.

In vitro studies examined the aptitude of SC-66110 as a substrate for the p-glycoprotein transporter and the ability of eplerenone to inhibit the transporter. Under the conditions of the studies reported, eplerenone was a poor substrate and also appeared to have little capacity to inhibit the P-GY transporter.

Distribution: Distribution of radiolabeled material was studied in mice, rats and dogs. The Vd ranged from [redacted] L/kg, indicating movement into tissues. The greatest concentrations of radiolabeled drug were reported in the liver and kidneys. The lowest levels of drug-associated radioactivity were located in the eye (without lens) and the brain. Radiolabeled studies also showed that the drug was secreted into milk, crossed the placenta and could be found at levels approaching maternal exposure in the fetal tissues.

Table 9. Comparison of Plasma Concentrations of Eplerenone in Maternal and Fetal Blood

Dose (mg/kg)	Study Day	Hours (h) post dose	Maternal (ng/mL)	Fetal (ng/mL)
[redacted]	19	24	[redacted]	BS
[redacted]	20	1	[redacted]	[redacted]
[redacted]	19	24	[redacted]	[redacted]
[redacted]	20	1	[redacted]	[redacted]
[redacted]	19	24	[redacted]	[redacted]
[redacted]	20	1	[redacted]	[redacted]

BS = Below sensitivity

Table 15. Tissue Distribution of Radioactivity in the Pregnant Rabbit

	µg equivalents/g			
Maternal Tissue	1 h	8 h	24 h	72 h
Adrenal glands	4.88	0.468	0.228	ND
Amniotic Fluid	1.09	0.580	0.333	0.109
Blood	6.10	0.544	0.264	ND
Brain	0.781	0.076	ND	ND
Heart	4.98	0.484	0.193	0.014
Kidneys	38.3	5.27	2.15	0.206
Liver	16.6	3.31	1.99	0.477
Lungs	5.11	0.467	0.209	ND
Ovaries	4.46	0.429	0.226	ND
Placenta	2.72	0.406	0.634	ND
Plasma	8.52	0.730	0.362	0.048
Uterus	4.16	0.512	0.227	ND
	µg equivalents/g			
Fetal Tissues	1 h	8 h	24 h	72 h
Fetus	2.15	0.339	0.127	ND
Blood	2.47	0.344	0.184	0.070
Brain	1.68	0.195	0.083	ND
Heart	2.11	ND	ND	ND
Kidneys	2.82	0.387	0.117	ND
Liver	2.05	0.165	ND	ND
Lungs	2.03	0.255	0.128	ND
ND = Not Detected Data from reference (14)				

Partitioning into red blood cells was determined in vivo in several studies in mice, rats, rabbits, dogs and humans. The concentrations of radioactivity in plasma were greater than or equal to those in RBC regardless of dosage, route of administration or duration of dosing. This suggests little partitioning.

Protein binding was low to moderate across species with the greatest degree of protein binding in humans.

Table 16. Binding of Eplerenone to Plasma Proteins

	Eplerenone (µg/mL)							
	0.02	0.2	1	5	20	60		
Species	% bound						C_{max} (µg/mL) **	Ref
P53 +/- KO mouse *	6.72	6.31	6.00	2.28	1.83	3.41	72.9	(67)
P53 KO WT mouse *	13.1	9.52	8.44	10.8	7.26	8.27	43.3	(67)
CD-1 mouse *	9.25	10.2	9.07	9.39	12.6	9.07	38.7	(67)
Rat *	18.9	15.1	14.2	17.3	15.2	17.8	23.6	(67)
Rat	25.1	25.2	18.9	16.7	ND	13.1	23.6	(66)
Rabbit *	20.0	17.8	17.6	17.4	16.3	15.7	18.8	(67)
Dog	21.6	15.8	13.3	13.4	ND	14.2	23.7	(66)
Human *	27.8	29.0	24.8	25.9	25.4	32.1	1.90	(67)
Human	60.6	59.0	38.2	33.3	ND	16.8	1.90	(66)
Human Albumin	11.5	5.92	6.53	7.13	7.63	8.34	1.90	(68)
Human AAG	53.7	53.0	53.3	41.2	31.1	10.5	1.90	(68)

* Study conducted using esterase inhibitor
** Highest C_{max} of bound + unbound eplerenone obtained in Toxicology studies or after 100 mg dose to man.

In vitro binding to powdered and granular forms of activated charcoal was determined from pH1.5 to 9.0 and concentrations of 100-500µg/ml. The binding of total radioactivity to activated charcoal was >98% and >81% for powdered and granular forms respectively, independent of concentration and pH.

Metabolism:

Overview

Metabolites were determined in vitro and in vivo for mice, rats and dogs. Induction of hepatic metabolism appeared to be a rodent phenomenon with consistent dose-dependent increases in liver weight correlating with centrilobular hepatocellular hypertrophy. Also consistent with induction of metabolism, plasma level exposure to SC-66110 tended to decrease with repeated dosing in rodents. In dogs, induction of hepatic metabolism was inconsistent at best, occurring primarily at high dosages and not to the extent seen in rodents.
Another rat specific phenomenon was the induction of UDPGT-2B1, the rate limiting enzyme in the hepatic metabolism of T4. This will be discussed at greater length in the toxicology section.

The lactone ring of eplerenone can be opened chemically at basic pH or biologically to form the inactive SC-70303 free acid. An equilibrium between SC-66110 and SC-70303 was demonstrated in canine plasma.

The major metabolite of eplerenone appears to be 6β-OH eplerenone (SC-71597), the formation of which is mediated by CYP3A. Nine predominant metabolites were identified in human urine or feces. These were either hydroxylated metabolites or from reduction of the keto group.

Mice: There were no apparent sex-related differences and no major strain-related differences between CD-1, P53N5-W and P53N5-T KO strains. The major plasma metabolites were 6β-OH eplerenone and eplerenone. The major urinary and fecal metabolites were 6β-OH eplerenone, 21-OH eplerenone, eplerenone and 2 unidentified metabolites. The major human metabolites were represented as well as several murine-specific metabolites that will be discussed later under "Carcinogenicity" in the toxicology section.

Rats: A sex-related difference in metabolism was reported for rats. Oral bioavailability was reported as 26% for males and 66% for females. Females consistently showed greater body burden of drug than males at the same dosages. When plasma samples were acidified, samples from females showed greater amounts of parent drug than did samples from males. Urine samples when acidified showed <12% eplerenone in males but 41-72% eplerenone in female samples. Acidified feces indicated < 19% eplerenone in males and 7-60% eplerenone in female samples. Thus, females show a greater body burden of eplerenone with much less metabolism than males. A slightly greater induction of CYP3A was demonstrated for male rats compared to female rats, possibly contributing to the differences in metabolism.

The major metabolites in rat urine were 6β-OH eplerenone and 3α, 6β-OH eplerenone. The nine major metabolites identified in human feces were also identified in rat feces. The major radioactive components were eplerenone and 6β-OH eplerenone.

Dogs: There were no obvious or consistent sex-related differences in metabolism. The 6β-OH and 21-OH metabolites were also identified in canine matrices.

Table 24. Comparison of Identified Metabolites Across Species

Compound	Human	CD-1 Mouse	p53 KO mouse	Rat	Dog	Rat Liver S9
Eplerenone	+	+	+	+	+	+
SC-70303 free acid	+	+	+	+	+	+
6β -OH	+	+	+	+	+	+
21-OH	+	+	+	+	+	+
3β, 6β, 21-OH	+	+	+	+	-	-
2α, 3β, 6β-OH	+	+	+	+	-	-
6β, 21-OH	+	-	-	+	+	-
3α, 6β, 21-OH	+	-	-	+	-	-
6β, 15α-OH	+	-	-	+	-	-
3β, 6β-OH	+	-	-	+	-	-
3α, 6β-OH	+	-	-	+	-	-
6α-OH	-	-	-	-	+	-
6β -OH glucuronide	-	-	-	-	+	-
Reference	(80)	(74)	(74)	(75, 76)	(75, 76)	(82)
+ = metabolite positively identified - = positive identification of the metabolite was not obtained						

Excretion: Excretion was primarily in feces after both IV and oral administration to mice, rats and dogs. Excretion from rabbits was primarily via the urine which was of lesser importance in the other species. Single doses of radiolabeled eplerenone given to human volunteers were primarily excreted in the urine. The mean percentages of dose excreted as total radioactivity in urine and feces were 67 and 32% respectively.

Other studies:

In vitro metabolism of [^{14}C]eplerenone, [^{14}C]spironolactone and [^{14}C] canrenone was compared in hepatocytes, liver S9 and hepatic microsomes of the dog and human. Metabolism was also compared for selected cDNA expressed CYP450 from both species. Comparative reaction phenotyping showed that the three tested compounds were metabolized by cells expressing human CYP3A4 or canine CYP3A12 (the isozyme associated with steroid metabolism in that species). The effect of CYP3A inhibitors on canine metabolism was also examined by combination treatment of eplerenone and ketaconazole, bergmottin, fluconazole or grapefruit juice. The AUC for SC-66110 was increased over control values with the concurrent presence of known the CYP3A inhibitors.

PK/TK summary: Eplerenone is well absorbed following oral administration to the preclinical species with good oral bioavailability. Protein binding is low to moderate across species. The major human metabolites were represented in each of the preclinical species. Induction of hepatic metabolism was a rodent phenomenon manifested by increased hepatic weights with a histologic correlate of centrilobular hypertrophy. Reaction phenotyping indicated primary involvement of CYP3A in metabolism.

Studies using [^{14}C]SC-66110 showed that radio-label was widely distributed. The greatest concentrations of drug-associated radioactivity were reported for the liver and kidneys. The lowest levels of drug-associated radioactivity were reported for the eye (without lens) and brain. Eplerenone was also shown to be secreted into the milk of lactating female rats, crossed the placenta (rats and rabbits) and could be found in fetal tissues (rats and rabbits) at levels approaching maternal exposure.

After IV and oral administration to mice, rats and dogs, eplerenone was excreted primarily in the feces. Excretion from rabbits was primarily via the urine, a route of lesser importance in the other species. Single dosages of radiolabeled eplerenone given to human volunteers were excreted primarily in the urine.

PK/TK conclusions: Findings for a given species were consistent across studies. Eplerenone is well absorbed after oral dosing, low-moderately protein bound and widely distributed, including milk and fetal tissues. Eplerenone is highly metabolized with significant involvement of hepatic CYP3A. Excretion is via the urine and feces.
The studies support exposure and dose exaggeration in the toxicology studies. The major human metabolites are represented in the preclinical species.
The most significant finding to be presented in the NDA is the calculation of free eplerenone across species, including humans. Prior to presentation of this data, calculations had been based

upon total eplerenone: eplerenone + the open ring lactone (SC-70303 free acid). The open ring lactone was determined to be pharmacologically inactive due to a very low affinity for the mineralocorticoid receptor. The sponsor provided for each species and each study the fractional contribution of eplerenone to total eplerenone. These recalculations of exposure will be presented *in situ* for each study in this review.

IV. GENERAL TOXICOLOGY:

Study title: A single dose oral toxicity study of SC-66110 in the rat

Key study findings: Lethal dose ≥2000 mg/kg.

Study no: — 78-01
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: May 22, 1996
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:RCT10016, 97.2%
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in dH2O

Methods (unique aspects):
Dosing:
Species/strain: Crj:CD(SD) rats
#/sex/group or time point (main study): 5 male, 5 female
Satellite groups used for toxicokinetics or recovery: na
Age: 5 weeks
Weight: 133-149g males, 117-127 g females
Observations and times:
Following single oral doses of 500, 1000 or 2000 mg/kg, the animals were observed for 14 days for signs and body weight changes. Following euthanasia, visual inspection of gross changes was performed.

Results:
No unscheduled mortality and no clinical signs other than the excretion of compound-colored feces (white) from the HD group were reported. There were no differences in body weights or food consumption.Gross pathology, organ weights and histopathology were not determined. Conclusion was that lethal dose is > 2000 mg/kg.

Study title: Acute Oral Tolerance in Beagle Dogs

Key study findings: 100 mg/kg given orally was tolerated by the one dog.

Study no — 30-083
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: not stated
GLP compliance:no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity
Formulation/vehicle: gelatin capsule

Methods (unique aspects):
One 16-month old Beagle dog was given a single 100 mg/kg dose of SC-66110 in a gelatin capsule. Body weight, food consumption and clinical signs were monitored for 14 days following the dosing.

Observations and times:
Observed for body weight, food consumption and signs for 14 days following the dose.
Results:
The animal did not die after the single oral dose of 100 mg/kg. There were no changes in body weight or food consumption.

Study title: A single dose oral toxicity study of SC-66110 in the dog

Key study findings: lethal dose was concluded to be > 2000 mg/kg

Study no: — **78-00**
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation:May 2, 1996
GLP compliance:yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:RCT10016, 97.2%
Formulation/vehicle: directly weighed into gelatin capsules

Methods (unique aspects):
Dosing:
Species/strain:Beagle dogs, male
#/sex/group or time point (main study): 2/group
Satellite groups used for toxicokinetics or recovery:
Age: 9-12 months old
Weight: 9.3-12.2 kg
Observations and times:
Male Beagles, 2/group, 9-12 months old were given single oral doses of 500, 1000 and 2000 mg/kg. The dogs were checked for clinical signs once an hour for 6 hours from days 1-13. Body weights were determined pre-treatment and days 0, 1,4,8 and 13. Food consumption was monitored daily. At euthanasia, gross observations were made and selected organs weighed. No histopathology was done.
Results:
There was no unscheduled mortality and no effects on body weight although food consumption was decreased on dosing day. Signs were reported for each dose and included:

500 mg/kg vomiting, salivation and tremors within 4 hours after dosing
1000 mg/kg, vomiting, salivation, tremors, decreased activity; 2000 mg/kg, vomiting, salivation, tremors,tonic convulsions, tachypnea, sedation , rigid muscle tone

Study title: 2-week feasibility study with SC-66110 dietary admix and oral gavage in mice

Key study findings: Despite apparent palatability problems, the animals on the dietary admix study received target amounts of drug. In both dosing regimens there were no reported clinical signs. There were dose-related increases in liver weight (114-187%) and decreases in heart and kidney weight. Body weight gain was decreased in the HD dietary study group. The increased liver weights were characterized histologically as centrilobular hepatocellular hypertrophy.

Study no: EX4466/ — 6127-284, doc P30E4466
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: January 31, 1996
GLP compliance:no
QA report: yes ( ) no ( )
Drug, lot #, radiolabel, and % purity:SC-66110, lot RCT9967
Formulation/vehicle: 0.5% methyl cellulose and 0.1% polysorbate 80

Methods (unique aspects):
Six week old CD-1 (ICR)BR VAF/plus mice (15/sex in control group, 35/sex in gavage groups, 30/sex dietary admix groups) were given gavage doses of 0, 100, 500 and 1000 mg/kg. The drug in the dietary admix study was the equivalent of 1000 and 3000 mg/kg dosages. The first 10 animals per group were designated for toxicokinetics. The animals were observed for clinical signs, body weight and food consumption. Gross pathology was noted for male euthanized moribund and all toxicology animals in week 3. Selected organs were weighed and histopathology examined for control and HD groups. Toxicokinetic sampling was performed day 16.

Results:
One control animal was euthanized moribund after a gavage accident and a TK animal was found dead. No clinical signs were reported.In the HD dietary admix group, body weight decreased from Day 4 in both sexes.The decrease was significant in the males from Day 4 and in the females at the last day. A great deal of spillage in the dietary administration groups indicated a palatability problem. However, intended exposure was achieved. Dose-related increases in liver weight were seen were both forms of dosing, both sexes. Decreased heart weight and renal weight were seen in both sexes in the dietary HD group. The primary gross pathology finding was mottling of the liver in MD and HD dietary admix males. Hepatocellular centrilobular hypertrophy was reported for the HD groups of both dosing methods.

Roughly proportional increases in exposure with increasing dose were seen in the gavage arm of the study. Lower mean exposure was seen with dietary administration as well as non-proportional increases in exposure.

Study title: Thirteen –week range-finding gavage toxicity study of SC-66110 in the mouse (SA4513/ — 96065)

Key study findings: Treatment of mice with SC-66110 for 13 weeks resulted in changes affecting primarily the liver. These included increased ALT levels and increased triglycerides in MD and HD males and females. Bilirubin levels were decreased in HD mice of both sexes. These changes correlated grossly with increased liver weight and microscopically with hepatocellular hypertrophy. Systemic exposures (AUCs) decreased over the course of the study, suggesting that the increased liver weight and hepatocellular hypertrophy could be from enzyme induction and increased metabolism. These drug-induced liver changes may be considered adaptive. The high dose of 1000 mg/kg produced ~8X the human therapeutic exposure (M3001079, p. 27).

Study no: SA4513
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: August 6, 1996
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:SC-66110, lots RCT10055 and E90178
Formulation/vehicle: 0.5% methyl cellulose and 0.1% polysorbate 80 in deionized water
Methods (unique aspects):
Dosing:

Species/strain: CD-1 mice
#/sex/group or time point (main study):25/sex, 15/sex necropsied
Satellite groups used for toxicokinetics or recovery: additional 50 animals per dose for tk
Age: 7 weeks
Weight: 21-34 g
Doses in administered units: 100, 250, 500 and 1000 mg/kg/day
Route, form, volume, and infusion rate:oral gavage

Observations and times:

Mice were observed for clinical signs. Body weights and food consumption were recorded weekly. Clinical chemistry and hematology parameters were determined just before necropsy. Blood was collected for determination of plasma drug levels from 5 mice/sex/dose in the toxicokinetic groups at several time points up to 24 hours after dosing on Day 8, week 6 and 13. Week 14, mice in the toxicology groups were euthanized and necropsy performed. Major organs were weighed and 40 tissues collected for microscopic examination.

Results: Several accidental (gavage-related) deaths were noted but none were drug-related. No abnormal clinical signs were reported. Weight gains in mid and high-dose males decreased 14-29%, respectively, relative to the controls during the course of the study but were similar to control weights by the end of the study. No drug-related effects on body weights in female mice were found. No significant hematologic changes were noted.

Clinical chemistry parameters showed several drug-related changes. ALT levels were increased from 132-213% in the male and female mid and high dose groups. Cholesterol levels were increased 22-24% and triglycerides were increased 44-101% in the HD males and females. Total bilirubin decreased 39% and 67% in the MD and HD males respectively.

A drug-related increase in liver weights was found in both sexes. Male and female liver weights were increased up to 40% and 30% respectively over control values. Histopathology showed hepatocyte hypertrophy in 10/15 HD males and 5/15 HD females (histopathology not determined for LD and MD groups).

Toxicokinetics indicated no sex-related differences. Systemic exposures by the end of the study for the MD and HD groups were decreased when compared to those at the beginning of the study, possibly related to enzyme induction, causing hepatocyte hypertrophy and the observed increase in liver weights.

Study title: Two week oral tolerability study in rats

Key study findings: A significant dose-dependent increase in liver weights was reported for both sexes. Biochemical analysis of male gonadal tissue showed a 36% increase in 17α-hydroxylase and an 80% increase in testicular CYP450 at both doses. A 63% decrease in plasma testosterone was reported for the HD.

Study no: [redacted] 30 083
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation:June 11, 1984
GLP compliance: not mentionec
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:CGP 30 083, lot 800184, 99.9%
Formulation/vehicle:

Methods (unique aspects):
Dosing:
Species/strain:Rat [redacted]
#/sex/group or time point (main study): 5 per sex per group
Satellite groups used for toxicokinetics or recovery:
Age: 5-6 weeks
Weight: 164-280
Doses in administered units: 0, 100, 300 mg/kg
Route, form, volume, and infusion rate: 1% methycellulose, p.o., once a day for 14 days

Observations and times:
Clinical signs: daily
Body weights:daily
Food consumption:twice a week

Hematology: yes
Clinical chemistry: yes
Gross pathology:yes
Organs weighed:no
Histopathology: yes
Toxicokinetics:no
Other: hearing test

Results:
No deaths or altered clinical signs were reported. There was a slight increase (2-9%) in serum protein concentration in both sexes at the high dose (300 mg/kg). There was a significant (positive trend test) dose-dependent increase in liver weights in both sexes when compared to controls. Microscopically there was no correlation (hypertrophy). Evaluation of gonadal tissue from the male rats showed a 36% increase in the activity of the testosterone synthetic enzyme 17 α-hydroxylase and an 80% increase in testicular cytochrome P450 content at both doses. At 300 mg/kg there was a 63% reduction in plasma testosterone levels. These changes are consistent with induction of microsomal enzymes in both liver and testes with resultant decreases in circulating testosterone levels and increases in testosterone synthetic activity.

Study title :Eight day range-findings gavage toxicity study of epoxymexrenone (SC-66110) in the male rat (EX4431)

Key study findings: Decreases in plasma levels from day 1 to day 8 were noted, suggesting induction of metabolism. Consistent with this were dose-dependent increases in liver weight and increased mRNA for hepatic CYP3A1. The high dose of 1000 mg/kg produced plasma AUC ~14X the human therapeutic exposure (M3001079, p. 28)

Study no: EX4431
Volume #, and page #:
Conducting laboratory and location: GD Searle, Skokie, IL
Date of study initiation:Sept 7, 1995
GLP compliance:no
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:SC-66110, GDS4652-158A
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80

Methods (unique aspects):
Dosing:
Species/strain: [redacted] CD
#/sex/group or time point (main study): 10 per sex(control and HD) 6 per sex all other groups
Satellite groups used for toxicokinetics or recovery: 4 per sex per group, 100, 200, 500 mg/kg
Age: 7 weeks
Weight: 200-250 g
Doses in administered units:0, 100, 200, 500, 1000 mg/kg/day

Route, form, volume, and infusion rate: oral gavage once a day for 8 days

Observations and times:

Clinical signs: daily
Body weights:pre-treatment, days 1,5 and 8
Food consumption: 3 times to provide cumulative amounts
Hematology: prior to necropsy
Clinical chemistry: prior to necropsy
Urinalysis:12 hour period prior to necropsy
Gross pathology:
Organs weighed:
Histopathology:
Toxicokinetics:0.5, 1,3,5,8 and 24 hour samples
Other: On Days 1 and 8, ^{14}C labeled test article was given to satellite animals dosed at 100, 200 and 500 mg/kg., Blood was collected from the animals at the pre-determined times and replacement fluid administered if necessary. Feces and urine samples were collected from the same animals for approximately 18 hours before and for seven days after each ^{14}C administration (Days 1 and 8).
Three animals per group were sampled for analysis for hepatic CYP450 mRNA and UDPGT.

Results:

No deaths were reported and there were no observed clinical signs or effects of body weights or food consumption. There was a slight increase (17%) in platelet counts and APTT (20%) at the highest dose (1000 mg/kg/day). There were no gross or microscopic lesions related to drug treatment. Liver weights were increased 18% and 41% at the two top doses (500 and 1000 mg/kg/day, respectively). This increased liver weight was correlated with hepatocellular hypertrophy which was thought to be due to induction of microsomal enzymes. This was confirmed by a 2.4-fold increase in the steroid-inducible enzyme CYP3A1. Also, thyroid weights increased 40% at the 1000 mg/kg dose. This increased thyroid weight was associated with a 1.8-fold induction of the thyroxine catabolic enzyme UDPGT-PB in the liver which would result in increased TSH levels and thyroid stimulation. SC-66110 did not induce overt toxicity in rats at doses up to 1000 mg/kg/day for 8 days.

Study title: 13Week Gavage tox study of SC-66110 (epoxymexrenone) in the rat

Key study findings: Drug was systemically available at all doses albeit non-proportionally. Steady state was reported to occur between Days 24 and 37. Females showed higher plasma levels at all doses. Findings were pharmacological (increased Na/K urinary ratio) and adaptive (adrenal hypertrophy, hepatocellular hypertrophy) and toxicological (increased incidence of chronic progressive nephropathy. This study formed the basis for the dose selection for the two-year carcinogenicity study. Multiples of human exposure were 9X in males and 18x in females given the high dose of eplerenone (M3001079, p 29, 30).

Study no:SA4453 — 95115
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: Dec 12, 1995
GLP compliance:yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:SC-66110 lot RCT9937
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80
Methods (unique aspects):
Dosing:

Species/strain: ——— CD albino rats
#/sex/group or time point (main study):20/sex
Satellite groups used for toxicokinetics or recovery:19/sex for tk, 10 for recovery (HD only)
Age: 6 weeks
Weight:127-177 g
Doses in administered units: 0, 20, 100 and 500 mg/kg/day
Route, form, volume, and infusion rate: oral gavage once a day for 13 weeks followed by a 4-week reversal period

Observations and times:

Clinical chemistry and hematology were performed at weeks 4, 8 and 14, and at the end of the 4 week reversal period. Blood was analyzed for TSH, T3, T4, estradiol, testosterone, FSH, LH and aldosterone. At weeks 14 and 18 necropsies were performed, organ weights determined and histopathologic examination performed. Liver samples were also analyzed for mRNA for HMG CoA reductase, CYP450 and UDPGT. Testes were examined for microsomal metabolic enzymes and testosterone biosynthetic and catabolic enzymes.

Toxicokinetics were determined day 1, weeks 6 and 13: 0.5, 1,2,3,4,6,8 and 24 hours after dosing.

Results:

There were two instances of unscheduled mortality: 1 20 mg/kg/male (urinary calculi), 1 100 mg/kg female(eye injury).

There were no reported changes in HMG CoA reductase mRNA. Increased mRNA levels were found for CYP3A, CYP2B and UDPGT. There were also increases in testicular expression of testosterone synthesizing enzymes and a decrease in expression of aromatase at the HD.

Minimal changes were found in serum hormones (testosterone, LH, FSH, estradiol, T3, T4, and TSH). However, aldosterone concentrations showed marked dose-related elevations with aldosterone in the high dose males and females reaching 9X and 14X their respective controls (Table 30). The zona glomerulosa cells, the principle site of aldosterone production, were vacuolated and hypertrophied reflecting their increased activity.

Table 30

Serum Aldosterone Concentrations (pg/ml) at Week 13 (Mean ± SD)

Dose (mg/kg/day)	Males	Females
0	97 ± 75	273 ± 267
20	422 ± 606	1719 ± 1963
100	675 ± 955	915 ± 651
500	848 ± 985	3776 ± 3672

Serum cholesterol levels in the high dose group (500 mg/kg/day) increased 40% and 107% in males and females, respectively. Serum triglycerides increased 97% in the high dose females. No other significant changes in clinical pathologic parameters were noted.

The urine sodium:potassium ratio was increased in all drug-treated animals which was due to increased sodium and decreased potassium excretion. This reflects the pharmacological activity associated with aldosterone receptor antagonism. However, urine protein content was also increased in the high dose animals (57% in males and 839% in females) and was considered a possible toxic effect due to altered glomerular filtration. This proteinuria, the first clinical indicator of chronic progressive nephropathy (CPN), correlated with an increased histological incidence of CPN in the high dose animals (Table 31).

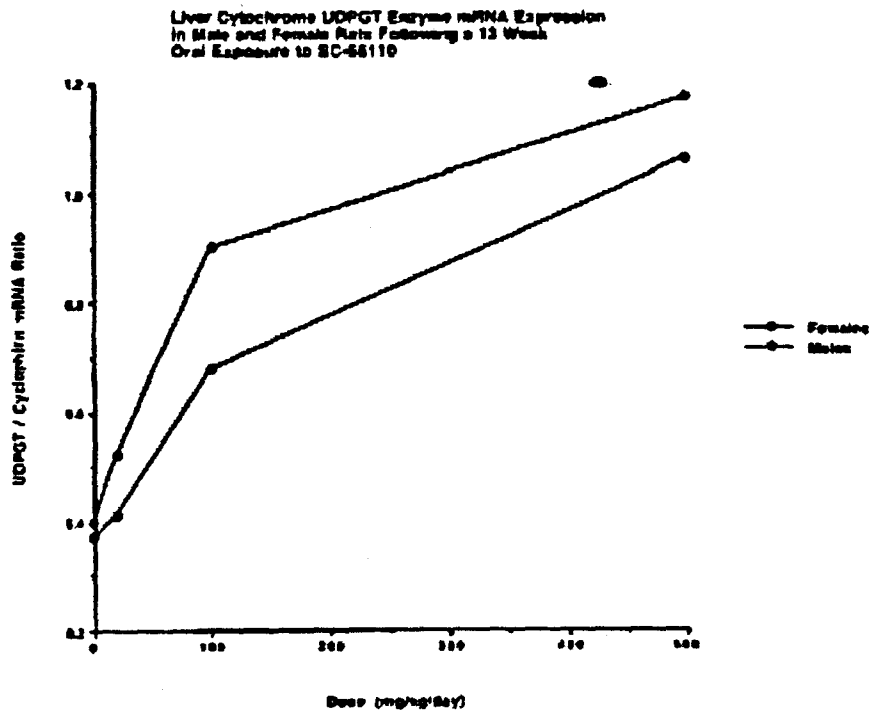
Table 31

CPN Incidence in Rats from Week 14 Sacrifice

Dose (mg/kg/day)	Males	Females
0	0/15	0/15
20	1/14	0/15
100	2/15	0/14
500	6/15	6/15

Liver weights were increased 34% in the high dose (500 mg/kg/day) males and 99% in the high dose females. Similarly, thyroid weights increased 24% in the high dose males and 93% in the high dose females. The increased weights were associated with histological evidence of hepatocellular hypertrophy and thyroid follicular cell hypertrophy. These changes were considered consistent with hepatic microsomal enzyme induction and with secondary thyroid enlargement from increased hepatic catabolism of thyroid hormones. Although thyroid hormone levels and TSH levels were not increased at 13 weeks, mRNA levels of uridine diphosphate glucuronsyl transferase (UDPGT), a thyroxin catabolic enzyme system in the liver, was increased by 186% in females and 193% in males (Figure 2). Also, there was induction of a steroid-inducible form of cytochrome P-450 (CYP3A) in liver. This suggests that thyroxin clearance would be increased with resulting feedback stimulation of the thyroid gland by TSH leading to the observed increase in thyroid weight and hypertrophy. A similar mechanism has been suggested for spironolactone.

Figure 2



Toxicokinetic data showed that steady state levels of SC-66110 was reached between days 24 and 37 (Table 32). Females had about 2X higher AUC levels than males. AUCs were dose proportional between 20 and 100 mg/kg/day, but less than dose proportional between 100 and 500 mg/kg/day suggesting saturation of absorption.

Table 32

SC-66110 Area Under the Plasma Concentration Time Curve
(AUC - µg/ml x hr)

Day	Sex	20 mg/kg	100 mg/kg	500 mg/kg
1	M	7.23	49.7	61.8
	F	15.1	97.4	106
24	M	5.36	22.7	110
	F	12.7	52.7	224
37	M	7.27	43.6	152
	F	15.1	60.7	207
86	M	9.64	44.2	109
	F	19.8	87.4	230

In summary, oral administration of SC-66110 to rats for 13 weeks caused several changes that were categorized as either pharmacological, adaptive, or toxic:

(A) pharmacological (aldosterone receptor blockage):
- (1) increased urine sodium:potassium ratio
- (2) increased serum aldosterone concentration
- (3) vacuolization and hypertrophy of adrenal zona glomerulosa cells

(B) adaptive (related to induction of liver microsomal enzymes and subsequent increased thyroid hormone catabolism):
- (1) hepatocellular enlargement with:
  - (a) increased UDPGT mRNA levels
  - (b) induction of a steroid-inducible form of cytochrome P450 (CYP3A)
- (2) thyroid gland hypertrophy
- (3) increased serum cholesterol and triglyceride

(C) toxic:
- (1) increased incidence of chronic progressive nephropathy (CPN)
- (2) increased urinary protein

Based on these changes, 500 mg/kg/day was regarded as the maximally tolerated dose (MTD) of SC-66110 in both male and female rats.

Study title: 13-Week oral *toxicity* and impurity qualification study in the rat

Key study findings: This comparison of three batches of SC-66110 showed no significant difference in the response of the animals to the different lots of drug.

Study no:P&T2001-0160/SA5143
Volume #, and page #:
Conducting laboratory and location: Pharmacia UpJohn, Kalamazoo, MI
Date of study initiation:May 1, 2001
GLP compliance:yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: SC-66110, lots 96K020-F1A, 00K007-F5A, SP12626 With purities of 99.9%, 98.9% and 100.0% respectively
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80

Methods (unique aspects)**:comparison of three bulk drug lots to qualify impurities**
Dosing:
Species/strain:Rat Crl:CD(SD)IGSBR

Key findings: Under the conditions of the study an increase in micronuclei was not detected.

Study no: SA4674
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: GD Searle, Skokie, IL
Date of study initiation:
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110, lot 96K018-F2B

Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in distilled water

Methods: Rats were orally gavaged with SC-66110 at doses of 0, 500, 1000 and 2000 mg/kg. The bone marrow was sampled at 24 and 48 hours after dosing. The bone marrow for the positive control (cyclophosphamide) was sampled at the 24-hour time point. Slides of marrow cells were prepared from 5 rats/sex/timepoint for each group. The results are presented as the individual readers' assessments of individual animals rather with no compilation and summary.

Summary of individual study findings: Under the conditions of the study an increase in micronuclei was not detected.

Study title: In vivo/in vitro unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints with SC-66110 (SA4670)

Key findings: Unscheduled DNA synthesis was not reported for rat hepatocytes following in vivo treatment with SC-66110.

Study no: SA4670/18781-0-494
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 8/21/1997
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110 lot 96K018-F2B

Formulation/vehicle: 0.5% methylcellulose plus 0.1% polysorbate 80

Methods: Male Sprague-Dawley rats (4/group) were given SC-66110 at 500, 1000 or 2000 mg/kg by oral gavage. Controls received vehicle or the positive control of N-dimethylnitrosamine (DMN). Hepatocyte cultures were prepared at two timepoints after drug administration, 2-4 hours and 15-16 hours, by perfusion of livers with collagenase. Viable hepatocytes were then cultured

#/sex/group or time point (main study):10/sex/group, 6/sex for group 2 from Day 23
Satellite groups used for toxicokinetics or recovery: 7/sex/group, 6/sex/group for group 5from day 23 due to shortage for that lot.
Age:8-9 weeks
Weight: males 221-271g, females154-196g
Doses in administered units: 0, 100(females) and 200(males) mg/kg/day
Route, form, volume, and infusion rate: given daily as an oral gavage for 13 weeks

Observations and times:
Clinical signs:daily
Body weights:pre-test and weekly
Food consumption: pre-test and weekly
Ophthalmoscopy: near end of treatment period
EKG:no
Hematology: samples collected at time of necropsy
Clinical chemistry:samples collected at time of necropsy
Urinalysis: approximately 16 hour sample prior to termination of study
Gross pathology:yes
Organs weighed:yes
Histopathology: yes
Toxicokinetics:Day 79, 1,2,3,5,8 and 24 hours after dosing
Other:no

Results:
Mortality: none unscheduled
Clinical signs:no
Body weights: there were no significant differences in gain between the batches
Food consumption:no difference between batches
Ophthalmoscopy: no findings reported
Electrocardiography:
Hematology: ↑PT and APTT in male groups 3 and 4
Clinical chemistry:↑Chol, ↑tprot, ↑globulin, ↑Ca and ↑K (m+f)
Urinalysis: no significant findings
Organ weights:↑liver, thyroid and kidney (m+f),↓mean relative adrenal weights (m+f)
Gross pathology: no gross lesions
Histopathology: hypertrophy of adrenal zona glomerulosa
Toxicokinetics:Exposure was greater in females than males. No differences in exposure between the batches based on AUC_{0-24} and Cmax .

Study title:Two Week Oral Tolerability Study in Beagle Dogs

Key study findings: Administering the powdered crystalline drug in gelatin capsules to male dogs for 2 weeks produced no mortality or clinical signs or findings of toxicological significance but did increase testosterone catabolism in testicular tissue. Organ weight increases reported for heart, liver, thyroid, kidney, adrenal and prostate(slight ↑ in both absolute and relative). No histologic correlates reported.

Study no:84-5072
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation:June 25, 1984
GLP compliance: NA
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:CGP083, lot 800184, 99.9%
Formulation/vehicle: gelatin capsules

Methods :
SC-66110 was given in gelatin capsules to 3-male Beagle dogs at 100 mg/kg for 14 days. Control (3 dogs) received empty capsules. Dogs were monitored for clinical signs. Hematology, clinical chemistry and urinalysis were conducted pre-test and on Day 14. Also, ophthalmologic, neurologic and ECG exams were conducted pre-test and on Day 14. Blood samples were taken on days 1 and 12 for determination of plasma drug levels. At necropsy, selected organs were weighed, examined and processed for histopathologic examination. Plasma levels of testosterone and estrogen were determined and organ hormonal and enzymatic analysis (17α hydroxylase) was performed in testes and liver.

Results:
SC-66110 appeared to be well tolerated. The mean plasma concentrations at Tmax of 2 hours on day 12 was 15.4 μg/ml. There were no gross or microscopic findings considered related to treatment. After 14 days, testicular cytochrome P450 content increased 53% and the 17α-hydroxylase activity increased 72% when compared to controls, indicating increased catabolism with corresponding increased synthesis of testosterone. However, there were no changes in plasma levels of either testosterone or estradiol.

Study title: Thirteen week oral capsule toxicity study of SC-66110 in the dog (SA4451)

Key study findings: 2 dogs given 300 mg/kg were euthanized early for humane reasons. They showed emaciation, salivation, convulsions (1 dogs) and blood chemistry findings of hyponatremia and other severe electrolyte imbalances. The changes found at the other dosages were referable to exaggerated pharmacology and while not always consistent and dose-related included decreased Na, increased K and increases in cholesterol and bile acids. Serum aldosterone levels were dose-relatedly increased in both sexes. Serum testosterone levels were decreased in treated males and was possibly connected to the decreased size of the prostate gland seen . Hypertrophy of the adrenal zona glomerulosa was also reported. Increased size of the thymus had no histologic correlates and is unexplained. Most signs were resolved by the end of the 4-week recovery period. Exposure to SC-66110 at dosages between 15 and 300 mg/kg/day for 13 weeks resulted in altered expression of several liver drug metabolizing and testosterone synthetic pathway genes. The predominant effects occurred at the HD where male and female dogs showed ~27X and ~42X human exposure(M3001079, p32).

Study no:SA4451

Volume #, and page #:
Conducting laboratory and location:GD Searle, Skokie, IL
Date of study initiation: November 28, 1995
GLP compliance:yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:SC-66110, lot RCT9938
Formulation/vehicle: weighed directly into gelatin capsule

Methods (unique aspects):
Dosing:

Species/strain: Dog, Beagle
#/sex/group or time point (main study): 4/sex/group; 2 dogs per sex from the control and HD groups entered a 4-week reversal period.
Satellite groups used for toxicokinetics or recovery:3/sex/group at 15 and 100 mg/kg/day
Age:9-12 months
Weight:7-11kg
Doses in administered units:0,15,100, 300 mg/kg/day
Route, form, volume, and infusion rate: gelatin capsule per os once a day for 91-93 days
2 additional groups (15 and 100 mg/kg, 3/sex/group) were given ^{14}C-labelled test article on days 1, 16, 38 and 85. Urine and feces were collected for up to 7 days after radiolabel administration.

Observations and times:

Clinical signs:daily
Body weights:pre-test then weekly
Food consumption:pretest then twice a week except for weeks 3,5 and 6 when consumption wasn't measured
Ophthalmoscopy:pre-test and week 12
EKG:pre-test and weeks 4,8 and 13 of dosing
Hematology:pre-test, day 3, weeks 4,8 and 12 of tx and week 4 of recovery
Clinical chemistry:
Urinalysis: 16 hour samples collected pre-test, weeks 4, 8 and 12 of tx and week 4 of recovery
Gross pathology:yes
Organs weighed:yes
Histopathology: yes

Hormones (aldosterone, estradiol and testosterone) measured week 12. Analysis of dog liver and testis mRNA levels for genes coding for metabolizing enzymes relative to cyclophilin.

Results

Two of the HD dogs were euthanized for humane reasons.They showed marked decrease in food consumption, weight loss, decreased fecal output, emaciation, ptyalism, hunched posture, reduced activity, vomiting and convulsions. These findings were regarded to be due to the pharmacological activity of the drug with the effects secondary to dehydration, emaciation and stress. The urinalysis showed hyponatremia and hypochloremia. The other animals showed no

clinical signs of toxicity that could be attributed to drug treatment. There were no test article-related ophthalmic or ECG findings.

Clinical chemistry parameters showed changes that could be attributed to the pharmacological activity of the drug. These included decreased serum sodium, chloride and bicarbonate. Increases were noted in serum potassium, calcium, total protein and urea nitrogen. Creatinine was not affected suggesting that the urea nitrogen changes were not due to toxic effects on the kidney but rather due to loss of hydration associated with electrolyte changes. These effects were not apparent after the one month reversal period. Serum cholesterol and triglyceride increased but were also reversed after one month. There were no test article-related changes apparent in hematological or urinalysis parameters.

Serum hormone analysis showed a marked dose-related increase in aldosterone levels in both males and females. These increases were no longer apparent after the one month drug-free period.
Weights of adrenal, liver and thymus were slightly increased. Thyroid weights were unchanged. Histologic examination of the adrenal glands showed thickening of the zona glomerulosa. This correlated with the increased serum levels of aldosterone and correlated with increased synthetic activity of the gland. This can be attributed to the pharmacological inhibition of aldosterone function leading to feedback stimulation of the adrenal cortex. The most marked effect was the decreased prostate weight in males which was significant at the 100 and 300 mg/kg doses. Histologically this was described as atrophy and correlated with decreased serum testosterone levels. There was a slight reversal of this effect after 30 days of no treatment.

Below is a table from Dr Papoian's original review.

Dose (mg/kg/day)	Prostate weight (gms)	Prostate/final body weight	Serum testosterone (ng/ml)
0	8.0±2.3	7.1±1.5	2.4±2.5
15	4.7±2.3	4.5±2.4	2.3±2.1
100	2.8±1.1	2.8±1.1	0.6±1.1
300	1.9±0.4	1.8±0.3	0.4±0.4
300 (4-wk reversal)	3.5	3.3	0.6

Study title: Thirteen week oral capsule toxicity and reversibility study of SC-66110 in the male dog (SA4512)

Key study findings: Detectable plasma levels at all doses. Dose-related decrease in prostate weight was reported that at the HD included histologic evidence of decreased glandular development. The decreased prostate weight resolved in the three month recovery period. Comparable effect with spironolactone treatment. The NOEL for the prostate effects was 5 mg/kg (2.6X human exposure levels, M3001079, p32).

Study no:SA4512
Volume #, and page #:
Conducting laboratory and location: Searle R&D, Skokie, IL

Date of study initiation:May 29, 1996
GLP compliance:yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:SC-66110, lot 10015 and SC-09420, lot E90060
Formulation/vehicle: gelatin capsule

Methods (unique aspects)**:Purpose was to find a NOEL for prostate effects and to compare to dogs treated with 5 mg/kg/day spironolactone (SC09420)**

Dosing:

Species/strain:dog, beagle
#/sex/group or time point (main study):6
Satellite groups used for toxicokinetics or recovery:
Age:8-9 months
Weight:8.6-13.7kg
Doses in administered units:SC-66110 :0, 1.5,5.0, 25 mg/kg/day and SC09420: 5 mg/kg/day
Route, form, volume, and infusion rate: orally administered for 91-93 days

Observations and times:

Clinical signs:daily
Body weights:weekly
Food consumption:twice weekly
Ophthalmoscopy:not done
EKG:not done
Hematology:pretreatment, day 3, weeks 2,4,8 and 12 of treatment and weeks 1,5,9 and 13 of recovery
Clinical chemistry: pretreatment, day 3, weeks 2,4,8 and 12 of treatment and weeks 1,5,9 and 13 of recovery
Urinalysis:for 16 hours pretreatment, weeks 2 and 12 of treatment and week 13 of recovery
Gross pathology:yes
Organs weighed:yes
Histopathology: adrenal, prostate, epididymides and testes.
Toxicokinetics:samples at 1,2,3,5,8 and 24 hours after dosing days 1 and 85
Other:aldosterone, cortisol, estradiol, testosterone, dihydrotestosterone, FSH, LH

Results:

Mortality: no unscheduled deaths
Clinical signs: no signs reported
Body weights: no meaningful differences
Food consumption: increased in reversal period in HD group
Ophthalmoscopy:not done
Electrocardiography:not done
Hematology: recovery period↓RBC ↓Hb, ↓HCT,↓MCH that increased over recovery period
Clinical chemistry: no toxicologically significant findings

Urinalysis:increased sodium and chloride excretion consistent with pharmacology, ↑Ca+P excretion at end of recovery?

Organ weights:dose-dependent ↓in prostate weight(absolute and normalized) that resolved by the end of recovery period. Seen also w/ spironolactone comparison group.

Dose(mg/kg/day)	Prostate weight (g)	Prostate/final body weight
0	8.1±2.72	6.9±2.14
SC-66110 1.5	7.5±3.50	6.1±2.78
5.0	5.2±2.17	4.5±1.54
25.0	4.7*±0.57	3.98*±0.48
Spironolactone 5.0	3.9**±2.11	3.1**± 0.41
*, ** significantly different from control at 5% or 1% respectively.		
0 (end of reversal phase)	10.3±3.69	7.5±2.09
SC-66110 25.0	9.5±3.30	7.2±2.68

Gross pathology:

Histopathology: decreased glandular development of prostate with both drugs, adrenal ZG hypertrophy. Adrenal changes were not completely resolved by the end of the recovery period.

Toxicokinetics:AUC for SC-66110 was 3-6X > SC-70303.Plasma levels found at all doses, albeit non-proportionally with decreased levels at HD.Mean AUC ranged from 0.5 -6.3X the human therapeutic exposure(M3001079, p.32).

Hormones: dose-related increase in serum aldosterone and serum DHT but not tissue hormone levels

Study title: Twenty-six week gavage toxicity study of SC-66110 in the rat (SA4516MSE-N96085)

Key study findings: Findings in both sexes included hepatomegaly, thyroid hypertrophy/hyperplasia and increased incidence and severity of chronic progressive nephropathy. Serum aldosterone and TSH were increased in both sexes. Changes in hematology were probably referable to effects on kidneys. Changes in electrolytes and clinical chemistry were consistent with pharmacology. Females consistently had twice the plasma exposure of males at the same dosages. Plasma exposure at the HD in both sexes decreased over the course of the study. Plasma exposure in males and females was 6.6X and 16.5X the human values (M3001079, p 30).

Study no: SA4516 — N96085
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:July 16, 1996
GLP compliance:yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and %

Dosage (mg/kg/day)	No./Sex	Endocrine Screen	Wk 27 Necropsy	Wk 40 Necropsy
Toxicology Animals				
0	50	25	15	10
30	35	20	15	
100	35	20	15	
500	50	25	15	10
Pharmacokinetic Animals				
0	5			
30	20			
100	20			
500	20			

purity:SC-66110, lots RCT10015 and RCT10056
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80

Methods (unique aspects):
Dosing:

Species/strain: Rat, Sprague-Dawley
#/sex/group or time point (main study): see table
Satellite groups used for toxicokinetics or recovery:see table
Age: 6 weeks
Weight:131-183
Doses in administered units: 0,30,100 and 500 mg/kg/day
Route, form, volume, and infusion rate: daily oral gavage

			Group (Target Number of Animals)			
Type of Exam	Period	Study Interval	N	1	2	3
Hematology, Clotting Potential, Clinical Chemistry	3	Wk 12/13	25	15	15	25
	4	Wk 26/27	25	15	15	25
	5	Wk 40	10	-	-	10
Abbreviated Urine Chemistry	1	Wk 5	10	10	10	10
	2	Wk 7/8	25	15	15	25
Complete Urine Analyses	3	Wk 12/13	25	15	15	25
	4	Wk 26/27	25	15	15	25
	5	Wk 40	10	-	-	10
Endocrine Assays	-	Day 8	5	5	5	5
	-	Wk 6	5	5	5	5
	-	Wk 12	5	5	5	5
	-	Wk 26	5	5	5	5
	-	Wk 40	5	-	-	5

Note 1: Animals for the endocrine assays were predesignated at the start of the study. All survivors were sampled at each interval for the remaining clinicopathologic exams except abbreviated urine chemistries were performed on 10 randomly selected animals at Week 5.
Note 2: Study Weeks (Wk) correspond to Days of Study in tables as follows: Wk 5 (Days 30-32); Wk 7/8 (Days 49-53); Wk 12/13 (Days 83-88); Wk 26/27 (Days 181-186); Wk 40 (Days 275-277).

Observations and times:

Clinical signs: daily
Body weights: pretreatment, twice weekly first month, weekly thereafter.
Food consumption:weekly
Ophthalmoscopy: pre-test, near end of treatment and end of recovery
EKG: not done
Gross pathology:
Organs weighed: yes
Histopathology: yes
Toxicokinetics: Day 1 and 177, 0.5, 1,2,3,4,6,8 and 24 hours after dosing
Other: EM analysis of liver, testes, uterus adrenal and thyroid
Clinical chemistry, urinalysis, hematology and endocrine assays: see table above.

Results:

Mortality: 1mC; 1mLD; 2mMD; 2mHD; 1fHD, most attributed to blood sampling and other causes. 2 HD males not determined.
Clinical signs: none attributed to drug
Body weights: no effect in males, significant ↓ HD f that did not reverse in recovery
Food consumption:↑in males in recovery
Ophthalmoscopy: end of tx, unilateral choroidal degeneration 1 MD m, 2/35 HD f and 3/33HD m: probably not drug-related
Hematology: microcytic, hypochromic anemia HD f; ↑ platelets m+f HD
Clinical chemistry: ↑chol + trig (m+f), tp, globulin, Ca, P (m+f),
Urinalysis:↑Ca, ↑Na, ↑Na/K ratio,↑P, ↑prot excretion (m+f)
Organ weights:↑liver and thyroid m+f, ↑kidney (f), ↑cecum + colon (f)

Gross pathology: enlarged liver, enlarged uterus

Histopathology: thyroid follicular cell hypertrophy/hyperplasia, pancreatic islet cell hyperplasia, chronic nephropathy

Hormones: ↑serum aldosterone,↑TSH m+f,

Toxicokinetics: AUC_{0-24} for total SC-66110

	Male		Female	
Dosage (mg/kg/day)	Day 1	Day 177	Day 1	Day 177
30	9.06	16.4	22.2	37.1
100	40.4	34.0	82.9	82.6
500	251	86.5	440	207

Toxicokinetics: the increase in exposure was slightly greater than proportional Day 1 and slightly less than proportional day 177. The exposure at the HD decreased over the course of the study.

Study title: Final Report for the 52-Week Capsule toxicity study with SC-66110 (SA4507)

Key study findings: Decreased prostate weights and decreased development histologically were reported. Increased adrenal weights at all male dosages and in HD f were associated with histologic evidence of hypertrophy of the zona glomerulosa. Dose-related increases in serum aldosterone and cortisol were reported for both sexes. There were no obvious sex-related differences in plasma drug levels. The final AUC values in males, from LD to HD, ranged from 0.63 – 20.3X the human values. The female exposure, from LD to HD, ranged from 0.7 – 24.6X the human value(M3001079, p33).

Study no: SA4507, [redacted]6127-305
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: June 27, 1996
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity:SC-66110, RCT 10055
Formulation/vehicle: gelatin capsules

Methods (unique aspects)**: dosed daily for 28 or 52 weeks with 12-week recovery period**
Dosing:

Species/strain: dog/beagle
#/sex/group or time point (main study):
Satellite groups used for toxicokinetics or recovery:
Age:
Weight:

Group	Dosage Level mg/kg/day	Number of Animals* Male	Female
1 (Control)	0	12	12
2 (Low)	1.5	8	8
3 (Mid)	5	12	12
4 (High)	100	12	12

*After 28 weeks of treatment, the first four dogs/sex/group from Groups 1 to 4 were sacrificed (interim sacrifice). The next four dogs/sex/group in Groups 1 to 4 were sacrificed following 52 weeks of treatment and the next four dogs/sex from Groups 1, 3 and 4 were sacrificed following at least a 12-week nontreatment recovery interval (recovery sacrifice).

Doses in administered units:
Route, form, volume, and infusion rate:

Observations and times:
Clinical signs: daily
Body weights:weekly
Food consumption:weekly
Ophthalmoscopy: pre-tx, week 26 and week 52
EKG: not done
Hematology: pre-tx and weeks 13, 26, 39, 52, 53, 57, 61 and 64
Clinical chemistry: pre-tx, weeks 13, 26, 39,52, 53, 57, 61 and 64
Urinalysis: pre-tx, weeks 12,25, 38 and 51
Gross pathology: yes
Organs weighed: yes
Histopathology: yes
Toxicokinetics: day 1, weeks 13,26 and 52 at 1,2,3,5,8 and 24 hours
Other: aldosterone, cortisol, estradiol, FSH, LH, testosterone and DHT day 3, weeks 4,13,26,39, 51 and recovery weeks 53, 57, 61 and 64.

Results: The major findings from this study were decreased prostate weight due to diminished development and increased adrenal weight due to hypertrophy of the zona glomerulosa cells. The latter may be due to compensatory response to drug-induced aldosterone receptor blockade. The effect on the prostate is incompletely understood. Based on the findings of reduced prostate development the NOEL from this study was 5 mg/kg/day in male dogs and 100 mg/kg/day in female dogs. The AUC for the NOEL of 5 mg/kg in male dogs = 14.1 µg.hr/ml. This is a multiple of 2.2X the human therapeutic exposure based on free eplerenone.

Summary of individual study findings: see above

Toxicology summary: General toxicology studies were conducted in the mouse, rat and dog. The response to eplerenone was pharmacologic (increased urinary Na/K ratio), adaptive (adrenal hypertrophy, hepatocellular hypertrophy) or toxicologic (increased incidence of chronic progressive nephropathy, prostatic atrophy).

Rats and mice showed hepatic adaptive changes related to induction of microsomal enzymes, CYP3A in particular and uridine diphosphate glucuronosyl transferase (UDPGT), the rate limiting enzyme in the conjugation of thyroxine for subsequent clearance, and inconsistently HMG CoA reductase, the rate limiting enzyme for cholesterol biosynthesis. There appeared to be several consequences to these hepatic changes. Serum cholesterol, triglyceride and total protein were increased in rodents. Increased thyroid weight with histologic correlates of hypertrophy/hyperplasia was reported in rats, ostensibly due to the increased clearance of T4 and responsive increase in TSH levels. This effect was observed consistently at dosages of ≥250 mg/kg/day. Studies that included drug-free recovery periods supported that this was a reversible phenomenon (discussed under Carcinogenicity). Thyroid hyperplasia and neoplasia in the rat has been reported in pre-clinical studies for a number of marketed drugs with similar hepatic/hormonal effects and have not been shown to be a problem in humans. There are species

differences that account for this, the foremost being the presence of a thyroxine binding protein in humans that gives T4 a half-life of several days in humans. Thyroxine in rats has a half-life of ≤12 hours, due to the lack of a binding protein. The thyroid hypertrophy is not expected to be relevant to humans.

Hepatocellular hypertrophy was noted consistently in rodents. Higher dosages (≥500 mg/kg) produced effects in a shorter time period than lower dosages given chronically.

Adrenal zona glomerulosa hypertrophy was also consistently noted in rodents and dogs. The adrenal ZG is the site of aldosterone synthesis. Hypertrophy of this area is probably an adaptive response to the pharmacological action.

The primary adverse effect in male dogs was prostate atrophy seen down to a NOEL of 5 mg/kg (approximately 2-3X the human therapeutic AUC_{0-24}). While not itself an endocrine organ, the prostate gland is under endocrine regulation. The proliferation and differentiation of the prostatic epithelium are primarily directed by androgens. Testosterone is locally converted to dihydrotestosterone (DHT) by the action of 5-α reductase. DHT has a greater affinity for the androgen receptor than does testosterone and is the primary mediator of prostatic growth.

Decreased prostatic size can be due to direct cytotoxicity, disruption of the hormonal regulation or indirectly through stress or loss of condition. Disruption of hormonal regulation may include decreased testosterone synthesis, inhibition of testosterone action or increased metabolism of testosterone. Drug-induced atrophy in laboratory animals has been reported for drugs such as goserelin (GnRH analogue), flutamide (non-steroidal anti-androgen), cimetidine (histamine H2 receptor blocker), cyproterone acetate, spironolactone and several benzodiazepines (Cheeke, Histopathology of Preclinical Toxicity Studies, 2nd Ed. 2000). Spironolactone, the first marketed aldosterone antagonist possessed anti-androgenic effects that in humans manifested clinically as gynecomastia and impotence. One clinical oncology text also notes that spironolactone, infrequently used even as a second line therapy in the management of prostate cancer, blocks steroidogenesis in the testes and adrenals by inhibitng the enzymes 17-αhydroxylase and 17,20-desmolase (Comprehensive Textbook of Genitourinary Oncology, 2nd ed.). Spironolactone was in fact used as a comparator compound in some of the studies conducted to elucidate eplerenone's effects on the prostate gland.

The sponsor evaluated 4 major hypotheses for the observed effects on the prostate.

1. Interference with conversion of testosterone to DHT (eplerenone inhibition of 5-α reductase)
2. Competitive antagonism of the androgen receptor
3. Decreased synthesis or release of testosterone
4. Aldosterone inhibition of 5α-reductase

Points not examined included the ratios of free and protein bound testosterone and effects upon the stability/activity of other enzymes involved in steroid synthesis and conversion. In a telephone conversation with the sponsor on 5/30/2002, this reviewer specifically asked whether eplerenone had been compared to spironolactone for effects on 17,20-desmolase. The sponsor

said no. The sponsor also confirmed that free and protein-bound testosterone ratios had not been evaluated.

To this end the sponsor examined in vitro the effects of eplerenone, spironolactone, canrenone, aldosterone and finasteride on 5-αreductase activity in homogenized dog prostate. The IC50 values generated from this study are shown in the sponsor's table below. As may be seen, eplerenone seems to have relatively low capacity to inhibit this enzyme when compared to 2 endogenous ligands and several currently marketed drugs.

Table 19. IC_{50} Values for Inhibition of Dog Prostate 5α-Reductase (P3097017)

Compound	IC_{50} in nM
finasteride	< 100
testosterone	120
aldosterone	2200
canrenone	3,500
spironolactone	30 000
eplerenone	>100 000 nM

Relative binding of the same compounds as above and SC-71597, (6β-OH eplerenone, a major metabolite) to the androgen receptor was also analyzed. The results from this are summarized in the sponsor's table.

Table 20. Relative Binding Affinity to Dog Prostate Androgen Receptors (P3196046)

	IC_{50}		Estimated Ki	RBA*
	3.5 nM ^{3}H-testosterone	2.5 nM ^{3}H-DHT		
DHT	7.9 nM	2.5 nM	3.6 nM	100
testosterone	20 nM	32 nM	18 nM	21
spironolactone	133 nM	100 nM	88 nM	4.3
canrenone	1,000 nM	1,200 nM	720 nM	0.53
aldosterone	60 000 nM	31,000 nM	33 000 nM	0.012
eplerenone	69 000 nM	160,000 nM	55 000 nM	0.0062
SC-71597 (6β-OH eplerenone)	>1,000,000 nM	>1,000,000 nM	>630,000	<0.001
*Relative binding affinity				

A study using isolated human androgen receptors showed eplerenone did not bind to the receptor at up to 1000 nM. Spironolactone and canrenone had IC50 values of 220 nM and 380 nM respectively.

In summary, eplerenone shows the ability to bind to the pertinent receptors and to displace DHT from its receptor. However, eplerenone's in vitro binding affinity to the various receptors appears to be lower than spironolactone's or that of any of the endogenous ligands.

A small in vivo study used GnRH to examine integrity of the endocrine status and pooled serum samples to decrease some of the variability due to pulsatile testosterone release (P31E4554, discussed later in this review). While there was variability in the results, mean DHT after 12 days of treatment was lower than the pre-treatment values. The animals showed appropriate hormonal responses to GnRH stimulation.

Dihydrotestosterone (DHT) (pg/mL)

Study Day / Animal No.	8.1*	8.2*	8.3*	8.4*	fold increase
1101					2
1102					2
1103					2
1104					4
1105					6
1106					4
1107					9
1108					6
Average	60.49	74.85	199.92	138.70	4
STD	27.85	38.70	63.11	55.38	2

Study Day / Animal No.	22.1*	22.2*	22.3*	22.4*	fold increase
1101					3
1102					2
1103					7
1104					7
1105					7
1106					8
1107					2
1108					4
Average	40.88	60.57	161.33	89.60	6
STD	31.00	40.73	64.08	46.12	5

DHT ND ≤ 0.38 pg/mL

* where x = Study Day and x.1 = value prior to GnRH stimulation, x.2 = value 15 minutes after GnRH stimulation, x.3 = value 60 minutes after stimulation, x.4 = value 90 minutes after stimulation.

** 'fold increase' is the multiple factor of the respective study day's highest value relative to the lowest value

As the prostate is the main source of DHT, the decrease in mean values may be reflective of the changes occurring in the prostate. While there is variability in the test system it seems reasonable to assume that the decrease in un-stimulated DHT on Day 22 is a real change. It is not clear if the duration of the study was sufficient to detect all changes.

An early 13-week study (4451) showed a decrease in absolute and normalized prostate weight in conjunction with decreased mean testosterone levels. The sponsor noted that the sampling did not take into account the pulsatile nature of testosterone release and therefore the change in testosterone levels could not be associated with drug treatment. However, random pulsatility producing the reported dose-related decrease in mean serum testosterone seems unlikely. The table below is reproduced from the original review.

Table 26

Effect of SC-66110 (15-300 mg/kg) for 13 Weeks on Prostate Weight in Male Dogs (Including 4-Week Reversal Period of High Dose Group)

Dose (mg/kg/day)	Prostate Weight (gms)	Prostate/Final Body Weight	Serum Testosterone (ng/ml)
0	8.0 ± 2.3	7.1 ± 1.5	2.4 ± 2.5
15	4.7 ± 2.3	4.5 ± 2.4	2.3 ± 2.1
100	2.8 ± 1.1	2.8 ± 1.1	0.6 ± 1.1
300	1.9 ± 0.4	1.8 ± 0.3	0.4 ± 0.4
300 (4-Wk Reversal)	3.5	3.3	0.6

A later 13-week study in dogs examined reproductive behavior, scrotal width, semen volume, semen protein content, sperm motility, serum hormones, histopathology and tissue (testes and prostate) hormones. Ultrasound examination indicated detectable prostate atrophy from week 2 in the 25 mg/kg/day group. Decreasing organ size in this group was accompanied by a decrease in semen volume. Prostatic levels of aldosterone were increased at this dosage as were testicular levels of aldosterone and testosterone.

Prostate Hormone Levels

	Group 1		
	T (ng/g)	Ald (pg/g)	DHT (ng/g)
Mean	5.95	92.34	1.72
S.D	3.22	19.83	0.50

	Group 2		
	T (ng/g)	Ald (pg/g)	DHT (ng/g)
Mean	3.52	128.35	1.48
S.D	1.99	56.13	0.56

	Group 3		
	T (ng/g)	Ald (pg/g)	DHT (ng/g)
Mean	4.185	232.83	1.51
S.D	1.97	119.78	0.64

Testis Hormone Levels

	Group 1		
	T (ng/g)	Ald (pg/g)	DHT (ng/g)
Mean	1775.46	232.96	19.33
S.D	1369.96	152.19	15.86

	Group 2		
	T (ng/g)	Ald (pg/g)	DHT (ng/g)
Mean	821.67	135.89	9.65
S.D	1545.34	154.67	17.00

	Group 3		
	T (ng/g)	Ald (pg/g)	DHT (ng/g)
Mean	2165.18	445.06	24.81
S.D	2459.86	257.68	27.97

The tissue levels of hormones are more critical than the serum levels in the maintenance of function. Therefore the above results provide support that neither testosterone synthesis nor 5α-reductase activity were affected.

The 1-year dog study(SA4507) used dosages of 0, 1.5, 5 and 100 mg/kg/day. Organ weight data showed decreased mean prostate weight and prostate-to-body-weight ratios in the HD group males in Weeks 26 and 52. After the 12-week recovery period there were no differences in prostate weights between controls and HD males.

The hormonal data for this study was presented as the "inverse of the transformed mean" (ITM) due to the variability of results and numerous values below the sensitivity of the assay. The ITM data was derived at each time point by 1) transforming the individual data points to normal scores (a function of their ranks in the data set); 2) calculating the mean of the normal scores for each group; 3)re-transforming the calculated mean back to the original data scale.

Cortisol at weeks 1,4,13, 26, 39 and 51 (all points of measurement) showed dose-dependent, statistically significant ($p<0.001$) increase with drug-treatment in both sexes. This may be related to the effects of mineralocorticoid receptor antagonists upon a subset of GR as will be discussed later.

FSH at week 4 showed significant ($p<0.05$) dose-related increases in the MD and HD groups and non-significant increases week 13. FSH was not measured after week 13 due to problems with the reagents.

At weeks 13 and 26 there was approximately a doubling of the LH value in HD males.

Testosterone was significantly ($p<0.05$) increased in all drug-treated groups at week 13 and again ($p<0.05$) at week 53 in the MD and HD males.

DHT was significantly ($p<0.05$) increased in all drug-treated males at week 13 and non-significantly increased in all drug-treated males at week 26. A significant ($p<0.01$) decrease was reported week 39 in the HD males. By week 51 the MD and HD males showed slightly lower levels than the control group. Non-significant fluctuations were reported for the recovery period.

The variability of results makes interpretation difficult but several possibilities exist. The striking dose-related increase in cortisol presents the possibility of stress-related atrophy although there were no clinical signs to indicate hypercortisolism. Another possibility concerns the subtypes of glucocorticoid receptors. It has been demonstrated that mineralocortcoid antagonists can block glucocorticoid receptors leading to changes in the hypothalamic-pituitary-adrenal axis (HPA). Human studies have demonstrated that single doses of spironolactone can cause significant increases in plasma cortisol. Multiple doses have been reported to produce sustained elevations in cortisol. Stress-induced cortisol secretion can further affect the GR. Age of the patient also seems to influence magnitude of the response (Young et al, 1998, J Clin End Metab pp3339-3345; Heuser et al., 2000 Neurobiol Aging, pp585-589). The changes in testosterone DHT and LH becoming apparent at the same time suggest the possibilities of 1) a new hormonal equilibrium or 2) process error in the sample handling. It is possible that the new hormonal

equilibrium is in some measure secondary to the increased cortisol levels. Because decreased prostatic size has been reported after 2 weeks of treatment, it seems more probable that this is a cortisol related effect rather than directly associated with androgen changes. One could also make a case for the local effects of increased tissue aldosterone on the androgen receptor. Note: In the May 30, 2002 telephone conversation the sponsor was asked about their hypothesis of eplerenone rather than aldosterone causing prostatic atrophy by competitively binding to the androgen receptor . The basis of this hypothesis was that the in vitro tissue levels of aldosterone were considered by the sponsor to be un-reflective of the in vivo situation. The data does not point to a definitive mechanism for the prostate effect. However, there are other biological control points that could be examined. On a mg/kg basis, eplerenone is less potent than spironolactone in causing prostate atrophy.

Hormonal and reproductive organ effects were reported for the rat. An early study conducted at Ciba-Geigy produced changes discussed in the original review:

> Evaluations of gonadal tissue from the male rats showed a 36% increase in the activity of the testosterone synthetic enzyme 17α-hydroxylase and an 80% increase in testicular cytochrome P450 content at both doses. At 300 mg/kg, there was a 63% reduction in plasma testosterone levels. These changes are consistent with induction of microsomal enzymes in both liver and testes with resultant decreases in circulating testosterone levels and increases in testosterone synthetic activity.

Another early study in dogs also indicated an induction of increased testosterone catabolism in testicular tissue. After 14 days of 100 mg SC-66110/kg/day, canine testicular CYP 450 content was increased 53% and 17α-hydroxylase 72% compared to controls. Increased mRNA expression was subsequently demonstrated.

Prostate atrophy in rats was also noted in a 13-week ad lib feeding study and a 4-week restricted diet study. Other hormonal and reproductive organ effects were reported for rats. For example, in the standard 2-year rat carcinogenicity assay, there were non-significant increases in testosterone in all drug-treated groups at 1 month. At 3 months, there was a non-significant increase in the LD group (20 mg/kg/day), and significant increases in the MD ($p<0.05$) and HD groups ($p<0.01$).
At 6 months there were no differences from control and at 10 months there was a non-significant decrease in values. Organ weight changes were recorded for seminal vesicles, epididymides and testes.

Reviewer's summary of male reproductive organ weight changes for standard 2-year CA study

Organ affected	Dosage mg/kg/day			
	0	20	75	250
Small/atrophied epididymides	3/85	4/85	6/85	9/85
Atrophied seminal vesicles	17/85	13/85	19/85	28/85
Atrophied testes	10/85	7/85	12/85	13/85

The two-year CA study with dietary restrictions also showed effects upon the male reproductive system.

Reviewer's summary of male reproductive organ weight changes for 2 year CA with dietary restriction study.

Organs affected	Dosage (mg/kg/day)	
	0	250
Atrophied prostate	1/85	6/85
Atrophied seminal vesicles	3/85	11/85

The above effects have also been reported for spironolactone.
The data overall indicate the disruption of the hormonal axis and perhaps establishment of a new equilibrium. Studies with recovery periods have shown the effects to be reversible.

A rat-specific toxicological effect was the increased incidence and severity of chronic progressive nephropathy. This was used as one of the determinants of the maximally tolerated dose in the carcinogenicity study. Increased incidence and severity of CPN was especially evident in female rats. A dose of 250 mg/kg/day (~14X the human exposure) for 1 year produced 26% and 33% increases in absolute and relative renal weight compared to control animals. The incidence was 7/10 control females versus 9/9 drug-treated females. One may ask whether the sex-related differences in metabolism contributed to this effect. Female rats had higher oral bioavailability than males, 66% vs 26% respectively and greater plasma exposure as measured by AUC than male rats at the same dosages. Average body burden for females was approximately 2-3X that measured in males at the same dosages in the carcinogenicity study. Male rats also metabolized ~75% of a given dose while female rats excreted ~75% unmetabolized parent drug.

Toxicology conclusions: The primary toxicological effect in rats was increased incidence and severity of CPN. An effect seen in both dogs and rats was prostatic atrophy. Both species showed adjustment of the hormonal axes.
It appears that eplerenone has the potential to cause the same side effects in humans as caused by spironolactone. However, there is data to suggest that eplerenone is less potent than spironolactone at producing these effects.

Histopathology Inventory for NDA #21437

Study	EX4466	SA4513	845093	EX4431	SA4453	SA5143	845072	SA4451	P30S4512	SA4516	SA4507
Species	Mice	Mice	Rats	Rats	Rats	Rat	Dog	Dog	Dog	Rat	Dog
Adrenals	X	X*	X*	X	X*	X*	X*	X*	X*	X*	X*
Aorta		X	X		X	X	X	X	X	X	X
Bone Marrow smear	X	X	X		X	X	X	X	X	X	X
Bone (femur)			X		X			X	X	X	X
Brain	X	X*	X*		X*	X*	X*	X*	X	X*	X*
Cecum	X	X*	X		X*			X*	X*	X*	X*
Cervix	X				X					X	X
Colon	X	X*	X		X*	X	X	X*	X	X*	X*

Duodenum	X	X	X	X	X	X	X	X	X	X	X
Epididymis		X*	X		X*	X	X	X*	X*	X*	X*
Esophagus		X	X		X	X	X	X	X	X	X
Eye		X	X		X	X	X	X	X	X	X
Fallopian tube										X	
Gall bladder						X	X	X	X		X
Gross lesions	X	X	X		X	X	X		X		X
Harderian gland			X								
Heart	X*	X*	X	X	X*	X*	X*	X*	X	X*	X*
Ileum	X	X	X	X	X	X	X	X	X	X	X
Injection site											
Jejunum	X	X	X	X	X	X	X	X	X	X	X
Kidneys	X*	X*	X*	X	X*	X*	X*	X*	X	X*	X*
Lachrymal gland											
Larynx											
Liver	X*		X*	X	X*	X*	X*	X*	X*	X*	X*
Lungs	X	X*	X		X*	X	X	X*	X	X*	X*
Lymph nodes, cervical											
Lymph nodes mandibular					X	X					
Lymph nodes, mesenteric		X	X		X	X	X	X	X	X	X
Mammary Gland		X			X	X	X	X		X	X
Nasal cavity											
Optic nerves		X			X		X				
Ovaries	X*	X*	X*		X*	X*	X*	X*		X*	X*
Pancreas		X	X		X	X	X	X	X	X	X
Parathyroid	X	X				X	X	X			X
Peripheral nerve											
Pharynx											
Pituitary	X	X*	X*	X	X*	X*	X*	X*	X	X*	X*
Prostate	X*	X*	X*		X*	X*	X*	X*	X*	X*	X*
Rectum		X				X					
Salivary gland		X	X		X	X		X	X	X	X
Sciatic nerve		X	X		X	X	X*	X	X	X	X
Seminal vesicles		X	X*		X	X				X	
Skeletal muscle		X	X		X	X	X	X	X	X	
Skin		X	X		X	X	X	X	X	X	X
Spinal cord		X			X	X	X	X	X	X	X
Spleen	X	X*	X*		X*	X*	X	X*	X	X*	X*
Sternum	X	X								X	X
Stomach		X*	X		X*	X*	X	X*	X	X*	X*
Testes	X*	X*	X*	X	X*	X*	X*	X*	X*	X*	X*

Thymus	X*	X*	X*	X	X*	X*	X*	X*	X*	X*	X
Thyroid	X*	X*	X*	X	X*	X*	X*	X*	X	X*	X*
Tongue		X			X	X		X	X	X	X
Trachea		X	X		X	X	X	X	X	X	X
Urinary bladder		X	X		X	X	X	X	X	X	X
Uterus	X*	X*	X		X*	X*	X	X		X*	X
Vagina		X			X	X		X		X	X
Zymbal gland											
Standard List											

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

Study title: Bacterial mutagenicity test for CGP 30083

Key findings: No positive findings of mutagenicity.

Study no:SP577
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: unknown
GLP compliance: no
QA reports: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity:

Formulation/vehicle: DMSO

Methods:

Strains/species/cell line: TA 100, TA1537, TA98, E. coli WP2 uvrA
Dose selection criteria:
Basis of dose selection: solubility
Range finding studies: not shown
Test agent stability:
Metabolic activation system: S9
Controls:
Vehicle: DMSO
Negative controls:
Positive controls:N-methyl-N'-nitro-N-nitrososguanidine, N-ethyl-N'-nitro-N-nitrososguanidine, benzo (a)pyrene, 3-methylcholanthrene and 2-aminoanthracene

Comments:
Exposure conditions:
Incubation and sampling times: 2-3 days
Doses used in definitive study: 5, 15.8, 50, 158, 500 and 1580 µg/plate

Study design:
Analysis:
No. of replicates:2-3
Counting method: unknown
Criteria for positive results:

Results: The study would not be considered valid by today's standards. However, the compound was negative for mutagenicity under the conditions of the assay.

Study title: Evaluation of the mutagenic potential of SC-66110 in the Ames Salmonella/microsome assay

Key findings: SC-66110 was not considered mutagenic in this test system

Study no: SA4475
Study type (if not reflected in title)**:**
Volume #, and page #:
Conducting laboratory and location: GD Searle and Co
Date of study initiation: 1/31/96
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110 lot RCT-9967

Formulation/vehicle: DMSO

Methods:
Strains/species/cell line: TA97a, TA98, TA100, TA1935, TA102
Doses used in definitive study: 10-5000 µg/plate

Results: SC-66110 was not considered mutagenic in this test system.

Study title: An evaluation of the mutagenic potential of SC-66110 for impurity qualification in the bacterial reverse mutation assay (SA5099)

Key findings: SC-66110 was not considered mutagenic under the conditions of the assay

Study no: SA5099
Study type (if not reflected in title)**: Ames**
Volume #, and page #:

Conducting laboratory and location: GD Searle and Co., Skokie, IL
Date of study initiation: March 6, 2001
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110, lot 00K007-F5A
Formulation/vehicle: *DMSO*
Methods:

Strains/species/cell line: E. coli WP2uvrApKM101 and S. typhimurium TA97a, TA98, TA100, TA1535
Dose selection criteria: solubility and prior experience
Basis of dose selection:
Metabolic activation system: S9
Controls:
Vehicle: DMSO
Positive controls: Acridine, nitrofluorene, sodium azide, N-ethyl-N-nitro-N-nitrosoguanidine, aminoanthracene
Doses used in definitive study: 10, 50, 100, 500, 1000 and 5000µg/plate
Criteria for positive results: 2-3X vehicle control

Results: SC-66110 was not considered mutagenic under the conditions of the assay.

Study title: Ames/Salmonella mutagenicity assay of SC-66110 (epoxymexrenone) spiked with 5% of SC-67246

Key findings: The substance(s) were negative under the conditions of the assay.

Study no: EX-4388/ — 95039
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: [redacted]

[redacted]

Date of study *initiation*: 5/2/95
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110 lot GDS-4268-187B spiked with the synthetic immpurity SC-67246 , to a final concentration of 5%

Formulation/vehicle:

Methods:

The above material was tested in S.typhimurium strain TA102 ±S9. Results showed a 1.3 fold increases over control, which is not, considered significant (<2-fold increase). Results of studies (EX-4444) using SC-66110 spiked with SC-67246 at 0.5% in the other four strains (TA97a, TA98, TA100 and TA1535) were negative as well. Appropriate positive controls were used.

Results: The assay showed a 1.3-fold increase over control, which was not considered significant (less than a 2-fold increase).

Study title: Final Report Amendment No 1: Evaluation of the mutagenic potential of SC-66110 spiked with 0.5% of SC-67246 in the Ames Salmonella/microsome assay

Key findings: The material tested was negative under the conditions of the assay.

Study no: EX4444
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: GD Searle and Co., Skokie, IL
Date of study initiation: October 3, 1995
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: lot GDS4790-058A

Formulation/vehicle:

Methods:

SC-66110 spiked with the impurity SC-67246 at 0.5% was tested in S. typhimurium strains TA97a, TA98, TA100, TA102 and TA1535 ±S9 activation at concentrations of 10, 50, 100, 500, 1000 and 5000 µg/plate.

Results: The material tested was negative under the conditions of the assay.

Study title: Searle Abstract Amendment No.1: Mutagenicity test on SC-66110 in the L5178Y TK+/- mouse lymphoma forward mutation assay with duplicate cultures

Key findings: Due to solubility limitations the level of cytotoxicity usual for this type of assay was not achieved. However, under the conditions of the assay the test substance was negative for mutagenicity.

Study no: EX4138
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: August 9, 1993
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, radiolabel and % purity: B90081

Formulation/vehicle: culture medium

Methods:

SC-66110 at concentrations from 125 µg/ml to 750 µg/ml were tested ±S9 activation. Appropriate positive controls were used.

Results: Due to solubility limitations the level of cytotoxicity usual for this type of assay was not achieved. However, under the conditions of the assay the test substance was negative for mutagenicity.

Study title:L5178YTK+ Mouse lymphoma forward mutation assay for SC-66110

Key findings: SC-66110 when tested at concentrations exceeding the limits of solubility did not induce an increase in mutant frequencies in L5178Y cells when tested ±S9 activation.

Study no: SA4672/ [redacted] 18781-0-431
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 8/11/97
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: lot 96K018-F2B

Formulation/vehicle: culture medium

Methods: Preliminary studies were conducted to determine cytotoxic concentrations. When tested at up to 2500 µg/ml for four hours, SC-66110 showed weak cytotoxic activity (30-40% cytotoxicity ±S9). The highest concentration tested was determined by limits on solubility. L5178Y cells in duplicate cultures were treated with SC-66110 for four hours at concentrations ranging from 31.3 to 750 µg/ml. Negative controls were treated with vehicle (medium) and positive controls were treated with methyl methane sulfonate (MMS, -S9) or with methyl cholanthrene (+S9). A positive response was defined as a 2-fold increase in mutant frequency over background.

Results: Cytotoxicity was not observed at any of the concentrations tested. The mutation frequency of SC-66110- treated cells was increased less than 2-fold compared to the negative control cultures. The positive controls MMS and MCA induced approximately 6 to 7-fold increases in mutation frequencies. SC-66110 when tested at concentrations exceeding the limits of solubility did not induce an increase in mutant frequencies in L5178Y cells when tested ±S9 activation.

Study title: An evaluation of the potential of SC-66110 to induce chromosome aberrations in vitro in Chinese hamster ovary (CHO) cells (SA4474)

Key findings:

Study no: PSA96S-SA4474
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: GD Searle and Co., Skokie, IL
Date of study initiation: 2/5/96
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: lot RCT-9967

Formulation/vehicle: McCoy's medium

Methods: SC-66110 was tested up to concentrations of 625 µg/ml ±S9. The highest concentration was determined by solubility. Appropriate positive controls were used.

Results: At the highest concentration there was 19% cytotoxicity. There were no increases in chromosomal aberrations at any dose level (200 metaphase cells were analyzed).

Study title: An evaluation of the potential of SC-66110 to induce chromosome aberrations in Chinese Hamster Ovary (CHO) cells

Key findings: When tested to the limits of solubility, the drug did not produce an increase in chromosomal aberrations under the conditions of the assay.

Study no: SA5135 and 22373-0-437
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: April 11, 2001
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110, lot 00k007-F5A, with impurities PHA-439698 and PHA-430700

Formulation/vehicle: McCoy's 5a culture medium

Methods: Duplicate cultures of cells were treated with concentrations of 125, 250, 375, 500, 625 and 800 µg/ml for 3 and 17.5 hours without S9 and for 3 hours with S9. Concentrations ≥375 µg/ml contained a suspension.

Summary of individual study findings: When tested to the limits of solubility the compound did not produce an increase in chromosomal aberrations under the conditions of the experiment.

Study title: An evaluation of the potential of SC-66110 to Induce micronucleated polychromatic erythrocytes in the bone marrow of rats (micronucleus test)

Key findings: Under the conditions of the study an increase in micronuclei was not detected.

Study no: SA4674
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: GD Searle, Skokie, IL
Date of study initiation:
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110, lot 96K018-F2B

Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in distilled water

Methods: Rats were orally gavaged with SC-66110 at doses of 0, 500, 1000 and 2000 mg/kg. The bone marrow was sampled at 24 and 48 hours after dosing. The bone marrow for the positive control (cyclophosphamide) was sampled at the 24-hour time point. Slides of marrow cells were prepared from 5 rats/sex/timepoint for each group. The results are presented as the individual readers' assessments of individual animals rather with no compilation and summary.

Summary of individual study findings: Under the conditions of the study an increase in micronuclei was not detected.

Study title: In vivo/in vitro unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints with SC-66110 (SA4670)

Key findings: Unscheduled DNA synthesis was not reported for rat hepatocytes following in vivo treatment with SC-66110.

Study no: SA4670/18781-0-494
Study type (if not reflected in title):
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 8/21/1997
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel and % purity: SC-66110 lot 96K018-F2B

Formulation/vehicle: 0.5% methylcellulose plus 0.1% polysorbate 80

Methods: Male Sprague-Dawley rats (4/group) were given SC-66110 at 500, 1000 or 2000 mg/kg by oral gavage. Controls received vehicle or the positive control of N-dimethylnitrosamine (DMN). Hepatocyte cultures were prepared at two timepoints after drug administration, 2-4 hours and 15-16 hours, by perfusion of livers with collagenase. Viable hepatocytes were then cultured

and labeled with ^{3}HTdR(tritiated thymidine) for 4 hours. After a cold thymidine chase, cells were cultured for an additional 17-19 hours. Cells were fixed and nuclear labeling analyzed by autoradiography. A positive response was defined as (1) a mean increase of 3 grains per nucleus above control counts or (2) a 10% increase in the mean percent of nuclei with five or more grains per nucleus above control counts.

Results: When compared to control cultures, SC-66110 did not increase either the mean net nuclear grain count or the percentage of cells with ≥5 mean net nuclear grains when tested at either the 2-4 hour or 15-16 hour timepoints. The positive control produced approximately a 20-30-fold increase in grain counts using both criteria.

Genetic toxicology summary: SC-66110 has been tested in the Ames assay, the mouse lymphoma assay, the chromosome aberration (CHO cell) assay and Unscheduled DNA synthesis assay. The impurity SC-67246 has been tested in the Ames assay at concentrations up to 5%. The impurities PHA-439689 and PHA-439700 have been tested in the chromosome aberration assay. Under the conditions of the various assays, neither SC-66110, the metabolites produced by the S9 activation system or the impurities have tested positive.

Genetic toxicology conclusions: In the assays listed above, SC-66110, the metabolites produced by S9 activation and the 3 impurities listed, have not produced genotoxic results.

Labeling recommendations: Acceptable as written.

VI. CARCINOGENICITY:

Study title: Twenty-Six week gavage toxicity study of SC-66110 in the C57BL/6TacfBR-[KO]p53

Key study findings: This alternative model was chosen with prior concurrence of the CAC because of murine-specific metabolites of unknown genotoxicity. The sensitivity of this model to genotoxins made it an appropriate alternative choice. There were no findings of significance in the study.

Study number: SA4848
Volume #, and page #:
Conducting laboratory and location: GD Searle, Skokie, IL
Date of study initiation: December 15, 1998
GLP compliance: yes
QA report: yes (x) no ()
Drug, lot #, and % purity:SC-66110 lots 96K018-F3A, SP3804, Heumann lot98-12060
CAC concurrence: yes

Study Type (2 yr bioassay, alternative model etc.): **alternative model**