Species/strain: mice, p53 knockouts (heterozygous)
Number/sex/group; age at start of study:15/sex/group in main group, 47/sex in tk satellite, 15/sex in positive control group
Animal housing: individual suspended stainless steel mesh
Formulation/vehicle: 0.5% methylcellulose and 0.1% Tween®80 in distilled water.
Drug stability/homogeneity: evaluated prior to study initiation at concentrations higher and lower than those used.

Methods:

Doses: 100, 300 and 1000 mg/kg/day
Basis of dose selection: MTD
Restriction paradigm for dietary restriction studies:
Route of administration: oral
Frequency of drug administration: daily
Dual controls employed:
Interim sacrifices:
Satellite PK or special study group(s): yes
Deviations from original study protocol: no
Statistical methods: unconventional. See Biostatistician's review.

Observations and times:

Clinical signs: daily
Body weights: pre-tx and weekly
Food consumption: weekly
Hematology: no
Clinical chemistry: no
Organ weights: yes
Gross pathology: yes
Histopathology: yes
Toxicokinetics: day 1, week 14 and week 26. Samples collected 1,2,4,8 and 24 hours post-dose

Results: Detailed discussion in original review

Genotype analysis confirmed the p53 status of the mice. The positive control of p-cresidine produced a robust neoplastic effect of transitional cell carcinoma in 13/15 males and 7/15 females. The doses of eplerenone used caused decreased weight gain of 7% in the HD males and 10% in the HD females. Plasma levels of drug were found at all dosages. The increases in exposure with increasing dose were not proportional. There was evidence of decreased exposure from Day 1 to Week 26 at all doses, consistent with induction of metabolism.

Unbound eplerenone, Day 177

Dosage Mg/kg/day	Females		Males	
	AUC µg.hr/ml	Multiple of human exposure	AUC µg.hr/ml	Multiple of human exposure
100	15.9	1.9X	10.8	1.3X
300	35.1	4.2X	38.6	5.0X
1000	59.3	7.1X	88.8	10.6X

AUC for humans at the therapeutic dose of 100 mg was listed as 6.48 µg.hr/ml

Absolute and normalized weight of the liver, thyroid, adrenal and pituitary glands were increased in both sexes in a dose related manner as shown in the sponsor's table. The same organs were also affected in the positive control group.

SC-66110-Related Organ Weight Changes

Dosage: (mg/kg/day)	0 mg/kg/day		100 mg/kg/day		300 mg/kg/day		1000 mg/kg/day	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Males								
Liver	1.44	4.73	1.46	4.75	1.48	5.29***	1.85***	6.53***
Thyroid Gland	0.003	0.093	0.003	0.110	0.003	0.118	0.004*	0.144**
Adrenal Glands	0.005	0.159	0.005	0.184	0.007***	0.249***	0.006***	0.228***
Pituitary Gland	0.0017	0.0560	0.0021	0.0668	0.0024	0.0845	0.0012**	0.0413*
Females								
Liver	1.16	4.67	1.26	4.90	1.26	5.22**	1.38*	6.10**
Thyroid Gland	0.004	0.156	0.004	0.151	0.004	0.179*	0.006***	0.257***
Adrenal Glands	0.008	0.336	0.008	0.303	0.010**	0.426***	0.011***	0.457***
Pituitary Gland	0.0028	0.1100	0.0027	0.1033	0.0021*	0.0904	0.0023*	0.1006

Abs. = absolute organ weight (g), Rel = relative organ weight (to body weight, %)
*p<0.05; **p<0.01; ***p<0.001

CDER statistical analysis indicated no neoplastic findings of significance.

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: study was adequate in design and conduct

Evaluation of tumor findings: CDER statistical analysis indicates no significant findings.

Study Title: Four-Week Oral Gavage Range Finding Toxicity Study of SC-66110 With Dietary Restrictions in the Rat (P30S4566)

Key Study Findings: Dietary restriction did not significantly alter either the toxicokinetic profile or the toxicology when compared to a previously conducted 13-week study with ad libitum feeding. Some of the males showed atrophic prostates, females showed decreased uterine weights. The thyroid hypertrophy observed and the possible mechanism of increased TSH resulting from drug-induced increased catabolism is significant of potential thyroid neoplasias.

Study Number: P30S4566
Conducting laboratory and location: GD Searle & Co; Skokie, IL
Date of Study Initiation: November 11, 1996
GLP Compliance: yes
QA report:
Drug, lot # and % purity: SC-66110, lot RCT 10016,

Purpose: This study was conducted to determine whether dietary restriction in the rat would alter plasma concentrations and/or alter (lessen) any toxicity profiles as a prelude to conduct a two

year carcinogencity study with dietary restriction in rats in addition to conducting a carcinogenicity study with ad libitum feeding. Results were compared to a previously conducted 13-week toxicity study in rats without dietary restriction (reported in the general toxicology section).

Methods: Male and female [redacted] CD rats (115-250 grams, 10/sex/group) were given SC-66110 at 250, 500, 750 or 1000 mg/kg/day by oral gavage for 4 weeks. Controls received vehicle (0.5% methylcellulose and 0.1% polysorbate 80). An additional 16 rats/sex/dose were used for toxicokinetic determinations with 4 rats/sex used as untreated controls.

Observations: Rats were observed daily for clinical signs. Body weights were measured 2-3X per week. Days 29-31, rats in the toxicology groups were sampled for hematology and clinical chemistry and urine was collected for urinalysis. Rats were then euthanized and necropsy performed. Several organs were weighed and histopathology performed from control and HD groups on liver, kidney, thyroid, adrenal, prostate, epididymus, testis, ovary, uterus and vagina. Blood was collected from the toxicokinetic groups at several timepoints up to 24 hours after drug administration on days 1 and 28.

Results: Three deaths were attributed to gavage accidents. There were no significant drug-induced changes on clinical signs, body weights or hematology parameters when compared to controls.

Clinical chemistry results showed increases in cholesterol of 122% of control in HD females. Compared to the 13-week study, the current results showed some variability but were generally within observed ranges. Urinalysis in the present study showed decreased sodium excretion in HD females and decreased potassium excretion in females given ≥ 500 mg/kg. In the previous 13-week study no such changes were observed.

Organ weight data included drug-induced changes in liver, prostate, epididymus and uterus. Liver weights (absolute and liver: BW ratios) showed a dose-dependent increase in both sexes, particularly females with a 91% increase in HD females. Similar results were found in the 13-week study. Mean prostate weights were decreased 19-36%. Similar results on prostate weights were found in the 13-week study. Epididymus weights were decreased ~10% relative to controls. Uterine weights decreased ~30%. Similar effects on uterine weights were found in the 13-week study. There were no changes in thyroid or pituitary weights in the present study whereas weights of these organs increased in the 13-week study.

Histopathology indicated hepatocellular hypertrophy in 6/9 and 9/9 HD males and females respectively, correlating with increased liver weight. The sponsor suggested that the results were compatible with P450 enzyme induction, an effect known to occur with SC-66110 in rats although evidence for this was not presented in the present study. One prostate was considered atrophic, similar to observations from the 13-week study. Evidence for chronic progressive nephropathy was not apparent, opposite to findings from the 13-week study. The thyroid follicular cells showed a slight hypertrophy, similar to that observed in the 13 week study, and was proposed to be due to increased catabolism of thyroid hormone, without presenting evidence here to support this. The adrenal zona glomerulosa cells, the site of aldosterone production,

showed a slight hypertrophy as was found in the 13-week study and may reflect a compensatory response to inhibition of aldosterone by SC-66110.

Toxicokinetic analysis showed a dose-proportional increase in AUC up to 750 mg/kg followed by a plateau from 750 to 1000 mg/kg indicating saturation of absorption. Plasma drug levels were higher in female rats than in male rats. Day 1 values were higher than Day 28 values and similar to those seen in the 13-week study, indicating that dietary restriction had no effect on systemic exposures.

Study title: Two-year oral gavage combination chronic toxicity and carcinogenicity study of SC-66110 in the rat (MSE-N 97129/SA4663)

Key study findings: Thyroid tumors appeared that were anticipated based upon structural and mechanistic similarity of eplerenone to spironolactone. Ancillary studies were provided to support a mechanism of action that makes this phenomenon unlikely to be relevant to humans. The sponsor was asked to provide further data regarding the finding of renal tumors in females.

Study number: [redacted] -N97129/SA4663
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: June 25, 1997
GLP compliance: yes
QA report: yes (x) no ()
Drug, lot #, and % purity: SC-66110 lots 96K018-F2B and 96K020-F1A
CAC concurrence: yes

Study Type (2 yr bioassay, alternative model etc.): **2-year bioassay**
Species/strain: **rat, [Crl:CD(SD)BR]**
Number/sex/group; age at start of study: **85/sex/group, 6 weeks**
Animal housing: **individually housed in suspended steel cages. Waffle bottom cages for those who developed foot problems**
Formulation/vehicle: **0.5% methylcellulose and 0.1% polysorbate 80 in distilled water**
Drug stability/homogeneity: **upfront determinations of homogeneity with visual inspection during study. Stability and concentration tested during study at weeks 1,4,6,8,12 and every 12 weeks thereafter until the end of the study.**

Methods:

Doses: 0, 20. 75 or 250 mg/kg/day
Basis of dose selection: **MTD**
Restriction paradigm for dietary restriction studies:
Route of administration: oral gavage
Frequency of drug administration: daily
Dual controls employed: no
Interim sacrifices: week 53, 10rats/sex/group designated
Satellite PK or special study group(s): PK, 6 control rats, 16rats/sex/dose
Deviations from original study protocol: none that affected the interpretation of the study

Statistical methods: unconventional. See CDER statistical review

Observations and times:

Morbidity/mortality: ≥ once daily during the pretreatment period; twice a day during the treatment period. Detailed examinations were performed once pre-test and weekly during the study.

Body weight: determined twice during pretreatment, weekly during first 26 weeks, every other week from weeks 27-52, every 4 weeks thereafter and the week before scheduled completion of the study. Final fasted body weight was determined just prior to necropsy.

Food consumption: measured once pretreatment, weekly during the first 26 weeks of the study, every other week from weeks 27-52, every 4 weeks thereafter, and the week before scheduled completion.

Ophthalmic examinations: performed on all rats pretest and all survivors weeks 25 and 50.

Clinical Pathology: toxicology groups only

First 10 animals of each group were sampled for hormone assays at months 1,3,6 and 10 and for clinical pathology and euthanasia at week 53.
Samples for the hormone assays were taken from the retro-orbital sinus except at week 53 when blood was collected from the posterior vena cava prior to necropsy.

Endocrine parameters: aldosterone
Triiodothyronine (T3)
Thyroxine (T4)
Testosterone
Thyroid stimulating hormone (TSH)

Hematology:

Hematocrit (HCT)
Hemoglobin (Hb)
MCH, MCHC
MCV
Mean platelet volume (MPV)
Platelet counts (PLT)
RBC
WBC and differential

Clotting Parameters:

Prothrombin time (PT)
Activated partial thromboplastin (APTT)
Fibrinogen(FIB)

Clinical Chemistry:

ALT	CA	Phos	trig
ALB	Cl	K	total bile acids
Albumin/globulin	Chol	Na	
ALP	Crea	SDH	

AST Glob TBIL
BUN GLU Tprot

Urinalysis was conducted week 53
Parameters from 4-hour urine sample:

Volume	urobilinogen*
Osmolality	sediment
pH*	Bilirubin*
Protein*	Occult blood*
Glucose*	Ketones*

*semi-quantitative determination

Parameters from 22-hour urinalysis determination

Calcium	chloride
Creatinine	phosphorus
Potassium	protein
Sodium	volume

Toxicokinetics
Blood was collected after the first dose and again during week 52 for toxicokinetic evaluation. Sampling times were 0.5, 1,2,3,4,6, 8 and 24 hours after dosing. Each TK animal was sampled at two different sampling points.

Necropsy Procedures
Ten animals/sex/group were predesignated at the start of the study to be euthanized at week 53. All surviving Toxicology group animals were euthanized week 104. Organ weights were determined at week 53 only.

Results:

See original review for detailed discussion.

High dose females showed 9% lower survival at week 104 compared to controls. Survival in males was unaffected. There were significant decreases in body weight gain in both sexes indicating that maximally tolerated dosages had been used.

Summary of exposure based upon

Dosage mg/kg/day	Females		Males	
	AUC µg.hr/ml	Multiple of human exposure	AUC µg.hr/ml	Multiple of human exposure
20	16.2	1.9X	4.68	0.5X
75	45.0	5.3X	18.1	2.1X
250	100	11.7X	59.2	6.9X

SC-66110 was tested in rats at levels ~2X-12X the human AUC in females and ~0.5-7x the human AUC in males. C_{max} in females and males was ~4-15X and ~1.5-7X human values. The toxicokinetic results in this study are consistent with past results.

Expected pharmacologic changes of increased urinary sodium were reported. Eplerenone blocks aldosterone receptors as its primary pharmacologic action. Therefore, the increased aldosterone levels seen with drug-treatment are an expected response as is hypertrophy of the adrenal zona glomerulosa, the site of aldosterone synthesis.

Adaptive changes appeared in the liver as manifested by increased liver weight and hepatocellular hypertrophy.

The incidence and mean severity of chronic progressive nephropathy (CPN) as determined by histologic criteria increased in the MD and HD groups of both sexes. Data from hematology, clinical chemistry, urinalysis and histopathology of other organs further support declining functional capability of the kidneys.

Two other noteworthy non-neoplastic findings were degeneration/atrophy of the seminal vesicles and degeneration of the seminiferous tubules of the male rats. Serum testosterone levels taken at the interim euthanasia were slightly below the control values but not significantly so. Serum testosterone at the time of final (2 year) euthanasia would have been useful in distinguishing between a primary adverse effect of the drug and an effect secondary to decreased hormone levels. Pancreatic islet cell hyperplasia was noted to be increased in incidence in drug-treated females.

There were two neoplastic findings of interest in the study 1) A statistically significant, dose-related increase in the incidence of thyroid follicular cell adenoma and 2) renal tubular carcinoma in 2 control males and 2 HD females (p=0.045 by the sponsor's calculations).
Prior to initiation of the study, the sponsor stated to the CAC that thyroid tumors would not be unexpected given the structural similarity of eplerenone to spironolactone and proposed a hypothesis that the carcinogenicity was due to a hormonal mechanism. In support of this, the sponsor undertook to do studies simultaneously with the conduct of the carcinogenicity study. These ancillary studies, discussed below, provided further data on serum TSH, T_4, T_3, rT_3 clearance of T_4, activity of T_4-UDPGT, activity of CYP3A and mRNA for UDPGT.

Because the renal tumors are rare, the occurrence was of interest. The sponsor was asked (telephone, 9/18/2001) to provide the background rate. They report from 18 studies the following incidences:

Incidences of renal carcinoma in control animals from 18 studies

	Malignant	Benign and malignant
Males	3/1045	11/1045
Females	1/1051	3/1051

Another source of background rate for renal carcinoma was provided on the Charles River website in their document "Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD®(SD)BR Rats from Control Groups," March 2001. Twenty-four studies initiated between 1991 and 1997 at six different commercial testing facilities were surveyed and compiled. The reported incidences are summarized below:

Summary of Renal Carcinoma in Control Rats. Compiled by Charles River

	Adenocarcinoma/Tubular adenocarcinoma	Adenoma/tubular adenoma
Males	5/1531	8/1531
Females	2/1729	Not listed

The histopathology data was specifically examined by the reviewer for any listing of renal tubular hyperplasia which would be indicative of a continuum of potentially neoplastic changes.

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: adequate
Evaluation of tumor findings: The appearance of thyroid tumors had been anticipated due to the structural similarity to spironolactone. With the concurrence of the CAC the sponsor conducted several studies to support the species-specific hormonal mechanism of the thyroid tumors, reported under the "Special Toxicology" section. The sponsor was asked to do further investigation into the appearance of renal carcinomas in the female HD group. This involved step-sectioning of the kidneys from the rats in this study and further evaluation of the kidneys from the rats in the restricted diet study. A more detailed discussion will be provided below in the overall Carcinogenicity summary.

Study title: Two-year oral gavage combination chronic toxicity and carcinogenicity study of SC-66110 with dietary control in the rat (MSE-N97140/SA4664)

Key study findings: No histopathology was performed. The sponsor was asked to perform step sections in light of the renal tumors found in the standard (ad lib feeding) study.

Study number:. N97140/SA4664
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: July 29, 1997
GLP compliance: yes
QA report: yes (x) no ()
Drug, lot #, and % purity: SC-66110 lots 96K018-F2B and 96K018-F3A
CAC concurrence: yes

Study Type (2 yr bioassay, alternative model etc.)**: 2-year bioassay**
Species/strain: rat, [Crl:CD(SD)BR]
Number/sex/group; age at start of study:85/sex/group, 6 weeks
Animal housing: individually housed in suspended steel cages. Waffle bottom cages for those who developed foot problems
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in distilled water
Drug stability/homogeneity: upfront determinations of homogeneity with visual inspection during study. Stability and concentration tested during study at weeks 1,4,6,8,12 and every 12 weeks thereafter until the end of the study.

Methods:

Doses: 0 or 250 mg/kg/day
Basis of dose selection: MTD
Restriction paradigm for dietary restriction studies: animals given 80% of the food usually consumed by rats in the test facility
Route of administration: oral gavage
Frequency of drug administration: daily
Dual controls employed: no
Interim sacrifices: week 53, 10rats/sex/group designated
Satellite PK or special study group(s): PK, 6 control rats, 16rats/sex/dose
Deviations from original study protocol: none that affected the interpretation of the study
Statistical methods: originally, no tissues from the study were examined microscopically so the original analysis examined parameters other than tumorigenicity.

Observations and times:

Survival and Signs: Animals were checked at least once a day during pretreatment and twice a day during treatment.

Detailed examinations: performed once during pretest and weekly for the toxicology animals during the study.

Ophthalmic examinations: These exams were conducted on all rats pretest. Due to lack of effects in the companion study, no additional examinations were performed.

Food Consumption: as described above, this was not measured. It was noted when an animal did not consume at least 2/3 of the food made available to it.

Body weights: Determined 3 times during the pretreatment period, weekly during the first 26 weeks of the study, every other week from weeks 27-52 and every 4 weeks thereafter. Fasted body weights were measured for the toxicology animals prior to the week 53 interim euthanasia.

Clinical Pathology: toxicology animals only.

The last 10 animals of each toxicology group were predesignated for hormone assays at months 1,3,6 and 10 and for clinicopathological sampling and euthanasia in week 53. There were no substitutions for unscheduled deaths. Animals were anesthetized with halothane prior to sample collection.

Urine was collected week 53 from the same animals used for blood collection.

Endocrine screen parameters: aldosterone, triiodothyronine (T3), thyroxine (T4)
Testosterone, thyroid stimulating hormone (TSH)

Hematology (EDTA coagulant): hematocrit (HCT), hemoglobin (Hb), MCH, MCHC, MCV, MPV, PLT, RBC, WBC and differential.

Clotting parameters (sodium citrate anticoagulant): PT, APTT, Fibrinogen (FIB)

Clinical Chemistry: ALT, ALB, albumin/globulin, ALP, AST, BUN, Ca, Cl, Chol, Crea, Glob, Glu, Phos, K, Na, SDH, total bile acids (BILE), total protein (TP), TRIG

Parameters from 4-hour urine sample: volume, osmolality, pH*, protein*, glucose*, ketones*, occult blood*, bilrubin*, urobilinogen*, microscopic examination of centrifuged sediment. *= semiquantitative determination.

Parameters from 22 hour urine sample: Ca, Cl, Crea, Phos, K, protein, Na, total urine volume (including the removed 4 hour amount).

Toxicokinetics: Blood was collected after the first dose and again during week 52 for TK evaluation. Samples were taken at 0.5, 1,2,3,4,6, 8 and 24 hours after dosing. On each of the two collection days, each toxicokinetic animal was sampled twice. Plasma was collected and sent to [redacted] for analysis.

Results:

A detailed discussion is presented in the original review.

In this 2-year controlled diet study, rats were dosed with 250 mg/kg of eplerenone, the high dose from the standard 2-year carcinogenicity study. The plasma exposures achieved, compared to human exposures, were ~6X in male rats and ~10X in female rats. As controlled feeding studies have been shown to decrease the spontaneous incidence of neoplasias, the present study was conducted to increase sensitivity of detection of drug effects in case of equivocal results from the companion study. The sponsor felt that there were no equivocal results in the companion study. Therefore, no histopathologic analysis of tissues was performed.

The pharmacologic properties of the drug were evident in the increased urinary sodium and decreased urinary potassium as well as the increased serum aldosterone levels. The increased osmolarity of the urine may have been due to the increased excretion of sodium.

There were no new findings in this study compared to previous toxicologic evaluations of eplerenone.

Alterations in renal function were supported by the urinalysis results showing an increase in the incidence and severity of urinary protein excretion in both sexes of drug-treated rats.

The gross lesions reported included atrophy/degeneration of the prostate glands and seminal vesicles of male rats. This was noted also in the companion study. Gross alterations in kidney morphology were suggestive of CPN. Masses/nodules were reported for the thyroid, a finding to be expected based upon results of the companion study.

TSH, T3 and T4 levels were consistently elevated in females and inconsistent in males. This study does not present as clear a picture of the hormonal changes as some other studies with the compound.

Overall the present study does not provide any new findings of significance but it does highlight the difference in body burden of drug in male versus female rats and the different incidences of gross renal lesions. Female plasma levels of eplerenone were approximately 2X that of males in terms of AUC and Cmax. There was approximately 10% incidence of gross renal lesions in the females compared to 0 in the males.

Summary of individual study findings: Consistent with other studies, the plasma exposures in female rats were twice the level found in male rats. Originally the sponsor felt that there was no need to perform histopathological analysis on the tissues from this study. In light of the renal findings in the standard 2-year bioassay, the sponsor was requested to step section the kidneys from this study and conduct a histological evaluation of the tissues. This is discussed below.

Carcinogenicity summary:

The sponsor presented data for SC-66110 (eplerenone) in a two-year rat bioassay and a 26-week study in p53 knockout mice. The rationale for the use of the alternative mouse model was the existence of murine-specific metabolites of unknown genotoxicity. The p53KO mouse has been demonstrated to have enhanced susceptibility to genotoxic agents. Therefore it was reasoned that a positive result in this model would provide more mechanistic information than a positive result in a traditional mouse strain. Both studies had prior approval of the CAC. Also provided was a study examining the effect of SC-66110 on radiolabeled thyroxine clearance and an ancillary study of the thyroid hormone effects of SC-66110.

	MALES		FEMALES	
Tumor/Tissue	Rare/ Common	p-Value (Exact/ Asymptotic)	Rare/ Common	p-Value (Exact/ Asymptotic)
Follicular Cell Adenoma/ Thyroid	Rare	0.0352 (Exact)*	Rare	0.0001 (Exact)**
Follicular Cell Carcinoma/Thyroid	Rare	0.8904 (Exact)	Rare	0.4552 (Exact)
Follicular Cell Adenoma and Carcinoma	Common	0.1129 (Exact)	Rare	0.0001 (Exact)**
Tubular Cell Adenoma/Kidney	No tumors	—	Rare	0.3750 (Exact)
Tubular Cell Carcinoma/Kidney	Common	1.000 (Exact)	Rare	0.0042 (Asymptotic)***
Tubular Cell Adenoma and Carcinoma	Common	1.000 (Exact)	Rare	0.0072 (Asymptotic)***

* Stat. significant at 0.05
** Stat. significant at 0.01
*** Stat. significant at 0.01 with asymptotic test and at 0.05 with exact test

In the p53 transgenic mouse study, no tumor findings reached the level of statistical significance according to the CDER statistical analysis. Plasma level exposure in the mice was from 15X(LD) to 95X (HD) the human exposure based on $AUC_{0\text{-}24}$ ratios of total eplerenone. Using unbound eplerenone and the recalculation provided by the sponsor, table 11, p 27., report M3001079, the multiples become 2 – 7X for the females and 1-11X for the males.

In the rat study, CDER statistical analysis showed that the incidence of thyroid follicular cell adenoma was significant in both sexes of rats and the combined follicular cell adenoma and carcinoma was significant in the females. The renal tubular cell carcinoma and combined tubular cell adenoma and carcinoma were significant in females only (Both at $p \leq 0.01$ with the asymptotic test and ≤ 0.05 with the exact test). This is summarized in the statistical reviewer's (R. Kelley) table shown here.

The CDER statistical analysis also showed that contrary to the sponsor's assertion, there was a statistically significant increase in mortality with dose (p=0.0422 by Cox's method and p= 0.0188 by Kruskal-Wallis) for the female rats. Of concern to the CDER statistical reviewer were the unconventional statistical methods used by the sponsor, some of which were inappropriate for the submitted studies. The reader is referred to Ms. Kelly's review and to the draft guidance available on the internet.

It may also be noted that in the CDER statistical analysis the thyroid tumors reached a greater significance in female rats compared to the males. It should be reiterated that at each dose the plasma drug levels in female rats were 2-3X the values seen in the males. The study "Isolation and identification of [^{14}C]SC-66110 metabolites in rat and dog feces" (Document number 3001063, dated May 17, 2001) examined the urine and feces from a 13 week oral toxicity study in rats and dog samples. It was demonstrated that approximately 80% of the administered dose was metabolized in male rats while only 25% of the dose in females was metabolized.

Prior to initiating the studies, the sponsor had postulated that because of the structural and pharmacologic similarities to spironolactone, eplerenone could be expected to behave as a rodent carcinogen. In particular, it was anticipated that the induction of hepatic UDPGT and subsequent accelerated metabolism of thyroxine would disrupt the hypothalamic-pituitary-thyroid axis. It was anticipated that chronically elevated TSH levels would cause thyroid hypertrophy/hyperplasia, followed by neoplasia. The association of induction of hepatic UDP-GT and thyroid tumorigenesis in the rat has been found for a number of chemicals including spironolactone and also nicardipine, bepridil, benzodiazepines and chlorinated hydrocarbons to name a few. One of the metabolic differences between rats and humans is the presence of a T4-binding protein (thyroxine binding globulin) found in humans and non-human primates but not in rodents. This protein increases the half-life of T4 in humans to 5-9 days compared to 12-24 hours in rats. Therefore, induced metabolism of thyroxine and subsequent elevations in TSH followed by the progression through hypertrophy, hyperplasia and neoplasia would most likely be a species (rodent)-specific effect.

Concurrent with the carcinogenicity studies, the sponsor conducted studies to examine the effect of eplerenone treatment on thyroxine clearance and overall thyroid economy.
A 13 week study examining the effect of dosing on the clearance of radiolabeled T4 compared doses of 250 mg/kg/day and 750 mg/kg/day doses of eplerenone to phenobarbital (50 mg/kg/day for 3 days followed by 100 mg/kg/day). The 250 mg/kg/day dose caused approximately 29% increases in mean plasma T_4 clearance in both sexes. The 750 mg/kg/day dose increased plasma T_4 clearance by 62% and 87% in male and female rats respectively. The increases produced by PB were 112% and 88% compared to control in males and females respectively.

A separate study focusing on the thyroid hormone effects was submitted. The review is cited here for the sake of cohesion of the data. The thyroid hormone study examined the activity of specific enzymes associated with thyroid metabolism as well as the hormonal levels. Thyroxine UDPGT activity in pmol/min/g/liver was increased over control values at all points of determination during the dosing period, significantly so in males, in the HD females at all treatment points and in the low dose females at 13 weeks. The LD and HD males showed 86%-103% and 150%-123% increases in activity respectively compared to the control group. The LD and HD females showed 59%-125% and 227%-203% increases in activity respectively compared

to the controls. The values in both sexes were not significantly different from the control groups at the end of the drug-free recovery period. The messenger RNA for UDPGT-2B1 was increased in males but at most time points was below the 3-fold level set as the level indicative of induction. Messenger RNA in the 750 mg/kg group was increased up to 4.5X during treatment. There were no differences from control at the end of the recovery period. In females, the messenger RNA for UDPGT-2B1 was mildly increased relative to the control group in the 750 mg/kg group. No differences from control were apparent at the end of the recovery period. The increased activity of the T4-metabolizing enzyme does not seem to be related to synthesis of new protein. Messenger RNA levels for other microsomal proteins (CYP3A) were substantially elevated compared to the internal standard (cyclophilin) suggesting the induction of other metabolizing enzymes to account for hepatic weight increases.

During treatment, T4 values in the eplerenone groups were the same or slightly below control values. At the end of the recovery period the HD-f group showed a mean 360% increase over the control values.

TSH levels in the males were 68%-56% greater than control from week 2 to week 13 in the LD group and 111%-43% greater than control in the HD group over the same time period. Elevated TSH levels in females showed a different pattern in that the difference from control increased over the treatment period. The LD and HD females showed changes of 58%-105% and 150%-300% respectively compared to the control group. At the end of the recovery period, both HD males and HD females showed decreases in TSH of 27% and 37% respectively compared to the control levels.

In the 2-year carcinogencity study, TSH levels were shown to be consistently elevated in MD and HD females and HD males and less consistently elevated in MD males. T4 values progessively fell below those of the control group from 3 months onward. These findings are consistent with those of the thyroid hormone study.

The sponsor has demonstrated increased clearance of radiolabeled T4, sustained increases in TSH levels, inconsistently decreased T4 plasma levels, and increased activity of hepatic UDPGT, the rate limiting enzyme for clearance of T4. Induction of CYP3A activity has also been shown. Correlating with the above changes are increases in liver and thyroid weight and histologic evidence of hepatocellular hypertrophy as well as thyroid follicular hypertrophy/hyperplasia. The inconsistency in the T4 levels may be due to the establishment of a new homeostasis.

It could be argued that supplementing T4 in an attempt to prevent the observed changes might have strengthened the sponsor's case. However, overall, the sponsor presents reasonable evidence that there is a hormonal mechanism of action causing the thyroid tumors. It is suggestive that the thyroid effects seen in rats are species specific.

Given the positive findings for the renal tumors in the CDER statistical analysis of the standard 2-year rat study, the Division requested that the sponsor examine this issue more closely. The Division made the following requests:

1. A blinded or coded re-evaluation of the existing (standard or std) histopathology sections of the kidneys from all rats in the ad lib feeding study.

2. Preparation of step sections of the kidneys from all animals in all dosage groups of both sexes in the ad lib feeding study.
3. Blinded evaluation of those step sections.
4. Preparation of standard and step sections from the kidneys of all animals, all groups, both sexes of the restricted diet study.
5. Blinded evaluation of the standard and step sections from the restricted diet study.
6. Re-analysis of the new data.
7. Provide a dataset of the new material for analysis by the CDER biostatistician.

The methods described below were presented in a May 17, 2002 report from the sponsor.

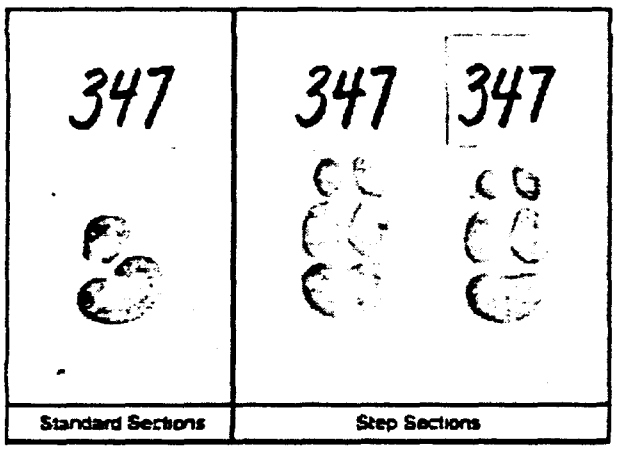
Histological Slide Preparation

The left kidney was sectioned longitudinally and the right kidney was sectioned transversely.

"Standard sections", i.e., the ones prepared and examined for the original report of Study SA4663, were made from the approximate midline of each kidney using the central calotte of the right kidney (cross section) and the larger half of the left kidney (longitudinal section). These two pieces usually were embedded together and a single section of the block was prepared and examined. In some cases the kidneys were too large to embed together, so separate slides were made for left and right kidneys. Inadvertently, duplicate standard sections were made for 35 rats, all of which were examined originally and again in this re-evaluation.

"Step sections" for the new evaluation were prepared as follows (modified from Eustis et al. (3)): the two remaining poles of the right kidney each were cut transversely (yielding 4 pieces total). The remaining piece of left kidney also was embedded. Depending on the size of the tissues, the pieces of right and left kidneys were embedded together or separately. Two sections were prepared from the blocks, the first at the initial facing and the second approximately 1 mm deeper. Therefore, the step sections typically included eight new pieces of right kidney and two of left kidney. Thus, for this re-evaluation there were 12 sections of kidney from each animal, three from the left kidney and nine from the right. For the dietary optimization study, step sections were prepared by the same procedure as for the *ad libitum* study.

The sponsor's pictorial representation of the standard and step sections are shown here.



The available slides were coded so that the study pathologist, [redacted] was unaware of the animal's identity. The study pathologist reviewed the slides and identified those animals with proliferative renal lesions

Pathology Peer Review Procedures

A pathology peer review was conducted for each study, also with the pathologists blinded to the group identity of the animals. For the *ad libitum* study, the peer review was done jointly by [redacted] and [redacted]

Dr. [redacted] reviewed the "standard sections" from 100% of the females and the "standard sections" from all males having any proliferative lesion, plus 20% of the other males (randomly selected). [redacted] reviewed "step sections" from all animals with a proliferative lesion, plus 20% of the other animals (randomly selected). For the dietary optimization study, [redacted] reviewed all sections from 20% of the animals of both sexes (randomly selected) in addition to all proliferative lesions. [redacted] and [redacted] then conferred to reach consensus on the proliferative lesions from both studies.

After these peer reviews, a PWG was assembled to review all the slides containing proliferative lesions confirmed during the peer reviews. The PWG members also were blinded to the animals' group identity. The PWG moderator was [redacted], who was no longer blinded at the time. The PWG diagnoses were accepted as the final diagnoses for proliferative lesions and are the ones reported here. The PWG's report is attached as Appendix 1. The PWG members were:

The sponsor was contacted by telephone by this reviewer May 30, 2002 for clarification of the methods. A telecon was held June 10, 2002 for further clarification of methodological details. Present from the FDA were Mr. Daryl Allis, Al DeFelice Ph.D. (Supervisory Pharmacologist),

this reviewer and Roswitha Kelly, M.S. (Biostatistician). Ms Kelley also pointed out problems with the dataset that made it unanalyzable by the standard computer program and requested revision of the dataset to comply with the guidance on electronic submissions. The details of the discussion are available in the official minutes of the telecon.

The incidences of neoplasia submitted after the re-evaluation of the studies is shown in the sponsor's tables below:

SA4663 Ad Lib Feeding Study

Table 2. **Incidence of Tubular Cell Proliferative Lesions for Standard Sections from SA4663 (*ad libitum* Feeding Study) (Based on PWG Review)**

	Control	20 mg/kg/day	75 mg/kg/day	250 mg/kg/day
Males				
Hyperplasia	0	2	1	0
Adenoma	2	0	1	1
Carcinoma	1	0	0	0
Total affected animals	3	2	2	1
Females				
Hyperplasia	0	3	0	1
Adenoma	1	0	0	2
Carcinoma	0	0	0	1
Total affected animals	1	2	0	4

Table 3. **Incidence of Tubular Cell Proliferative Lesions for All Sections Combined from SA4663 (*ad libitum* Feeding Study) (Based on PWG Review)**

	Control	20 mg/kg/day	75 mg/kg/day	250 mg/kg/day
Males				
Hyperplasia	0	3	2	2
Adenoma	4	1	2	2
Carcinoma	1	0	0	0
Total affected animals	5	4	4	4
Females				
Hyperplasia	0	4	0	5
Adenoma	2	2	0	8**
Carcinoma	0	0	0	1
Total affected animals	2	6	0	12

SA4664 Restricted Diet Study

Table 6. Incidence of Tubular Cell Proliferative Lesions for Standard Sections from SA4664 (Dietary Optimization Study)

	Control	250mg/kg/day
Males		
Hyperplasia	0	0
Adenoma	0	0
Carcinoma	0	0
Affected animals	0	0
Females		
Hyperplasia	0	0
Adenoma	0	0
Carcinoma	0	0
Affected animals	0	0

Table 7. Incidence of Tubular Cell Proliferative Lesions for All Sections Combined from SA4664 (Dietary Optimization Study)

	Control	250mg/kg/day
Males		
Hyperplasia	0	3
Adenoma	1	1
Carcinoma	0	0
Affected animals	1	4
Females		
Hyperplasia	0	1
Adenoma	0	3
Carcinoma	0	0
Affected animals	0	3

The sponsor proposed correlation of the tumors to Chronic Progressive Nephropathy (CPN) and provided the following tables:

Table 2.7. Incidence of Females with Renal Tubular Cell Proliferative Lesions at Each CPN Grade (All Dose Groups) in SA4663 (*ad libitum* Feeding Study)

	CPN Grade	0	1	2	3	4	5
Hyperplasia	Incidence	0/25	2/147	0/61	1/58	1/22	5/27
	Percent	0	1.4	0	1.7	5	19
	p (unadjusted)					.21	.00005***
	p (time adjusted)					.24	.0004***
Adenoma (and Carcinoma)	Incidence	0/25	2/147	0/61	4/58	2/22	4/27
	Percent	0	1.4	0	7	9	15
	p (unadjusted)			.86	.024*	.004**	.00006***
	p (time adjusted)			.91	.015*	.006**	.0001***
Adenoma, Carcinoma, and Hyperplasia	Incidence	0/25	4/147	0/61	5/58	3/22	6/27
	Percent	0	3	0	9	14	30
	p (unadjusted)			.84	.037*	.003**	7x10^{-6}***
	p (time adjusted)			.92	.031*	.004**	2x10^{-6}***

*p≤.05 **p≤.01, ***p≤.001

Table 2.11. Incidence of Females with Renal Tubular Cell Proliferative Lesions at Each CPN Grade (All Dose Groups Combined) in SA4664 (Dietary Optimization Study)

	CPN Grade	0	1	2	3	4	5
Hyperplasia	Incidence	0/62	0/81	0/18	0/6	1/3	0/0
	Percent	0	0	0	0	33	–
	p (unadjusted)				1.0	.018*	
	p (time adjusted)				1.0	.034*	
Adenoma and Hyperplasia	Incidence	0/62	2/81	0/18	0/6	1/3	0/0
	Percent	0	2.5	0	0	33	–
	p (unadjusted)				.50	.043*	
	p (time adjusted)					.065	

*p≤.05. **p≤.01. ***p≤.001

Table 2.8. Incidence of Males with Renal Tubular Cell Proliferative Lesions at Each CPN Grade (All Dose Groups Combined) in SA4663 (*ad libitum* Feeding Study)

	CPN Grade	0	1	2	3	4	5
Hyperplasia	Incidence	0/18	0/71	0/73	2/86	2/55	3/37
	Percent	0	0	0	2	4	8
	p (unadjusted)				.12	.029*	.002**
	p (time adjusted)					.079	.017*
Adenoma and Carcinoma	Incidence	0/18	0/71	1/73	2/86	1/55	6/37
	Percent	0	0	1	2	2	16
	p (unadjusted)					.17	.0002***
	p (time adjusted)					.57	.0008***
Adenoma, Carcinoma, and Hyperplasia	Incidence	0/18	0/71	1/73	4/86	3/55	9/37
	Percent	0	0	1	5	5	24
	p (unadjusted)			.45	.025*	.015*	$1x10^{-4}$***
	p (time adjusted)					.061	$9x10^{-4}$***

*p≤.05, **p≤.01, ***p≤.001

Table 2.10. Incidence of Males with Renal Tubular Cell Proliferative Lesions at Each CPN Grade (All Dose Groups Combined) in SA4664 (Dietary Optimization Study)

	CPN Grade	0	1	2	3	4	5
Hyperplasia	Incidence	0/25	1/116	0/20	1/5	1/2	0/0
	Percent	0	1	0	20	50	
	p (unadjusted)				.063	.005**	
	p (time adjusted)*				.14	.027**	
Adenoma	Incidence	0/25	1/116	0/20	1/5	0/2	0/0
	Percent	0	1	0	20	0	–
	p (unadjusted)					.065	
	p (time adjusted)*					.17	
Adenoma and Hyperplasia	Incidence	0/25	2/116	0/20	2/5	1/2	0/0
	Percent	0	2	0	40	50	
	p (unadjusted)			.75	.007**	.0006***	
	p (time adjusted)*			.89	.024*	.003**	

*p≤.05 **p≤.01 ***p≤.001

The sponsor cited furosemide, hydrochlorthiazide and monochloro-1,2-propanediol (a trace by-product in acid hydrolyzed vegetable protein) as chemicals that caused an increased incidence and/or severity of CPN. The literature references provided by the sponsor are summarized below.

Monochloro-1,2-propanediol (Lynch et al., Carcinogenicity of monochloro-1,2-propanediol (α-chlorohydrin, 3-MCPD. International Journal of Toxicology,17:47-76, 1998).

Based on the available data concerning dermal application to ICR/HA Swiss mice, 3-MCPD does not appear to be carcinogenic to mice.

Survival in one rat study was significantly increased in males and completely unaffected in another study. In the author's words:

> A highly significant dose-dependent increase in atypical renal tubular hyperplasia was also reported in both sexes of the intermediate and high-dose-groups. In addition, chronic progressive nephropathy was significantly increased in incidence, and particularly in severity, in males and females of the intermediate- and top-dose groups, with females more adversely affected than males. There were clear correlations between the severity of the nephropathy and the incidence/degree of renal tubular hyperplasia and presence of renal tubular adenomas.

Table 4. Histological findings in the Bannbare et al (1993) 2-yr rat carcinogenicity study

Organ/finding	Dose group (ppm in drinking water)			
	0	30	100	300
Males				
Testes				
Number examined	50	50	50	50
Leydig-cell hyperplasia	39	27*	4*	0*
Leydig-cell adenoma	35	42	50*	47*
Leydig-cell carcinoma	0	0	0	3
Mammary gland				
Number examined	45	48	47	49
Glandular hyperplasia	4	13	39*	42*
Fibroadenoma	0	0	2	10*
Adenoma	0	0	1	1
Adenocarcinoma	0	0	1	1
Preputial gland[a]				
Number examined	5	13	18	11
Adenoma	1	2	6	8
Carcinoma	0	0	1	2
Kidneys				
Number examined	50	50	50	50
Nephropathy	36	40	45*	45*
Tubular hyperplasia	3	6	15*	34*
Tubular adenoma	0	0	1	5
Females				
Kidneys				
Number examined	50	50	50	50
Nephropathy	24	23	42*	48*
Tubular hyperplasia	2	4	20*	31*
Tubular adenoma	0	1	0	9*

[a]The preputial gland was a congenital organ. It was either incidentally present on the skin section or was collected at autopsy if it contained a visible nodule. Since this organ was not examined in all animals, meaningful statistical analysis of the tumor incidence cannot be conducted.
*Statistically significant at $p < .05$ (pairwise Fisher's test between treated and controls).
*Statistically significant at $p < .01$.
*Statistically significant at $p < .001$

Furosemide (Bucher et al. Toxicology and Carcinogenicity Studies of Diuretics in F344 Rats and B6C3F1 Mice 2. Furosemide. J. Appl Tox, Vol 10(5), 369-378 (1990)).

There were no significant differences in survival in any groups of either sex in the 2-year rat studies. Nephropathy was observed at similar incidences but with increased severity in dosed male rats relative to controls. Incidence and severity were not affected in female rats. The following incidences were provided in the reference:

Number of rats with the selected lesion

Renal Tubular cell lesions	Dose ppm					
	Males			Females		
	0	350	700	0	350	700
# examined	50	50	50	50	50	50
Hyperplasia	6	5	10	1	0	0
Adenoma	3	4	5	0	0	0
Adeno-carcinoma	0	1	1	0	0	0

Hydrochlorthiazide (Bucher et al. Toxicology and Carcinogenicity Studies of Diuretics in F344 rats and B6C3F1 Mice 1. Hydrochlorthiazide) J. Appl Tox., Vol 10(5), 359-367 (1990)).

No significant differences in survival were observed between any groups of either sex. Severity of CPN was increased in dosed groups relative to controls. The following incidences of tumors were provided in the reference:

Renal Tubular cell lesions	Dose ppm							
	Males				Females			
	0	250	500	2000	0	250	500	2000
# examined	50	49	50	50	50	50	49	50
Hyperplasia	0	0	1	0	0	0	0	0
Adenoma	3	1	0	1	0	0	1	1

Carcinogenicity conclusions:
After evaluation of the additional information submitted by the sponsor, the Executive CAC concluded that the renal neoplasia in HD females was not a biologically significant finding.

Labeling Recommendations: Acceptable as written.

Addendum/appendix listing:

Dose-ranging study report: Included under General Toxicology
CAC report: minutes available in DFS
Alternative study protocols and CAC report: NA
Sponsor's incidence of histopathology findings: Submitted to CAC and Biometrics
List of organs and tissues examined: See original reviews
Body weight changes versus dose level: See original Reviews
Group body weight summary: See original reviews
Individual data listing: see original reviews

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

N.B.For the following studies, ratios of animal to human AUC based on free eplerenone (rather than total) are from the sponsor's calculations presented in P6001171.

Study title: Study of fertility and early embryonic development to implantation in rats with orally administered SC-66110

Key study findings: SC-66110 at doses up to 300 mg/kg did not affect the following reproductive parameters in rats: mating behavior, sperm production and viability, conception, implantation and in-utero survival.

Study no.: SA4455/700-342
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation:12/5/1995
GLP compliance: yes
QA reports: yes (x) *no* ()
Drug, lot #, radiolabel, and % purity: SC-66110 lot GDS-6050-001
Formulation/vehicle: 0.5% methylcellulose and 0.1% Tween 80

Methods: Sprague-Dawley Crl: CD rats (25/sex/group; 6-7 weeks old) were treated by oral gavage at doses of 0, 20, 100 or 300 mg/kg. An additional group of males were treated with the high dose (300 mg/kg) and were mated with untreated females. Males were dosed at least 27 days before mating and throughout the mating period until termination. Females were dosed for at least 13 days before mating, throughout the mating period and through gestation day 7.

On day 13 of gestation, females were euthanized and examined grossly. The uterus was examined for number of implantation sites, live and dead fetuses, early and late resorptions and abnormalities of the uterus or embryonic sacs. The ovaries were examined for number of corpora lutea. Males were euthanized on days 71-73 and examined grossly. The left testis, left epididymus, seminal vesicles, coagulation gland and prostate were fixed for histopathological examination. Epididymal sperm were collected for examination of sperm morphology.

Results:

No treatment related deaths were reported. There were minimal changes in body weight gains or food consumption (a transient decrease in gain from GD3-GD7 in HD females). There was no treatment-related difference in reproductive performance (male/female copulation index). In the females, the number of corpora lutea and implantation sites were similar between groups. There were no dead fetuses. In the males, there was no evidence of reduced sperm motility or in mean sperm count.

SC-66110 at doses up to 300 mg/kg did not affect the following reproductive parameters in rats: mating behavior, sperm production and viability, conception, implantation and in-utero survival.

Study title: A study of the effects of oral administration of SC-66110 on fertility and early embryonic development to implantation in male and female rats and Searle Container report amendment no.1

Key study findings: Both HD males and females gained less weight than did the control groups. There were no significant effects upon the treated females. Untreated females mated with the HD males produced a decrease in viable fetuses and increased absolute and percentages of pre-implantation loss. The HD males used for this mating showed decreased weight of seminal vesicles and epididymides as well as minor alterations in sperm morphology The NOAEL for male fertility effects in this study was 300 mg/kg/day, a dose that gave ~8X the predicted human exposure. The NOAEL for female fertility was 1000 mg/kg/day (~30X human exposure).

Study no.: SA4997/ [redacted] 111116
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: January 20, 2001
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: SC-66110 lot 97K017-F1A
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in deionized water.

Methods:

Sprague-Dawley rats were orally gavaged with 0, 100, 300 or 1000 mg/kg/day of SC-66110. Females were treated for 2 weeks prior to mating through gestation day 7 then mated with untreated males. After this mating period, the untreated males were then treated for10 weeks prior to the second mating period. The treated males were paired with untreated females. Mating and fertility indices were examined, including vaginal smears for cytological evaluaton. GD15 laparotomies were performed on females following euthanasia. Factors examined included corpora lutea, number and location of embryos, early resorptions, total implantation sites, viability of embryos. Following euthanasia, males were assessed for sperm number, motility and morphology. The following tissues were collected for histopathology: adrenal, epididymus, heart, kidney, liver, pituitary, prostate, seminal vesicle and coagulating gland, testes and vas deferens. Toxicokinetics were assessed for male rats only.

Results:

Both HD males and females gained less weight than did the control groups. In the first two weeks of dosing this was significant at $p<0.01$. There were no apparent differences in rate of gain during gestation. There were no significant reproductive effects upon the treated females. Treated males showed some changes in sperm morphology such as normal sperm heads with absent flagella and absent head with normal flagella. Untreated females mated with the HD males produced a decrease in viable fetuses and increased absolute and percentages of pre-implantation loss. The HD males used for this mating showed decreased weight of seminal vesicles and epididymides as well as minor alterations in sperm morphology (discussed above). Based upon the free eplerenone values as provided in M3001079, table 6, plasma values obtained after 10 weeks of dosing for the males showed AUC_{0-24} values from ~3X-23X the values

achieved in humans following repeat dosing. The NOAEL for male fertility effects in this study was 300 mg/kg/day, a dose that gave ~8X the predicted human exposure. The NOAEL for female fertility was 1000 mg/kg/day. See original review for further details.

GROUP		VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
1	TOTAL	340	0	12	0	12	352	399	47	23
	MEAN	14.8	0.0	0.5	0.0	0.5	15.3	17.3	2.0	
	S.D.	2.28	0.00	0.67	0.00	0.67	2.12	2.60	1.72	
2	TOTAL	364	0	15	0	15	379	426	47	25
	MEAN	14.6	0.0	0.6	0.0	0.6	15.2	17.0	1.9	
	S.D.	1.56	0.00	0.76	0.00	0.76	1.72	1.90	1.54	
3	TOTAL	350	0	10	0	10	360	390	30	24
	MEAN	14.6	0.0	0.4	0.0	0.4	15.0	16.3	1.3	
	S.D.	1.72	0.00	0.65	0.00	0.65	1.64	2.19	1.75	
4	TOTAL	281	0	11	0	11	292	359	67	21
	MEAN	13.4	0.0	0.5	0.0	0.5	13.9	17.1	3.2	
	S.D.	2.91	0.00	0.81	0.00	0.81	2.74	2.74	2.06	

None significantly different from control group
MEAN NUMBER OF VIABLE EMBRYOS, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST

1- 0 mg/kg/day 2- 100 mg/kg/day 3- 300 mg/kg/day 4- 1000 mg/kg/day

GROUP NUMBER:	1	2	3	4
TOTAL RESORPTIONS (%)				
MEAN	3.6	3.8	2.8	4.1
S.D.	4.54	4.78	4.29	6.12
N	23	25	24	21
PRE-IMPLANTATION LOSS (%)				
MEAN	11.3	10.7	7.0	18.3*
S.D.	9.11	8.37	9.25	12.37
N	23	25	24	21
POST-IMPLANTATION LOSS (%)				
MEAN	3.6	3.8	2.8	4.1
S.D.	4.54	4.78	4.29	6.12
N	23	25	24	21

1- 0 mg/kg/day 2- 100 mg/kg/day 3- 300 mg/kg/day 4- 1000 mg/kg/day

PROPORTIONAL (%) DATA COMPARED USING THE KRUSKAL-WALLIS TEST
CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
* = Significantly different from the control group at 0.05

Study title: Embryo-fetal developmental toxicity study of SC-66110 in rats (segment II) and final report amendment No.1.

Key study findings: Female rats were dosed at levels of 20, 100 or 300 mg/kg/day with no reported maternal clinical signs or effect on mean body weight. PK data showed good systemic availability. An increased incidence of 14th rudimentary ribs was considered to be a developmental variation. No other external, visceral or skeletal effects were reported. The ratios of rat:human AUCs based on free eplerenone at the NOEL of 300 mg/kg were 26X and 11X on GD6 and GD17 respectively.

Study no.: SA4468

Volume #, and page #:
Conducting laboratory and location: GD Searle and Co., Skokie, IL
Date of study initiation: 2/12/1996
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: SC-66110 lot number RCT-9938
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80

Methods:

Female [redacted] (VAF) CD rats were given SC-66110 by gavage at 20, 100 or 300 mg/kg from GD6-17. Control rats (25) received the vehicle. Additional groups of rats (12/test group and 2 controls) received SC-66110 for assessment of bioavailability on days 7 and 18.

Observations:

Signs and survival were checked daily. Body weights were determined GD0, 6,8,10, 13 and 15. Maternal observations included number of corpora lutea, implantations, resorptions and live or dead fetuses. Placentas were examined grossly. Fetal observations included the weighing and external examination of each. Live fetuses were euthanized. The viscera of every other fetus were examined; the head was removed and preserved. Heads and skeletons of the control and HD groups were examined.

Results:

Examination of the reproductive status of females euthanized GD20 showed no adverse effects on the average number of corpora lutea, implantations, resorptions and live or dead feti.

Table 4. Results of Skeletal Fetal Examination

SC-66110 (mg/kg/day)	Control	20	100	300
FETUSES: Live				
No. Examined	360	-*	1*	389
No. with Malformations	2	-	1	2
No. of Malformations	2	-	1	2
No. with Variations	29	-	1	54
No. of Variations	32	-	1	56
LITTERS				
No. Examined	22	-	1	24
No. with Fetal Malformations	2	-	1	2
No. with Fetal Variation	10	-	1	19
Average Sacral Caudal vertebrae count	7.97	-	3.00*	7.88
TYPE AND NUMBER OF FETAL ALTERATIONS: (No. of Fetuses/No. of Litters)				
Bone Slit - skull (M)	1:1	-	0	0
Bone Missing - skull (M)	1:1	-	0	0
Fused/Shortened Lower Jaw (M)	0	-	1:1	0
Ribs Kinked (M)	0	-	0	2/2

M = Malformation; V = Variation
* Low and Medium dose groups not evaluated (study design)
* Skeletal exam performed on Maternal 44682311 Fetus #12 due to External Finding of shortened lower jaw.

One fetus in the control group had a missing skull bone. One fetus in the MD group showed a shortened lower jaw. This finding was considered incidental because there were no similar findings in the HD group. Skeletal examinations showed an increased incidence of 14th rudimentary ribs in both the control group (19fetuses/8 litters) and in the HD group (48 fetuses/17 litters).

These findings were considered developmental variations and not related to drug treatment. They may also be related to maternal toxicity except that no maternal toxicity was reported. There was a transient decrease in food consumption GD6-GD10 for all dosage groups. No other findings on external, visceral or skeletal effects were reported. Fetal weights were unaffected. PK data showed good systemic bioavailability,

with decreased exposure after repeated administration, similar to previous findings. Maternal weight was not affected and no clinical signs were reported.

Table 4. Results of Skeletal Fetal Examination

SC-66110 (mg/kg/day)	Control	20	100	300
TYPE AND NUMBER OF FETAL ALTERATIONS: (No. of Fetuses/No. of Litters)				
Skull Bone				
Reduced Ossification (V)	1/1	-	0	1/1
Sternebrae Unossified (V)	1/1	-	1/1	1/1
Sternebrae Displaced (V)	1/1	-	0	0
Vertebral Centrum Bipartite Cleaved (V)	3/3	-	0	3/3
Vertebral Centrum Unilaterally Ossified (V)	1/1	-	0	0
Vertebral Centrum Unossified (V)	1/1	-	0	2/2
Pubis Unossified (V)	1/1	-	0	0
14th Rudimentary Rib(s) (V)	19/8	-	0	48/17
14th Shortened Rib(s) (V)	4/1	-	0	1/1

M = Malformation
V = Variation

Study title: A study of the effects of SC-66110 on embryo/fetal development in rats

Key study findings: When female rats were dosed to the point of maternal toxicity as manifested by decreased weight gain, the only apparent effect on the pups was decreased weight. Toxicokinetic data indicated fetal as well as maternal exposure. The fetal NOAEL in this study was 300 mg/kg, with AUC values 31X and 15X the expected human therapeutic exposure on GD6 and GD17 respectively. This repeat Seg II study, using the MD and HD from study SA4468 and the inclusion of a new higher dose, provide additional support that the skeletal effects of the previous study were in fact variations and not drug-related.

Study no.: SA4990
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: November 9, 1999
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: SC-66110 lot 97K017-F1A
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80

Methods:

Female Crl: CD®(SD)IGSBR rats were orally dosed with 100, 300 or 1000 mg/kg of SC-66110 from GD6-17. An additional 9 rats per dose were used for toxicokinetic analysis of both fetal and maternal plasma levels on GD20. Dams in the main study were euthanized Day20 and examined for corpora lutea per ovary, number and location of fetuses, early and late resorptions, total number of implantation sites. Fetuses were weighed and examined for external, visceral and skeletal anomalies.

Results:

See original review for detailed discussion. Maternal toxicity was evidenced by decreased weight gain (6%, $p<0.01$) in the HD group compared to the controls. The only apparent effect on the fetuses was a significant ($p<0.01$) decrease in average weight of male and female pups from HD dams. This effect may have been secondary to maternal toxicity.

TABLE 9

PROJECT NO.. 111115 STUDY OF SC-66110 ON EMBRYO/FETAL DEVELOPMENT IN RATS PAGE 1
SPONSOR:SEARLE SUMMARY OF MEAN FETAL DATA AT THE SCHEDULED NECROPSY
SPONSOR NO.:SA 4990

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	186	184	370	0	16	1	17	387	422	35	NA	25
MEAN	7.4	7.4	14.8	0.0	0.6	0.0	0.7	15.5	16.9	1.4	3.6	
S.D.	2.69	2.86	2.86	0.00	0.86	0.20	0.90	1.98	2.26	1.67	0.21	
2 TOTAL	189	182	371	0	15	2	17	388	445	57	NA	24
MEAN	7.9	7.6	15.5	0.0	0.6	0.1	0.7	16.2	18.5	2.4	3.5	
S.D.	2.36	2.84	1.84	0.00	0.71	0.28	0.81	1.52	2.90	2.89	0.21	
3 TOTAL	192	182	374	0	14	1	15	389	429	40	NA	25
MEAN	7.7	7.3	15.0	0.0	0.6	0.0	0.6	15.6	17.2	1.6	3.6	
S.D.	2.32	2.37	1.40	0.00	0.71	0.20	0.76	1.58	2.43	1.96	0.19	
4 TOTAL	150	181	331	0	19	1	20	351	402	51	NA	23
MEAN	6.5	7.9	14.4	0.0	0.8	0.0	0.9	15.3	17.5	2.2	3.3**	
S.D.	2.56	2.30	2.59	0.00	2.61	0.21	2.01	1.79	2.95	1.91	0.27	

** - Significantly different from the control group at 0.01
NA - NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST
MEAN FETAL BODY WEIGHTS COMPARED USING ANALYSIS OF COVARIANCE WITH STUDENT'S T-TEST

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Toxicokinetic analysis showed that the fetuses were exposed to the drug *in utero*.
Day 20, the maternal and fetal levels of total SC-66110 were equivalent at the approximate Tmax. Consistent with other studies, AUC_{0-24} decreased over time, most likely due to induction of hepatic metabolism. The highest dosage in the present study gave plasma levels ~31X higher than expected in humans based upon total eplerenone. The lowest dosage level gave plasma levels over 5 times the expected human exposure. The fetal NOAEL in this study was 300 mg/kg, with plasma levels 15X those expected at the human therapeutic dosage. The maternal NOEL was 100 mg/kg based upon body weight.

Using the tables provided in M3001079, the ratios of free eplerenone AUC for rats compared to humans were ~6.5X(LD) and 20X(MD, 300 mg/kg/day).

Study title: A range-finding pre- and postnatal toxicity study of SC-66110 in rats (EX4855)

Key study findings: There was a decrease in maternal food consumption during the first few days of dosing, but overall the dosages were well-tolerated by the dams. The pharmacokinetic data showed a proportional increase in plasma levels from 500 to 750 mg/kg and essentially no change from 750 to 1000 mg/kg. AUC_{0-24} decreased over time, consistent with other studies and the induction of metabolism that has been shown.

Study no.: EX4855
Volume #, and page #:
Conducting laboratory and location: GD Searle and Co, Skokie, IL
Date of study initiation: November 3, 1998
GLP compliance: no
QA reports: yes () no (x)
Drug, lot #, radiolabel, and % purity: SC-66110, lot 96K018-F3A
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate

Methods:

SC-66110 at doses of 500, 750 and 1000 mg/kg/day were given to pregnant Crl:CD(SD)IGS BR rats from GD6 through lactation day 3 for toxicology. The same dosages were given to separate groups of animals from GD6 through 20 for pharmacokinetic analysis. The observations made in the toxicology portion of the range findings study were primarily weight, food consumption, mortality and clinical signs. Numbers of corpora lutea, implantations, litter size and pre-implantation loss were reported.

Results:

There was a decrease in food consumption but no significant effect upon maternal weight. Body weight of the pups was affected at all dosages. Decreased day 1 body weight as a percent of control pup weight was 6, 7 and 12% from lowest dosage to highest. Day 4 percent differences from mean control weight were 3, 9 and 13% from LD to HD. The pharmacokinetic data showed a proportional increase in plasma levels from 500 to 750 mg/kg and essentially no change from 750 to 1000 mg/kg. AUC_{0-24} decreased over time, consistent with other studies and the induction of metabolism that has been shown.

Study title: Study of effects on pre- and post-natal development in CD rat by oral gavage administration of SC-66110

Key study findings: The HD caused maternal toxicity in the form of decreased weight gain and food consumption with milder effects noted in the MD group. Reproductive indices for the F0 dams were unaffected. F1 male and female HD pups showed lower mean body weight than the control, LD or MD groups. The decrease in weight gain persisted in both sexes until euthanasia.

Slight growth delays (balanopreputial separation in the HD males) were also noted in these pups. Reproductive performance of the F1 offspring appeared to be unaffected as were behavioral, functional and learning tests. The maternal NOAEL based upon weight effects was 100 mg/kg in this study. The high dose of 1000 mg/kg produced maternal toxicity in decreased weight gain and pup effects of decreased weight and slight delay in development as indicated by time of balano-preputial separation.

Study no.: SA4891/ — 162/994319
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: April 6, 1999
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: SC-66110 lot 96K020-F1A
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80

Methods:

Female Crl:CD®BR rats were orally dosed with 0, 100, 300 or 1000 mg/kg/day of SC-66110 from GD6 to lactation day 20. Offspring were monitored for viability, survivability, body weights, auditory function, visual function, neuromuscular function, learning ability and reproductive capacity. Blood samples were collected from selected pups at 2 or 4 hours after maternal dosing on Day 4 of age. Details of this analysis were reported separately.

Results:

The high dose caused maternal toxicity in the form of decreased bodyweight gain and food consumption. The mid-dose, 300 mg/kg caused slightly less body weight effects and the low dose caused no discernible weight effects. All treated F0 females showed dose-dependent increases in absolute and normalized liver weights, which appears to be the primary treatment related finding in the F0 maternal group. The percent increases from control for absolute liver weight were 5-59% and 7-58% for normalized liver weight. Reproductive indices of gestation, post-implantation survival, live birth and viability were unaffected as reported.

Both male and female HD offspring from birth to day 28 showed lower body weight than the control group or the offspring of the LD and MD groups (males 18-9%,$p<0.01$; females 14-8%, $p<0.01$). From 5 weeks of age to euthanasia, the male offspring of the HD group showed lower weight gain than the other three groups who were essentially indistinguishable. The HD male offspring were from 7-10% lower than control mean body weights ($p<0.1$). Female offspring of the HD group also showed slightly lower (4-6%) weight gain than the control and MD groups from 5-9 weeks of age. F1 males also showed a slight delay in the balano-preputial separation although this may be due to the overall delay in growth. A similar delay in growth was noted in the females.

Reproductive performance of the F1 generation appeared to be unaffected as evidenced by litter size, offspring survival to day 7 and offspring bodyweight.

Behavioral tests of the F1 generation showed no significant differences from the control offspring.

The maternal NOAEL for this study was 100 mg/kg/day. Doses up to 300 mg/kg/day did not appear to have an affect on development of the F1 offspring as assessed by the parameters described in the report.
Plasma levels of both SC-66110 and SC-70303 were detectable in neonates at all doses.

Study title: Embryo-fetal development study in New Zealand White Rabbits with SC-66110

Key study findings: There was maternal toxicity evidenced by dose-dependent decreases in food consumption and weight loss which were significant at the HD (300 mg/kg/day). While there was no evidence of teratogenicity, there were increases in early resorptions and post-implantation losses in the HD group.

Study no.: SA4479
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: February 26, 1996
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: SC-66110, lot number not given
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80

Methods:

SC-66110 was given once a day by oral gavage to groups of 25 pregnant NZW rabbits at dosages of 20, 100 or 300 mg/kg/day from GD7-20. Six additional rabbits per dosage were used for a pharmacokinetic evaluation on GD7 and GD19. Following euthanasia on GD29, the uteruses were weighed. Number and location of fetuses, viability, early and late resorptions, total number of implants and corpora lutea were recorded. Both viable and non-viable fetuses were examined externally, viscerally and skeletally. Data from the non-viable feti was not included in the summary tables.

Results:

No deaths or altered clinical signs were reported. There was a dose-dependent effect on body weights and food consumption which was significant at the 300 mg/kg group from gestation day 19-21. These values reached control values by gestation day 30. Examination of females euthanized GD29 showed no adverse effects on corpora lutea or number of implantations. However, mean gravid uterine weight was decreased in the HD does. In the 300 mg/kg group there was a significant increase (mean of 0.42/doe in controls vs 1.11 /doe in the HD) in early resorptions and an increase in post-implantation loss (mean of 0.58/doe in the controls vs 1.47/doe HD) which was interpreted as being due to developmental toxicity.

Table 5 Cont.

Summary of Maternal and Developmental Observations at Laparohysterectomy - Toxicology Groups

		Group 1 0 mg/kg/day (Vehicle Control)	Group 2 20 mg/kg/day	Group 3 100 mg/kg/day	Group 4 300 mg/kg/day
Males	TOTAL	88	54	78	66
No. per animal	MEAN	3.63 d	2.84	3.39	3.47
	S.D.	1.86	2.43	1.85	1.31
	N	19	19	23	19
	MEANS	50.43 w	39.58	46.09	66.92
	S.D.	13.71	22.66	21.56	10.37
Females	TOTAL	68	60	93	56
No. per animal	MEAN	3.58 d	3.16	4.04	2.84
	S.D.	1.89	1.50	2.10	1.57
	N	19	19	23	19
	MEANS	49.57 w	55.15	53.91	44.08
	S.D.	13.71	24.50	21.56	10.37
Postimplantation Loss	TOTAL	11	17	17	28
No. per animal	MEAN	0.58 d	0.89	0.74	1.47
	S.D.	0.90	1.85	0.86	1.58
	N	19	19	23	19
% impl. per animal	MEANS	9.87 w	16.74	9.16	18.24
	S.D.	16.73	25.21	13.15	10.93

196-842 Statistical key: d=ANOVA + Dunnett-test w=Kruskal-Wallis

Table 5 Cont.

Summary of Maternal and Developmental Observations at Laparohysterectomy - Toxicology Groups

		Group 1 0 mg/kg/day (Vehicle Control)	Group 2 20 mg/kg/day	Group 3 100 mg/kg/day	Group 4 300 mg/kg/day
Dead Fetuses	TOTAL	0	1	1	0
No. per animal	MEAN	0.00 d	0.05	0.04	0.00
	S.D.	NA	0.23	0.21	NA
	N	19	19	23	19
% of impl. per animal	MEANS	0.00 w	0.48	0.48	0.00
	S.D.	NA	2.09	2.32	NA
Resorptions: early+late	TOTAL	11	16	16	28
No. per animal	MEAN	0.58 d	0.84	0.70	1.47
	S.D.	0.90	1.01	0.76	1.58
	N	19	19	23	19
% of impl. per animal	MEANS	9.87 w	16.26	8.67	18.24
	S.D.	16.73	25.27	10.47	10.93
Resorptions: Early	TOTAL	8	11	7	21
No. per animal	MEAN	0.42 d	0.58	0.30	1.11*
	S.D.	0.77	0.77	0.56	1.20
	N	19	19	23	19
% of impl. per animal	MEANS	7.68 w	13.91	4.49	14.97
	S.D.	14.00	25.13	9.45	17.20
Resorptions: Late	TOTAL	3	5	9	7
No. per animal	MEAN	0.16 d	0.26	0.39	0.37
	S.D.	0.37	0.73	0.58	1.16
	N	19	19	23	19
% of impl. per animal	MEANS	2.19 w	2.75	4.19	3.27
	S.D.	5.58	7.12	6.10	9.58

196-842 Statistical key: d=ANOVA + Dunnett-test w=Kruskal-Wallis

Fetal body weights were unaffected. There were no fetal external, visceral, skeletal or head

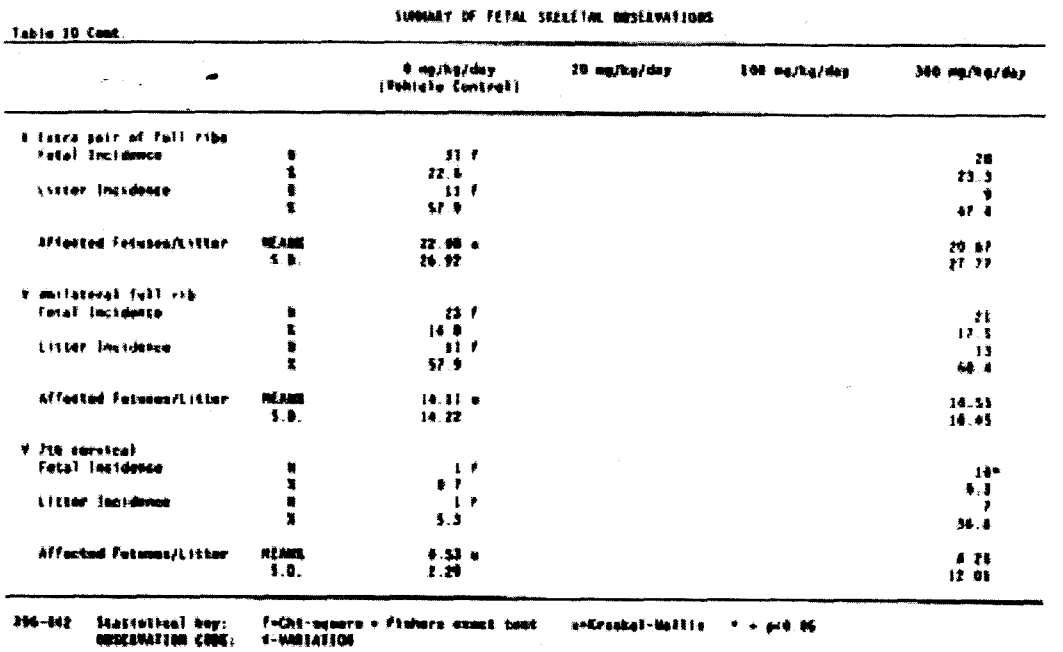


Table 10 Cont. SUMMARY OF FETAL SKELETAL OBSERVATIONS

		0 mg/kg/day (Vehicle Control)	20 mg/kg/day	100 mg/kg/day	300 mg/kg/day
8 fetus pair of full ribs					
Fetal Incidence	N	11 F			28
	%	22.6			23.3
Litter Incidence	N	11 F			9
	%	57.9			47.4
Affected Fetuses/Litter	MEANS	22.90 a			20.67
	S.D.	26.92			27.77
9 unilateral full rib					
Fetal Incidence	N	23 F			21
	%	14.8			17.5
Litter Incidence	N	11 F			13
	%	57.9			68.4
Affected Fetuses/Litter	MEANS	14.11 a			14.53
	S.D.	14.22			16.65
7 7th cervical					
Fetal Incidence	N	1 F			10*
	%	0.7			8.3
Litter Incidence	N	1 F			7
	%	5.3			36.8
Affected Fetuses/Litter	MEANS	0.53 a			0.76
	S.D.	2.29			12.01

396-842 Statistical key: F=Chi-square + Fishers exact test a=Kruskal-Wallis * = p<0.05
OBSERVATION CODE: 4-VARIATION

abnormalities reported that the sponsor attributed to drug treatment. Hydrocephaly was reported for 2 fetuses, one in each of 2 different HD litters versus 0 in any other treatment group or the controls. Five litters in the MD group and 5 litters in the HD group showed hypoplasia of the gall bladder. One litter in the control and LD groups was similarly affected. Seven HD litters were reported to have 7th cervical rib effects compared to 1 control litter ($p<0.05$ by Chi Square and Fisher's exact test). Data for this effect was not provided for the LD and MD groups so an evaluation of a dose-response effect is not possible.

Pharmacokinetic data showed that the drug was systemically available at all dose levels and did not change with repeated exposure. AUC levels in rabbits were 0.82 – 20X the anticipated human therapeutic level for free eplerenone based upon values from the M3001079, table 7. A maternal NOEL was not identified based upon weight effects.

Summary of individual study findings: See above

Reproductive and developmental toxicology summary:
The reproductive toxicology assessment included fertility studies in the rat, embryo-fetal development in the rat and rabbit and post-natal development in the rat. PK and metabolism studies indicated maternal and fetal exposure through the blood. Neonates were also exposed via the dam's milk.

Fertility:
Fertility in female rats was unaffected except at those doses producing maternal toxicity with a NOAEL of 1000 mg/kg/day.
Males given 1000 mg/kg/day for 10 weeks prior to mating showed significantly decreased seminal vesicle weight (82% of control values) and epididymus weights (90% of control). Breeding these males to untreated females resulted in a slight increase in pre-implantation loss:

18% vs 11% in the control group. One possibility is that the seminal vesicles were producing smaller or poorer quality copulatory plugs, a factor that may contribute to infertility and is species specific. There was also material from this study to indicate a decrease in the quality of the sperm. There were some very slight changes in sperm analysis parameters. In particular, normal sperm heads with absent flagellum was 0.6±1.06% in control males and 0.7±1.33% in HD males. An absent head with a normal flagellum was listed as 0.1±0.42 for the control group v. 0.8±2.21 for the HD group. Both parameter values for the HD males are outside the historical ranges provided by the laboratory (the historical value for normal head, absent flagellum is 0.6±0.32 and for absent head, normal flagellum is 0±0%). Although there were no significant differences in the numbers of homogenization–resistant sperm, this value says nothing about the adequacy or normality of release from the Sertoli cells. The dosage used in this study produced ~23X estimated human exposure based upon unbound plasma eplerenone values.

It should also be noted that lower dosages producing lower multiples of human exposure have also affected male reproductive organ weight, as demonstrated in the carcinogenicity studies. These changes were already addressed in the toxicology and carcinogenicity sections of this review.

Embryo-fetal development:

Rats

There was no discernible effect upon rat fetuses except at dosages causing maternal toxicity. Toxicokinetic analysis indicated that the fetuses were exposed to drug *in utero*. Day 20, the maternal and fetal levels of total SC-66110 were equivalent at the approximate Tmax. Plasma levels of parent compound were detected in the fetuses at C_{min} except in the LD group. Consistent with other studies, AUC_{0-24} values decreased over time, most likely due to induction of metabolism. From lowest dosage to highest, the plasma levels in this study were 5-31X those produced in humans at the therapeutic dosage based upon free eplerenone. The fetal NOAEL in this study was the MD of 300 mg/kg. The mean plasma levels of eplerenone at this dosage were 15X the expected human plasma levels. The maternal NOEL was 100 mg/kg.

Rabbits

Maternal toxicity was evidenced by dose-dependent decreases in food consumption and body weight of 9% ($p<0.05$) at the HD (300 mg/kg/day). Early resorptions at the HD were significantly increased as were post-implantation losses. There were no fetal external, visceral, skeletal or cranial anomalies reported that could be attributed to drug treatment although there were increased litters with gallbladder effects, 7th cervical rib effects and hydrocephaly when compared to the control groups. Pharmacokinetic data showed that the drug was systemically available at all dosages and exposure did not change with repeated dosing. AUC in the rabbits were 0.82 – 20X of the anticipated human exposure based on free eplerenone. A maternal NOEL was not identified based upon weight loss in all treatment groups.

Pre and Post-natal development

Both the MD of 300 mg/kg and the HD of 1000 mg/kg caused decreased food consumption and weight gain in the adult rats with a more severe effect in the HD group. Reproductive indices for the F0 dams were unaffected. The F1 male and female pups of the HD group showed lower mean body weight than the pups of the 0, LD or MD groups. The decrease in weight gain persisted in both sexes until euthanasia. A developmental delay was indicated by slight increase in the time to balano-preputial separation. Based upon weight effects, the NOAEL for developmental toxicity in this study was 100 mg/kg/day, which produces ~8X the human plasma exposure.

Reproductive and developmental toxicology conclusions:
Rabbits appear to be very sensitive to the effects of eplerenone. No maternal NOEL was identified based upon body weight effects. At the dosages tested, there were increases in resorptions and post-implantation losses in conjunction with decreased weight and clinical signs of decreased urination and defecation.
Epelerenone given orally to rats appears to affect female fertility, embryo-fetal development and post-natal development only at dosages causing maternal toxicity. There are indications from the reproductive toxicity studies that eplerenone may have the capacity to alter male rat fertility at ~23X the human exposure without evidence of paternal toxicity.

Labeling recommendations: Acceptable as written.

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: Comparison of hepatic activities of eplerenone (SC-66110) and spironolactone in rats

Key study findings: Drug-treatment produced increases in liver weight in this non-GLP study. Both drugs caused some induction of hepatic drug-metabolizing enzymes.

Study no:EX4107
Volume #, and page #:
Conducting laboratory and location: GD Searle, Skokie, IL for in life portion and some analysis
Date of study initiation: August 18, 1993
GLP compliance: no
QA reports: yes () no (x):
Drug, lot #, radiolabel, and % purity: eplerenone lot B-90081, spironolactone lot 91-H0797
Formulation/vehicle: 0.5% methylcellulose

Methods: [redacted] CD rats (10/sex/group) received either eplerenone or spironolactone. Animals were then euthanized and necropsied. The effects of the two drugs were assessed using body and liver weight changes, serum ALT and SDH, serum total bile acids, CYP450 and fatty

acyl CoA oxidase activities, hepatic protein content, light microscopy, EM analysis (males only), PCNA labeling and in situ end labeling analysis for apoptosis.
Dosing: oral gavage of 100 mg/kg/day for 2 weeks

Observations and times: see above

Results: Liver weights were increased in both sexes treated with spironolactone (17%m, 31%f) and males treated with eplerenone (13%). Both drugs caused an increase in testosterone oxidation associated with CYP3A1/2,and in females an increase in testosterone 16βhydroxylase activity which may be catalyzed by either CYP3A or CYP2B. In both cases, spironolactone produced a greater change than eplerenone. In male rats a slight decrease in the number of apoptotic cells and a slight increase in PCNA labeling were noted for rats treated with either drug, consistent with the slight increase in cellularity for treated vs control livers. There were no reported changes in serum enzymes or other parameters.

Study title: The effect of SC-66110 on the clearance of thyroxine in male and female rats

Key study findings: The 250 mg/kg dose of SC-66110 caused approximately 29% increase in mean plasma T4 clearance in both sexes. The 750 mg/kg/day dose increased plasma T4 clearance by 62% and 87% in male and female rats respectively. The increases produced by Phenobarbital were 112% and 88% compared to control in males and females respectively.
Study no: EX4880/1 — N00056
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: May 16, 2000
GLP compliance: no
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110 lot SP12626
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in distilled water

Methods: [redacted] Sprague-Dawley rats (male and female) were orally dosed with vehicle or SC-66110 daily for 14 weeks. After 14 weeks, all animals received a single intravenous dose of radiolabelled T4 at a target dose of 1 µg T4/kg (50 µCi/kg). The clearance of the radioactivity from the plasma was determined by [redacted]
Dosing: 0, 250, 750 mg/kg/day

Observations and times: see original review

Results: The 250 mg/kg dose of SC-66110 caused approximately 29% increase in mean plasma T4 clearance in both sexes. The 750 mg/kg/day dose increased plasma T4 clearance by 62% and 87% in male and female rats respectively. For in depth discussion see original review or the Carcinogenicity section of this review.

Study title: Thyroid hormone effect of SC-66110 in the rat (— N 00036/SA5027)

Key study findings: This study showed sustained, significant increases in circulating levels of TSH in drug-treated animals causing increased thyroid weights and association with histologic evidence of thyroid follicular cell hypertrophy/hyperplasia. The activity of hepatic UDPGT was shown to be increased, indicative of increased turnover of T4 (consistent with the previous study using radiolabelled T4) and plausibly leading to increased pituitary secretion of TSH. Most of the changes found during the treatment period were no longer evident at the end of the recovery phase. The changes in thyroid weight and histology were partially reversed.

Study no: SA5027, — N 00036
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: March 29, 2000
GLP compliance: yes
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110 lot 0000955
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in distilled water

Methods: Male and female Sprague-Dawley rats were dosed by oral gavage with either SC-66110, vehicle or phenobarbital (positive control). Duration of dosing was 14, 28 or 91 days. One group of animals received 13 weeks of dosing followed by a 13-week drug-free recovery period.
Dosing: 0, 250, 750 mg/kg/day SC-66110

Observations and times: see original review

Results: Absolute and normalized liver weights were increased in both sexes of drug-treated animals. Both sexes also showed dose-related increases in thyroid weight that were significant at the 750 mg/kg dosage. A dose-related increase in the incidence of thyroid follicular hypertrophy/hyperplasia was also reported for the dosing period but markedly decreased by the end of the recovery period, indicating reversibility. Thyroxine-UDPGT activity in both sexes of eplerenone-treated animals was increased over control values at all points of determination during the dosing period, significantly so in the males as well as the females in week 13. Values did not differ significantly from the controls at the end of the recovery period. Plasma T4 values were decreased week 2 in all drug-treated males and the LD-eplerenone females. At the end of the recovery period the HD-f showed a 360% increase over controls, possibly due to a rebound effect. TSH levels were consistently and significantly elevated in all drug-treated groups during the dosing period. Microsomal CYP3A activity was increased in both sexes. Messenger RNA levels were also increased in both sexes at the end of the dosing period but not at the end of the recovery period. Messenger RNA for UDPGT-2B1 was increased slightly if at all although activity was increased. See original review for more detailed discussion.

Study title: Effects of SC-66110 on GnRH stimulated LH and testosterone release in the male Beagle dog

Key study findings: SC-66110 given to male dogs at 25 mg/kg/day for 12 days did not affect the ability of the dogs to produce an appropriate response to GnRH and thus does not explain the reduction in prostate size in dogs treated with SC-66110.

Study no: EX4554
Volume #, and page #:
Conducting laboratory and location: GD Searle, Skokie, IL
Date of study initiation: October 21, 1996
GLP compliance: no
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110 lot RCT10015
Formulation/vehicle: gelatin capsules

Methods: Weeks 1-2 constituted a 2-week baseline period. 8 male Beagles (15-17 kg) were treated with SC-66110 in gel caps at 25 mg/kg/day for 12 days (weeks 3-4). Blood samples were taken 3X/week during weeks 1 and 3 to determine baseline serum concentrations of LH, testosterone and dihydrotestosterone (DHT). At the beginning of weeks 2 and 4, the dogs were stimulated with GnRH and blood taken to determine resulting concentrations of LH, testosterone and DHT.

Dosing: see above.

Observations and times: see above

Results: While there were some individual animal responses, overall there were no changes in GnRH-stimulated LH release, testosterone production/secretion or DHT concentrations after 12 days of drug treatment when compared to baseline levels. There was a great deal of variability inherent in the system as demonstrated in the individual animal data. As shown in the table below, following 8 days of eplerenone treatment there appears to have been a decrease in mean baseline values of DHT before GnRH stimulation. This was discussed in the toxicology summary.

Dihydrotestosterone (DHT) (pg/mL)

Study Day	1	3	5	8.1*	8.2*	8.3*	8.4*	10
Animal No.								
1101								
1102								
1103								
1104								
1105								
1106								
1107								
1108								
Average	165.69	107.48	55.06	60.49	74.85	199.97	136.7	56.91
STD	375.89	59.86	73.69	77.85	38.7	63.11	55.38	79.36

Study Day	13	15	17	19	22.1*	22.2*	22.3*	22.4*	24	26
Animal No.										
1101										
1102										
1103										
1104										
1105										
1106										
1107										
1108										
Average	91.66	34.98	37.46	32.07	40.88	60.57	161.33	89.6	47.55	41.7
STD	83.32	28.08	29.15	21.61	31	40.73	64.08	46.12	26.67	29.44

DHT NO ≤ 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

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Study title: Effects of SC-66110 on Reproductive Function and Onset of Prostate Size Change in the Male Beagle Dog

Key study findings: Ultrasound showed decreases in prostatic volume in dogs treated with 25 mg/kg/day beginning from week 2. Prostate size in dogs treated with 5 mg/kg/day was unaffected. Prostatic levels of aldosterone were increased in the drug-treated animals while tissue level of DHT and testosterone were slightly decreased. Absolute and relative epididymal weight were decreased in both drug-treated groups. Sperm counts and production were unaffected. Morphology was not assessed.

Study no: EX4541 — 4541
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: November 11, 1996
GLP compliance: no
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110 lot RCT-10056
Formulation/vehicle: gelatin capsules

Methods: Male Beagles ~ 25 months of age were given either empty gelatin capsules (vehicle) or 5 or 25 mg/kg/day of SC-66110, once a day for 13 weeks. Blood for pharmacokinetics was collected at start and completion of the study. Blood for hormonal analyses was collected weeks 1,2,4,6,9 11,12 and at euthanasia. Prostate measurements were made by transrectal ultrasound at weeks 1,2,4,6,9, 11 and 12. Libido and semen were assessed every 2 weeks during treatment. After euthanasia, organ weights and histopathologic analysis were conducted. Daily sperm production and epididymal transit time were also measured. Hormone content of the prostate and testicular tissue were also measured.

Table 11. Mean Hormone Concentrations in Tissue

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	1775.45	232.96	19.33
S.D	1368.96	152.19	15.86
		Group 2	
	T (ng/g)	Ald (pg/g)	DHT (ng/g)
Mean	821.67	135.88	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

Dosing: see above

Observations and times: see above

Results: Ultrasound showed a decrease in prostate size in the 25 mg/kg group from week 2 with weight significantly reduced at necropsy ($p<0.001$). Volume of ejaculate was also decreased (NS) in this group and histopathology showed drug-related atrophy of the secretory epithelium. Testicular weight and sperm production were unaffected by drug-treatment. Epididymal weight was significantly ($p<0.05$) decreased in both drug-treated groups. Serum aldosterone was increased in the HD group while cortisone, DHT, LH and testosterone were unaffected. Prostatic and testicular levels of aldosterone were increased in both drug-treated groups. Prostatic DHT and testosterone were slightly decreased in both drug-treated groups. Testicular testosterone and DHT were increased in the HD group.

Study *title*: In vitro 5alpha-reductase inhibition to support SC-66110

Key study findings: All compounds tested (finasteride, spironolactone, canrenone and aldosterone) except for SC-66110 inhibited 5α-reductase activity in the dog prostate. Only testosterone produced positive results in the human foreskin samples. SC-66110 was shown not to inhibit 5α-reductase activity when tested in dog prostate homogenates. However, the sponsor postulated that inhibition of 5α-reductase activity may still be the mechanism for the prostatic atrophy observed in previous studies. Beagles treated with SC-66110 showed marked elevations in serum aldosterone concentrations and decreased prostate weight. Aldosterone when tested in dog prostate homogenates was shown to inhibit 5α-reductase activity with an IC_{50} of ~2μM.

Study no: P3097017
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: not specified
GLP compliance: no
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: lot numbers not specified
Formulation/vehicle: all compounds dissolved in ethanol

Methods: Activity of 5αreductase was assessed in the pelleted fraction of homogenized dog (untreated) prostate and in human foreskin fibroblasts in the presence of SC-66110, spironolactone, canrenone, aldosterone and finasteride. [^{3}H]-testosterone was incubated with the prostatic or foreskin preparations then analyzed by [redacted] All compounds tested at concentrations from 100 nM to 100 μM.
Dosing: NA

Observations and times: NA

Results: All compounds tested (finasteride, spironolactone, canrenone and aldosterone) except for SC-66110 inhibited 5α-reductase activity in the dog prostate. Only testosterone produced positive results in the human foreskin samples.
There seems to be an error in the reporting of the human foreskin fibroblast results. Why were the compounds not tested above 2 μM?

Reported IC50 values

Compound	IC50 for 5 α-reductase	
	Dog prostate	Human foreskin fibroblasts
Spironolactone	30 μM	
SC-66110	>100 μM	>2μM
Canrenone	2-3.5 μM	>2 μM
Finasteride	<0.1 μM	
Aldosterone	2 μM	

Study title: *In vitro* androgen receptor binding analysis to support SC-66110

Key study findings: All compounds tested showed binding to the androgen receptor. Eplerenone had an IC50 value of 160,000 nM compared to spironolactone's IC50 of 100 nM. SC-71597 had an IC50 value estimated at >1,000,000 nM. In the human foreskin fibroblast assay, SC-66110 exhibited essentially no binding to the androgen receptor.

Study no: P96046
Volume #, and page #:
Conducting laboratory and location: not apparent
Date of study *initiation*: not apparent
GLP compliance: no
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: lot numbers not apparent
Formulation/vehicle: all steroids dissolved in ethanol

Methods: Cytosolic fractions were prepared from the prostates of untreated dogs and used for analysis of competitive androgen receptor binding of [^{3}H]testosterone by eplerenone, SC-71597 (the 6β-OH metabolite of SC-66110) spironolactone, canrenone, DHT and aldosterone. Data were also generated using cultured human foreskin fibroblasts to identify receptor binding potential of SC-66110 to the human androgen receptor.
Dosing: concentrations of 0.05 through 50,000 nM used.

Observations and times: NA

Results: All compounds tested showed binding to the androgen receptor of the canine prostate. Eplerenone had an IC50 value of 160,000 nM compared to spironolactone's IC50 of 100 nM. SC-71597 had an IC50 value estimated at >1,000,000 nM. In the human foreskin fibroblast assay, SC-66110 exhibited essentially no binding to the androgen receptor.

	IC_{50}			
	3.5 nM ^{3}H-testosterone	2.5 nM ^{3}H-DHT	estimated Ki	RBA*
DHT	7.9 nM	2.5 nM	3.8 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
SC-66110	69.000 nM	160.000 nM	55.000 nM	0.0062
SC-71597	>1.000.000 nM	>1.000.000 nM	>630.000	<0.001
*relative binding affinity				

Study title: Human Androgen Receptor Transcriptional Activation Dose-Response Studies with Various lots of SC-66110

Key study findings: There were no significant differences between the lots in their ability to bind the hAR.

Study no: R01317
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: April 23, 2001
GLP compliance: no
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110 lots RCT10055 (A), RCT9937 (B), 00K007-F3A(C), 00K007-F5A(D)
Formulation/vehicle: dissolved in ethanol

Methods: The ability of various lots of SC-66110 to inhibit 5α-dihydrotestosterone-induced transcription was assessed in an in vitro transcriptional assay using the human androgen receptor (hAR) and a luciferase reporter in monkey kidney CV-1 cells. The four lots listed above were tested in 6 separate experiments. Hydroxyflutamide was the positive control.
Dosing: concentrations of 0.03 – 100 μM for SC-66110

Observations and times: NA

Results: Despite suboptimal presentation of results, it appears that all lots of SC-66110 showed essentially the same IC50 of ~30μM for binding to the hAR . The positive control had an IC50 of ~ 0.1μM.

Study title: In vitro compatibility of a parenteral formulation of SC-66110 with canine blood

Key study findings: Significant hemolysis occurred with SC-66110 in this formulation down to dilutions of 33.3% of the original concentration. This formulation of the drug was determined to be incompatible with canine blood in this in vitro study.

Study no: EX4458
Volume #, and page #:
Conducting laboratory and location: GD Searle, Skokie, IL
Date of study initiation: November 21, 1995
GLP compliance: no
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110 lot GDS-6050-001
Formulation/vehicle: 15% hydroxypropyl β-cyclodextrin (HBCD)

Methods: The vehicle alone and with drug were tested for their potential to cause in vitro hemolysis of whole (heparinized) blood from untreated dogs. Both the formulation and the vehicle were tested in dilutions from 100% to 10% of the original concentration.
Dosing: NA

Observations and times: NA

Results: Significant hemolysis occurred with SC-66110 in hydroxypropyl β-cyclodextrin down to the 33.3% dilution. The next dilution was 10% for which no hemolysis was reported.

Study title: An antigenicity study of SC-66110 in multiple species
Key study findings: Under the conditions of the study, eplerenone did not exhibit antigenic properties.

Study no: [redacted] 78-02
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation:
GLP compliance: yes
QA reports: yes (x) no ():
Drug, lot #, radiolabel, and % purity: SC-66110, lot RCT10016
Formulation/vehicle: 0.5% methylcellulose and 0.1% polysorbate 80 in distilled water. Ovalbumin was dissolved in saline as the positive control.

Methods: The antigenicity of SC-66110 was investigated using guinea pigs, mice and rats. To sensitize the guinea pigs, SC-66110 was administered orally five times weekly and subcutaneously with Freund's complete adjuvant (FCA) once weekly for 3 weeks. To sensitize the mice, SC-66110 was administered orally 5X/week and intraperitoneally with 3% aluminum hydroxide gel once weekly for 3 weeks. 0.5% methylcellulose and 0.1% polysorbate 80 in dH2O was administered orally five times weekly for 3 weeks as the negative control. Ovalbumin with

adjuvant was administered subcutaneously to guinea pigs and intraperitoneally to mice once weekly for 3 weeks as the positive control.
Dosing: see above. Doses for sensitization in all species were 2 and 20 mg/kg po, sc and ip. The same doses were used for the iv challenge for both elicitation of systemic anaphylaxis and the passive cutaneous anaphylaxis response.

Observations and times: Appropriate for the tests

Results:
ASA response with guinea pigs-no abnormalities reported for the SC-66110 or negative control gps. Positive control animals died within 4 minutes after the challenge.

4hr PCA response in guinea pigs-None of the passively sensitized guinea pigs or negative control animals showed evidence of PCA. GPs sensitized with sera from the positive control groups showed evidence of PCA.

48 hour PCA response with Mice and Rats- Rats passively sensitized with sera from the SC-66110 group did not show evidence of PCA while the positive control groups did.

Study title: Dermal Sensitization Study of SC-66110 in Guinea Pigs-Maximization Test

Key study findings: None of the test animals exhibited a dermal reaction to the challenge application of SC-66110

Study no: EX4754
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: January 21, 1998
GLP compliance: yes
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110, lot 96K018-F1A
Formulation/vehicle: petrolatum

Methods:

conducted. Twenty males were assigned to the SC-66110 group and ten males were assigned to the control group. On Day 1, animals in the test and control groups received the following duplicate 0.1-mL intradermal injections in the shoulder area.

Description of Injections		
	Test Group	Control Group
Anterior Sites	1:1 dilution of Freund's Complete Adjuvant (FCA) in sterile water	
Medial Sites	5% w/v suspension of test material in mineral oil	Vehicle (mineral oil)
Posterior Sites	5% w/v suspension of test material in FCA in sterile water	1:1 dilubon of mineral oil in FCA

On Day 7, the animals in the test and control groups were pretreated with 10% w/w sodium lauryl sulfate suspension in petrolatum applied topically at the injection sites. On Day 8, a 25% w/w mixture of test material in petrolatum was topically applied over the injection sites of the animals in the test group, and petrolatum was applied topically over the injection sites on the animals in the control group. All induction sites were then occluded for 48 hours.

Two weeks after topical application, all animals received a challenge dose of SC-66110. A 15% w/w mixture of SC-66110 in petrolatum was topically applied to the right side and the control material (petrolatum) was applied to the left side of each animal in the test and control groups. All test and control sites were occluded for 24 hours and then wiped clean. The challenge sites were examined for dermal reactions at 24 and 48 hours after patch removal. The ratings for dermal sensitization are based solely on the number of animals responding. Clinical observations were performed daily throughout the study

Dosing: As described under methods

Observations and times: As described above

Results: There were no recorded dermal reactions.

Study title: Primary Dermal Irritation Study of SC-66110 in Rabbits
Key study findings: A primary dermal irritation index of 0.04 was reported. Under the conditions of the test, SC-66110 was slightly irritating to rabbit skin.

Study no: EX4752
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: January 21, 1998
GLP compliance:yes
QA reports: yes ( ) no ( ):

Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: distilled water.

Methods: 0.5g of test material was moistened with distilled water and applied to a single intact site on each of 3 male and 3 female NZW rabbits. The drug was then covered with a semi-occlusive dressing. After a 4-hour exposure period the test material patch was removed and the area washed. Observations of dermal irritation according to the Draize system were made at 30 – 60 minutes after removal of the test material (referred to as the 4 hour observation) and at 24, 48 and 72 hours after application.
Dosing: As described in methods.

Observations and times: As described in methods.

Results: A slight erythema was reported for one animal at the 4-hour observation.

Study title: Primary Eye Irritation Study of SC-66110 in Rabbits
Key study findings: Under the conditions of the study, the drug was considered to be minimally irritating to the treated eyes of rabbits that were unwashed after treatment and to eyes that were washed after instillation of test material.

Study no: EX4753
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: January 21, 1998
GLP compliance: yes
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: SC-66110, lot 96K018-F1A
Formulation/vehicle: administered as received (a white powder)

Methods: SC-66110 (28 mg) in a volume of 0.1ml was placed into the lower eyelid of the right eye. The left eye of each rabbit served as the untreated control. The eyes of Group 1 rabbits were not flushed after instillation with test material. The eyes of Group 2 rabbits were flushed with water approximately 30 seconds after drug application. Eyes were observed and graded according to the Draize system at 1,24,48 and 72 hours after treatment.
Dosing: As described above.

Observations and times: As described above.

Results: In Group 1 unwashed eyes, slight iritis was reported for 1/3 rabbits (cleared by 24 hours) and slight to moderate conjunctival irritation in 3/3 animals. The conjunctivitis was cleared within 48 hours. In Group 2 washed eyes, slight conjunctival irritation was reported for 3/3 rabbits. This was reported as cleared within 24 hours.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: There are no Pharmacology/Toxicology issues that preclude approvability

General Toxicology Issues: None relevant to clinical use

Recommendations: The reviewer recommends approval

Labeling with basis for findings: The labeling is acceptable as written

X. APPENDIX/ATTACHMENTS:

Addendum to review: Appendix I. Sponsor's Summary of Impurity Qualification

Other relevant materials (Studies not reviewed, appended consults, etc.): Studies Not reviewed are attached.

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