CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-437/S-002

Pharmacology Review(s)

NDA # 21,437/ S002

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

INSPRA®
For the indication of heart failure after myocardial infarction

Pharmacia-Pfizer
Chicago, Illinois

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Division of Cardio-Renal Drug Products (HFD-110)
Center for drug evaluation and Research
FDA
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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability: There are no preclinical findings to preclude approval

1.2 Recommendation for nonclinical studies: None

1.3 Recommendations on labeling: Lines — regarding findings — — should be removed.

2. Summary of nonclinical findings

2.1 Brief overview of nonclinical findings: This is a supplemental application to NDA21437, approved October, 2002. The review of that NDA is referenced. No new toxicology studies were presented

2.2 Pharmacologic activity: Inspra® (eplerenone) is a mineralocorticoid receptor antagonist. Clinically, plasma aldosterone has been shown to correlate to left ventricular hypertrophy, vascular stiffness and mortality in heart failure.

2.3 Nonclinical safety issues relevant to clinical use: none. The review of NDA 21-437 is referenced.

PHARMACOLOGY/TOXICOLOGY REVIEW

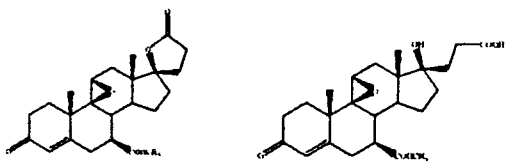
3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21437/S002
Review number: 1
Sequence number/date/type of submission: SE1-002
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Pharmacia
Manufacturer for drug substance:

Reviewer name: **Elizabeth Hausner, DVM**
Division name: Cardio-Renal
HFD #: 110
Review completion date: July 10, 2003

Drug:

Trade name: Inspra™
Generic name: eplerenone
Code name: SC-66110
Chemical name: Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo-,γ-lactone,methyl ester, (7α, 11α,17α)
CAS registry number:
Molecular formula/molecular weight: $C_{24}H_{30}O_6$/414.50
Structure:



SC-66110 SC-70303 free acid

Relevant INDs/NDAs/DMFs: IND51780, NDA21-437

Drug class: mineralocorticoid antagonist

Indication: treatment of heart failure after myocardial infarction

Clinical formulation: tablets contain 50mg or 100 mg. Inactive ingredients are lactose, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl methylcellulose, sodium lauryl sulfate, talc, magnesium stearate, titanium dioxide, polyethylene glycol, polysorbate 80, iron oxide yellow and iron oxide red (25 mg tablet) and iron oxide red (50 and 100 mg tablets).

Route of administration: oral

Proposed use: [redacted]

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:
DO2CM0061 *Effect of Eplerenone (SC- 66110) in a Transgenic Mouse Model of Aldosterone- Mediated Heart Failure*
D02CM0024: *Effect of Eplerenone (SC- 66110), Enalapril, and Coadministration Therapy in Rats Post Myocardial Infarction*
D02CM0036: *Effect of Eplerenone (SC-66110), Enalapril and Coadministration Therapy in Mice Post Myocardial Infarction*
BRD01D2120 *AMENDMENT 1 - Effects of Eplerenone (SC-66110) on the Progression of Myocardial Dysfunction and Injury in Dogs with Heart Failure*
D02CM0025: *Effects of eplerenone(SC-66110), enalapril or eplerenone coadministered with enalapril on the progression of heart failure in SHHF rats.*
D02CM0083: *Long-term eplerenone (SC-66110) treatment in the SHHF rat.*
BRD01D2139: *Pharmacokinetics of eplerenone (SC-66110) in rats: eplerenone administered in the chow*

M2001172: *Tissue distribution following single oral admin to female Sprague-Dawley rats*
M2002021: *[^{14}C]SC-66110 whole body autoradiography in rats following oral administration*
M2002020: *[^{14}C]SC-66110 mass balance, metabolic profile, partial distribution following oral admin*
M2001173: *[^{14}C]SC-66110 mass balance and metabolic profile oral admin in beagle dogs*
PK0144: *Biliary excretion and enterohepatic circulation of [^{14}C]SC-66110 in the rat*

Studies not reviewed within this submission:
None

3.2 PHARMACOLOGY

3.2.1 Brief summary: The sponsor presents a transgenic mouse model of over-expression of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), a surgical model of myocardial infarction (coronary ligation) in rats and mice, an induced heart failure model in dogs (repeated coronary microembolizations) and a congenital model of heart failure using Spontaneously Hypertensive Heart Failure Prone rats (SHHF) in both acute and chronic studies.

The 11β-HSD2 genotype allows for over-conversion of glucocorticoids to receptor-inactive 11-keto analogs, allowing aldosterone to bind to the mineralocorticoid receptor. The present study showed significant induction of the gene expression in

the heart, lung and liver with significant increases in the gene product's enzymatic activity in the heart. Eplerenone treatment mitigated both anatomic factors (less of an increase in heart weight compared to untreated transgenic mice) and some functional parameters such as ejection fraction (48±4 % vs 32±4 for the TG control group, p<0.05) and left ventricular areas at systole (0.11±0.01 cm2 vs 0.21±0.02 cm2 for the control group, p<0.05) and diastole (0.16±0.01 cm2 vs 0.26±0.02 cm2 , p<0.05).

Eplerenone and enalapril were compared alone and in combination in mechanically-induced heart failure mice and rats. Treatment lasted for 8 weeks in the rats and 12 weeks in mice. In both species, the drug-treated animals gained as much or more body weight than did the sham operated or untreated controls. Both eplerenone and enalapril produced slight increases in ±dp/dt in rats suggesting increased contractility. This is summarized in the reviewer's table below.

Summary of Hemodynamic Data for rats

	Sham n=20	MI Untreated N=11	MI Eplerenone N=13	MI Enalapril N=13	MI Epler+enalap N=15
+dp/dt mm Hg/sec	6444±112	5698±121	6309±189	6894±162	6773±162
-dp/dt mm Hg/sec	6317±93	4771±152	5052±156	5755±133	5529±149

In rats both drugs slightly decreased the interstitial collagen fraction in viable myocardium with no apparent difference between the drugs(untreated 2.8±0.6%, eplerenone 2.3±0.4%, enalapril 2.4±0.7%, eplerenone + enalapril 2.7±0.6). Each treated group was different from the control by p<0.05. Infarct size was smaller in enalapril-treated rats (25.0± 1.0% vs 30.0±1.0% for eplerenone and 32.0±2.0% for the untreated, p<0.05 vs eplerenone). There were no significant differences between the groups with respect to plasma ANP or OPN. Enalapril produced a striking decrease in urinary protein. Overall, in this model in rats, eplerenone alone or with enalapril attenuated some of the progression of cardiac dysfunction. There were some parameters where enalapril alone produced a greater effect than eplerenone or the combination with eplerenone. In mechanically-injured mice, there were minor effects on the progression of the cardiac pathology for the first 6 weeks of treatment (8 weeks post-injury). Enalapril had more of an effect than eplerenone or the combination of enalapril and eplerenone. By the end of the study, myocyte cross sectional area and interstitial collagen fraction were somewhat decreased following eplerenone treatment, but not to the same extent as seen with enalapril treatment. This is shown in the sponsor's table below.

Table 21. Effect of Eplerenone After 12 Weeks of Treatment

	Sham (n=13)	MI-Untreated (n=11)	MI-Eplerenone (n=16)	MI-ACEi (n=13)	MI-Eplerenone + ACEi (n=12)
At_{WT}/BW (mg·100/g BW)	26.1±1.7	61.4±8.4*	55.6±4.3*	36.9±3.9*†‡	34.5±2.6*†‡
LV_{WT}/BW (mg·100/g BW)	309.0±8.3	492.1±29.8*	418.1±12.1*†	386.2±20.0*†‡	387.8±18.1*†
RV_{WT}/BW (mg·100/g BW)	74.5±2.9	105.0±10.5*	106.7±6.3*	84.5±6.7*‡	80.3±3.2†‡
HW/BW (mg·100/g BW)	409.5±9.0	658.5±45.0*	580.3±19.9*	507.6±28.1*†‡	502.6±20.2*†‡
$Liver_{WT}$/BW (mg·100/g BW)	4052.0±114.4	4595.0±92.1*	4956.4±95.6*†	4308.3±113.2†‡	4862.4±169.3*†§
$Lung_{WT}$/BW (mg·100/g BW)	537.3±23.3	677.7±73.2*	626.5±25.0*	585.1±23.1	550.4±22.2†‡
Infarct Size (%)	--	43±2	42±2	38±3	42±3
MCSA (μm^2)	173.5±6.2	345.0±9.1*	290.5±6.8*†	264.2±9.4*†‡	264.5±10.0*†‡
ICF (%)	4.68±0.17	12.69±0.60*	9.48±0.46*†	9.20±0.49*†	8.44±0.46*†‡

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated, ‡ $p<0.05$ vs. MI-Eplerenone, § $p<0.05$ vs. MI- ACEi. At_{WT}, atrium weight, BW, body weight; LV_{WT}, left ventricle weight; RV_{WT}, right ventricle weight; HW, heart weight; MCSA, myocyte cross-sectional area; ICF, interstitial collagen fraction; --, no infarction.

Overall, enalapril produced greater effects than eplerenone in the parameters studied.

Repeated coronary microembolizations were used to induce heart failure in otherwise healthy dogs. Under the conditions of the study, use of eplerenone in dogs with induced cardiac functional deficits caused less replacement fibrosis and interstitial collagen. Drug treatment was also reported to cause an increase in capillary density. The study would be stronger for the use of positive controls or a comparator compound, either historical or concurrent. The methodological details

of functional determinations were without sufficient detail to allow for independent evaluation of the data.

The SHHF-Gmi-/fa^{CD} is a congenital model of dilated cardiomyopathy with hypertension that progresses to decompensated heart failure. Echocardiographic evaluation indicated that eplerenone attenuated some of the effects of the progression of heart failure but that enalapril produced a greater effect. The combination of enalapril and eplerenone produced a slightly greater effect than enalapril alone.

Table 1. Effects of 13 Weeks of Treatment on Echocardiography Parameters

Parameters Evaluated	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
LVAd (cm^2)	1.39±0.06	1.28±0.05 (0.14±0.04)	1.22±0.02 (0.11±0.04)	1.11±0.03 (-0.07±0.03)*†‡	1.07±0.05 (-0.01±0.04)*†‡
LVAs (cm^2)	1.15±0.05 (0.35±0.05)	0.94±0.06 (0.17±0.04)*	0.86±0.02 (0.13±0.04)*	0.71±0.03 (-0.09±0.02)*†‡	0.58±0.04 (-0.04±0.03)*†‡
EDV (mL)	0.97±0.06 (0.25±0.05)	0.83±0.05 (0.14±0.04)	0.74±0.02 (0.12±0.03)*	0.63±0.03 (-0.06±0.03)*†‡	0.60±0.04 (-0.03±0.04)*†‡
ESV (mL)	0.65±0.05 (0.29±0.01)	0.47±0.05 (0.14±0.04)*	0.39±0.02 (0.10±0.03)*	0.30±0.02 (-0.06±0.02)*†‡	0.22±0.02 (-0.02±0.02)*†‡
EF (%)	33±2 (-17±2)	45±3 (0.9±2)*	47±1 (-6±2)*	53±2 (4±3)*†‡	63±1 (0.9±2)*†‡
LVLd (cm)	1.72±0.05 (0.07±0.04)	1.72±0.04 (0.06±0.05)	1.69±0.03 (0.08±0.04)	1.63±0.03 (-0.06±0.03)*†‡	1.60±0.04 (-0.02±0.05)
HR (beats/min)	336±9 (3±12)	341±8 (-11±13)	357±7 (11±13)	354±8 (-0.1±10)	334±5 (3±14)
CO (L/min)	0.11±0.01 (-0.01±0.01)	0.12±0.01 (0.00±0.01)	0.13±0.01 (0.01±0.01)*	0.12±0.01 (0.00±0.01)	0.13±0.01 (0.00±0.01)
SV (mL)	0.32±0.03 (-0.05±0.03)	0.33±0.02 (-0.02±0.02)	0.35±0.01 (0.02±0.02)*	0.34±0.02 (-0.01±0.03)	0.37±0.02 (-0.02±0.03)
FS (%)	28±3 (-5±3)	33±2 (0.5±2)	33±0.9 (0±2)	36±2 (6±3)*§	37±2 (-1±3)§
FAC (%)	17±1 (-17±2)	27±2 (-5±2)*	30±2 (-5±2)*	36±2 (4±2)*†‡	46±2 (2±2)*†‡
IVSd (cm)	0.22±0.01 (0.00±0.01)	0.21±0.01 (-0.03±0.01)*	0.21±0.01 (-0.03±0.01)*	0.20±0.01 (-0.02±0.01)	0.21±0.02 (0.02±0.03)§
IVSs (cm)	0.35±0.02 (0.01±0.03)	0.35±0.02 (-0.02±0.02)	0.33±0.01 (-0.03±0.01)	0.32±0.01 (-0.02±0.01)	0.35±0.02 (0.02±0.03)
LVIDd (cm)	1.07±0.03 (0.14±0.03)	1.00±0.03 (0.11±0.02)*	0.94±0.01 (0.06±0.02)*	0.86±0.02 (-0.03±0.04)*†‡	0.86±0.02 (-0.01±0.03)*‡
LVIDs (cm)	0.77±0.03 (0.14±0.03)	0.68±0.04 (0.07±0.03)	0.63±0.01 (0.04±0.02)*	0.53±0.02 (-0.07±0.04)*†‡	0.54±0.03 (0.00±0.03)*
LVPWd (cm)	0.20±0.01 (-0.02±0.01)	0.22±0.01 (0.00±0.01)	0.18±0.02 (-0.04±0.01)‡	0.19±0.01 (-0.02±0.01)	0.20±0.01 (-0.02±0.01)
LVPWs (cm)	0.35±0.02 (0.01±0.03)	0.32±0.01 (-0.01±0.02)	0.28±0.01 (-0.05±0.01)	0.29±0.01 (-0.02±0.02)	0.33±0.01 (-0.02±0.02)

Data reported as mean ± SEM.

* $p<0.05$ vs. Untreated, † $p<0.05$ vs. Eplerenone, ‡ $p<0.05$ vs. Enalapril, § $p<0.05$ vs. Eplerenone + Enalapril

SHHF, spontaneously hypertensive heart failure prone rat; SD-Ctrl, age matched Sprague Dawley control rats; LVAd, left ventricular area diastole, LVAs, left ventricular area systole; EDV, end diastolic volume; ESV, end systolic volume; EF, ejection fraction; LVLd, left ventricular length in diastole; HR, heart rate; CO, cardiac output; SV, stroke volume; FS, fractional shortening; FAC, fractional area change; IVSd, interventricular septum diastole; IVSs, interventricular septum systole; LVPWd, left ventricular posterior wall diastole; LVPWs, left ventricular posterior wall systole.

Eplerenone had no effect on systolic blood pressure while enalapril caused a decrease that was enhanced by the addition of eplerenone.

Table 2. Effects of Treatment on Systolic Blood Pressure

SBP (mm Hg)	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
Baseline	199±6	196±6	195±3	200±7	140±4*
6 Weeks	189±8	204±7	152±6*†	127±5*†‡	129±5*
12 Weeks	192±6	188±7	163±6*†	151±6*†	135±2*

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated; † $p<0.05$ vs. Eplerenone; ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat. SD-Ctrl, age matched Sprague Dawley control rats; SBP, systolic blood pressure.

Left ventricular collagen content was slightly increased by eplerenone, slightly decreased by enalapril and somewhat more decreased by the combination of drugs. For most of the parameters studied, enalapril produced more of an effect than eplerenone. The combination of eplerenone and enalapril produced slightly more of an effect than enalapril alone. The same animal model was used in a study lasting 56 weeks, conducted using one dose of eplerenone and no comparator compound. Under the conditions of the longer study, there was no effect upon cardiac collagen content. Eplerenone provided some mitigation of the effects of congenital spontaneous hypertensive heart failure of rats. A decrease in plasma osteopontin levels was maintained throughout the study and became statistically significant at the last points of determination (48 and 56 weeks).

Table 6. Effect of Eplerenone Treatment on Plasma Osteopontin Levels

OPN (ng/ml.)		
Weeks of Treatment	Untreated	Eplerenone
Baseline (0)	44.24±3.57	38.51±2.13
16	41.54±3.64	37.99±2.90
32	37.29±2.68	26.64±3.01
48	107.47±19.70	67.53±8.21*
56	133.98±15.93	86.39±12.10*

* $p<0.05$ vs. Untreated.
Values represent mean ± SEM.
OPN, osteopontin.

There were decreases in cardiac gene expression of osteopontin (1.84±0.53 for control vs 0.86±0.10 for eplerenone, $p<0.05$) and COX-2 (1.22±0.17 for control vs

0.76±0.11 for eplerenone, p<0.05). The study would have been stronger with the inclusion of a comparator compound and the use of several doses to allow for determination of dose-response effects.

Overall, in those studies where a comparator compound was used, eplerenone provided some mitigation of the cardiac deterioration but was less effective than the comparator. In the acute SHHF rat study, eplerenone actually worsened one of the parameters (left ventricular collagen content). However, each animal model is at best an approximation for different facets of a complex, multi-factorial condition of humans. One cannot necessarily extrapolate the results of animal models to predict efficacy or the lack thereof in the clinical setting. A number of factors were analyzed that are indicative of damage (ANP, β-MHC, collagen III) or considered to indicate inflammatory or degenerative processes (the pro-inflammatory cytokines MCP-1, OPN, IL-1β and IL-6). While these markers may be of interest in examining potential mechanisms in the non-clinical species, they are not yet correlated with a clinical outcome in humans. Therefore, the section of the proposed labeling from lines 77 – 81 that refers to the non-clinical models of heart failure should be deleted.

3.2.2 Primary pharmacodynamics

Mechanism of action: mineralocorticoid receptor antagonist

Drug activity related to proposed indication: Clinically, plasma aldosterone concentrations have been shown to correlate positively with left ventricular hypertrophy, vascular stiffness and increased mortality in heart failure. In the RALES (Randomized Aldactone Evaluation Study) trial in NY Heart Association Class III/IV heart failure patients, a survival benefit was achieved when the aldosterone receptor antagonist spironolactone was added to standard therapy for congestive heart failure.

Effect of Eplerenone (SC- 66110) in a Transgenic Mouse Model of Aldosterone-Mediated Heart Failure D02CM0061. January 17, 2003

The effects of eplerenone (SC-66110), a selective aldosterone blocker, were examined in transgenic (TG) mice overexpressing 11 - β hydroxysteroid dehydrogenase type 2 (11 β-HSD2) selectively in cardiomyocytes. These TG (C57BL/6, 11β-HSD2, founder line 326) developed cardiac hypertrophy, fibrosis, and died prematurely between 4 and 6 months of age on a normal salt diet without exogenous aldosterone. In this study, one month old, male TG mice and their nontransgenic littermates were randomized to the following 4 treatment groups: 1) nonTG control (n=8, normal chow); 2) non TG eplerenone (n=9, eplerenone, 200 mg/kg/day in food); 3) TG control (n=12, normal chow); and 4) TG eplerenone (n=12, eplerenone, 200 mg/kg/day in food and studied from 1 to 3.5 months of age. Twenty-four hours prior to study termination, animals were placed in metabolism cages for the collection of urine. Blood samples were collected at time of euthanasia. Hearts and kidneys were collected for histological analysis. Various

tissues were collected for molecular analysis. Echocardiograms were acquired when the animals were 2.5 and 3.5 months of age using a ——— system with a 15 MHz linear array transducer. Blood pressure measurements were obtained at 3.5 months of age by indirect methods after the mice were trained to the techniques.

The 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts glucocorticoids to their receptor-inactive 11-keto analogs and thus allows aldosterone to bind to mineralocorticoid receptors(MR). Under physiologic conditions, cardiac MR are tonically inactivated by corticosterone. The current study evaluated the hypothesis that selective overexpression of cardiomyocyte 11β-HSD2 would result in aldosterone activation of cardiac MR and thus pathological consequences.

Results: Expression of 11β-HSD2 gene was normalized to cyclophilin. There was significant induction of expression in the heart, lung and liver. Expression in the spleen, brain and muscle were slightly above the wild type levels.

Table 2. Tissue Specific Expression of the 11β-HSD2 Gene*

	NonTG Mice	TG Mice
Heart	0.90±0.34	3406.60±526.65
Lung	1.20±0.21	81.80±22.86
Liver	1.00±0.07	12.40±4.45
Spleen	1.00±0.13	2.30±0.25
Brain	1.00±0.06	2.20±0.80
Muscle	1.00±0.18	3.80±1.17
Colon	1.10±0.15	1.10±0.14
Kidney	0.90±0.12	1.00±0.06

TG, transgenic.
* Relative fold induction of 11β-HSD2 mRNA normalized to cyclophilin.
Values expressed as mean ± SEM.

Activity of the enzyme was shown to be markedly increased over the wild type levels in the heart.

Table 3. 11β-HSD2 Activity in Heart and Kidney

	Activity (fmol/mg/min)	
	NonTG Mice (n=2)	TG Mice (n=2)
Heart	0.10±0.04	6.87±0.91
Kidney	3.95±0.60	3.76±0.13

TG, transgenic.
Values expressed as mean ± SEM.

After 2.5 months of treatment with eplerenone, there were no significant differences between the groups with regard to body weight. Heart weight was significantly increased in both groups of TG mice but less so in the drug-treated group. Eplerenone treatment also modified some of the functional parameters of the cardiovascular system. Ejection fraction (EF) was greater in TG mice who received epelerenone compared to the controls. The left ventricular areas at diastole and systole of the drug-treated TGs were significantly smaller than those of the untreated TGs. Drug-treatment also mitigated the effects of the 11β-HSD2 activity on several other parameters as shown below.

Table 4. Effect of 2.5 Months of Eplerenone Treatment on Phenotypic Parameters

	NonTG Control	NonTG Eplerenone	TG Control	TG Eplerenone
BW (g)	24.19±0.68	23.30±0.39	23.79±1.09	22.97±0.72
HW (mg)	152±10	148±8	340±30*†	227±18*†‡
HW/BW (mg/g)	6.35±0.46	6.35±0.34	14.41±1.63*†	9.85±0.66*†‡
KW (g)	0.17±0.00	0.15±0.01*	0.14±0.01*	0.15±0.01
KW/BW (mg/g)	7.18±0.26	6.41±0.33*	5.88±0.32*	6.49±0.33
EF (%)	73±4	72±2	32±4*†	48±4*†‡
LVAd (cm^2)	0.12±0.00	0.11±0.01	0.26±0.02*†	0.16±0.01*†‡
LVAs (cm^2)	0.05±0.01	0.05±0.01	0.21±0.02*†	0.11±0.01*†‡
EDV (mL)	0.02±0.00	0.02±0.00	0.08±0.01*†	0.03±0.00‡
ESV (mL)	0.01±0.00	0.01±0.00	0.06±0.01*†	0.02±0.00‡
SV (mL)	0.014±0.002	0.014±0.002	0.024±0.002*†	0.016±0.002‡
FS (%)	54±3	54±2	19±2*†	31±3*†‡
LVIDd (cm)	0.33±0.01	0.31±0.02	0.49±0.01*†	0.38±0.02*†‡
LVIDs (cm)	0.15±0.01	0.14±0.01	0.40±0.02*†	0.26±0.02*†‡
HR (beats/min)	524±12	514±10	495±12	484±11
SBP (mmHg)	110±1	106±2*	105±2*	104±2*

TG, transgenic; BW, body weight; HW, heart weight, KW, kidney weight; EF, ejection fraction; LVAd, left ventricular area diastole; LVAs, left ventricular area systole; EDV, end diastolic volume; ESV, end systolic volume; SV, stroke volume; FS, fractional shortening; LVIDd, left ventricular internal dimensions diastole; LVIDs, left ventricular internal dimensions systole; HR, heart rate; SBP, systolic blood pressure.

Values expressed as mean ± SEM.

* $p<0.05$ vs. NonTG Control, † $p<0.05$ vs. NonTG Eplerenone, ‡ $p<0.05$ vs. TG Control.

Table 7. Effect of 2.5 Months of Treatment on Cardiac Gene Expression**

	NonTG Control	NonTG Eplerenone	TG Control	TG Eplerenone
11β-HSD2	1.04±0.12	1.04±0.14	5964.69±619.11*†	10530.04±1031.34*†‡
ANP	1.03±0.06	0.83±0.13*	11.27±1.48*†	8.92±0.85*†
β-MHC	1.11±0.13	2.06±0.73	40.93±4.84*†	32.20±2.52*†
α-MHC	1.05±0.09	1.34±0.10*	0.56±0.02*†	0.75±0.04*†‡
Collagen I	1.05±0.11	0.94±0.12	2.92±0.23*†	2.33±0.25*†
Collagen III	1.09±0.12	1.20±0.15	2.71±0.18*†	1.90±0.18*‡
OPN	1.20±0.27	1.43±0.29	14.12±0.62*†	10.92±1.47*†‡
MMP-9	1.01±0.06	0.78±0.03*	0.39±0.03*†	0.49±0.04*†‡
AS	1.04±0.18	1.59±0.34	0.92±0.15†	1.74±0.28*‡
EGR-1	1.07±0.19	0.72±0.11	0.78±0.06	1.03±0.06†‡

11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; ANP, atrial natriuretic peptide; MHC, myosin heavy chain; OPN, osteopontin; MMP-9, matrix metalloproteinase; AS, aldosterone synthase; EGR, early growth response.

** Relative fold induction of mRNA normalized to cyclophilin.

* $p<0.05$ vs. NonTG Control, † $p<0.05$ vs. NonTG Eplerenone, ‡ $p<0.05$ vs. TG Control.

Values expressed as mean ± SEM.

Plasma sodium was lower in the eplerenone-TG (135.6±7.6 mmol/l) group compared to the control group (148.7±1.6 mmol/l) . BNP was highest in the eplerenone-TG animals: 2.07±0.39 ng/ml vs 2.00 ±0.47ng/ml for the controls. The morphology analysis showed that all TG animals regardless of treatment showed myocardial hypertrophy and interstitial fibrosis. Eplerenone treatment caused a striking up-regulation of the 11β-HSD2 regulation as shown below.

Over-expression of 11β-HSD2 in the murine heart lead to cardiac hypertrophy and death of number of the mice, presumably from heart failure. Under the conditions of the study, 2.5 months of eplerenone treatment mitigated some of the physical and functional effects of the over-expression on the heart. An interesting finding was that treatment of the TG mice with epelernone lead to still further increased over-expression of the gene. Perhaps analogous to the up-regulation of the renin-angiotensin-aldosterone system as a consequence of blockade.

Effect of Eplerenone (SC- 66110), Enalapril, and Coadministration Therapy in Rats Post Myocardial Infarction D02CM0024, November 25, 2002
Male Sprague-Dawley rats underwent surgical infarction of the left ventricle (LV) or sham MI. Following surgery, sham MI rats received normal chow and rats with MI received either eplerenone in chow (100 mg/kg/day, n=21), enalapril in drinking water (10 mg/kg/day, n=20), or eplerenone plus enalapril for 8 weeks (n=21). The sham operated group (n=20) received regular rodent chow. Left ventricular structure and function were monitored using serial echocardiography (— 15 MHz transducer) and LV hemodynamic function was evaluated at the termination of the study (— catheter transducer). Blood samples were collected at time of euthanasia. Heart and kidneys were also collected. The heart was perfusion fixed after weighing and normalization of weight to tibial length. The kidneys were sectioned and snap frozen in liquid nitrogen.

Plasma osteopontin levels were determined as well as atrial natriuretic peptide, plasma aldosterone and urinary protein. Image analysis included picrosirius red staining to quantitate interstitial collagen fractions and infarct sizes.

Results:
After 2 weeks of treatment, eplerenone, enalapril and the combination produced statistically significant differences from the MI-untreated group in left ventricular area, left ventricular systolic end volume, left ventricular length, ejection fraction, ejection velocity and mitral valve deceleration time.

The effect was maintained at 6 weeks and 8 weeks although there was progression of the treated animals and increased difference from the sham operated animals.

Table 2. Effect of Treatment at 2 Weeks

	Sham (n=20)	MI-Untreated (n=11)	MI-Eplerenone (n=13)	MI-Enalapril (n=12)	MI-Eplerenone + Enalapril (n=15)
LVAs (cm^2)	0.44±0.01	0.84±0.03*	0.74±0.02*†	0.77±0.03*†	0.74±0.02*†
LVEVs (mL)	0.16±0.01	0.44±0.02*	0.36±0.02*†	0.39±0.04*	0.36±0.01*†
LVAd (cm^2)	0.87±0.01	1.12±0.03*	1.05±0.02*†	1.04±0.03*†	1.05±0.02*†
LVEVd (mL)	0.48±0.01	0.70±0.03*	0.64±0.02*†	0.64±0.02*†‡	0.64±0.02*†
LVLd (cm)	1.32±0.02	1.51±0.04*	1.45±0.03*†	1.44±0.02*†	1.45±0.02*†
HR (BPM)	336±4	336±9	344±6	330±7	324±5
EF (%)	67±0.8	37±2*	44±2*†	40±2*‡	44±1*†
SV (mL)	0.32±0.01	0.26±0.02*	0.28±0.01*	0.26±0.01*	0.28±0.01
CO (L/min)	0.11±0.00	0.09±0.01*	0.10±0.01*	0.09±0.00*	0.09±0.00
E-Velocity (cm/sec)	82.0±1.9	94.9±4.0*	93.5±5.5*	98.2±3.2*	85.5±2.7†§
A-velocity (cm/sec)	53.3±2.4	18.4±2.5*	31.4±5.3*†	30.3±5.1*	37.6±6.2*†
E/A Ratio	1.6±0.1	6.0±0.8*	4.0±0.6*†	3.9±0.5*†	3.6±0.8*†
Decel T (sec)	0.04±0.00	0.03±0.00*	0.03±0.00*	0.03±0.00*	0.03±0.00*

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated; ‡ $p<0.05$ vs. MI-Eplerenone.
Values represent mean ± SEM.
LVAs, systolic left ventricular area; LVEVs, systolic left ventricular end volume; LVAd, diastolic left ventricular area; LVEVd, diastolic left ventricular end volume; LVLd, diastolic left ventricular length; HR, heart rate; EF, ejection fraction; SV, stroke volume; CO, cardiac output; Decel T, mitral valve deceleration time.

Table 5. Effect of Treatment at 8 Weeks

	Sham (n=20)	MI-Untreated (n=11)	MI-Eplerenone (n=13)	MI-Enalapril (n=12)	MI-Eplerenone + Enalapril (n=15)
LVAs (cm^2)	0.47±0.01	1.09±0.05*	0.94±0.04*†	0.92±0.03*†	0.94±0.03*†
LVEVs (mL)	0.17±0.01	0.67±0.05*	0.53±0.04*†	0.52±0.02*†	0.53±0.03*†
LVAd (cm^2)	0.91±0.01	1.35±0.05*	1.22±0.03*†	1.20±0.02*†	1.21±0.03*†
LVEVd (mL)	0.51±0.01	0.94±0.06*	0.82±0.03*†	0.80±0.02*†	0.81±0.03*†
LVLd (cm)	1.36±0.01	1.60±0.04*	1.52±0.02*†	1.54±0.02*†	1.53±0.02*†
HR (BPM)	343±5	323±5*	350±5†	334±6‡	336±6
EF (%)	67±0.6	30±2*	37±2*†	36±2*†	35±2*†
SV (mL)	0.34±0.01	0.28±0.02*	0.30±0.01*	0.28±0.02*	0.28±0.01*
CO (L/min)	0.12±0.00	0.09±0.01*	0.10±0.00*†	0.10±0.01*	0.10±0.01*
E-Velocity (cm/sec)	83.2±2.6	101.9±2.4*	95.5±3.8*	91.3±3.8*†	83.8±2.6†‡§
A-velocity (cm/sec)	52.3±2.4	33.9±9.7*	36.4±6.7*	26.9±4.5*	41.7±6.3
E/A Ratio	1.6±0.1	5.1±1.0*	3.6±0.5*	4.3±0.5*	3.3±0.9*†
Decel T (sec)	0.04±0.00	0.03±0.00*	0.03±0.00*	0.03±0.00*	0.03±0.00*†

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated; ‡ $p<0.05$ vs. MI-Eplerenone, § $p<0.05$ vs. MI-Enalapril.

Values represent mean ± SEM.

LVAs, systolic left ventricular area; LVEVs, systolic left ventricular end volume; LVAd, diastolic left ventricular area; LVEVd, diastolic left ventricular end volume; LVLd, diastolic left ventricular length; HR, heart rate; EF, ejection fraction; SV, stroke volume; CO, cardiac output; Decel T, mitral valve deceleration time.

The MI-untreated animals gained less weight than the sham controls. All drug-treated groups gained on average less weight than the MI-untreated group. Absolute and normalized heart weight was less in the drug-treated animals compared to the MI-untreated group. Enalapril and eplerenone together produced a greater effect than eplerenone alone.

The hemodynamic data indicated that heart rate was increased in the drug-treated compared to the MI-untreated while left ventricular end diastolic pressure was decreased. Both + and – dP/dt were increased with drug-treatment suggesting increased contractility.

Table 7. Hemodynamic Data

	Sham (n=20)	MI-Untreated (n=11)	MI-Eplerenone (n=13)	MI-Enalapril (n=13)	MI-Eplerenone + Enalapril (n=15)
HR (BPM)	333±6	319±6	332±6†	337±6†	347±4*†‡
LVESP (mm Hg)	121±0.3	119±0.4*	120±0.5	120±0.4*	120±0.6
LVEDP (mm Hg)	11±0.4	20±0.9*	18±1.6*	16±0.7*†	15±0.7*†
+dP/dt (mm Hg/sec)	6444±112	5698±121*	6309±189†	6894±162*†‡	6773±162†‡
-dP/dt (mm Hg/sec)	6317±93	4771±152*	5052±156*	5755±133*†‡	5529±149*†‡
PDP (mm Hg)	165±6	144±4*	144±10*	143±4*	148±5*
SBP (mm Hg)	128±2	131±4	131±3	113±2*†‡	112±2*†‡

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated; ‡ $p<0.05$ vs. MI-Eplerenone.
Values represent mean ± SEM.
HR, heart weight; LVESP, left ventricular end systolic pressure; LVEDP, left ventricular end diastolic pressure; +dP/dt, positive developed pressure over time; -dP/dt, negative developed pressure over time; PDP, peak developed pressure.

The interstitial collagen fraction in viable myocardium was slightly decreased in the drug-treated animals. There was no significant difference between the groups for collagen in the infarcted areas. Infarct size was significantly smaller in the enalapril group.

Plasma aldosterone levels did not differ between the sham group, MI-untreated and eplerenone group. Aldosterone was slightly decreased with enlapril treatment and slightly increased when enalapril and eplerenone were combined. There were no significant differences between the groups with respect to ANP and OPN. Urinary protein was markedly decreased with enalapril treatment and not as markedly decreased when eplerenone was added to enalapril.

Table 8. Histology Data

	Sham (n=20)	MI-Untreated (n=11)	MI-Eplerenone (n=13)	MI-Enalapril (n=13)	MI-Eplerenone + Enalapril (n=15)
ICF-VM (%)	1.3±0.2	2.8±0.6*	2.3±0.4*	2.4±0.7*	2.7±0.6*
ICF-IM (%)	--	48.6±3.5	46.1±2.7	43.5±2.4	44.0±2.6
Infarct Size (%)	--	32.0±2.0	30.0±1.0	25.0±1.0†‡	32.0±1.0§

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated; ‡ $p<0.05$ vs. MI-Eplerenone; § $p<0.05$ vs. MI-Enalapril.
Values represent mean ± SEM.
ICF, interstitial collagen fraction; VM, viable myocardium; IM, infarcted myocardium;
--, no infarction.

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Table 9. Biochemical Assays

	Sham (n=20)	MI-Untreated (n=11)	MI-Eplerenone (n=13)	MI-Enalapril (n=13)	MI-Eplerenone + Enalapril (n=15)
Plasma Aldosterone (ng/mL)	0.86±0.06	0.85±0.08	0.86±0.08	0.71±0.04*	0.92±0.06§
Plasma ANP (ng/mL)	6.08±0.77	7.34±1.45	7.37±1.19	5.54±1.52	6.00±1.04
Plasma OPN (ng/mL)	44.67±3.28	45.28±6.31	41.91±4.74	44.24±5.90	38.26±4.67
Urinary Prot (mg/24h)	44.59±7.42	48.77±8.98	49.42±8.63	18.51±2.31*†‡	27.63±4.02*†‡

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated; ‡ $p<0.05$ vs. MI-Eplerenone; § $p<0.05$ vs. MI-Enalapril.

Values represent mean ± SEM.

ANP, atrial natriuretic peptide; OPN, osteopontin; Prot, protein excretion.

In this model, administration of eplerenone alone or with enalapril attenuated some of the progression of cardiac dysfunction. There were parameters such as urinary excretion of protein where enalapril alone produced a greater change than eplerenone or the combination of enalapril with eplerenone.

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Effect of Eplerenone (SC-66110), Enalapril and Coadministration Therapy in Mice Post Myocardial InfarctionD02CM0036, 25 November 2002

Male 12 week old [redacted] mice were either sham operated or the LAD was ligated to induce an MI. Two weeks after the procedure, the mice were assigned to one of the following treatment groups:

1.untreated, n=11, normal chow and water
2. MI-eplerenone n=16, eplerenone-containing chow, 200 mg/kg/day
3. MI-ACEi-enalapril, n=13, 20 mg/kg/day in the water
4. MI-eplerenone + ACEi, n=12, eplerenone containing chow and drinking water + enalapril.
5. Sham control , n=13, normal chow and tap water.

Mice were treated for 2-14 weeks post MI. Systolic blood pressure was measured using tail-cuff plethysmography in conscious mice. Measurements were taken prior to infarction and at 2,3,4,5,6,8,10,12 and 14 weeks post-infarction. Mice were trained pre-study for echocardiographic measurements. Echocardiographic evaluation was made using an [redacted] machine with a 15-MHz linear transducer. B and M mode images were obtained as were pulsed-wave Doppler ultrasound scans. Measurements were taken prior to infarction and at 2,3,4,5,6,7,10, 12 and 14 weeks post infarction.

The study was terminated at 14 weeks post-MI. Heart, liver and lungs were weighed. Hearts were assessed for infarct size, myocyte cross sectional area and interstitial collagen fraction.

Results

There were minor effects on the progression of the cardiac pathology for about six weeks of treatment (8 weeks post-MI). The ACEi had more of a detectable effect than eplerenone alone or in combination. Changes from the 12th week of treatment are shown here.

Table 19. Effect of Eplerenone on Cardiac Structure After 12 Weeks of Treatment at 14 Weeks Post-MI

	Sham (n=13)	MI- Untreated (n=11)	MI-Eplerenone (n=16)	MI-ACEi (n=13)	MI-Eplerenone + ACEi (n=12)
BW (g)	29.5±0.4 (4.4±0.3)	29.6±0.7 (3.1±0.5)*	30.5±0.4 (4.8±0.4)†	30.8±0.6 (4.3±0.4)	30.2±0.5 (3.5±0.4)*‡
SBP (mm Hg)	107±3 (-1±3)	99±4 (1±5)	100±4 (-6±4)	88±3 (-19±3)*†‡	83±5 (-23±3)*†‡
LVAs (mm^2)	1.17±0.03 (0.43±0.04)	15.26±2.48 (4.36±1.43)	12.36±1.92 (0.62±1.41)*†	8.95±1.54 (-0.45±0.79)*†	6.17±1.42 (-2.42±0.69)*†‡
LVAd (mm^2)	3.47±0.10 (0.36±0.19)	18.72±2.70 (4.76±1.68)*	17.98±2.04 (2.64±1.65)	14.29±2.07 (2.02±1.05)	10.47±1.54 (-0.64±0.78)†
IVSTs (mm)	1.23±0.02 (-0.05±0.02)	0.68±0.02 (0.12±0.03)*	0.64±0.01 (-0.07±0.02)†	0.78±0.03 (0.12±0.05)*‡	0.72±0.03 (0.09±0.05)*‡
LVDs (mm)	0.89±0.02 (-0.05±0.03)	4.27±0.39 (0.24±0.22)	3.75±0.29 (-0.31±0.16)†	2.82±0.38 (-0.35±0.12)*†	2.47±0.32 (-0.95±0.21)*†‡§
PWTs (mm)	1.28±0.02 (-0.08±0.06)	1.14±0.07 (0.07±0.08)	0.97±0.03 (0.00±0.04)	1.12±0.07 (-0.02±0.04)	1.03±0.03 (0.11±0.04)*‡§
IVSTd (mm)	0.77±0.02 (-0.05±0.02)	0.58±0.03 (0.07±0.03)*	0.54±0.01 (0.00±0.02)†	0.58±0.03 (-0.01±0.03)†	0.60±0.02 (0.04±0.04)*
LVDd (mm)	2.63±0.05 (0.13±0.07)	5.33±0.36 (0.45±0.20)	4.89±0.27 (-0.18±0.13)†	4.14±0.36 (0.01±0.11)	3.65±0.32 (-0.74±0.19)*†‡§
PWTd (mm)	1.03±0.02 (-0.07±0.04)	0.95±0.07 (0.06±0.07)*	0.77±0.03 (0.00±0.03)	0.92±0.06 (-0.07±0.04)†	0.82±0.02 (0.08±0.03)*‡§
Aorta D (mm)	1.48±0.01 (0.03±0.02)	1.47±0.02 (-0.02±0.04)	1.62±0.03 (0.15±0.05)*†	1.54±0.02 (0.07±0.03)	1.53±0.04 (0.12±0.04)†
VTI (mm/sec)	18.0±0.6 (-0.3±1.1)	10.5±0.7 (-5.0±2.2)	15.2±1.0 (0.9±1.0)†	15.5±0.6 (3.2±1.0)*†	15.0±0.9 (1.5±1.2)†

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated; ‡ $p<0.05$ vs. MI-Eplerenone; § $p<0.05$ vs. MI-ACEi.
BW, body weight; SBP, systolic blood pressure; LVAs, left ventricular area systole; LVAd, left ventricular area diastole; IVSTs, systolic interventricular septal thickness; LVDs, systolic left ventricular diameter; PWTs, systolic posterior wall thickness; IVSTd, diastolic interventricular septal thickness; LVDd, diastolic left ventricular diameter; PWTd, diastolic posterior wall thickness; Aorta D, aortic dimension; VTI, velocity-time integral.
Numbers in parentheses represent change from week 2 (mean ± standard error mean).

Table 21. Effect of Eplerenone After 12 Weeks of Treatment

	Sham (n=13)	MI-Untreated (n=11)	MI-Eplerenone (n=16)	MI-ACEi (n=13)	MI-Eplerenone + ACEi (n=12)
At_{WT}/BW (mg·100/g BW)	26.1±1.7	61.4±8.4*	55.6±4.3*	36.9±3.9*†‡	34.5±2.6*†‡
LV_{WT}/BW (mg·100/g BW)	309.0±8.3	492.1±29.8*	418.1±12.1*†	386.2±20.0*†‡	387.8±18.1*†
RV_{WT}/BW (mg·100/g BW)	74.5±2.9	105.0±10.5*	106.7±6.3*	84.5±6.7†‡	80.3±3.2†‡
HW/BW (mg·100/g BW)	409.5±9.0	658.5±45.0*	580.3±19.9*	507.6±28.1*†‡	502.6±20.2*†‡
$Liver_{WT}$/BW (mg·100/g BW)	4052.0±114.4	4595.0±92.1*	4956.4±95.6*†	4308.3±113.2†‡	4862.4±169.3*†§
$Lung_{WT}$/BW (mg·100/g BW)	537.3±23.3	677.7±73.2*	626.5±25.0*	585.1±23.1	550.4±22.2†‡
Infarct Size (%)	--	43±2	42±2	38±3	42±3
MCSA (μm^2)	173.5±6.2	345.0±9.1*	290.5±6.8*†	264.2±9.4*†‡	264.5±10.0*†‡
ICF (%)	4.68±0.17	12.69±0.60*	9.48±0.46*†	9.20±0.49*†	8.44±0.46*†‡

* $p<0.05$ vs. Sham; † $p<0.05$ vs. MI-Untreated, ‡ $p<0.05$ vs. MI-Eplerenone; § $p<0.05$ vs. MI- ACEi. At_{WT}, atrium weight; BW, body weight; LV_{WT}, left ventricle weight; RV_{WT}, right ventricle weight; HW, heart weight, MCSA, myocyte cross-sectional area; ICF, interstitial collagen fraction; --, no infarction.

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Table 20. Effect of Eplerenone on Cardiac Function After 12 Weeks of Treatment at 14 Weeks Post-MI

	Sham (n=13)	MI-Untreated (n=11)	MI-Eplerenone (n=16)	MI-ACEi (n=13)	MI-Eplerenone + ACEi (n=12)
FS (%)	66.0±0.7 (2.9±1.3)	20.7±2.0 (1.2±1.7)	25.2±2.9 (4.3±2.6) ●	35.3±3.2 (9.2±2.1)*†‡	34.5±2.9 (11.0±2.5)*†‡
HR (beats/min)	696±14 (20±15)	708±10 (83±39)	702±10 (45±17)	723±9 (33±16)	695±12 (50±16)
EF (%)	67±0.6 (-8±1)	20±2 (-6±3)	37±4 (11±3)*†	39±2 (14±2)*†	46±5 (21±4)*†‡
CO (mL/min)	21.91±0.88 (1.07±1.62)	11.82±0.88 (-3.88±1.85)	22.19±1.74 (5.92±1.66)*†	20.69±0.65 (6.29±1.00)*†	19.14±1.43 (5.67±1.73)*†
CO/10g BW (mL/min/10g)	7.44±0.31 (-0.85±0.61)	3.98±0.28 (-1.96±0.68)	7.26±0.56 (0.92±0.57)*†	6.68±0.22 (1.26±0.34)*†	6.35±0.46 (1.29±0.62)*†

* p<0.05 vs. Sham; † p<0.05 vs. MI-Untreated; ‡ p<0.05 vs. MI-Eplerenone.
FS, fractional shortening; HR, heart rate; EF, ejection fraction; CO, cardiac output; BW, body weight.
Numbers in parentheses represent change from week 2 (mean ± standard error mean)

Myocardial cross sectional area and interstitial collagen fraction were somewhat decreased with eplerenone treatment but not to the extent seen with the ACEi.

AMENDMENT 1 - Effects of Eplerenone (SC-66110) on the Progression of Myocardial Dysfunction and Injury in Dogs with Heart Failure BRD01D2120-A1, 4 February 2003
The amendment adds a third study group (n=7) to the dataset. Some animals were renumbered in the TaqMan and zymography assays. Details were added to the description of methodology.

Heart failure was induced in dogs with multiple sequential coronary microembolizations over a period of approximately 6-7 weeks. After a two week recovery period after the last microembolization, dogs received either eplerenone(20 mg/kg/day as 10 mg/kg b.i.d.) or no treatment for 3 months. Cardiac structure and function were evaluated (hemodynamics, angiography and echocardiography) prior to assignment to groups and at time of euthanasia. Description of the methodology for these measurements was not sufficient to allow for independent evaluation of the data.
At time of euthanasia, the dogs were weighed and the heart collected after appropriate measures. The heart was processed for histopathology, evaluation of myocyte cross-sectional area, interstitial fibrosis and capillary density.

Results

Eplerenone treatment increased contractility and LV ejection fraction post-MI. Interstitial collagen and fibrosis were also decreased compared to the untreated control while capillary density was increased. The sponsor's results are shown below.

Table 2. Effects of eplerenone on myocardial fibrosis and hypertrophy and microvascular remodeling

	CONTROL (n=7)	EPLERENONE (n=7)
Interstitial Collagen (%)	16.1±0.3	10.1±0.5†
Replacement Fibrosis (%)	19.8±2.0	13.0±0.7†
Cross-Sectional Area (μm^2)	809.1±30.2	582.4±14.3†
Capillary Density ($\#/m^2$)	1775.4±63.4	2190.9±57.2†

All values are mean±SEM.

† $p<0.05$ vs. Control.

Table 1. Effects of eplerenone on LV dysfunction

	CONTROL (n =7)		EPLERENONE (n=7)	
n	Pre	Post	Pre	Post
Body Weight (kg)	23.7±1.0	24.8±0.8*	26.7±1.0	27.5±1.5*†
Heart Rate (beats/min)	82±5	86±4	87±4	83±4
Systolic Blood Pressure (mmHg)	108±7	107±3	116±9	116±8
Diastolic Blood Pressure (mmHg)	81±8	84±3	83±7	87±6
Mean AoP (mmHg)	91±8	92±3	94±7	99±8
+dP/dt (mmHg/sec)	1846±131	1521±86*	1861±140	1990±131*†
-dP/dt (mmHg/sec)	1564±124	1266±59*	1564±146	1989±145*†
PA Systolic Blood Pressure (mmHg)	22±1	22±2	20±1	22±3
PA Diastolic Blood Pressure (mmHg)	8±2	9±1*	7±0	10±1*
Mean PA Pressure (mm Hg)	12±1	13±1	12±0	14±1
Mean PAWP (mmHg)	8±1	7±1	7±1	9±2
RAP (mmHg)	3±1	4±1*	3±1	2±1*
LVEDP (mmHg)	16±1	15±1	15±1	9±2*†
CO (L/min)	2.46±0.17	2.68±0.13	2.58±0.26	2.51±0.30
BSA (m^2)	0.82±0.02	0.85±0.02*	0.89±0.02	0.91±0.03*†
SV (mL)	31±2	31±3	30±3	30±3
CI ($L/min/m^2$)	3.0±0.2	3.2±0.1	2.9±0.3	2.7±0.3
SVR	2948±336	2659±136	3040±435	3170±317
ED Wall Stress	67±9	81±7*	58±7	33±6*†
LV EDV (mL)	62±4	68±4*	73±5	72±5†
LV ESV (mL)	38±3	47±3*	46±3	45±4
ES Ratio	1.65±0.08	1.43±0.08*	1.42±0.05	1.49±0.07*†
ED Ratio	1.46±0.06	1.34±0.05*	1.31±0.03	1.38±0.03*
LV EF (%)	38±1	31±2*	37±1	38±1†

All Pre- and Post- values are mean±SEM.

*$p<0.05$ vs. Pre value for group, † $p<0.05$ vs. Control Post value

AoP, aortic blood pressure; PA, pulmonary artery; PAWP, pulmonary artery wedge pressure; LVEDP, left ventricular end-diastolic pressure; CO, cardiac output, BSA, body surface area; SV, stroke volume; CI, cardiac index; SVR, systemic vascular resistance; ED wall stress, LV end-diastolic circumferential wall stress; EDV, end-diastolic volume; ESV, end-systolic volume; ES ratio, ratio of major-to-minor dimension at end-systole; ED ratio, ratio of major-to-minor dimension at end-diastole; EF, ejection fraction.

Under the conditions of the study, use of eplerenone in dogs with induced cardiac functional deficits caused less replacement fibrosis and interstitial collagen. Drug treatment also caused an increase in capillary density. The hemodynamic data suggests some functional benefit from eplerenone. The methods for the echocardiography were not presented in sufficient detail to allow for independent evaluation of the data. As presented, the data suggest slight improvement in function. Given the variability inherent in the technology used, it is not possible to assess whether the changes are real. The study would be stronger for the inclusion of dogs who have not undergone induction of cardiac failure.

Effects of eplerenone(SC-66110), enalapril or eplerenone coadministered with enalapril on the progression of heart failure in SHHF rats. D02CM0025, November 4, 2002

Fourteen month old SHHF rats were randomized into each of the following treatment groups for 13 weeks of treatment:
1)untreated
2)eplerenone 100 mg/kg/day
3)enalapril 10 mg/kg/day
4) eplerenone (100 mg/kg/day) and enalapril (10 mg/kg/day)
5) age matched Sprague-Dawley rats

The SHHF-Gmi-/fa^{CD} is a congenital model of dilated cardiomyopathy with hypertension progressing to decompensated heart failure. Homozygous cp/cp and heterozygous +/cp rats were used in this study. Baseline assessment included genotyping, echocardiography and blood pressure. The animals were assigned to treatment groups so that average ejection fraction was 50±1.5% with an equal number of heterozygous and homozygous animals per group. Eplerenone-treated animals received the drug in the chow. Enalapril was given in the water. Untreated rats received standard rodent chow. Echocardiography was performed at baseline and after 4, 9 and 13 weeks (termination) of study. Systolic blood pressure was measured by non-invasive tail cuff at baseline, 6 and 12 weeks.
At the end of the study period, animals were anesthetized then exsanguinated with a needle and syringe. Hearts and kidneys were then also collected. Part of the heart was processed for histopathology while the remainder was saved for molecular analysis. The kidneys were likewise saved in part for histopathology and in part for other analyses. The left tibia was also collected and length determined. Cardiac collagen was quantitated. Arterial and myocardial changes were assessed on separate scales of 1-4. Urine samples collected from metabolism cages were analyzed for albumin. Plasma osteopontin, aldosterone and serum albumin, blood urea nitrogen, creatinine, sodium, potassium and chloride were also assessed. TaqMan analysis was also used for unspecified target genes which were normalized to cyclophilin. RNA was extracted from heart and kidney.

Results

Four animals either died from dehydration in the first 3 weeks or were euthanized for humane reasons. No data from these animals was incorporated into the analysis.

The echocardiography parameters indicated that eplerenone attenuated some of the effects of the progression of cardiac failure. Enalapril alone produced a greater effect. The combination of enalapril and eplerenone produced a slightly greater effect than enalpril alone. None of the treatments returned the animals to the values of the age-matched Sprague-Dawleys.

Table 1. Effects of 13 Weeks of Treatment on Echocardiography Parameters

Parameters Evaluated	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
LVAd (cm^2)	1.39±0.06	1.28±0.05 (0.14±0.03)	1.22±0.02 (0.11±0.01)	1.11±0.03 (-0.07±0.03)*†‡	1.07±0.05 (-0.01±0.04)*†‡
LVAs (cm^2)	1.15±0.05 (0.35±0.05)	0.94±0.06 (0.17±0.04)*	0.86±0.02 (0.13±0.04)*	0.71±0.03 (-0.07±0.02)*†‡	0.58±0.04 (-0.01±0.03)*†‡
EDV (mL)	0.97±0.06 (0.25±0.05)	0.83±0.05 (0.14±0.03)	0.74±0.02 (0.12±0.03)*	0.63±0.03 (-0.06±0.03)*†‡	0.69±0.04 (-0.03±0.04)*†‡
ESV (mL)	0.65±0.05 (0.29±0.04)	0.47±0.05 (0.14±0.04)*	0.39±0.02 (0.10±0.03)*	0.30±0.02 (-0.06±0.02)*†‡	0.22±0.02 (-0.02±0.02)*†‡
EF (%)	33±2 (-17±2)	45±3 (0.9±2)*	47±1 (-6±2)*	53±2 (4±3)*†‡	63±1 (0.9±2)*†‡
LVLd (cm)	1.72±0.05 (0.07±0.01)	1.72±0.03 (0.06±0.05)	1.69±0.03 (0.08±0.04)	1.63±0.03 (-0.06±0.03)*†‡	1.60±0.04 (-0.02±0.05)
HR (beats/min)	336±9 (3±12)	341±8 (-11±13)	357±7 (11±13)	354±8 (-0.1±10)	334±5 (3±14)
CO (L/min)	0.11±0.01 (-0.01±0.01)	0.12±0.01 (0.00±0.01)	0.13±0.01 (0.01±0.01)*	0.12±0.01 (0.00±0.01)	0.13±0.01 (0.00±0.01)
SV (mL)	0.32±0.03 (-0.05±0.03)	0.33±0.02 (-0.02±0.02)	0.35±0.01 (0.02±0.02)*	0.34±0.02 (-0.01±0.03)	0.37±0.02 (-0.02±0.03)
FS (%)	28±3 (-5±3)	33±2 (0.5±2)	33±0.9 (0±2)	36±2 (6±3)*‡	37±2 (-1±3)§
FAC (%)	17±1 (-17±2)	27±2 (-5±2)*	30±2 (-5±2)*	36±2 (4±2)*†‡	46±2 (2±2)*†‡
IVSd (cm)	0.22±0.01 (0.00±0.01)	0.21±0.01 (-0.03±0.01)*	0.21±0.01 (-0.03±0.01)*	0.20±0.01 (-0.02±0.01)	0.21±0.02 (0.02±0.03)‡
IVSs (cm)	0.35±0.02 (0.01±0.03)	0.35±0.02 (-0.02±0.02)	0.33±0.01 (-0.03±0.01)	0.32±0.01 (-0.02±0.01)	0.35±0.02 (0.02±0.03)
LVIDd (cm)	1.07±0.03 (0.14±0.03)	1.00±0.03 (0.11±0.02)*	0.94±0.01 (0.06±0.02)*	0.86±0.02 (-0.03±0.04)*†‡	0.86±0.02 (-0.01±0.03)*‡
LVIDs (cm)	0.77±0.03 (0.14±0.03)	0.68±0.04 (0.07±0.03)	0.63±0.01 (0.04±0.02)*	0.55±0.02 (-0.07±0.04)*†‡	0.54±0.03 (0.00±0.03)*
LVPWd (cm)	0.20±0.01 (-0.02±0.01)	0.22±0.01 (0.00±0.01)	0.18±0.02 (-0.04±0.01)‡	0.19±0.01 (-0.02±0.01)	0.20±0.01 (-0.02±0.01)
LVPWs (cm)	0.35±0.02 (0.01±0.03)	0.32±0.01 (-0.01±0.02)	0.28±0.01 (-0.05±0.01)	0.29±0.01 (-0.02±0.02)	0.33±0.01 (-0.02±0.02)

Data reported as mean ± SEM.

* $p<0.05$ vs. Untreated, † $p<0.05$ vs. Eplerenone, ‡ $p<0.05$ vs. Enalapril, § $p<0.05$ vs. Eplerenone – Enalapril

Blood pressure was increased by eplerenone treatment alone at 6 weeks of treatment then slightly decreased at 12 weeks. Enalapril by itself significantly decreased blood pressure while the combination of enalapril and eplerenone produced a further decrease.

Table 2. Effects of Treatment on Systolic Blood Pressure

SBP (mm Hg)	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
Baseline	199±6	196±6	195±3	200±7	140±4*
6 Weeks	189±8	204±7	152±6*†	127±5*†‡	129±5*
12 Weeks	192±6	188±7	163±6*†	151±6*†	135±2*

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated, † $p<0.05$ vs. Eplerenone; ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat, SD-Ctrl, age matched Sprague Dawley control rats; SBP, systolic blood pressure.

Heart weight did not increase as much with eplerenone treatment compared to the untreated controls. Enalapril produced a greater organ weight effect and the combination of enalapril and eplerenone caused a still greater effect. Kidney weight was unchanged by eplerenone alone, increased by enalapril and somewhat decreased by the combination of the two drugs.

Table 3. Effects of 13 Weeks of Treatment on Body, Heart, and Kidney Weights

Parameters Evaluated	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
BW (g)	487±14	492±10	498±8	473±12	722±34*
HW (g)	2.15±0.11	1.96±0.07	1.62±0.03*†	1.36±0.04*†‡	1.63±0.07*
HW/BW (g/kg)	4.46±0.31	4.01±0.21	3.26±0.06*†	2.88±0.03*†‡	2.19±0.10*
TL (cm)	4.05±0.03	4.03±0.01	4.05±0.02	4.06±0.02	4.48±0.04*
HW/TL (g/mm)	5.31±0.29	4.86±0.17	4.00±0.08*†	3.36±0.10*†‡	3.62±0.15*
KW (g)	1.74±0.09	1.72±0.06	1.82±0.04	1.57±0.04†‡	1.86±0.06*
KW/BW (g/kg)	3.60±0.20	3.49±0.07	3.66±0.05†	3.32±0.04*†‡	2.49±0.07*
KW/TL (g/mm)	4.30±0.21	4.28±0.16	4.50±0.13	3.86±0.10*†‡	4.13±0.11

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated, † $p<0.05$ vs. Eplerenone; ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat, SD-Ctrl, age matched Sprague Dawley control rats; BW, body weight, HW, heart weight; TL, tibia length; KW, kidney weight.

The greatest effects on arterial and myocardial damage were produced by the combination of eplerenone and enalapril.

Table 4. Effects of 13 Weeks of Treatment on Myocardial Histopathology

Myocardial Injury	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
Arterial Damage	2.40±0.27	2.42±0.19	2.55±0.21	1.82±0.18*†‡	0.10±0.10*
Myocardial Changes	2.60±0.22	2.58±0.15	2.36±0.20	1.36±0.20*†‡	0.20±0.13*

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated; † $p<0.05$ vs. Eplerenone, ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat; SD-Ctrl, age matched Sprague Dawley control rats.

Table 5. Effects of 13 Weeks of Treatment on Myocardial Collagen Content

Collagen: Tissue Area	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
LV	15.61±1.72	16.00±1.50	14.53±1.05	13.99±1.21	3.85±0.43*
RV	10.80±1.10	8.45±0.68	10.39±1.21	8.89±1.09	4.44±0.69*

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated; † $p<0.05$ vs. Eplerenone; ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat; SD-Ctrl, age matched Sprague Dawley control rats. LV, left ventricular; RV, right ventricular.

Left ventricular collagen content was slightly increased by eplerenone treatment, slightly decreased by enalapril and somewhat more decreased by the combination of drugs. Right ventricular collagen was decreased by eplerenone, increased by enalapril and not decreased to the same extent by the combination.

Eplerenone had slight mitigating effects upon renal histopathology. Enalapril had a greater effect while the combination of drugs had the greatest effect.

The same pattern was noted for the effect on urinary albumin. Plasma aldosterone was non-significantly decreased by eplerenone and significantly decreased by enalapril at 4 weeks of treatment. By 9 weeks of treatment eplerenone had significantly increased plasma aldosterone while enalapril maintained a decrease. Urinary albumin was decreased to a greater extent by enalapril. By the end of the study the same patterns seen at 9 weeks were still in place.

Table 8. Urine Albumin and Plasma Osteopontin and Aldosterone After 4 Weeks of Treatment

Parameters Evaluated	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
ALB (mg/24hr)	347.04±32.00	254.51±39.35*	238.16±59.22*	117.24±15.00*†‡	40.53±26.16*
OPN (ng/mL)	41.84±1.72	40.78±3.33	38.59±2.26	41.44±2.23	31.58±1.86*
Aldo (pg/mL)	358.64±42.71	341.10±40.50	252.34±28.51*†	356.69±26.36‡	284.03±49.48

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated; † $p<0.05$ vs. Eplerenone; ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat. SD-Ctrl, age matched Sprague Dawley control rats. ALB, albumin; OPN, osteopontin; Aldo, aldosterone.

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Table 10. Urine Albumin and Plasma Osteopontin and Aldosterone After 13 Weeks of Treatment

Parameters Evaluated	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
ALB (mg/24hr)	408.42±84.18	342.64±78.08	257.29±15.94*	257.28±62.86*	133.12±34.77*
OPN (ng/mL)	51.83±12.05	36.54±5.63	25.26±3.42*	17.38±1.93*†	18.76±2.21*
Aldo (pg/mL)	309.46±37.08	405.26±48.34	287.62±35.67†	465.27±44.95*‡	291.15±27.47*

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated; † $p<0.05$ vs. Eplerenone; ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat, SD-Ctrl, age matched Sprague Dawley control rats; ALB, albumin; OPN, osteopontin, Aldo, aldosterone, --, measurement not taken.
Note. ANP not measured after 13 weeks of treatment

Enalapril by itself caused a slight decrease in serum sodium. Enalapril combined with eplerenone slightly increased the sodium effect. The combination of drugs caused a slight increase in potassium.

Table 11. Effects of 13 Weeks of Treatment on Plasma and Serum Chemistry

Parameters Evaluated	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
BUN (mg/dL)	20.79±2.67	20.95±1.27	20.54±1.31	25.45±2.28	14.01±0.53*
CRE (mg/dL)	0.40±0.04	0.40±0.03	0.39±0.02	0.35±0.03	0.29±0.02*
Na^+ (mmol/L)	140.25±2.11	140.45±0.37	137.70±1.10†	136.09±0.59*†	140.11±0.87
K^+ (mmol/L)	4.03±0.17	4.38±0.14	4.23±0.06	4.45±0.09*	3.90±0.07
Cl^- (mmol/L)	101.88±1.77	102.27±0.45	101.30±0.75	100.00±0.36	102.78±0.80

Data reported as mean ± SEM.
* $p<0.05$ vs. Untreated, † $p<0.05$ vs. Eplerenone; ‡ $p<0.05$ vs. Enalapril.
SHHF, spontaneously hypertensive heart failure prone rat; SD-Ctrl, age matched Sprague Dawley control rats; BUN, blood urea nitrogen; CRE, creatinine; Na^+, sodium, K^+, potassium; Cl^-, chloride.

Both drugs produced slight decreases in the expression of pro-inflammatory genes in cardiac tissue. Renal gene expression showed the same phenomenon.

Table 12. Effects of 13 Weeks of Treatment on Proinflammatory Cardiac Gene Expression

Gene Expression	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
OPN	18.62±5.37§	10.96±3.45§	8.69±1.72§	3.55±0.73*‡§	1.22±0.31
MCP-1	1.01±0.16	0.65±0.05§	1.15±0.20†	0.56±0.08*†‡§	0.98±0.12
IL-6	2.09±0.43§	1.18±0.17	1.74±0.32§	0.86±0.15*‡	0.98±0.17
IL-1β	0.69±0.14§	0.48±0.06§	0.76±0.10†	0.47±0.04‡§	1.23±0.36
11β HSD2	1.197±0.14	1.48±0.11*§	1.02±0.14†	0.97±0.06†	0.98±0.07

Data reported as mean ± SEM.

* p<0.05 vs. Untreated; † p<0.05 vs. Eplerenone; ‡ p<0.05 vs. Enalapril, § p<0.05 vs. SD-Ctrl

SHHF, spontaneously hypertensive heart failure prone rat, SD-Ctrl, age matched Sprague Dawley control rats, OPN, osteopontin; MCP-1, monocyte chemoattractant protein-1, IL-6, interleukin-6, IL-1β, interleukin-1β, 11β HSD2, 11β hydroxysteroid dehydrogenase type 2.

Table 13. Effects of 13 Weeks of Treatment on Renal Gene Expression

Gene Expression	SHHF Rats				SD-Ctrl Rats
	Untreated	Eplerenone	Enalapril	Eplerenone + Enalapril	
OPN	25.32±3.80§	20.42±2.46§	18.46±4.63*†§	6.45±0.67*†‡	1.1±0.15
MCP-1	3.50±0.61§	2.34±0.30§	3.28±0.97	1.30±0.14*†‡	1.27±0.24
IL-6	9.14±1.49§	5.23±0.64*§	5.30±1.27*§	1.92±0.19*†‡	1.08±0.15
IL-1β	4.25±0.84§	2.99±0.35§	4.01±1.25§	1.54±0.16*†‡	1.04±0.10
11β HSD2	1.85±0.14§	1.59±0.07§	2.09±0.61*	1.12±0.06*†‡	1.02±0.05

Data reported as mean ± SEM.

* p<0.05 vs. Untreated; † p<0.05 vs. Eplerenone; ‡ p<0.05 vs. Enalapril; § p<0.05 vs. SD-Ctrl

SHHF, spontaneously hypertensive heart failure prone rat, SD-Ctrl, age matched Sprague Dawley control rats; OPN, osteopontin; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6, IL-1β, interleukin-1β; 11β HSD2, 11β hydroxysteroid dehydrogenase type 2.

For most of the parameters studied, enalapril produced more of an effect than eplerenone. The combination of eplerenone and enalapril produced slightly more of an effect than enalapril alone.

Long-term eplerenone (SC-66110) treatment in the SHHF rat. D02CM0083. November 4, 2002.

Lean male SHHF-Gm1-/fa^{CD} (spontaneously hypertensive heart failure) rats were used. Animals were assigned to two treatment groups so that the average ejection fraction was similar between the two groups with an equal number of homozygous and heterozygous animals in each group. The rats received either standard rat chow or chow with eplerenone incorporated to give a target dose of 100 mg/kg/day. The treatment was started 1 week after assignment into groups. The study was conducted over 56 weeks. Baseline measurements were made for genotyping, echocardiography and systolic blood pressure. Periodic re-assessments were made throughout the study.

At time if euthanasia the animals were anesthetized and the blood collected. The heart, kidneys and tibia were collected. Portions of the heart were processed for histopathology, collagen quantitation, and assessment of arterial and myocardial changes. The kidney was also processed for histopathology and semi-qualitative grading.

Gene expression of COX-2, OPN and MCP-1 was assessed by TaqMan methodology. Target genes were normalized against cyclophilin expression. Plasma osteopontin levels were also determined.

Results: Treatment with eplerenone produced a slight mitigation of some of the echocardiographic changes. Eplerenone had no effect on blood pressure over the duration of the study. There was however a slight decrease in the mean myocardial damage score. There was a non-significant decrease in LV collagen content and no difference in RV collagen content. The kidney damage score was unchanged to slightly increased by eplerenone.

Table 5. Effect of 56 Weeks of Eplerenone Treatment on Myocardial Collagen Content

	Untreated	Eplerenone
Collagen to LV tissue (%)	18.35±2.26	16.80±1.46
Collagen to RV tissue (%)	7.22±0.61	7.27±0.98

Values represent mean ± SEM.
LV, left ventricular; RV, right ventricular.

Plasma osteopontin levels were decreased compared to untreated animals in the eplerenone group. Cardiac gene expression was minimally affected.

Table 6. Effect of Eplerenone Treatment on Plasma Osteopontin Levels

OPN (ng/mL)		
Weeks of Treatment	**Untreated**	**Eplerenone**
Baseline (0)	44.24±3.57	38.51±2.13
16	41.54±3.64	37.99±2.90
32	37.29±2.68	26.64±3.01
48	107.47±19.70	67.53±8.21*
56	133.98±15.93	86.39±12.10*

* $p<0.05$ vs. Untreated.
Values represent mean ± SEM.
OPN, osteopontin.

Table 7. Effect of 56 Weeks of Eplerenone Treatment on Cardiac Gene Expression

	Untreated	Eplerenone
COX-2	1.22±0.17	0.76±0.11*
OPN	1.84±0.53	0.86±0.10*
MCP-1	1.03±0.08	0.85±0.04*
IL-1β	1.04±0.10	0.93±0.10
IL-6	1.11±0.23	0.80±0.10
ANP	0.92±0.09	0.79±0.08
11β HSD2	0.99±0.05	0.96±0.04

* $p<0.05$ vs. Untreated
Values represent mean ± SEM.
COX-2, cyclooxygenase-2; OPN, osteopontin; MCP-1, monocyte chemoattractant protein-1; IL-1β, interleukin-1β; IL-6, interleukin-6; ANP, atrial natriuretic peptide; 11β HSD2, 11β hydroxysteroid dehydrogenase type 2.

Under the conditions of the study, eplerenone produced some mitigation of the effects of spontaneous hypertensive heart failure of rats. The decrease in plasma osteopontin levels was maintained throughout the study and became statistically significant at the last points of determination (48 and56 weeks). There were decrease in cardiac gene expression of osteopontin and COX-2.

The study could have been stronger for the inclusion of several doses to allow for determination of dose-response effects.

Pharmacokinetics of eplerenone (SC-66110) in rats: eplerenone administered in the chow. BRD01D2139. December 21, 2001.

The rats used in the study were maintained in a room lighted from 6am to 6 pm. Eplerenone was incorporated into —— rodent diet at a concentration of 0.1 g eplerenone/100g chow (0.1% to deliver approximately 100 mg/kg/day). Male Sprague-Dawley rats were given the drug-containing diet for 7 days. The rats were limited to 10 g chow/100 g body weight. Food consumption was monitored each day from days 2-6. The rats were euthanized day7 at 6 pm, 10 pm, 2 am, 6 am, 10 am and 2 am (n=4 per timepoint). Blood was collected and the samples were analyzed for drug levels and aldosterone.

Results: Food consumption increased in the last 3 days of the study by roughly 20%. Average plasma aldosterone levels ranged from a high of 2081±530 (10 am) to a low of 643±85 (2 pm). There were no untreated animals for comparison.

Table 2. Plasma aldosterone levels after 6 days of eplerenone administration in the chow.

Time	6pm (n=4)	10pm (n=4)	2am (n=4)	6am (n=4)	10am (n=4)	2pm (n=4)
rat 1 (pg/mL)						
rat 2 (pg/mL)						
rat 3 (pg/mL)						
rat 4 (pg/mL)						
MEAN	**1657.7**	**1449.6**	**1067.4**	**1898.2**	**2081.2**	**643.0**
SEM	308.5	283.3	277.1	298.7	529.6	84.6

SEM= standard error mean.

Plasma eplerenone levels ranged from a mean of 0.59±0.09 µg/ml (10 pm) to 0.17±0.01 µg/ml (2pm). Detectable levels were present at each point of determination. The sponsor's summary of results is shown below.

Table 3. Plasma eplerenone levels after 6 days of eplerenone administration in the chow.

Time	6pm (n=4)	10pm (n=4)	2am (n=4)	6am (n=4)	10am (n=4)	2pm (n=4)
rat 1 (µg/mL)						
rat 2 (µg/mL)						
rat 3 (µg/mL)						
rat4 (µg/mL)						
MEAN	**0.43**	**0.59**	**0.43**	**0.30**	**0.42**	**0.17**
SEM	0.10	0.09	0.05	0.12	0.08	0.01

SEM = standard error mean.

Table 4. Eplerenone exposure after 6 days of eplerenone administration in the chow.

Rat Number	Cmax (µg/mL)	Cmin (ug/mL)	AUC (µg•hr/mL)
rat 1			
rat 2			
rat 3			
rat 4			
MEAN	**0.612**	**0.154**	**9.35**
SEM	0.09	0.01	1.29

SEM= standard error mean.

Dietary administration of eplerenone for a 7 day period provided detectable levels of drug at all points of determination.

3.2.3 Secondary pharmacodynamics Secondary pharmacodynamics would be expected to be due to any relative non-specificity of binding to the steroid receptors. Non-clinically, this drug appears to have greater specificity for the mineralocorticoid receptor than spironolactone, the original drug in this class. The review of original NDA-21437 is referenced.

3.2.4 Safety pharmacology No new safety pharmacology was submitted with this application. There were no safety pharmacology concerns in the original NDA. Please see review for NDA 21-437

3.2.5 Pharmacodynamic drug interactions: No new issues have been presented. See review of original NDA 21-437

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief summary: The non-clinical absorption, distribution, metabolism and excretion were well described in the original eplerenone NDA 21-437. [redacted] **These studies extended tissue distribution in male Long-Evans rats to female Sprague-Dawley rats, added a whole body autoradiography study in male and female Sprague-Dawley rats and added a confirmatory mass balance/metabolism/excretion study in male and female rats and dogs. New information was reported on the biliary excretion and enterohepatic recirculation of eplerenone in male and female rats.**

3.3.3 Absorption: No new studies conducted.

3.3.4 Distribution:

8.4. Mean Recovery of Drug-Derived Radioactivity (% of Dose) in Tissue (Mean ± SD) From Female Sprague Dawley Rats One, Eight and Ninet Six Hours Following Administration of a Single Oral Dose ([^{14}C]SC-66110 (20 mg/kg)

Tissues/Organs	Recovery of Drug-Derived Radioactivity (% of Dose)		
	1 hr	8 hr	96 hr
Blood[c]	2.37 ± 0.25	0.04 ± 0.02	BLQ
Plasma[f]	0.10 ± 0.01	<0.005	BLQ
Adrenal glands[a]	0.02 ± 0.00	<0.005	BLQ
Urinary bladder[a]	0.02 ± 0.01	<0.005	BLQ
Bone[f]	0.01 ± 0.00	<0.005	BLQ
Bone marrow[f]	0.01 ± 0.00	<0.005	BLQ
Brain[a]	0.07 ± 0.01	<0.005	BLQ
Carcass[a]	32.51 ± 4.28	0.77 ± 0.23	0.06 ± 0.05
Eyes[a]	0.02 ± 1.21	<0.005	<0.005
Brown fat[b]	4.50 ± 0.97	0.08 ± 0.04	BLQ
Peritoneal fat[b]	1.86 ± 0.23	0.04 ± 0.01	BLQ
Harderian glands[a]	0.06 ± 0.01	<0.005	BLQ
Heart[a]	0.16 ± 0.01	<0.005	BLQ
Kidneys[a]	0.51 ± 0.02	0.01± 0.00	BLQ
Large intestine[a]	0.38 ± 0.04	0.80 ± 0.45	BLQ
Large intestinal contents[a]	1.16 ± 0.05	35.46 ± 15.16	0.01 ± 0.01
Liver[a]	5.37 ± 0.14	0.27 ± 0.12	0.01 ± 0.00
Lungs[a]	0.22 ± 0.00	<0.005	BLQ
Mesenteric lymph nodes[f]	0.01 ± 0.00	<0.005	BLQ
Skeletal muscle[d]	20.08 ± 2.19	0.37 ± 0.21	BLQ
Pancreas[a]	0.11 ± 0.01	<0.005	BLQ
Pituitary[a]	<0.005	<0 005	BLQ
Skin[a]	7.11 ± 0.67	0.17 ± 0.07	BLQ
Small intestine[a]	9.36 ± 1.40	0.25 ± 0.11	BLQ
Small intestine contents[a]	25.18 ± 4.66	1.84 ± 0.68	<0.005
Spinal cord[f]	0.01 ± 0.00	<0.005	BLQ
Spleen[a]	0.07 ± 0.01	<0.005	BLQ
Stomach[a]	1.27 ± 0.14	0.01 ± 0.00	BLQ
Stomach contents[a]	5.72 ± 2.53	0.08 ± 0.01	BLQ
Ovaries[a]	0.02 ± 0.00	<0.005	BLQ
Uterus[a]	0.07 ± 0.00	<0.005	BLQ
Thymus[a]	0.04 ± 0.01	<0.005	BLQ
Thyroid[a]	<0.005	<0.005	BLQ
Vena cava[a]	<0.005	BLQ	BLQ

BLQ: Values Below Limit of Quantitation
<0.005: Values below 0.005(actual values of mean and SD were calculated)
[a]: Whole tissue/organs used
[b]: Lookup value of 7.08% used for calculation
[c]: Lookup value of 4.95% used for calculation
[d]: Lookup value of 45.5% used for calculation
[e]: Lookup value of 18.0% used for calculation
[f]: Calculated from a portion of tissue/organ only
Untruncated values used for calculation purposes. Values reported to 2 decimal places. The conversion from DPM/g values to % of dose was based on a specific activity of 10.8 µCi/mg measured on the September 27, 2001 assessment.

SC-66110: A quantatitive tissue distribution study following a single oral administration of [^{14}C]SC-66110 to female Sprague-Dawley rats. M2001172. March 22, 2002.

Nine female Sprague-Dawley rats received a single oral administration of 20 mg/kg of [^{14}C]SC-66110 (~50-100µCi). One female received vehicle only and the tissues were harvested to determine background levels of radioactivity. Three drug-treated animals were euthanized at each time point of 1, 8 and 96 hours post-dose. Blood samples were collected at time of euthanasia. A complete set of tissues was collected as was the carcass and processed to determine radioactivity content by [redacted]

Results: At 1 hour after dosing, the highest concentrations of drug-derived radioactivity were located

in the contents of the gastrointestinal tract, liver, fat and skeletal muscle. By 8 hours, the majority of radioactivity was located in the large intestine. Levels had decreased in the tissues where radioactivity had previously been located. By 96 hours, drug-derived radioactivity was below quantifiable levels in almost all tissues. Blood to plasma ratios ranged from — to — in all animals at 1 and 8 hour post-dose. No radioactivity was measured in blood or plasma at 96 hours following a single oral dose of radiolabelled SC-66110. The drug was rapidly and widely distributed throughout the body after oral dosing and rapidly cleared.

A WHOLE BODY AUTORADIOGRAPHY STUDY IN RATS FOLLOWING ORAL ADMINISTRATION OF [^{14}C] SC- 66110 M2002021 March 8, 2002

A single oral dose of 20 mg/kg [^{14}C]SC-66110 (~450 µCi/kg) was given to male and female Sprague-Dawley rats (n=3 per sex). One animal per sex per time point was euthanized at 0.5, 8 and 24 hours after dosing. Whole body sections were processed for autoradiography. The results were analyzed by

Results: Half an hour after drug administration substantial amounts of drug-derived radioactivity were located in the small intestinal content, liver, and stomach contents. Radioactivity was also noted in the rest of the major organs with the exception of cerebral cortex, cerebellum, spinal cord, white fat, thymus and large intestinal contents. At this time point, maximum concentration of radioactivity following drug administration was found in the urinary bladder of the male rat (— time the blood concentration) while in the female the maximum concentration was measured in the stomach contents (— times the blood concentration).

At 8 hours post-dose, large amounts of radioactivity were found in the large intestinal contents. Lower amounts were seen in the small intestinal contents and still lower amounts were seen in the liver, stomach contents and stomach wall and small intestinal wall. The remaining tissues were close to background levels of <10 nCi/g wet tissue. At 8 hours post-dose, the highest concentration of radioactivity of was measured in the large intestinal content.

At 24 hours the largest concentration of radioactivity was in the large intestinal contents. Small amounts of radioactivity were found in the remainder of the GI tract. The remaining tissues were below background levels.

Following oral administration, radiolabelled SC-66110 was rapidly and widely distributed in male and female rats. The urinary and fecal routes of excretion were again demonstrated.

3.3.5 Metabolism: no new studies were submitted

3.3.5 Excretion

SC-66110: A STUDY OF THE MASS BALANCE, METABOLIC PROFILE AND PARTIAL DISTRIBUTION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [^{14}C] SC- 66110 IN RATS M2002020 March 20, 2002

Eighteen Sprague Dawley rats (9 males and 9 females) received a single oral dose of [^{14}C] SC- 66110 at a target dose level of 20 mg/ kg. Following dose administration, urine and feces were collected at selected intervals to 24 hr post- dose. Feces was collected pre-dose and at 0-24 hours post-dose. Urine was collected pre-dose, 0-8 and 8-24 hours post-dose. Cage washes (water and methanol) were collected at 24 hr following dose administration. Blood samples were collected from each animal at pre-dose and again at 1, 5 and 24 hr following dose administration. At the end of the collection period, animals were sacrificed and liver and kidneys were harvested for analysis of radioactivity content and metabolic profile analysis. All samples were analyzed for total radioactivity content by [ ]

3.1.9. Study Design

Group No.	ID	Gender	Dose	No Rats	Rat ID	Time of Euthanasia (hr)
1	Mass Balance	Males	20 mg/kg	3	1001-1003	1
				3	1004-1006	5
				3	1007-1009	24
		Females		3	1501-1503	1
				3	1504-1506	5
				3	1507-1509	24
2[a]	Control	Male	5 mL/kg	1	2001	ca 24
		Female		1	2501	ca 24

a. Animals in Group 2 received the vehicle only at a dose volume of 5 mL/kg. Samples collected from these animals were used to determine background levels of radioactivity (tissues only).

Results: For males, a total of 78.4% of the radioactive dose administered was recovered in the urine, feces and cage wash within 24 hours of dosing. Drug-derived radioactivity was recovered mainly in the feces (~67% of dose administered). Radioactivity in the urine accounted for ~12% of the dose administered. For female rats, 89% of the dose administered was recovered in the urine, feces and cage wash within 24 hours post-dosing. Approximately 75% of the drug-derived radioactivity was recovered in the feces. An additional 12.8% of the dose was recovered in the urine. For both sexes, negligible amounts were recovered in the cage washes.

At 24 hours post-dose, a total of 8.2 and 3.4% of drug-derived radioactivity was recovered from the carcass of males and females respectively. Less than 0.1% of the dose was recovered in the kidneys and liver.
Following single oral administration of radiolabelled eplerenone, the majority of drug-derived radioactivity was excreted in the feces with the remainder eliminated via the urine.

SC-66110: A STUDY OF THE MASS BALANCE AND METABOLIC PROFILE OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [^{14}C]SC-66110 IN BEAGLE DOGS M2001173 March 20, 2002

Six beagles (3 males and 3 females) received a single oral dose of [^{14}C]SC- 66110 at a target dose of 15 mg/kg. Urine, feces and cage debris were collected at selected intervals to 48 hr post-dose. Cage washes (water and methanol) were collected at the end of the collection period (48 hr following dose administration). In addition, blood samples were collected from each animal at pre- dose and again at 1, 5, 24 and 48 hr following dose administration. All samples (urine, feces, cage debris, cages washes, blood and plasma) were analyzed for total radioactivity content by [ ]

Results: for male dogs, 88% of the radioactive dose was recovered in the urine, feces cage debris and cage wash within 48 hours after dosing. The drug-derived radioactivity was mainly in the feces (~54% of the administered dose). The radioactivity recovered in the urine was 32% of the given dose. It was reported that total recovery might be low since the recovery for 1 animal was low for the 8-24 hour time point and urine spillage was recorded for the 0-8 and 8-24 hour collections. Approximately 76% of the drug-derived radioactivity was recovered in the urine and feces in the first 24 hours. Less than 1% of the dose was recovered in the cage wash and ~1.8% was found in the debris. For females, 86% of the dose was recovered within the first 48 hours. Approximately 49% of the radioactivity was found in the feces with an additional 35% in the urine. Less than 1% of the dose was recovered in the cage wash and ~1.5% was found in the debris.

The peak concentrations of radioactivity in blood and plasma were reported at 1 hour post-dose for both sexes. Blood to plasma ratios of radioactivity were comparable between the sexes. As the sponsor noted:

- Blood to plasma radioactivity concentration ratios were relatively constant (— to —) at 1 and 5 hr following dose administration in both genders.
- Drug-derived radioactivity was quantifiable in plasma to 24 hr post-dose (range: — to — µg equiv/g for males and — to — µg equiv/g for females). However, only one male dog showed quantifiable amounts of radioactivity in blood beyond 5 hr post-dose. These results suggest fairly rapid elimination.

Following single oral administration to dogs, the drug-associated radioactivity was excreted primarily in the feces with the remainder eliminated in the urine. There were no obvious sex-related differences in the excretion of radioactivity.

Pharmacokinetics of SC-66110: Biliary Excretion and Enterohepatic Circulation of [^{14}C]SC-66110 after Single Oral Administration of [^{14}C]SC-66110 in Male and Female Rats (Study No. PK0144) February 18, 2002

Male and female bile duct-cannulated rats (9 per sex) were given single oral doses of [^{14}C]SC-66110 at 20 mg/kg. The rats were assigned 3 per sex per group as subjects in the biliary excretion phase, as donor rats for enterohepatic recirculation study of radioactivity or as recipient rats for the enterohepatic circulation study of radioactivity.

Biliary excretion of radioactivity: 3 rats per sex were cannulated and 30 minutes after recovery from anesthesia were given the oral dose of radiolabelled SC-66110. Bile was collected during 0-4, 4-8, 8-24 and 24-48 hours after administration. Urine was collected during 0-8, 8-24 and 24-48 hours after administration. Feces were collected during 0-24 hours and 24-48 hours after dosing. Forty-eight hours after dosing, the rats were euthanized and the stomach, small intestines and large intestines were collected and the radioactivity measured. The carcass was also assayed for radioactivity.

Enterohepatic circulation of radioactivity: 3 rats per sex with bile duct cannulas were given the 20 mg/kg dose of [14C]SC-66110. The bile was colleted 0-8 hours after dosing. The radioactivity was measured and the samples pooled according to the sexes. Another 3 rats per sex were recipients of the pooled bile which was administered into the duodenum. Bile was then collected from the recipients at 0-4, 4-8, 8-24 and 24-48 hours after administration. Urine was collected 0-8, 8-24 and 24-48 after dosing. Feces were collected 0-24 and 24-48 hours after dosing. Feces were not collected for one of the rats from 0-24. At 48 hours after intra-duodenal administration the radioactivity of the gut and carcass were measured. The re-absorption ratio of enterohepatic circulation was calculated by the whole sum of biliary and urinary excretion and the radioactivity in the carcass with the exception of the stomach, small intestine and large intestine.

Radioactivity was measured by liquid scintillation counting.

Results

In the Phase I portion of the study, there was no significant difference between the male and female rats in the excretion of radioactivity. The sponsor's table is shown below.

Table 1 Cumulative excretion of radioactivity in bile, urine and feces and radioactivity in gastric contents and small intestinal contents, large intestinal contents and carcass after oral administration of [^{14}C]SC-66110 to bile-duct cannulated rats at 20 mg/kg

Time (h)	Cumulative excretion (% of dose)							
	Male				Female			
	Bile	Urine	Feces	Total	Bile	Urine	Feces	Total
0 – 4	65.5 ± 10.6	—	—	65.5 ± 10.6	70.6 ± 4.4	—	—	70.6 ± 4.4
0 – 8	73.5 ± 6.5	—	—	73.5 ± 6.5	75.1 ± 4.0	—	—	75.1 ± 4.0
0 – 24	78.7 ± 3.7	11.5 ± 1.9	4.1 ± 0.3	94.2 ± 2.2	77.3 ± 4.1	15.9 ± 4.6	2.2 ± 0.5	95.4 ± 1.1
0 – 48	79.6 ± 3.3	11.8 ± 2.2	4.5 ± 0.5	96.0 ± 1.6	77.5 ± 4.1	16.1 ± 4.6	2.5 ± 0.5	96.1 ± 1.0
Gastric contents				N.D.				N.D.
Small intestinal contents				N.D.				N.D.
Large intestinal contents				0.4 ± 0.8				0.3 ± 0.3
Carcass				N.D.				0.7 ± 0.6
Total				96.6 ± 1.0				97.1 ± 1.2

— : not collected, N.D. : not detected
Each value represents the mean ± S.D. of three rats.

Following intra-duodenal administration of radio-labeled bile, the females showed a greater cumulative excretion of radioactivity in the bile than did the males. The sponsor's table is shown below.

Table II Cumulative excretion of radioactivity in bile, urine and feces and radioactivity in gastric contents and small intestinal contents, large intestinal contents and carcass after intraduodenal administration of bile, which was collected in donor rats up to 8 hours after oral administration of [^{14}C]SC-66110 at 20 mg/kg, to recipient rats

Time (h)	Cumulative excretion (% of intraduodenal dose)							
	Male				Female			
	Bile	Urine	Feces	Total	Bile	Urine	Feces	Total
0 – 4	19.8 ± 5.7	—	—	19.8 ± 5.7	27.8 ± 10.7	—	—	27.8 ± 10.7
0 – 8	30.7 ± 12.5	—		30.7 ± 12.5	49.6 ± 13.4	—	—	49.6 ± 13.4
0 – 24	40.4 ± 10.5	4.7 ± 1.4	35.4 ± 16.6	80.6 ± 15.8	71.5 ± 6.8	10.1 ± 0.5	10.5 (n=2)*	88.5 ± 4.1
0 – 48	43.2 ± 10.1	5.9 ± 2.9	43.2 ± 13.7	92.3 ± 5.6	73.8 ± 7.2	12.1 ± 2.2	10.7 ± 7.6	96.5 ± 1.5
Gastric contents				N.D.				N.D.
Small intestinal contents				N.D.				N.D.
Large intestinal contents				3.7 ± 4.9				0.9 ± 0.2
Carcass				N.D.				N.D.
Total				96.0 ± 0.8				97.5 ± 1.3

— : not collected, N.D. : not detected
Each value represents the mean ± S.D. of three rats.
*: No feces during 0-24 hours after administration was collected in one of three rats.

The extent of re-absorption of radioactivity in male and female rats was ≥49% and ≥85% of the radioactivity in the dosed bile respectively. The study results suggest a sex-related difference in the enterohepatic metabolism of SC-66110 in rats.

3.3.7 Pharmacokinetic drug interactions No new studies submitted

3.3.10 Tables and figures to include comparative TK summary. For the complete cross-species comparison of toxicokinetics, the reader is referenced to NDA-21437.

The current studies include tissue distribution in female Sprague-Dawley rats (previously reported in male rats), a whole body radiography study in both sexes of rats, a confirmatory mass balance study/metabolism/excretion study in dogs and rats and a study

on the biliary excretion and enterohepatic recirculation of eplerenone in rats of both sexes.

As previously reported, the current studies showed that eplerenone was rapidly absorbed and widely distributed following oral administration with the highest concentrations in the gastrointestinal tract, fat, liver and muscle within 1 hour after dosing. The drug was also shown to be present in the heart. By 8 hours after dosing, the tissue concentrations were low in all tissues except for the gastrointestinal tract (large intestine). By 96 hours levels of radioactivity were below quantifiable limits in all examined tissues with the exception of the eye and liver. Whole body autoradiography also showed that at 0.5 hours after a single oral dose of 20 mg/kg radio-labeled eplerenone radioactivity was primarily in the gastrointestinal tract of both sexes and in the urinary bladder of males. Presence of radioactivity in the heart was also noted at this timepoint. At 8 and 24 hours after dosing, radioactivity was primarily associated with the large intestinal contents of both sexes. Radioactivity in the remaining tissues was below quantifiable limits.

An excretion study showed ~78% and 89% of the drug-derived radioactivity recovered in urine, feces and cage wash in male and female rats respectively within 24 hours of dosing. The majority of drug was recovered in the feces for both sexes: 67% in males and 75% in females. An additional 12% and 13% was recovered in the urine of males and females respectively. In dogs, 88% and 86% of the radioactivity was recovered from urine, feces and cage wash in males and females respectively within 48 hours of dosing. Again, the drug-derived radioactivity was mainly in the feces (49-54%) followed by 32-35% in the urine.

The examination of biliary excretion in rats indicated that the extent of reabsorption of radioactivity in male and female rats was ≥49% and ≥85% of radioactivity present in exogenously administered bile respectively. The results suggest a sex-related difference in the enterohepatic metabolism of eplerenone in rats.

The studies do not present any new safety concerns nor do they indicate the need for any re-evaluation of existing safety studies.

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary: No new toxicology studies were submitted. The toxicology for NDA 21437 is referenced. No new toxicology issues have been made apparent.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: There are no preclinical issues to preclude approvability.

Unresolved toxicology issues (if any): None.

Recommendations: none.

Suggested labeling: Lines [redacted] of the proposed labeling refer to findings in [redacted] and should be removed.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

3.7. APPENDIX/ATTACHMENTS

NONE

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

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