# CENTER FOR DRUG EVALUATION AND RESEARCH

## APPROVAL PACKAGE FOR:

## APPLICATION NUMBER
## 21-268

# Pharmacology Review(s)

**NDA # 21,268**

# REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

**Anthony G. Proakis, Ph.D.**
**4/17/01**

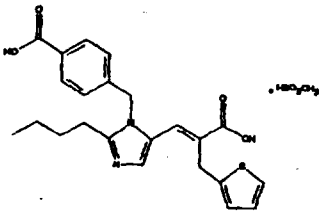
**ORIGINAL SUBMISSION DATED:** 8/30/00
**CENTER RECEIPT DATE:** 8/30/00
**REVIEWER RECEIPT DATE:** 9/01/00

**PRODUCT:** TEVETEN[redacted]

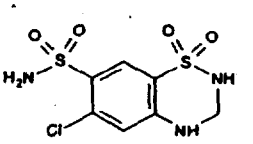
**ACTIVE INGREDIENTS:** Eprosartan mesylate/hydrochlorothiazide

**SPONSOR:** Unimed Pharmaceuticals, Inc.
Four Parkway North.
Deerfield, IL 60015-2544
(847) 282-5400

**CHEMISTRY:** Eprosartan mesylate (Code name: SK&F 108566-J; CAS No. 144143-96-4) is described as (E)-2-butyl-1- (p-carboxybenzyl)- -2-thienylimidazole-5-acrylic acid monomethane sulfonate. Its molecular weight is 520.63 and its empirical formula is $C_{23}H_{24}N_2O_4S.CH_4O_3S$. Hydrochlorothiazide (CAS No. 58-93-5) is described as 6-chloro-3, 4-dihydro-2H-1, 2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. Its molecular weight is 297.72 and its empirical formula is $C_7H_8ClN_3O_4S_2$.



Eprosartan mesylate



Hydrochlorothiazide

**IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:** IND #[redacted]

**PHARMACOLOGICAL CLASS:** Angiotensin II Antagonist/Diuretic

**PROPOSED INDICATION:** Treatment of Hypertension

**FORMULATION AND ROUTE OF ADMINISTRATION:** TEVETEN®[redacted] is formulated as tablets for oral use containing 735.8 mg of eprosartan mesylate (equivalent to 600 mg eprosartan) and 12.5 or 25 mg of hydrochlorothiazide per tablet; excipients include microcrystalline cellulose NF, lactose monohydrate NF, pregelatinized starch NF, crosprovidone, magnesium stearate NF and purified water. Hypromellose, polyethylene glycol 400, titanium dioxide, macrogol/PEG 3000, triacetin, iron oxide black, iron oxide red and iron oxide yellow are added to the 600/12.5 and 600/25 mg tablets as film coating and colorants.

**PROPOSED DOSAGE REGIMEN:** The recommended starting dose of TEVETEN®[redacted] is 600/12.5 mg once daily and may be increased to 600/25 mg once daily.

## TABLE OF CONTENTS

## INTRODUCTION

Teveten[redacted] (eprosartan mesylate/hydrochlorothiazide) is a combination of two marketed antihypertensive drugs. Eprosartan mesylate exerts its antihypertensive effect via blockade by eprosartan of vascular angiotensin II receptors. The preclinical pharmacology and toxicology of eprosartan mesylate have been reviewed previously under NDA # 20,738. Hydrochlorothiazide (HCTZ), a thiazide diuretic, acts directly on the kidney to increase the excretion of sodium chloride and other electrolytes. The antihypertensive effect of thiazides has been associated with thiazide-induced changes in sodium balance and consequent reduction in extracellular fluid volume. Thus, HCTZ reduces arterial blood pressure by decreasing the plasma volume and the cardiac output following diuresis/natriuresis; it also reduces vascular resistance by a direct action on vascular smooth muscle. The combination of eprosartan mesylate and hydrochlorothiazide is considered a rational approach to hypertension management by utilizing the antihypertensive properties of both drugs.

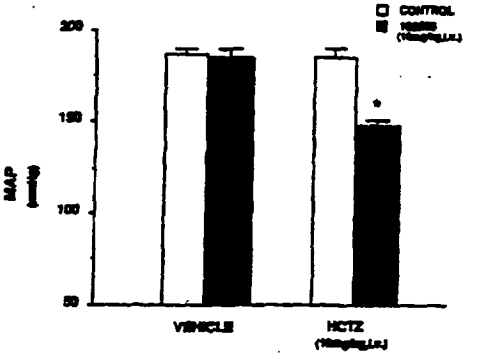
## PHARMACODYNAMICS

### *Effects Related to Proposed Therapeutic Indication*

Antihypertensive Effects in Spontaneously Hypertensive Rats

The antihypertensive effects of eprosartan mesylate and hydrochlorothiazide were evaluated in spontaneously hypertensive male rats (SHR). Blood pressure was measured directly from an implanted arterial cannula. A bolus intravenous dose of 10 mg eprosartan/kg was administered to six SHR and blood pressure was recorded for 2 hours. The experiment was repeated 3 days later in the same rats following 18 to 20 hours of fluid deprivation and the administration of 10 mg/kg IV of HCTZ. In euvolemic SHR, blood pressure was unchanged by treatment with eprosartan mesylate. Following HCTZ treatment (which by itself did not affect blood pressure), eprosartan caused a statistically significant ($p<0.05$) decrease in mean arterial blood pressure (from 186 to 148 mmHg). These results demonstrate that although eprosartan alone is not active in lowering blood pressure in the SHR, a low-renin model of hypertension, pretreatment with HCTZ to induce diuresis (which activates the renin-angiotensin system and creates a high-renin state) imparts antihypertensive efficacy to eprosartan in this SHR model (Fig 1).

Figure 1. Effect of eprosartan mesylate on mean arterial blood pressure (MAP) following vehicle or HCTZ treatment in conscious SHR; Values are the mean ± SE (n=6).



## PHARMACOKINETICS

The pharmacokinetics and biotransformation of eprosartan mesylate alone have been investigated in rats and dogs and were reviewed under NDA #20,738. Additional information provided under the current NDA is limited to plasma drug level data (for eprosartan and hydrochlorothiazide) from toxicity studies of the combination in dogs and mice (see Single Dose Toxicity and Repeat Dose Toxicity sections of the review).

## SINGLE DOSE TOXICITY

***Single Dose Toxicity Study of Oral Eprosartan mesylate/HCTZ in Female Dogs (Vol 22; pg 1)***

Study Facility: SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Study No.: D98040
Study Date: 3/31/98 (Day of treatment)

GLP Compliance: Compliance with GLP regulation attested.
QA Report: Yes

Animals: Female Beagle dogs (12-13 months old; 6.9-8.8 kg) were housed individually and fed 300 gm/day of solid diet [redacted] filtered tap water was available *ad libitum*.

Drug Administration: Eprosartan mesylate (Lot # 31-97UP) and hydrochlorothiazide (Lot # 7J5S56042(1)#1) were suspended in 1% aqueous carboxymethylcellulose and administered orally by gavage.

Dose Levels: 1000/0.3, 1000/1.0 and 1000/2.0 mg/kg of eprosartan /HCTZ (3/dose group).

Observations/Measurements: Dogs were observed for mortality and clinical signs of toxicity on the day of dosing and the following day. Body weight was measured predose, on the day of dosing and the day after dosing. Serial samples of venous blood were obtained from each dog predose and at 1, 2, 3, 4, 5, 6, 8, 12 and 24 hours after dosing for measurement of eprosartan and HCTZ plasma levels. At the end of day 2 of observations the animals were returned to the stock animal colony.

Results

*Mortality and Clinical Signs*
No dogs died during the study. Emesis occurred approximately 18 to 34 min after dosing in one dog from each dose group. Soft/mucoid feces were observed in one dog from the low dose group and 2 dogs from the high dose group.

*Body Weight*
Body weights after eprosartan mesylate/HCTZ treatment were comparable to pretreatment levels.

*Toxicokinetics*
Eprosartan and HCTZ were detectable in plasma of all dogs sampled (2 dogs/group; 1 dog/group experienced emesis and were excluded from data presented below). Maximum plasma concentrations of eprosartan and HCTZ were reached approximately 2 to 3 hours after dosing (Tables 1 and 2). Cmax and AUC values for HCTZ increased with increasing dose. Systemic exposure (AUC) to HCTZ increased in an approximately dose proportional manner (8-fold) from 0.3 to 3 mg/kg (10-fold increase in dose).

Table 1. Systemic Exposure to Eprosartan after Oral Eprosartan mesylate/HCTZ in Female Dogs

| Dose (mg/kg) | | Cmax [ug/mL] | AUC(0-t) [ug·h/mL] | Tmax [hour] |
|---|---|---|---|---|
| SKF-108566 | HCTZ | Day 1 | Day 1 | Day 1 |
| 1000 | 0.3 | 11493 (7683, 15302) | 38458 (31858, 45057) | 3.02 (2.03, 4.00) |
| 1000 | 1 | 5702 (5426, 5978) | 14264 (12103, 16425) | 2.00 (2.00, 2.00) |
| 1000 | 3 | 7180 (4806, 9553) | 30819 (22130, 39508) | 2.00 (2.00, 2.00) |

Table 2. Systemic Exposure to HCTZ after Oral Eprosartan mesylate/HCTZ in Female Dogs

| Dose (mg/kg) | | Cmax [ug/mL] | AUC(0-t) [ug·h/mL] | Tmax [hour] |
|---|---|---|---|---|
| SKF-108566 | HCTZ | Day 1 | Day 1 | Day 1 |
| 1000 | 0.3 | 0.159 (0.157 , 0.162) | 0.601 (0.495 , 0.706) | 2.52 (2.03 , 3.00) |
| 1000 | 1 | 0.581 (0.540 , 0.623) | 1.98 (1.86 , 2.10) | 2.50 (2.00 , 3.00) |
| 1000 | 3 | 0.742 (0.721 , 0.764) | 5.04 (3.28 , 6.80) | 2.50 (2.00 , 3.00) |

## REPEATED DOSE TOXICITY

***3-Month Toxicity Study of Oral Eprosartan/HCTZ in Mice (Vol. 22, pg. 38)***

Study Facility: SmithKline Pharmaceuticals, King of Prussia, PA
Study No: G96129
Study Dates: 5/08/97-5/28/97

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female mice (M=30.9-39.9 gm; F=22.2-30.2 gm). The mice were housed individually and fed [redacted] *ad libitum*; filtered tap water was available *ad libitum*.

Drug Administration: Eprosartan mesylate (Lot# 31-97UP) and hydrochlorothiazide (Lot# 7J5S56042(1)#1) were suspended in 1% aqueous carboxymethylcellulose and administered orally by gavage.

Dose Levels: Doses of HCTZ were chosen to achieve a 32:1 ratio of eprosartan:HCTZ which was considered to be the most likely clinical dose ratio. Selection of the high dose of eprosartan mesylate (2000 mg eprosartan/kg/day) was based on results of the 2-year mouse study which showed decreased survival in females and lower than control body weight in males and females given ≥ 1000 mg eprosartan/kg/day. Doses for eprosartan mesylate are expressed as amount of eprosartan (non-salt form).

| Group | Dose, mg/kg/day | Mice/sex/group | |
|---|---|---|---|
| | | Main Study | TK Satellite |
| Vehicle | 0/0 | 12 | None |
| Eprosartan mesylate/HCTZ | 300/9.375* | 12 | 48 |
| Eprosartan mesylate/HCTZ | 2000/62.5* | 12 | 48 |
| Eprosartan mesylate | 2000* | 12 | 48 |
| HCTZ | 62.5 | 12 | 48 |

* Doses expressed as amount of eprosartan or eprosartan/HCTZ

Observations/Measurements: Animals were observed daily for mortality and clinical signs of toxicity. Body weights were measured predose, daily for 28 days, then weekly throughout the remainder of the dosing period and on the day of necropsy. Food consumption was measured predose and once weekly during treatment. The eyes of all main study mice were examined prior to dosing and on day 90 of treatment. Blood samples (from the vena cava) were obtained from all surviving main study mice for hematology and clinical chemistry analyses. Blood samples were also obtained at 0.5, 1, 2, 4, 8, 12 and 24 hours after dosing from satellite mice (3 /sex/group/sample period) on days 1 and 28 of treatment for measurement of plasma eprosartan and/or HCTZ concentrations. At treatment termination surviving mice from the main study were killed (by exsanguination) and necropsied. The adrenals, brain, heart, kidneys, liver and thymus were weighed. Sections of major organs and tissues (Appendix A) from decedents in 2000 mg/kg eprosartan and 62.5 mg/kg HCTZ groups and from all mice (decedents and terminally-killed) from vehicle and 2000/62.5 mg/kg eprosartan/HCTZ group were fixed onto slides and examined microscopically. For terminally killed mice in the 300/9.375 mg/kg eprosartan/HCTZ, 2000 mg/kg eprosartan and 62.5 mg/kg HCTZ groups, only kidneys and tissues with macroscopic findings were examined microscopically. Statistical analysis for group differences was only performed for body weight, food consumption and organ weight values.

**Results**

*Mortality and Clinical Signs*

Four mice (2M, 2F) died during the study. These deaths were considered to be unrelated to drug treatment (Table 3). No clinical signs of toxicity were observed among surviving animals.

Table 3. Animal Deaths

| Group | # Mice, Sex | Treatment Day | Gross or Microscopic Finding |
|---|---|---|---|
| Vehicle | 1F | Day 7 | Red lungs and pleuritis (gavage error). |
| Eprosartan/HCTZ, 300/9.375 | - | | |
| Eprosartan/HCTZ, 2000/62.5 | 1M | Day 41 | Focal alveolitis and focal epicarditis (gavage error). |
| Eprosartan , 2000 | 1M | Day 43 | Penile ulcer, distension of urinary bladder, cystitis and urinary obstruction and deteriorated condition (sacrificed). |
| HCTZ, 62.5 | 1F | Day 18 | Intestinal distension, enlargement of spleen and mesenteric lymph nodes, malignant lymphoma. |

*Body Weight and Food Consumption*

Mean body weights for treated animals throughout the study were comparable to control (Table 4). Mean food consumption among treated groups was comparable to control.

Table 4. Mean Body Weights, gm.

| Treatment Group | Sex | Study Day | | | |
|---|---|---|---|---|---|
| | | Predose | Day 28 | Day 57 | Day 91 |
| Vehicle | M | 33.8 | 36.7 | 38.5 | 39.3 |
| Eprosartan/HCTZ 300/9.375 | | 33.8 | 36.4 | 37.8 | 37.9 |
| Eprosartan/HCTZ 2000/62.5 | | 33.9 | 36.3 | 37.9 | 38.7 |
| Eprosartan 2000 | | 33.8 | 36.7 | 36.9 | 37.3 |
| HCTZ | | 33.8 | 36.9 | 39.0 | 39.7 |
| | | | | | |
| Vehicle | F | 26.0 | 28.8 | 30.3 | 30.5 |
| Eprosartan/HCTZ 300/9.375 | | 26.0 | 29.6 | 31.4 | 31.5 |
| Eprosartan/HCTZ 2000/62.5 | | 26.1 | 29.1 | 30.5 | 29.7 |
| Eprosartan 2000 | | 26.1 | 29.2 | 30.7 | 31.0 |
| HCTZ | | 26.1 | 28.2 | 29.7 | 30.0 |

*Ophthalmology*

No treatment-related ocular effects were observed.

*Hematology and Blood Chemistry*

Slightly (~6%; no statistical analysis performed) lower than control erythrocyte parameters were noted at study termination for females treated with 2000 mg eprosartan/kg/day alone or in combination with 62.5 mg HCTZ/kg/day (Table 5).

Table 5. Hematology Findings

| Hematology Parameter | Sex | Eprosartan/HCTZ Dose Group, mg/kg/day | | | | |
|---|---|---|---|---|---|---|
| | | 0/0 (Vehicle) | 300/9.375 | 2000/62.5 | 2000/0 | 0/62.5 |
| RBC count, $x10^{12}$/L | M | 9.30 | 9.16 | 9.10 | 9.24 | 9.37 |
| | F | 9.24 | 8.91 | 8.67 | 8.68 | 9.03 |
| Hct, % | M | 49 | 48 | 48 | 49 | 49 |
| | F | 48 | 47 | 45 | 46 | 48 |
| Hb, gm/L | M | 147 | 144 | 144 | 147 | 147 |
| | F | 148 | 145 | 139 | 139 | 148 |

Mean serum urea values for males and females in the 2000/62.5 mg/kg/day eprosartan/HCTZ group or 62.5 mg/kg/day HCTZ group were higher than control (about 4% and 20% for the eprosartan/HCTZ and HCTZ groups, respectively; no statistical analysis, Table 6). No other treatment-related effects on blood chemistry were noted.

Table 6. Blood Chemistry Findings

| Blood Chemistry Parameter | Sex | Eprosartan/HCTZ Dose Group, mg/kg/day | | | | |
|---|---|---|---|---|---|---|
| | | 0/0 (Vehicle) | 300/9.375 | 2000/62.5 | 2000/0 | 0/62.5 |
| Serum urea, mmol/L | M | 8.6 | 10.9 | 11.0 | 8.9 | 10.7 |
| | F | 6.3 | 6.7 | 8.7 | 6.9 | 7.5 |

*Organ Weights*

The absolute and relative heart weights for females in the 2000/62.5 mg/kg/day eprosartan/HCTZ group were significantly lower (14% and 12%, respectively) than control. Also, the relative heart weight for females in the 300/9.375 mg/kg/day eprosartan/HCTZ group was significantly lower (11%) than control.

*Gross and Microscopic Pathology*
No drug-related gross lesions were observed. An increased incidence of tubular regeneration was observed in the kidneys of male and female mice given 2000/62.5 mg/kg/day of eprosartan /HCTZ or of male mice given 62.5 mg/kg/day of HCTZ (Table 7). JG cell hyperplasia was observed in kidneys of male and female mice given the high dose combination. Although JG cell hyperplasia is a common finding with angiotensin II antagonists, no JG cell hyperplasia was noted in mice treated with eprosartan mesylate alone.

Table 7. Renal Histopathology Findings (Incidence)

| Kidney Lesion | Sex | Eprosartan/HCTZ Dose Group, mg/kg/day | | | | |
|---|---|---|---|---|---|---|
| | | 0/0 (Vehicle) | 300/9.375 | 2000/62.5 | 2000/0 | 0/62.5 |
| Tubular regeneration (minimal to mild) | M | 1/12 | 3/12 | 6/11 | 2/11 | 6/12 |
| | F | 1/11 | 1/12 | 5/12 | 1/12 | 0/11 |
| JG cell hyperplasia (mild to moderate) | M | 0/12 | 0/12 | 9/11 | 0/11 | 0/12 |
| | F | 0/11 | 0/12 | 10/12 | 0/12 | 0/11 |

*Toxicokinetics*
Systemic exposure (AUC) to eprosartan increased with increasing dose of the combination. AUC values for eprosartan on day 1 were approximately 2-fold higher after administration of the high dose combination in both males and females versus eprosartan mesylate alone. On day 28, the eprosartan AUC values were similar for these eprosartan mesylate/HCTZ and eprosartan mesylate groups. No marked differences in Cmax or AUC were observed on day 28 compared to day 1 following the administration of low-dose or high dose combination (Table 8).

AUC values for HCTZ were similar following administration of the high dose combination versus 62.5 mg/kg HCTZ alone on both days 1 and 28. Nor were there remarkable differences in HCTZ AUCs between males and females for either of these treatment groups.

Table 8. Toxicokinetic Parameters

**SKF-108566**

| Dose (mg/kg/day) | | Sex | Cmax [ug/mL] | | AUC(0-t) [ug·h/mL] | | Tmax [hour] | |
|---|---|---|---|---|---|---|---|---|
| | | | Day | | Day | | Day | |
| SKF-108566-J | HCTZ | | 1 | 28 | 1 | 28 | 1 | 28 |
| 300 | 9.375 | M | 0.450 | 0.560 | 0.542 | 0.587 | 0.47 | 0.98 |
| 2000 | 62.5 | M | 6.54 | 3.49 | 3.99 | 3.83 | 0.99 | 0.47 |
| 2000 | 0 | M | 3.17 | 9.65 | 1.87 | 4.66 | 0.60 | 0.48 |
| 300 | 9.375 | F | 0.530 | 0.810 | 0.661 | 0.973 | 0.47 | 0.46 |
| 2000 | 62.5 | F | 4.81 | 4.03 | 8.37 | 9.63 | 1.00 | 0.47 |
| 2000 | 0 | F | 2.54 | 8.40 | 3.95 | 8.43 | 0.96 | 0.47 |

**HCTZ**

| Dose (mg/kg/day) | | Sex | Cmax [ug/mL] | | AUC(0-t) [ug·h/mL] | | Tmax [hour] | |
|---|---|---|---|---|---|---|---|---|
| | | | Day | | Day | | Day | |
| SKF-108566-J | HCTZ | | 1 | 28 | 1 | 28 | 1 | 28 |
| 300 | 9.375 | M | 1.87 | 1.22 | 2.48 | 2.02 | 0.98 | 0.47 |
| 2000 | 62.5 | M | 11.2 | 7.03 | 21.9 | 24.1 | 0.99 | 0.99 |
| 0 | 62.5 | M | 10.1 | 7.39 | 20.4 | 15.0 | 0.96 | 0.48 |
| 300 | 9.375 | F | 1.66 | 1.66 | 3.55 | 4.20 | 0.99 | 0.46 |
| 2000 | 62.5 | F | 13.8 | 8.01 | 35.4 | 29.4 | 1.00 | 0.97 |
| 0 | 62.5 | F | 9.57 | 8.51 | 28.4 | 20.8 | 0.97 | 0.98 |

Doses of eprosartan mesylate expressed in terms of eprosartan (SKF-108566)

***One-Month Toxicity Study of Oral Eprosartan Mesylate/Hydroclorothiazide in Dogs (Vol. 23, pg. 1)***

Study Facility: SmithKline Pharmaceuticals, King of Prussia, PA
Study No: G96130
Study Dates: First day of treatment, 6/17/97; Necropsy 7/15/97

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female Beagle dogs (M=9.1-12.5 kg; F=7.2-11.3 kg) were housed individually and fed 400 gm/day of solid diet each day [redacted] filtered tap water was available *ad libitum*.

Drug Administration: Eprosartan mesylate (Lot# 31-97UP) and hydrochlorothiazide (Lot# 7J5S56042(1)#1) were suspended in 1% aqueous carboxymethylcellulose and administered orally by gavage.

Dose Levels:

| Group | Dose, mg/kg* | Dogs/sex/group |
|---|---|---|
| Vehicle | 0 | 4 |
| Eprosartan mesylate | 1000 | 4[a] |
| HCTZ | 31.25 | 4 |
| Eprosartan mesylate/HCTZ | 100/3.125 | 4 |
| Eprosartan mesylate/HCTZ | 1000/31.25 | 4[b] |

* Doses of eprosartan mesylate expressed as amount of eprosartan
[a] A female dog was added on Day 11 to replace one sacrificed in moribund state.
[b] A male dog was added on Day 8 to replace one sacrificed in moribund state.

Observations/Measurements: Animals were observed daily for mortality and clinical signs of toxicity. Body weights were measured pretest, once weekly throughout dosing and prior to necropsy. Food consumption was measured pretest and once weekly during treatment. Venous blood samples were obtained pretest and on days 22 and 29 of treatment for hematology and clinical chemistry analyses. Urine was collected overnight pretest and on day 25 of treatment for urinalysis. Blood samples were also obtained (from the jugular vein) at 1, 2, 4, 8, 12 and 24 hours after dosing on days 1 and 28 of treatment for measurement of plasma eprosartan and HCTZ concentrations. At treatment termination, the dogs were killed (by exsanguination) and major organs were examined macroscopically. The adrenals, brain, heart, kidneys, liver, ovaries, testes and epididymides and thymus were weighed. Sections of major organs and tissues from all dogs (Appendix A) were fixed onto slides and examined microscopically. Statistical analysis was performed for group differences in body weight, food consumption and organ weights.

Results

*Mortality and Clinical Signs*

One male dog given eprosartan mesylate alone was killed in a moribund state on day 7 of treatment after accidental intratracheal administration (supported by macroscopic and microscopic findings); a replacement dog was added to this group on day 8 of the study period. One female and one male from the high dose combination group were killed in moribund states on days 10 (replacement added on day 11) and 27 (no replacement), respectively, with clinical signs and serum chemistry findings indicative of renal failure (renal tubular degeneration seen microscopically). Clinical signs of toxicity in these animals included emesis, loss of appetite, body weight loss, hypoactivity and hypothermia. Emesis was also noted in non-decedent dogs given eprosartan mesylate with or without HCTZ. Due to adverse drug-related clinical signs, the study was terminated after 28 days (3-month intended duration).

*Body Weight*

On day 28, male and female dogs treated with the high dose combination had mean body weights 4 to 5% below pretreatment weight (not statistically significant). No adverse effect on body weight was seen in any other group (Table 9).

Table 9. Mean Body Weights

| Group | Dose, mg/kg* | Sex | Mean Body Wt., Kg | |
|---|---|---|---|---|
| | | | Day 0 | Day 28 |
| Vehicle | 0 | M | 10.3 | 10.3 |
| | | F | 8.9 | 9.3 |
| Eprosartan mesylate | 1000 | M | 10.4 | 10.4 |
| | | F | 8.3 | 8.4 |
| HCTZ | 31.25 | M | 10.2 | 10.0 |
| | | F | 8.2 | 8.3 |
| Eprosartan mesylate/HCTZ | 100/3.125 | M | 10.3 | 10.4 |
| | | F | 8.4 | 8.5 |
| Eprosartan mesylate/HCTZ | 1000/31.25 | M | 10.4 | 10.0 |
| | | F | 8.5 | 8.1 |

* Doses of eprosartan mesylate expressed in terms of eprosartan

*Food Consumption*
A lower than control mean food consumption was noted for the high dose combination group (for females, 66% lower than concurrent control; for males, 28% lower than concurrent control).

*Hematology*
A lower than baseline mean reticulocyte count was noted for male dogs given eprosartan mesylate alone (no difference from concurrent control) and female dogs given the high dose combination of eprosartan mesylate/HCTZ (~36% lower than the concurrent control, Table 10). Other hematology parameters were not affected by treatment.

Table 10. Hematology Results

| Treatment Group, mg/kg* | Sex | Hematology Parameter | | | | | |
|---|---|---|---|---|---|---|---|
| | | Reticulocyte count, x $10^9$/L | | Hemoglobin g/L | | Hematocrit % | |
| | | Predose | Day 22 | Predose | Day 22 | Predose | Day 22 |
| Vehicle, 0 | M | 32.2 | 28.8 | 168 | 162 | 51 | 49 |
| Eprosartan mesylate, 1000 | | 52.4 | 29.0 | 166 | 156 | 50 | 47 |
| HCTZ, 31.25 | | 48.1 | 55.1 | 164 | 167 | 49 | 50 |
| Eprosartan mesylate/HCTZ, 100/3.125 | | 51.7 | 56.2 | 170 | 170 | 51 | 50 |
| Eprosartan mesylate/HCTZ, 1000/31.25 | | 39.5 | 41.0 | 163 | 160 | 49 | 49 |
| | | | | | | | |
| Vehicle, 0 | F | 41.1 | 42.4 | 159 | 159 | 48 | 48 |
| Eprosartan mesylate, 1000 | | 45.5 | 42.9 | 152 | 146 | 45 | 44 |
| HCTZ, 31.25 | | 34.4 | 36.1 | 164 | 163 | 49 | 50 |
| Eprosartan mesylate/HCTZ, 100/3.125 | | 33.7 | 23.5 | 157 | 157 | 47 | 47 |
| Eprosartan mesylate/HCTZ, 1000/31.25 | | 40.3 | 27.3 | 164 | 164 | 49 | 49 |

* Dose of eprosartan mesylate expressed in terms of eprosartan.

*Clinical Chemistry*
Serum urea levels for males and females treated with the high dose combination were higher on day 29 than at pretest and higher than concurrent control levels. Males and females given HCTZ alone or the high dose combination of eprosartan mesylate/HCTZ showed lower than concurrent control or pretest levels of serum potassium. Higher than pretest levels of ALP and ALT were noted in males and females, respectively, treated with the high-dose eprosartan mesylate/HCTZ combination (higher than concurrent control for ALT only, Table 11).

Table 11. Clinical Chemistry Results

| Parameter | Sex | Dosing Day | Group, mg/kg* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Vehicle 0 | Eprosartan mesylate 1000 | HCTZ 31.25 | Eprosartan mesylate/HCTZ 100/3.125 | Eprosartan mesylate/HCTZ 1000/31.25 |
| Urea, mmol/L | M | Pretest | 5.4 | 4.9 | 5.0 | 5.0 | 5.5 |
| | | Day 29 | 5.6 | 5.2 | 6.1 | 6.8 | 16.1 |
| | F | Pretest | 5.7 | 5.8 | 5.4 | 4.8 | 4.8 |
| | | Day 29 | 5.8 | 6.6 | 7.1 | 7.5 | 34.4 |
| Potassium, mmol/L | M | Pretest | 4.5 | 4.3 | 4.6 | 4.5 | 4.4 |
| | | Day 29 | 4.6 | 4.2 | 3.5 | 4.4 | 3.8 |
| | F | Pretest | 4.6 | 4.5 | 4.5 | 4.5 | 4.7 |
| | | Day 29 | 4.6 | 4.3 | 3.6 | 4.3 | 3.8 |
| ALP, IU/L | M | Pretest | 148 | 141 | 118 | 120 | 115 |
| | | Day 29 | 162 | 160 | 115 | 135 | 174 |
| | F | Pretest | 163 | 145 | 142 | 153 | 130 |
| | | Day 29 | 146 | 149 | 130 | 136 | 131 |
| ALT, IU/L | M | Pretest | 36 | 32 | 31 | 35 | 32 |
| | | Day 29 | 33 | 35 | 33 | 40 | 35 |
| | F | Pretest | 34 | 27 | 26 | 28 | 29 |
| | | Day 29 | 37 | 29 | 47 | 32 | 55 |

* Dose of eprosartan mesylate expressed in terms of eprosartan.

*Urinalysis*

Higher (2.4- to 4.2-fold) than concurrent control or predrug urine volumes were observed on day 25 in males and females given the high dose eprosartan mesylate/HCTZ combination (Table 12).

Table 12. Urinalysis Findings

| Parameter | Sex | Dosing Day | Group, mg/kg* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Vehicle 0 | Eprosartan mesylate 1000 | HCTZ 31.25 | Eprosartan mesylate/HCTZ 100/3.125 | Eprosartan mesylate/HCTZ 1000/31.25 |
| Urine Volume ( ml) | M | Predose | 74.9 | 78.0 | 65.6 | 80.5 | 52.5 |
| | | Day 25 | 59.2 | 116.3 | 73.8 | 83.6 | 124.0 |
| | F | Predose | 85.1 | 24.6 | 37.9 | 59.5 | 50.3 |
| | | Day 25 | 138.5 | 54.8 | 87.5 | 87.8 | 210.1 |

* Dose of eprosartan mesylate expressed in terms of eprosartan.

*Organ Weights*

Higher (24-31%) than control relative (but not absolute) kidney weights were noted in males and females given the high dose combination of eprosartan mesylate and HCTZ. No other significant differences in absolute or relative organ weights were observed.

*Macroscopic Examination*

Macroscopic observations in the female decedent given the high dose combination and killed on day 10 included multiple red areas on the gastric fundic mucosa, green raised areas on the ventral tongue and red thymic lymph nodes; these changes correspond with microscopic findings of ulcerative gastritis, glossitis and hemorrhage in the thymic lymph nodes.

Pale kidneys were observed in 5 of 7 surviving dogs of the high dose combination group.

*Microscopic Examination*

Drug-related microscopic findings were limited to the kidneys, upper digestive system, liver and hemolymphatic system of dogs (decedents and survivors) of the high dose eprosartan mesylate/HCTZ group (Table 13).

Table 13. Treatment Associated Histopathology (# with lesion/# examined).

| Lesion | Treatment Group, mg/kg* | |
|---|---|---|
| | Vehicle (0) | Eprosartan mesylate/HCTZ (1000/31.25) |
| Kidney | | |
| Tubular degeneration and regeneration | 0/4M, 0/4F | 2/4M, 3/5F |
| Upper GI System | | |
| Ulcerative gastritis | 0/4M, 0/4F | 0/4M, 1/5F |
| Ulcerative esophagitis | 0/4M, 0/4F | 0/4M, 2/5F |
| Glossitis | 0/4M, 0/4F | 0/4M, 1/5F |
| Hemolymphatic System | | |
| Cortical atrophy of thymus | 0/4M, 0/4F | 0/4M, 3/5F |
| Lymph node hemorrhage | 0/4M, 0/4F | 0/4M, 1/5F |

* Dose of eprosartan mesylate expressed in terms of eprosartan.

*Toxicokinetics*

Mean peak plasma concentrations (Cmax) of eprosartan increased with increasing doses of eprosartan mesylate (Table 14). Generally, the maximum plasma concentration was attained between 1 and 2 hours following dosing and there were no marked differences in systemic exposure between males and females. Mean peak plasma concentrations (Cmax) of HCTZ increased with increasing doses of the diuretic (Table 15). No marked differences in systemic exposure (AUC) to HCTZ between males and females were observed. Systemic exposure to HCTZ, based on AUC, was similar on days 1 and 28 in dogs receiving 100/3.125 mg/kg eprosartan/HCTZ or 31.25 mg/kg HCTZ alone. However, systemic exposure to HCTZ was 4.4-fold higher on day 28 than on day 1 in dogs receiving 1000/31.25 mg/kg eprosartan/HCTZ. This difference appears to reflect drug-induced renal damage or failure among dogs in this treatment group.

Table 14. Systemic Exposure to Eprosartan after Oral Eprosartan mesylate/HCTZ

| Dose (mg/kg/day) | | Sex (n=4) | Cmax [ng/mL] | | AUC(0-t) [ng·h/mL] | | Tmax+ [hour] | |
|---|---|---|---|---|---|---|---|---|
| | | | Day | | Day | | Day | |
| SKF-108566-J | HCTZ | | 1 | 28 | 1 | 28 | 1 | 28 |
| 100 | 3.125 | M | 1318* (237) | 848 (315) | 1912 ** | 2566 (659) | 1.00 (1.00-1.00) | 1.03 (1.00-1.03) |
| 1000 | 31.25 | M | 2051 (1333) | 5112* (1735) | 9430 (3826) | 23194* (13537) | 2.01 (2.00-4.00) | 1.98 (1.00-2.00) |
| 1000 | 0 | M | 1971 (743) | 1970 (225) | 7214 (2916) | 7849 (2572) | 2.00 (1.00-2.00) | 2.01 (2.00-2.02) |
| 100 | 3.125 | F | 2276 (779) | 1775 (757) | 4794 (840) | 5901 (2256) | 1.00 (1.00-1.00) | 1.01 (1.00-1.02) |
| 1000 | 31.25 | F | 2309 (1091) | 5418 (3545) | 10087 (3910) | 29852 (18512) | 2.00 (1.98-2.02) | 2.00 (1.98-4.00) |
| 1000 | 0 | F | 3404 (1719) | 2938 (1799) | 10138 (4193) | 11452 (8880) | 2.00 (1.00-2.00) | 2.00 (1.02-2.02) |

\+ Tmax expressed as median (range); * n = 3; ** n = 1.

Table 15. Systemic Exposure to HCTZ after Oral Eprosartan mesylate/HCTZ

| Dose (mg/kg/day) | | Sex (n=4) | Cmax [ug/mL] | | AUC(0-t) [ug·h/mL] | | Tmax+ [hour] | |
|---|---|---|---|---|---|---|---|---|
| | | | Day | | Day | | Day | |
| SKF-108566-J | HCTZ | | 1 | 28 | 1 | 28 | 1 | 28 |
| 100 | 3.125 | M | 2.12 (0.56) | 1.83 (0.14) | 3.44 (0.75) | 2.69 (0.45) | 1.00 (0.98-1.00) | 1.03 (1.00-1.03) |
| 1000 | 31.25 | M | 12.8 (1.9) | 18.7* (14.1) | 46.6 (10.9) | 200* (275) | 1.01 (1.00-2.00) | 2.00* (1.98-2.00) |
| 0 | 31.25 | M | 8.52 (3.21) | 9.14 (2.15) | 26.6 (3.8) | 20.5 (8.5) | 1.00 (1.00-1.00) | 1.00 (1.00-1.00) |
| 100 | 3.125 | F | 2.30 (1.04) | 1.85 (0.68) | 3.89 (1.23) | 3.22 (1.13) | 1.00 (1.00-2.02) | 1.01 (1.00-1.02) |
| 1000 | 31.25 | F | 12.4 (4.6) | 18.0 (14.3) | 43.7 (12.1) | 200 (233) | 1.50 (1.00-2.00) | 1.99 (1.00-2.00) |
| 0 | 31.25 | F | 8.76 (0.96) | 7.63 (1.62) | 27.9 (7.9) | 28.6 (2.8) | 1.01 (1.00-2.00) | 1.00 (1.00-1.00) |

+ Tmax expressed as median (range); * n = 3.

***Three-Month Toxicity Study of Oral Eprosartan Mesylate/Hydroclorothiazide in Dogs (Vol. 24, pg. 1)***

Study Facility: SmithKline Pharmaceuticals, King of Prussia, PA
Study No: G98015
Study Dates: Initiation of treatment, 6/03/98; Necropsy, 9/03/98

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female Beagle dogs (M=7.1-9.1 kg; F=6.3-8.4 kg) dogs were housed individually and fed 400 gm/day of solid diet each day [redacted] filtered tap water was available *ad libitum.*

Drug Administration: Eprosartan mesylate (Lot# 31-97UP) and hydrochlorothiazide (Lot# 7J5S56042(1)#1) were suspended in 1% aqueous carboxymethylcellulose and administered orally by gavage.

Dose Levels:

| Group | Dose, mg/kg/day* | Dogs/sex/group |
|---|---|---|
| Vehicle | 0/0 | 4 |
| Eprosartan mesylate/HCTZ | 1000/0.3 | 4 |
| Eprosartan mesylate/HCTZ | 1000/1.0 | 4 |

* Dose of eprosartan mesylate expressed in terms of eprosartan.

Observations/Measurements: Animals were observed daily for mortality and clinical signs of toxicity. Body weights were measured pretest, once weekly throughout dosing and on the day of necropsy. Food consumption was measured pretest and once weekly during treatment. ECG recordings were obtained pretest and on day 87 of treatment. The eyes of all dogs were examined pretest and on day 86 of treatment. Venous blood samples were obtained pretest and on days 29 and 91 for hematology and clinical chemistry analyses. Urine was collected overnight pretest and on days 29 and 91 of treatment for urinalysis. Blood samples were also obtained (from the jugular vein) at 1, 2, 4, 8, 12 and 24 hours after dosing on days 1 and 91 of treatment for measurement of plasma eprosartan and HCTZ concentrations. At treatment termination the dogs were killed by

exsanguination and major organs were examined macroscopically. The adrenals, brain, heart, kidneys, liver, ovaries, testes, epididymides and thymus were weighed. Sections of major organs and tissues from all animals (Appendix A) were fixed onto slides and examined microscopically. Statistical analysis was performed for group differences in body weight, food consumption and organ weights.

Results

*Mortality and Clinical Signs*
Gavage error, followed by respiratory distress resulted in the early sacrifice of two male dogs (on day 21 for dog given 1000/0.3 mg/kg/day and on day 74 for dog given 1000/1.0 mg/kg/day). The lungs of both these dogs were discolored at necropsy with extensive necrosis with vascular thrombosis and infarction seen microscopically, consistent with the intratracheal instillation of the drug substance.

Clinical signs of toxicity included emesis and abnormal (unformed or discolored) feces.

*Body Weight*
Group mean body weights of the eprosartan mesylate/HCTZ treated dogs over the course of the study did not significantly differ from vehicle control (Table 16).

Table 16. Mean Body Weights (kg)

| Group | Sex | Treatment Day | | | |
|---|---|---|---|---|---|
| | | Day 1 | Day 30 | Day 64 | Day 85 |
| Vehicle | M | 7.9 | 8.0 | 8.4 | 8.4 |
| | F | 7.3 | 7.6 | 7.8 | 8.1 |
| Eprosartan mesylate/HCTZ 1000/0.3* | M | 8.0 | 8.0[a] | 8.0[a] | 8.3[a] |
| | F | 7.0 | 7.1 | 7.4 | 7.6 |
| Eprosartan mesylate/HCTZ 1000/1.0* | M | 8.4 | 8.6 | 9.2 | 9.5[a] |
| | F | 7.3 | 7.1 | 7.5 | 7.7 |

* Dose of eprosartan mesylate expressed in terms of eprosartan. [a] n=3; all other values n=4

*Food Consumption*
Food consumption for treated groups was comparable to control.

*Electrocardiography*
No electrocardiographic effects related to drug treatment were observed.

*Ophthalmology*
No treatment-related ocular effects were observed.

*Hematology, Clinical Chemistry and Urinalysis*
Slightly lower than concurrent control erythrocyte parameters (RBC counts, hematocrit and/or hemoglobin) were noted on day 29, but not on day 91, for females receiving 1000/0.3 or 1000/1.0 mg/kg of eprosartan/HCTZ (Table 17). The statistical significance of these differences was not determined.

Table 17. Hematology Findings

| Hematology Parameters | Sex | Eprosartan mesylate/HCTZ Dose Group, mg/kg/day* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0/0 (Vehicle) | | | 1000/0.3 | | | 1000/1.0 | | |
| | | Pre-dose | Day 29 | Day 91 | Pre-dose | Day 29 | Day 91 | Pre-dose | Day 29 | Day 91 |
| RBC Counts, x $10^{12}$/L | M | 7.45 | 7.11 | 7.62 | 7.08 | 7.38 | 7.29 | 6.91 | 6.78 | 7.02 |
| | F | 6.93 | 7.02 | 7.34 | 6.39 | 6.10 | 6.74 | 6.99 | 6.61 | 7.24 |
| Hemoglobin, g/L | M | 175 | 164 | 179 | 167 | 171 | 171 | 161 | 156 | 165 |
| | F | 165 | 164 | 175 | 151 | 141 | 159 | 162 | 142 | 170 |
| Hematocrit, % | M | 52 | 48 | 52 | 49 | 50 | 50 | 48 | 46 | 48 |
| | F | 48 | 48 | 51 | 45 | 41 | 47 | 48 | 42 | 50 |

* Dose of eprosartan mesylate expressed in terms of eprosartan.

Mean serum potassium values were 9%-12% lower than control means (4.3 and 4.6 mmol/L respectively) on days 29 and 91 for females receiving 1000/1.0 mg/kg/day of the eprosartan/HCTZ combination and 4% to 9% lower than control means for males receiving 0.3 or 1 mg/kg/day of HCTZ in combination with 1000 mg eprosartan/kg/day (Table 18). Mean serum urea values were higher than control for males and females in both eprosartan mesylate/HCTZ combination groups on day 29 (15% and 52% higher) and day 91 (16% and 31% higher).

Table 18. Clinical Chemistry Findings

| Clinical Chemistry Parameters | Sex | Eprosartan mesylate/HCTZ Dose Group, mg/kg/day* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0/0 (Vehicle) | | | 1000/0.3 | | | 1000/1.0 | | |
| | | Pre-dose | Day 29 | Day 91 | Pre-dose | Day 29 | Day 91 | Pre-dose | Day 29 | Day 91 |
| Serum Potassium, mmol/L | M | 4.4 | 4.1 | 4.3 | 4.5 | 4.5 | 3.9 | 4.2 | 4.0 | 3.8 |
| | F | 4.5 | 4.3 | 4.6 | 4.4 | 4.2 | 4.4 | 4.6 | 3.9 | 4.2 |
| Serum Urea, mmol/L | M | 5.5 | 5.3 | 5.6 | 5.6 | 7.6 | 6.5 | 5.4 | 7.1 | 6.8 |
| | F | 4.6 | 5.4 | 4.8 | 5.0 | 6.2 | 6.1 | 5.4 | 8.2 | 6.3 |

* Dose of eprosartan mesylate expressed in terms of eprosartan.

No drug-related effects on urinalysis parameters were observed.

*Organ Weights*

Absolute and relative weights of organs from drug-treated animals were comparable to control.

*Gross and Microscopic Pathology*

Gross and microscopic findings (discolored lungs, pulmonary necrosis with vascular thombosis and infarction) associated with drug treatment were limited to the two dogs that were killed following gavage errors. There were no other drug-related microscopic observations.

*Toxicokinetics*

The maximum plasma concentration of eprosartan was generally attained by 2 hours after dosing. Systemic exposure for eprosartan, based on AUC, was slightly higher for females than for males; AUCs did not differ markedly between day 1 and day 91 (Table 19). Plasma concentrations of HCTZ increased with increasing doses (Table 20). The maximum plasma concentration was attained by 1 to 2 hours after dosing. Systemic exposure to HCTZ, based on AUC, was comparable for males and females for each respective dose. AUCs for HCTZ were similar when comparing day 1 to day 91 for the low-dose group; however, the AUCs for HCTZ for the high-dose group were nearly 2-fold higher on day 91 than on day 1.

Table 19. Systemic Exposure to Eprosartan after Administration of Eprosartan Mesylate/HCTZ

| Dose (mg/kg/day) | | Sex (n=4) | Cmax [ug/mL] | | AUC(0-t) [ug·h/mL] | | Tmax* [hour] | |
|---|---|---|---|---|---|---|---|---|
| SKF-108566 | HCTZ | | Day 1 | Day 91 | Day 1 | Day 91 | Day 1 | Day 91 |
| 1000 | 0.3 | M | 4575 (2519) | 2715** (862) | 16157 (9505) | 9959** (4143) | 2.03 (1.98-4.00) | 2.00** (1.98-2.02) |
| 1000 | 1.0 | M | 3401 (1291) | 3423** (2511) | 9405 (3318) | 9260** (5846) | 2.00 (1.98-2.00) | 2.00** (1.00-2.02) |
| 1000 | 0.3 | F | 9634 (9036) | 4268 (2273) | 25436 (16250) | 17827 (8139) | 2.00 (1.98-2.00) | 3.00 (2.00-4.00) |
| 1000 | 1.0 | F | 5099 (3431) | 3327 (1950) | 18222 (9784) | 19984 (12751) | 2.00 (1.98-2.00) | 2.00 (2.00-4.00) |

* Tmax expressed as median (range)

** n=3

Table 20. Systemic Exposure to HCTZ after Administration of Eprosartan Mesylate/HCTZ

| Dose (mg/kg/day) | | Sex (n=4) | Cmax [ug/mL] | | AUC(0-t) [ug·h/mL] | | Tmax* [hour] | |
|---|---|---|---|---|---|---|---|---|
| SKF-108566 | HCTZ | | Day 1 | Day 91 | Day 1 | Day 91 | Day 1 | Day 91 |
| 1000 | 0.3 | M | 0.097 (0.019) | 0.084** (0.021) | 0.291 (0.116) | 0.249** (0.089) | 1.51 (1.00-2.03) | 1.03** (1.03-2.00) |
| 1000 | 1.0 | M | 0.345 (0.074) | 0.482** (0.246) | 0.977 (0.155) | 1.69** (1.02) | 1.00 (0.98-2.00) | 2.00** (2.00-2.02) |
| 1000 | 0.3 | F | 0.116 (0.017) | 0.110 (0.022) | 0.310 (0.061) | 0.285 (0.073) | 1.49 (1.00-2.00) | 1.51 (1.00-2.00) |
| 1000 | 1.0 | F | 0.380 (0.201) | 0.539 (0.156) | 0.962 (0.410) | 1.71 (0.610) | 1.49 (1.00-2.00) | 1.01 (1.00-2.00) |

* Tmax expressed as median (range)

** n=3

## REPRODUCTIVE TOXICOLOGY

***Developmental Toxicity Study of Oral Eprosartan Mesylate/HCTZ Administered During Late-Gestation in Rabbits (Vol 26, pg 1)***

Study Facility: SmithKline Beecham Pharmaceuticals, King of Prussia, PA.
Study No: G98141
Study Dates: Study initiation: 10/08/98; Necropsy, 10/21/98

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Presumed pregnant New Zealand White rabbits (6 months old; approx 3 kg) were housed individually and provided 125 gm/day of [redacted] Tap water was available *ad libitum*.

Drug Administration: Eprosartan mesylate (Lot # 31-97J), hydrochlorothiazide (Lot # 7JS56042(1)#1) and a mixture of eprosartan mesylate/HCTZ were each suspended in aqueous 1% carboxymethylcellulose and administered orally by gavage on days 18 to 28 post coitus.

Dose Levels:

| Treatment | Dose (mg/kg/day)* | # of Mated Females |
|---|---|---|
| Vehicle | - | 24 |
| HCTZ | 3 | 24 |
| Eprosartan mesylate | 10 | 24 |
| Eprosartan mesylate/HCTZ | 3/1 | 24 |
| | 10/3 | 24 |

*Eprosartan mesylate doses expressed as amount of eprosartan

Observations/Measurements: Animals were observed daily for clinical signs of toxicity and mortality. Body weight was measured on days 6, 10, 15 post coitus (pc), and daily from day 17 to day 29 pc. Food consumption was measured daily from days 16 through day 29 pc. On day 23 pc (day 6 of treatment) blood was obtained from an ear vein (at varying intervals up to 24 hours post dose; n=3/each interval) from rabbits of drug-treated groups only for measurement of eprosartan and or HCTZ blood levels. On day 29 pc, the mated females were killed with sodium pentobarbital IV and necropsied. The ovaries were removed and the corpora lutea counted. The uterus was weighed and examined for implantation sites, resorptions and live and dead fetuses. A gross examination of each placenta was performed. Live fetuses were weighed and examined externally. Fetuses were killed and the abdominal and thoracic viscera were examined. All fetuses were eviscerated and stained for skeletal examination.

Results

*Mortality, Clinical Signs and Mating Results*
There were no unscheduled deaths and no clinical signs of toxicity were observed.

The incidence of pregnancy among the 24 rabbits/group that were mated is summarized in Table 21.

Table 21. Mating Results

| Treatment Group (mg/kg) | # Mated | #Pregnant |
|---|---|---|
| Vehicle | 24 | 23 |
| HCTZ (3) | 24 | 24 |
| Eprosartan mesylate (10) | 24 | 23 |
| Eprosartan mesylate/HCTZ (3/1) | 24 | 23 |
| Eprosartan mesylate/HCTZ (10/3) | 24 | 22 |

*Body Weight and Food Consumption*
Mean body weights did not differ significantly among groups (Table 22). Body weight gain for does treated with 10/3 mg/kg of eprosartan/HCTZ was lower than control during the first 3 days of drug treatment (days 18 to 21 pc) but there were no significant differences in body weight gain, thereafter.

Food consumption among drug-treated groups did not significantly differ from control.

Table 22. Maternal Mean Body Weights (kg).

| Study Days: | | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test Used : | | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- |
| Dose (mg/kg/day) | | SKF-108566-J/HCTZ | | | | | | | | | | | | |
| 0 | Mean | 3.60 | 3.60 | 3.60 | 3.60 | 3.61 | 3.63 | 3.65 | 3.67 | 3.70 | 3.71 | 3.73 | 3.75 | 3.78 |
| Control | SEM | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| | N | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 |
| 0/3 | Mean | 3.62 | 3.60 | 3.56 | 3.58 | 3.59 | 3.61 | 3.64 | 3.64 | 3.67 | 3.68 | 3.71 | 3.72 | 3.75 |
| | SEM | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| | N | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| 10/0 | Mean | 3.62 | 3.62 | 3.62 | 3.62 | 3.64 | 3.67 | 3.69 | 3.69 | 3.72 | 3.74 | 3.75 | 3.77 | 3.80 |
| | SEM | 0.06 | 0.07 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| | N | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 |
| 3/1 | Mean | 3.60 | 3.59 | 3.58 | 3.58 | 3.60 | 3.62 | 3.66 | 3.66 | 3.70 | 3.71 | 3.72 | 3.75 | 3.76 |
| | SEM | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.06 |
| | N | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 |
| 10/3 | Mean | 3.65 | 3.65 | 3.61 | 3.61 | 3.63 | 3.66 | 3.69 | 3.68 | 3.70 | 3.72 | 3.73 | 3.75 | 3.77 |
| | SEM | 0.06 | 0.06 | 0.05 | 0.06 | 0.06 | 0.06 | 0.05 | 0.04 | 0.04 | 0.05 | 0.05 | 0.05 | 0.06 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |

N=number of pregnant animals.

*Early Deliveries*

| Treatment Group (mg/kg) | Early Deliveries |
|---|---|
| Vehicle | - |
| HCTZ (3) | - |
| Eprosartan mesylate (10) | One doe delivered early (day 29 pc) with 9 non-viable fetuses |
| Eprosartan mesylate/HCTZ (3/1) | One doe delivered early (day 29 pc) with 9 non-viable fetuses |
| Eprosartan mesylate/HCTZ (10/3) | One doe delivered early (day 29 pc) with 3 viable and 8 non-viable fetuses. Another doe aborted its entire litter on day 23 to 24 pc. |

*Cesarean Section Results*

A higher than control incidence of resorptions (primarily late resorptions indicative of fetal death) was noted among animals receiving 10 mg eprosartan/kg or 10/3 mg/kg of eprosartan/HCTZ (Table 23). No other treatment-related effects on implantation parameters were observed.

Table 23. Cesarean Section Results

| Endpoints : | | No. of Corpora Lutea KN- | No. of Implants KN- | Percent Pre-Implant Loss KN- | ------Resorptions------ Early KN+ | Late KN+ | Total KN+ | Percent Implants Resorbed KN+ | No. of Live Fetuses KN+ | Total No. of Live Males KN- | Total No. of Live Females KN- | No. of Dead Fetuses KN- | Live Birth Index % PE- | Uterus Weight (g) KN- |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test Used : | | | | | | | | | | | | | | |
| Dose (mg/kg/day) | | SKF-108566-J/HCTZ | | | | | | | | | | | | |
| 0 | Mean | 8.8 | 8.7 | 2.1 | 0.7 | 0.1 | 0.1 | 10.0 | 7.8 | 3.8 | 4.0 | 0.0 | 100.0 | 459.2 |
| Cont- | SEM | 0.3 | 0.3 | 1.3 | 0.2 | 0.1 | 0.1 | 2.7 | 0.4 | 0.4 | 0.4 | 0.0 | 0.0 | 20.7 |
| rol | N | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 |
| 0/3 | Mean | 8.8 | 8.1 | 7.0 | 0.1* | 0.1 | 0.1 | 2.2* | 7.9 | 3.6 | 4.3 | 0.0 | 100.0 | 448.7 |
| | SEM | 0.4 | 0.5 | 3.8 | 0.1 | 0.1 | 0.1 | 1.3 | 0.5 | 0.4 | 0.4 | 0.0 | 0.0 | 23.3 |
| | N | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| 10/0 | Mean | 9.6 | 8.4 | 13.6 | 0.2* | 2.5* | 2.7* | 26.2* | 5.5* | 2.8 | 2.9 | 0.2 | 96.0 | 415.2 |
| | SEM | 0.6 | 0.7 | 4.9 | 0.1 | 0.7 | 0.7 | 5.6 | 0.6 | 0.3 | 0.3 | 0.1 | 2.2 | 23.3 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| 3/1 | Mean | 8.9 | 8.0 | 9.9 | 0.2 | 0.1 | 0.1 | 3.5 | 7.7 | 3.5 | 4.2 | 0.0 | 100.0 | 450.5 |
| | SEM | 0.3 | 0.4 | 2.7 | 0.1 | 0.1 | 0.1 | 1.4 | 0.3 | 0.3 | 0.4 | 0.0 | 0.0 | 18.4 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| 10/3 | Mean | 9.7 | 9.1 | 6.8 | 0.2 | 2.5* | 2.6* | 26.0 | 6.3* | 2.8 | 3.7 | 0.2 | 97.2 | 481.9 |
| | SEM | 0.6 | 0.5 | 3.0 | 0.1 | 0.6 | 0.7 | 5.9 | 0.5 | 0.3 | 0.4 | 0.1 | 2.1 | 17.7 |
| | N | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

N = Number of litters at term
Total = Late Resorptions + Dead Fetuses
Percent Pre-Implant Loss = 100 x (corpora lutea - implants)/corpora lutea
Percent Implants Resorbed = 100 x (total resorptions / implants)
Live Birth Index (%) = 100 x (Total Live Fetuses) / (Total Live + Dead Fetuses).

*Fetal Examination and Fetal Malformations*

No treatment-related effect on fetal body weight was observed (Table 24).

Table 24. Fetal Weight

| Sex : Test Used : | | Female NAV- | Male NAV- |
|---|---|---|---|
| Dose (mg/kg/day) SCH-108566-J/HCTZ | | | |
| 0 Control | Mean | 41.62 | 41.91 |
| | SEM | 0.91 | 1.17 |
| | N | 23 | 23 |
| 0/3 | Mean | 41.04 | 40.98 |
| | SEM | 1.07 | 0.96 |
| | N | 24 | 22 |
| 10/0 | Mean | 41.11 | 40.50 |
| | SEM | 1.54 | 1.54 |
| | N | 22 | 20 |
| 3/1 | Mean | 39.72 | 42.52 |
| | SEM | 1.25 | 1.14 |
| | N | 22 | 22 |
| 10/3 | Mean | 41.22 | 41.34 |
| | SEM | 1.05 | 1.28 |
| | N | 19 | 20 |

N = Number of litters at term.

Examination of fetuses for external, visceral and skeletal malformations revealed no drug-related effects (Table 25). A higher than control mean incidence of fetuses with malformations in the 10 mg/kg eprosartan group was not considered drug-related. The reason for the high value was that one litter contained only one fetus and this fetus had fused sternebrae. This resulted in a litter with 100% malformations, greatly inflating the group mean. In addition, the group receiving the same dose of eprosartan in combination with HCTZ did not show a higher than control incidence of malformed fetuses.

Table 25. Total Incidence of Fetal Malformations.
(mean +/- SEM)
(n = number of litters)
(includes all live and dead fetuses at term)

| Dose (mg/kg/day) SCH-108566-J/HCTZ Test Used: | Mean % of fetuses per litter with malformations NF- | Malformed fetuses/ total fetuses NF | Litters with at least 1 malformed fetus/ total litters PE- |
|---|---|---|---|
| 0 Control | 1.1 +/- 0.77 (n = 23) | 2 /180 | 2 /23 |
| 0/3 | 2.1 +/- 1.48 (n = 24) | 4 /190 | 2 /24 |
| 10/0 | 10.2 +/- 5.22 (n = 22) | 7 /126 | 5 /22 |
| 3/1 | 1.7 +/- 0.94 (n = 22) | 3 /170 | 3 /22 |
| 10/3 | 1.9 +/- 1.34 (n = 21) | 3 /131 | 2 /21 |

(a) = Malformations are those fetal observations judged to potentially affect survival, growth, development, functional competence or external appearance

*Toxicokinetics*

The composite Cmax and AUC values obtained for eprosartan were similar with and without the coadministration of HCTZ (Table 26). The mean eprosartan AUC for animals given 10 mg/kg of eprosartan mesylate alone was very similar to that obtained for animals given 10/3 mg/kg of eprosartan/HCTZ. Similarly, the AUCs for HCTZ were similar for groups given 3 mg/kg HCTZ or 10/3 mg/kg of eprosartan mesylate/HCTZ. Systemic exposure (AUC) to eprosartan increased, on average, 2.9-fold for a 3.3-fold increase in dose between 3 and 10 mg/kg.

Table 26. Systemic Exposure to Eprosartan and HCTZ after Oral Administration of Eprosartan Mesylate, HCTZ or Eprosartan Mesylate/HCTZ to Pregnant Rabbits During Late Gestation.

| Dose (mg/kg/day) | Cmax (ng/mL) | | AUC(0-t) (ng.h/mL) | |
|---|---|---|---|---|
| [SKF-108566-J/HCTZ] | SKF-108566 | HCTZ | SKF-108566 | HCTZ |
| 0/3.0 | - | 357 | - | 1528 |
| 3.0/1.0 | 563 | 91 | 5968 | 361 |
| 10.0/0 | 1337 | - | 16197 | - |
| 10.0/3.0 | 1523 | 218 | 17251 | 1376 |

Dose of eprosartan mesylate (SKF-108566-J) expressed in terms of eprosartan (SKF-108566)

***Developmental Toxicity Study of Oral Eprosartan Mesylate/HCTZ Administered During Mid-Gestation in Rabbits (Vol.25, pg. 114)***

Study Facility: SmithKline Beecham Pharmaceuticals, King of Prussia, PA.
Study No: G98149
Study Dates: Initiation of dosing, 10/20/98; Necropsy, 11/11/98

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Presumed pregnant New Zealand White rabbits (6 months old; approx 3 kg) were housed individually and provided 125 gm/day of [redacted] Tap waster was available *ad libitum*.

Drug Administration: Eprosartan mesylate (Lot # BCT-L-09Cmic), hydrochlorothiazide (Lot # 7JS56042(1)#1) and a mixture of eprosartan mesylate/HCTZ were each suspended in aqueous 1% carboxymethylcellulose and administered orally by gavage on days 6 to 17 post coitus.

Dose Levels:

| Treatment | Dose (mg/kg/day)* | # of Mated Females |
|---|---|---|
| Vehicle | - | 24 |
| HCTZ | 10 | 24 |
| Eprosartan mesylate | 30 | 24 |
| Eprosartan mesylate/HCTZ | 10/3 | 24 |
| | 30/10 | 24 |

* Doses of eprosartan mesylate expressed as amount of eprosartan

*Dose selection was based on results of an oral dose range-finding study in pregnant rabbits (#D98060; study initiated 4/26/98) that revealed one drug-related maternal death and depressed body weight gain in does receiving 30/10 mg/kg/day of eprosartan /HCTZ. This dose was considered to be a maximum tolerated dose.*

Observations/Measurements: Animals were observed daily for clinical signs of toxicity and mortality. Body weight was measured daily on days 5 through 18 and then on days 24 and 29 pc. Food consumption was measured daily from day 5 through day 29 pc. On day 11 pc (day 6 of treatment) blood was obtained from an ear vein (at varying intervals up to 24 hours post dose; n=3/each interval) from rabbits of only drug-treated groups for measurement of eprosartan and or HCTZ levels. On day 29 pc, the mated females were killed with sodium pentobarbital IV and necropsied (mated females that died prior to scheduled sacrifice were subjected to necropsy). The

ovaries were removed and the corpora lutea counted. The uterus was weighed and examined for implantation sites, resorptions and live and dead fetuses. A gross examination of each placenta was performed. Live fetuses were weighed and examined externally. Fetuses were killed and the abdominal and thoracic viscera were examined. All fetuses were eviscerated and stained for skeletal examination.

Results

*Mortality, Clinical Signs and Mating*

One control doe aborted and died on day 26 pc; the cause of death was unknown. Deaths associated with drug treatment occurred in the eprosartan mesylate group on days 17 pc and 25 pc., the low dose combination group on days 21 pc and 29 pc and the high dose combination group on days 16 pc, 16 pc, 21 pc, 21 pc and 22 pc. The fates of control and treated females and their litters are summarized in Table 27.

Table 27. Number and Fate of Female Rabbits and Their Litters

| | Treatment Group (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| | Vehicle | HCTZ 10 | Eprosartan 30 | Epro/HCTZ (10/3) | Epro/HCTZ 30/10 |
| # Females Treated | 24 | 24 | 24 | 24 | 24 |
| # Females not pregnant | 4 | 2 | 1 | 2 | 1 |
| # Maternal unscheduled deaths | 1 | 0 | 2 | 2[a] | 5 |
| # Pregnant Survivors to term. | 19 | 21 | 21 | 20 | 17 |
| # Viable litters | 19 | 21 | 21 | 20 | 17 |
| # Aborted litters | 1 | 1 | 1[b] | 0 | 1 |

[a] Both does pregnant

[b] Doe died after litter aborted

*Body Weight and Food Consumption*

Mean body weights on individual days of gestation did not differ significantly among groups (Table 28). Lower than control body weight gains between days 12 and 15 pc were noted for does given 30 mg/kg/day of eprosartan or 30/10 mg/kg/day of eprosartan/HCTZ.

Table 28. Maternal Body Weights (kg)

| Study Days: | | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 24 | 29 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test Used: | | AV- | XN- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | AV- | XN- | AV- |
| Dose (mg/kg/day) | | SKF-108566-J/HCTZ | | | | | | | | | | | | | | |
| 0 Control | Mean | 3.44 | 3.43 | 3.44 | 3.46 | 3.46 | 3.48 | 3.50 | 3.56 | 3.60 | 3.63 | 3.64 | 3.65 | 3.63 | 3.75 | 3.86 |
| | SEM | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.06 | 0.06 | 0.06 | 0.06 | 0.05 | 0.06 | 0.06 |
| | N | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 19 |
| 0/10 | Mean | 3.49 | 3.45 | 3.44 | 3.46 | 3.48 | 3.47 | 3.49 | 3.53 | 3.55 | 3.57 | 3.57 | 3.57 | 3.57 | 3.67 | 3.78 |
| | SEM | 0.08 | 0.07 | 0.08 | 0.08 | 0.08 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.09 | 0.09 | 0.10 | 0.10 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| 30/0 | Mean | 3.46 | 3.42 | 3.42 | 3.42 | 3.42 | 3.45 | 3.47 | 3.51 | 3.52 | 3.52 | 3.54 | 3.57 | 3.59 | 3.70 | 3.85 |
| | SEM | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 | 0.09 | 0.07 |
| | N | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 22 | 22 | 22 | 21 |
| 10/3 | Mean | 3.44 | 3.41 | 3.41 | 3.43 | 3.43 | 3.44 | 3.47 | 3.51 | 3.52 | 3.54 | 3.56 | 3.57 | 3.58 | 3.71 | 3.83 |
| | SEM | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.07 | 0.07 | 0.06 | 0.06 |
| | N | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 21 | 20 |
| 30/10 | Mean | 3.46 | 3.42 | 3.42 | 3.41 | 3.41 | 3.41 | 3.44 | 3.46 | 3.46 | 3.45 | 3.53 | 3.51 | 3.52 | 3.69 | 3.78 |
| | SEM | 0.07 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.05 | 0.06 | 0.06 | 0.07 | 0.06 |
| | N | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 21 | 21 | 21 | 18 | 18 |

N=number of pregnant animals.

A decrease in food consumption was evident in all drug-treated groups, particularly between days 13 and 15 pc. Does treated with 30/10 mg/kg/day of eprosartan/HCTZ consumed 26% less food than controls during the treatment period.

*Cesarean Section Results*

There were no significant differences among groups in the numbers of corpora lutea, implantations, resorptions, and dead or live fetuses (Table 29).

Table 29. Cesarean Section Results

| Endpoints: | | No. of Corpora Lutea | No. of Implants | Percent Pre-Implant Loss | Early | Late | Total | Percent Implants Resorbed | No. of Live Fetuses | Total No. of Live Males | Total No. of Live Females | No. of Dead Fetuses | Live Birth Index % | Uterus Weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test Used: | | NAV- | NAV- | NAV+ | NAV- | NAV- | NAV- | NAV- | NAV- | NAV- | NAV- | MT | MT | NAV- |
| Dose (mg/kg/day) | | SKF-108566-J/HCTZ | | | | | | | | | | | | |
| 0 Cont-rol | Mean | 9.4 | 9.1 | 0.7 | 0.2 | 0.1 | 0.3 | 2.5 | 9.1 | 5.1 | 4.0 | 0.0 | 100.0 | 522.6 |
| | SEM | 0.3 | 0.3 | 0.7 | 0.1 | 0.1 | 0.1 | 1.3 | 0.3 | 0.5 | 0.4 | 0.0 | 0.0 | 18.9 |
| | N | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 |
| 0/10 | Mean | 9.1 | 8.0 | 13.1* | 0.4 | 0.1 | 0.5 | 5.2 | 7.6 | 4.5 | 3.1 | 0.0 | 100.0 | 455.8 |
| | SEM | 0.3 | 0.6 | 4.6 | 0.2 | 0.1 | 0.2 | 2.3 | 0.5 | 0.4 | 0.4 | 0.0 | 0.0 | 28.3 |
| | N | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| 30/0 | Mean | 8.6 | 8.3 | 4.0 | 0.1 | 0.2 | 0.3 | 4.0 | 8.0 | 4.0 | 4.0 | 0.0 | 100.0 | 484.5 |
| | SEM | 0.3 | 0.3 | 1.5 | 0.1 | 0.1 | 0.1 | 1.5 | 0.4 | 0.3 | 0.3 | 0.0 | 0.0 | 21.4 |
| | N | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| 10/3 | Mean | 9.1 | 8.8 | 3.5 | 0.4 | 0.1 | 0.5 | 5.1 | 8.3 | 4.1 | 4.2 | 0.0 | 100.0 | 479.3 |
| | SEM | 0.4 | 0.4 | 1.7 | 0.2 | 0.1 | 0.2 | 1.7 | 0.4 | 0.4 | 0.4 | 0.0 | 0.0 | 30.4 |
| | N | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| 30/10 | Mean | 8.9 | 8.5 | 5.1 | 0.2 | 0.0 | 0.2 | 3.0 | 8.3 | 4.2 | 4.1 | 0.0 | 100.0 | 503.9 |
| | SEM | 0.4 | 0.4 | 2.0 | 0.1 | 0.0 | 0.1 | 1.3 | 0.4 | 0.4 | 0.4 | 0.0 | 0.0 | 19.7 |
| | N | 16 | 17 | 16 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 |

N = Number of litters at term
Percent Pre-Implant Loss = 100 x (corpora lutea - implants)/corpora lutea
Percent Implants Resorbed = 100 x (total resorptions / implants)
Live Birth Index (%) = 100 x (Total Live Fetuses) / (Total Live + Dead Fetuses).

*Fetal Examinations and Fetal Malformations*

Fetal body weights among treated groups did not significantly differ from control (Table 30).

Table 30. Fetal Mean Body Weights (gm)

| Sex: | | Female | Male |
|---|---|---|---|
| Test Used: | | NAV- | NAV- |
| Dose (mg/kg/day) | SKF-108566-J/HCTZ | | |
| 0 Control | Mean | 40.78 | 41.34 |
| | SEM | 0.82 | 0.75 |
| | N | 19 | 19 |
| 0/10 | Mean | 41.75 | 43.15 |
| | SEM | 1.24 | 1.19 |
| | N | 20 | 21 |
| 30/0 | Mean | 42.40 | 42.83 |
| | SEM | 0.80 | 0.91 |
| | N | 21 | 21 |
| 10/3 | Mean | 40.76 | 41.27 |
| | SEM | 0.89 | 1.21 |
| | N | 20 | 20 |
| 30/10 | Mean | 43.00 | 44.15 |
| | SEM | 0.76 | 0.96 |
| | N | 17 | 17 |

N = Number of litters at term.

External, visceral and skeletal examination of fetuses showed no drug-related fetal malformations (Table 31).

Table 31. Total Incidence of Fetal Malformations

(Mean +/- SEM)
(n = number of litters)
(Includes all live and dead fetuses at term)

| Dose (mg/kg/day) SKF-108566-J/HCTZ Test Used: | Mean % of fetuses per litter with malformations NT | Malformed fetuses/ total fetuses NT | Litters with at least 1 malformed fetus/ total litters PR- |
|---|---|---|---|
| 0 Control | 5.9 +/- 2.74 (n = 19 ) | 11 /172 | 5 /19 |
| 0/10 | 1.5 +/- 1.10 (n = 21 ) | 2 /159 | 2 /21 |
| 30/0 | 2.3 +/- 1.11 (n = 21 ) | 4 /167 | 4 /21 |
| 10/3 | 2.2 +/- 1.05 (n = 20 ) | 4 /166 | 4 /20 |
| 30/10 | 2.1 +/- 1.14 (n = 17 ) | 3 /141 | 3 /17 |

(a) = Malformations are those fetal observations judged to potentially affect survival, growth, development, functional competence or external appearance

*Necropsy Findings*

No drug related effects were noted for does examined at scheduled sacrificed. Alterations in the stomach (red mucosa and firm pyloric sphincter) were detected in animals that had stopped eating and were found dead.

*Toxicokinetics*

Systemic exposure (AUC) to eprosartan and HCTZ increased with increasing doses of the eprosartan mesylate/HCTZ combination (Table 32). The Cmax and AUC values obtained for eprosartan after a 30 mg/kg dose were not much different when HCTZ was co-administered.

Table 32. Systemic Exposure to Eprosartan and HCTZ After Oral Administration of Eprosartan Mesylate, HCTZ or Eprosartan Mesylate/HCTZ to Pregnant Rabbits During Mid Gestation.

| Dose (mg/kg/day) | | $C_{max}$ (ng/mL) | | $AUC_{(0-t)}$ (ng•h/mL) | |
|---|---|---|---|---|---|
| Eprosartan | HCTZ | Eprosartan | HCTZ | Eprosartan | HCTZ |
| 0 | 10.0 | - | 1311 | - | 6100 |
| 10.0 | 3.0 | 1034 | 298 | 12682 | 1514 |
| 30.0 | 0 | 3842 | - | 48865 | - |
| 30.0 | 10.0 | 4773 | NC | 62932 | NC |
| NC = Not Calculated, because of high variation of data. | | | | | |

APPEARS THIS WAY
ON ORIGINAL

## GENOTOXICITY

***Bacterial Mutagen (Ames) Test (Vol. 26, pg. 199)***

Study Facility: [redacted]
Study No: G98572
Study Dates: 5/15/98 to 8/21/98

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Bacterial Strains: *Salmonella typhymurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strains WP2 pKM101 and WP2 uvrA.

Procedure: Eprosartan mesylate (Lot # 31-97UP) and hydrochlorothiazide (Lot # 7JS56042 (1)#1 and 7JS56042 (1)#2) were dissolved in DMSO at a ratio of 48:1 (anticipated ratio of clinical product) and added to plates containing the bacterial tester strains in the presence and absence of metabolic activation (liver S-9 fraction obtained from [redacted]-treated rats). In a preliminary assay, this formulation was evaluated for solubility, and for toxicity to all tester strains at doses ranging from 8 to 5000 ug of eprosartan mesylate per plate. Based on the results of the preliminary assay, the mixture was tested at doses of 312.5, 625, 1250, 2500 and 5000 ug eprosartan mesylate/plate in two mutation assays. DMSO was used as the solvent control. The positive controls consisted of acriflavine and 2-anthramine in the presence of S-9 fraction and 2-nitroflurene, sodium azide, 9-aminoacridine and 4-nitroquinolone-1-oxide in the absence of S-9 fraction. The mean number of revertant colonies was determined for each dose of the eprosartan mesylate/HCTZ combination (3 plates/conc.) and for negative and positive controls in the presence and absence of metabolic activation. The test agent was judged to be genotoxic if the number of revertant colonies on the treated plates was dose-dependent and, for a single concentration, ≥3 times the negative control value (responses >1.5 to <3 fold higher than the negative control were considered positive only if the increase was statistically significant and concentration-related).

Results:

In two assays, in the absence of S9, the mixture of eprosartan mesylate/HCTZ did not cause an increase in the number of revertant colonies of any of the bacterial tester strains. The number of revertant colonies elicited by eprosartan mesylate/HCTZ in the absence of S-9 were less than 1.5 times the vehicle control level and the results of the test were judged to be negative (Table 33).

In the presence of S-9 with *S. typhymurium* strain TA1537, eprosartan mesylate/HCTZ at a dose of 625 ug eprosartan mesylate/plate, was associated with a number of revertant colonies that was more than 1.5 times the vehicle control value. This increase was neither statistically significant nor reproducible in a second assay and the result was judged to be negative. All other results with eprosartan mesylate/HCTZ in the presence of S-9 were judged to be negative (Table 34).

The positive controls utilized for each bacterial strain in the presence and absence of metabolic activation showed the expected increases in the number of revertant colonies. Thus a mixture of eprosartan mesylate/HCTZ in a ratio of 48:1 showed no evidence of induction of mutation in four strains of *Salmonella typhimurium* and two strains of *Escherichia coli*, either in the presence or absence of metabolic activation.

Table 39. Bacterial Mutagen Assays (Without S-9).

| Treatment Assay #1 | ug/plate | Mean # Revertant Colonies/Plate | | | | | |
|---|---|---|---|---|---|---|---|
| | | TA98 | TA100 | TA1535 | TA1537 | WP2pKM101 | WP2 uvrA |
| Vehicle | | 30.5 | 117 | 18.8 | 9.0 | 23.3 | 125 |
| Eprosartan mesylate /HCTZ | 312.5 | 36.3 (1.2) | 116 (1.0) | 16.3 (0.9) | 9.3 (1.0) | 24.3 (1.0) | 128 (1.0) |
| | 625 | 45.7 (1.5) | 116 (1.0) | 20.0 (1.1) | 8.0 (0.9) | 21.3 (0.9) | 131 (1.1) |
| | 1250 | 33.7 (1.1) | 106 (0.9) | 16.7 (0.9) | 9.0 (1.0) | 23.0 (1.0) | 122 (1.0) |
| | 2500 | 27.0 (0.9) | 127 (1.1) | 19.7 (1.0) | 7.3 (0.8) | 21.0 (0.9) | 125 (1.0) |
| | 5000 | 21.3 (0.7) | 104 (0.9) | 16.0 (0.9) | 5.3 (0.6) | 16.0 (0.7) | 86 (0.7) |
| 2NF | 5 | 835.0 (27.4) | | | | | |
| NaN3 | 2 | | 685 (5.9) | 404 (21.5) | | | |
| AAC | 50 | | | | 298 (33.2) | | |
| NQO | 2 | | | | | 540 (23.1) | 1244 (10.0) |
| Assay #2 | | | | | | | |
| Vehicle | | 21.2 | 85.2 | 13.3 | 7.2 | 30.5 | 143 |
| Eprosartan mesylate /HCTZ | 312.5 | 19.7 (0.9) | 91.7 (1.1) | 14.0 (1.1) | 8.0 (1.1) | 32.3 (1.1) | 156 (1.1) |
| | 625 | 23.7 (1.1) | 84.7 (1.0) | 13.7 (1.0) | 6.3 (0.9) | 37.7 (1.2) | 158 (1.1) |
| | 1250 | 19.0 (0.9) | 83.7 (1.0) | 12.7 (1.0) | 5.7 (0.8) | 35.3 (1.2) | 159 (1.1) |
| | 2500 | 21.3 (1.0) | 77.7 (0.9) | 9.7 (0.7) | 7.7 (1.1) | 31.3 (1.0) | 146 (1.0) |
| | 5000 | 11.7 (0.6) | 87.3 (1.0) | 12.0 (0.9) | 7.7 (1.1) | 28.3 (0.9) | 107 (0.8) |
| 2NF | 5 | 1152 (54.4) | | | | | |
| NaN3 | 2 | | 645 (7.6) | 437 (32.8) | | | |
| AAC | 50 | | | | 368 (51.4) | | |
| NQO | 2 | | | | | 833 (27.3) | 2163 (15.2) |

Values in parentheses indicate the fold increase above solvent control value. 2NF=2 nitrofluorene, NaN3= sodium azide, AAC= 9-aminoacridine, NQO=4-nitoquinolone-1-oxide.

Table 40. Bacterial Mutagen Assays (With S-9).

| Treatment Assay #1 | ug/plate | Mean # Revertant Colonies/Plate | | | | | |
|---|---|---|---|---|---|---|---|
| | | TA98 | TA100 | TA1535 | TA1537 | WP2pKM101 | WP2 uvrA |
| Vehicle | | 32.8 | 129 | 20.8 | 5.0 | 35.2 | 162 |
| Eprosartan mesylate /HCTZ | 312.5 | 30.3 (0.9) | 140 (1.1) | 21.0 (1.0) | 6.0 (1.2) | 36.7 (1.0) | 167.3 (1.0) |
| | 625 | 32.3 (1.0) | 109 (0.8) | 16.7 (0.8) | 6.0 (1.2) | 44.7 (1.3) | 163 (1.0) |
| | 1250 | 35.7 (1.1) | 122 (0.9) | 18.3 (0.9) | 4.7 (0.9) | 44.3 (1.3) | 166 (1.0) |
| | 2500 | 28.7 (0.9) | 123 (1.0) | 19.0 (0.9) | 6.0 (1.2) | 40.7 (1.2) | 166 (1.0) |
| | 5000 | 23.3 (0.7) | 116 (0.9) | 16.3 (0.8) | 4.3 (0.9) | 37.3 (1.1) | 143 (0.9) |
| ACF | 20 | 244 (7.4) | 413 (3.2) | | 52.7 (10.5) | | |
| | 40 | | | | | 64.7 (1.8) | |
| AAN | 5 | | | 142 (6.8) | | | |
| | 10 | | | | | | 776 (4.8) |
| Assay #2 | | | | | | | |
| Vehicle | | 29.7 | 94 | 13.5 | 7.8 | 58.0 | 255 |
| Eprosartan mesylate /HCTZ | 312.5 | 29.0 (1.0) | 97.3 (1.0) | 12.3 (0.9) | 9.0 (1.2) | 65.3 (1.1) | 236 (0.9) |
| | 625 | 28.7 (1.0) | 93.7 (1.0) | 12.7 (0.9) | 12.3 (1.6) | 64.3 (1.1) | 218 (0.8) |
| | 1250 | 30.7 (1.0) | 95.3 (1.0) | 18.3 (1.4) | 6.3 (0.8) | 67.3 (1.2) | 235 (0.9) |
| | 2500 | 30.0 (1.0) | 94.3 (1.0) | 17.3 (1.3) | 6.7 (0.9) | 53.3 (0.9) | 222 (0.9) |
| | 5000 | 23.7 (0.8) | 24.3 (0.3) | 17.0 (1.3) | 8.7 (1.1) | 69.3 (1.2) | 220 (0.9) |
| ACF | 20 | 484 (16.3) | 361 (3.8) | | 66.3 (8.5) | | |
| | 40 | | | | | 93.3 (1.6) | |
| AAN | 5 | | | 183 (13.6) | | | |
| | 10 | | | | | | 974 (3.8) |

Values in parentheses indicate the fold increase above solvent control value. ACF=acriflavine, AAN=2-anthramine.

***Chromosome Aberration Assay in Vitro with Cultured Human Lymphocytes (Vol 26, pg 255)***

Study Facility: [redacted]
Study No.: G98573
Study Date: 5/21/98

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Test System: Human lymphocytes in culture.

Procedure: Eprosartan mesylate ( Lot # 31-97UP) and hydrochlorothiazide (Lot # 7J5S56042 (1) #1 and # 7J5S56042 (1) #2) were dissolved in DMSO (48:1 ratio) and added to the human lymphocyte cultures in experiment #1 at eprosartan mesylate/HCTZ concentrations of 86.1/1.8 to 3625/75.5 ug/ml in the presence and absence of metabolic activation (S9 fraction obtained from livers of [redacted]-treated rats). In a second assay, concentrations of 230.2/4.8 to 2240/46.7 ug/ml of eprosartan mesylate/HCTZ were tested in the absence of metabolic activation and concentrations of 844.7/17.6 to 3100/64.6 ug/ml of eprosartan mesylate/HCTZ were tested in the presence of metabolic activation. The cells were exposed to the drug combination for 20 hrs in the absence of metabolic activation and for 3 hours (followed by a 17 hr drug free recovery) in the presence of metabolic activation (experiments 1 &2). In addition, the cultured cells were exposed to a single concentration of 1375/28.64 ug/ml of eprosartan mesylate/HCTZ for 44 hours in the absence of metabolic activation and to a single concentration of 2635/54.90 ug/ml for 3 hours (with 41 hr recovery) in the presence of metabolic activation. After drug treatment and prior to cell harvest, colchicine was added to the culture medium to arrest dividing cells at metaphase. Lymphocytes were fixed onto slides and stained for microscopic examination. All tests included a negative control (DMSO) and a positive control (cyclophosphamide with S9 activation and 4-nitroquinolone without S9 activation). Slides from all concentration levels were examined to determine the mitotic index (percentage of cells in mitosis) and whether drug-induced mitotic inhibition had occurred. The top dose for chromosome analysis from the 3 + 17 hour (+S9) and 20 + 0 hr (-S9) treatments in both experiments was one that produced 50%-80% reduction in mitotic index. Slides from this dose and the next two lower doses were analyzed for chromosome aberrations. If a negative or equivocal result was obtained for the treatments analyzed, a single dose from the delayed harvest (3 + 41 hr, +S9 and 44 + 0 hr, -S9 in Experiment #2) was scored. The dose level used was that which corresponded to the top dose scored following the 3 + 17 hr or the 20 + 0 hr treatment, unless mitotic inhibition was too severe in which case the highest scorable dose was selected. Approximately 1000 metaphase cells were analyzed and categorized as cells with structural aberrations including gaps, cells with structural aberrations excluding gaps, hyperdiploid cells and polyploid and endoreduplicated cells. The critical endpoints for determining structural chromosomal damage were structural aberrations excluding gaps and hyperdiploidy. Gaps, polyploidy and endoreduplication were recorded as accessory data and not used as a measure of drug-induced chromosomal damage. The test was considered to be positive if the frequency of cells with chromosomal aberrations was ≥14 times the vehicle control or if the frequency of cells with chromosomal aberrations was ≥8% with a zero control value. The test was considered negative if the frequency of aberrations was ≤3 times the vehicle control [multiples based on laboratory historical control data (Table 41)]. Assays in which the frequency of chromosomal aberrations was between 3 and 14 times the vehicle control were considered positive only if they showed a significant concentration-related increase in chromosomal aberrations and if the maximum frequency was above the upper 99% confidence limit established from historical negative control data. To be considered genotoxic, the sponsor

indicates that a test agent must be judged positive in two valid tests; to be considered non-genotoxic, it must be negative in 2 valid tests.

Results

A number (14-17/experiment) of rising concentrations of the test agent (ranging from 86.1 to 3625 ug/ml of eprosartan mesylate in Exp #1 and from 230.2 to 3100 ug/ml of eprosartan mesylate in Exp #2) were tested and, from those eliciting inhibition of mitotic index (≤80%), the following concentrations were selected for analysis of chromosome aberrations.

| Experiment # | Drug Exposure + Drug-Free Incubation Period, (hr) | S9 | Eprosartan Mesylate Concentration (ug/ml) | % Mitotic Inhibition at Highest Drug Conc. |
|---|---|---|---|---|
| #1 | 3 + 17 | + | 1529, 2039, 2719 | 53 |
| | 20 + 0 | - | 860.2, 1147, 1529 | 77 |
| #2 | 3 + 17 | + | 1904, 2240, 2635 | 72 |
| | 20 + 0 | - | 1169, 1375, 1618 | 69 |
| | 3 + 41 | + | 2635 | 19 |
| | 44 + 0 | - | 1375 | 73 |
| | 3 + 17 | - | 1831 | 50 |

Drug concentrations are expressed as concentration of eprosartan mesylate for the eprosartan mesylate/HCTZ combination tested.

For the two assays conducted, the positive controls gave acceptable increases in structural aberrations and the vehicle control results were within the historical control ranges (Table 41) for that laboratory.

Table 41. Historical Control Data

Negative control data

| Sex and S9 treatment | Category | Total number of cells scored | Aberrant cells scored per 100 cells | |
|---|---|---|---|---|
| | | | Mean | Range (min/max)* |
| Male -S9 | Structural aberrations including gaps | 14800 | 2.28 | 0-6 |
| | Structural aberrations excluding gaps | 14800 | 1.16 | 0-4 |
| | Polyploid cells | 14911 | 0.45 | 0-3 |
| | Numerical aberrations | 14911 | 0.72 | 0-3 |

| Sex and S9 treatment | Category | Total number of cells scored | Aberrant cells scored per 100 cells | |
|---|---|---|---|---|
| | | | Mean | Range (min/max)* |
| Male +S9 | Structural aberrations including gaps | 13592 | 1.91 | 0-6 |
| | Structural aberrations excluding gaps | 13592 | 0.99 | 0-4 |
| | Polyploid cells | 13658 | 0.28 | 0-2 |
| | Numerical aberrations | 16658 | 0.48 | 0-2 |

Calculated in September 1997
* 99% confidence limits

In the presence of S9 with the 3 hr exposure (with 17-hr drug-free period), the highest incidence of structural aberrations in Exp # 1 (11.5% at 2719 ug/ml) was ≥8% with a zero control value; in Exp #2, the highest incidences of these aberrations (11% and 9.5% at 2240 and 2635 ug/ml, respectively) were ≥14 times the vehicle control value. Thus, in both experiments the test was clearly positive (Table 42). The results from the 3+41 hr drug treatment in Exp.2 were negative (i.e., ≤2% with a zero control value). The reason for scoring the 3+41 hour test is unclear since the concentration (2635 ug/ml) tested showed a positive response in the 3+17 hour experiment (the criteria indicate that the test is scored only if the previous results were negative or equivocal).

Table 42. Chromosome Aberration Assay (Exp. #1 & 2) With Metabolic Activation

Experiment 1; (3+17 hours); +S9

| Treatment (ug/mL) | Cells with aberrations excluding gaps (%) | Hyperdiploid cells (%) | Polyploid cells (%) | Mitotic inhibition (%) |
|---|---|---|---|---|
| Solvent | 0 | 0.5 | 0.25 | - |
| 1529 | 1.0 | 0.5 | 0.20 | 24 |
| 2039 | 0.5 | 0.5 | 0.30 | 33 |
| 2719 | 11.5 | 0.5 | 2.60 | 54 |
| CPA, 12.5 | 20.0 | 2.5 | 0.05 | - |

Experiment 2; (3+17 hours); +S9

| Treatment (ug/mL) | Cells with aberrations excluding gaps (%) | Hyperdiploid cells (%) | Polyploid cells (%) | Mitotic inhibition (%) |
|---|---|---|---|---|
| Solvent | 0.5 | 0 | 0.40 | - |
| 1904 | 0 | 0 | 0.75 | 15 |
| 2240 | 11.0 | 0 | 3.05 | 53 |
| 2635 | 9.5 | 0 | 2.21 | 72 |
| CPA, 12.5 | 21.5 | 0 | 0 | - |

Experiment 2; (3+41 hours); +S9

| Treatment (ug/mL) | Cells with aberrations excluding gaps (%) | Hyperdiploid cells (%) | Polyploid cells (%) | Mitotic inhibition (%) |
|---|---|---|---|---|
| Solvent | 0 | 0 | 0.15 | - |
| 2635 | 2.0 | 0.5 | 1.05 | 19 |

In the absence of S9, small increases above vehicle control (>3-fold but <14-fold) in mean chromosome aberration frequencies were observed in Exp. #1 and #2 (20 + 0 hrs). Also, chromosome aberration frequencies >2% and < 8%, with a zero control value, were seen in Exp #2 (44 +0 hr and 3 + 17 hr). The increase in chromosome aberration frequency above vehicle control value in the 44 + 0 hr treatment did not achieve statistical significance. However, in the 3 + 17 hour treatment (with 1831 ug/ml) the mean chromosome aberration frequency was 5% (i.e., 10-fold above control value) and was statistically significant (in excess of upper 99% confidence limit of historical controls); therefore, the test agent was judged to be positive in this assay.

Table 43. Chromosome Aberration Assay (Exp. #1 & 2) Without Metabolic Activation

Experiment 1; (20+0 hours); -S9

| Treatment (ug/mL) | Cells with aberrations excluding gaps (%) | Hyperdiploid cells (%) | Polyploid cells (%) | Mitotic inhibition (%) |
|---|---|---|---|---|
| Solvent | 0.50 | 0 | 0 | - |
| 860.2 | 2.00 | 0.5 | 0 | 25 |
| 1147 | 4.00 | 0 | 0.10 | 42 |
| 1529 | 3.00 | 0.5 | 0.11 | 77 |
| NQO. 2.5 | 15.00 | 0.5 | 0.05 | - |

Experiment 2; (20+0 hours); -S9

| Treatment (ug/mL) | Cells with aberrations excluding gaps (%) | Hyperdiploid cells (%) | Polyploid cells (%) | Mitotic inhibition (%) |
|---|---|---|---|---|
| Solvent | 0.5 | 0 | 0.10 | - |
| 1169 | 1.0 | 0 | 0.14 | 53 |
| 1375 | 2.0 | 0.5 | 0.23 | 40 |
| 1618 | 5.45 | 0 | 0.23 | 69 |
| NQO. 2.5 | 12.0 | 0 | 0.05 | - |

Experiment 2; (44+0 hours); -S9

| Treatment (ug/mL) | Cells with aberrations excluding gaps (%) | Hyperdiploid cells (%) | Polyploid cells (%) | Mitotic inhibition (%) |
|---|---|---|---|---|
| Solvent | 0 | 0 | 0.10 | - |
| 1375 | 3.0 | 0.5 | 0.20 | 73 |

Experiment 2; (3+17 hours); -S9

| Treatment (ug/mL) | Cells with aberrations excluding gaps (%) | Hyperdiploid cells (%) | Polyploid cells (%) | Mitotic inhibition (%) |
|---|---|---|---|---|
| Solvent | 0.5 | 0 | 0.35 | - |
| 1831 | 5.0 | 0.5 | 2.25 | 50 |
| NQO. 5 | 19.5 | 0.5 | 0.36 | - |

Although the sponsor states that the positive responses for chromosome aberrations only occurred at high and extremely cytotoxic concentrations of the drug combination and have no biological importance, the ICH Guideline on Genotoxicity Tests for Pharmaceuticals (April, 1996) indicates that the desired level of inhibition of mitotic index should be >50%. It should be noted that the positive responses seen with the eprosartan mesylate/HCTZ combination in the presence and absence of metabolic activation (Exp. # 1 & 2) occurred at 50%-54% mitotic inhibition. Thus, the eprosartan mesylate/HCTZ combination is considered to be positive (both with and without metabolic activation) in this assay.

***Micronucleus Assay in Mice (Vol. 26, pg. 167)***

Study Facility:
Study No.: G98574
Study Dates: 5/19/98 -5/27/98

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female CD-1 mice (5-6 weeks old) were housed 3/cage and fed *ad libitum.* Tap water was available *ad libitum.*

Procedure: Eprosartan mesylate (Lot # 31-97UP) and hydrochlorothiazide (Lot # 7J5S56042(1) #1 and 7J5S56042(1) #2) were mixed in a ratio of 48:1, suspended in 1% carboxymethylcellulose aqueous solution and administered to mice orally by gavage for 2 consecutive days. Negative control animals received 1% carboxymethylcellulose aqueous solution (2 days) and positive control animals were given 60 mg cyclophosphamide/kg (dosed 1 day only). In the range finding test, doses (sponsor expresses doses in terms of amount of eprosartan) from 125 to 2000 mg eprosartan/kg/day were administered to mice (3/sex/dose group). Based on results of the range-finding test, a dose of 2000 mg eprosartan/kg/day (the high-dose limit described in the literature for this test) was administered to a group of 7 male mice for the definitive assay, which also included vehicle or positive control. Mice were killed 24 hours after the last dose of test article or negative or positive control. Both femurs from each animal were exposed and bone marrow was removed using a syringe and needle. The bone marrow was centrifuged, cells removed, resuspended and smeared onto slides and stained for microscopic examination. In the dose-rangefinding study, 500 polychromatic erythrocytes (PCE) were analyzed for the presence of micronuclei. In the definitive assay, at least 2000 PCE per animal were examined for the presence of micronuclei. The number of micronucleated PCE was expressed as a percentage of the total PCE analyzed per animal. The criteria established for a negative or positive response were as follows: 1) If the mean frequency of micronucleated PCE is $\leq$ 2-fold that of the concurrent vehicle control value, the test agent is considered negative. 2) If the mean frequency of micronucleated PCE is $\geq$ 4-fold that of the concurrent vehicle control value, the response is considered positive. 3) If the treatment yields a mean micronucleated PCE frequency >2-fold but < 4-fold the concurrent vehicle control value, the results are subjected to further analysis (assessment using historical control data and statistics).

Results

*Range Finding Test*

The frequencies of micronucleated PCE in mice treated with eprosartan mesylate/HCTZ at all doses were comparable to frequencies (sex combined) observed for concurrent vehicle control (Table 43). No significant differences between males and females for any treatment were observed. A greater than 4-fold increase above negative control frequency of micronucleated PCE was observed for cyclophosphamide.

Table 43. Dose-Range Finding Test Results

| Treatment group (mg/kg/day) | Sex | Group mean %PCE | Group mean frequency %MNPCE (SD) | | Group mean frequency %MNNCE (SD) | |
|---|---|---|---|---|---|---|
| | | | per sex | per group | per sex | per group |
| Positive control CPA (60) | M | 38±4.7 | 1.93±0.42 | 2.43±0.69 | 0.04±0.08 | 0.06±0.07 |
| | F | 33±5.6 | 2.93±0.50 | | 0.08±0.08 | |
| Negative control (0) | M | 44±9.4 | 0.07±0.12 | 0.10±0.17 | 0.17±0.21 | 0.08±0.16 |
| | F | 48±3.5 | 0.13±0.23 | | 0±0 | |
| SKF-108566-J/HCTZ (125) | M | 49±13.4 | 0.13±0.23 | 0.17±0.20 | 0.17±0.18 | 0.17±0.19 |
| | F | 48±22.6 | 0.20±0.20 | | 0.17±0.24 | |
| SKF-108566-J/HCTZ (250) | M | 49±12 | 0.20±0.20 | 0.13±0.16 | 0±0 | 0±0 |
| | F | 42±11.4 | 0.07±0.12 | | 0±0 | |
| SKF-108566-J/HCTZ (500) | M | 54±15 | 0.13±0.12 | 0.13±0.10 | 0.16±0.18 | 0.11±0.14 |
| | F | 46±12.8 | 0.13±0.12 | | 0.06±0.11 | |
| SKF-108566-J/HCTZ (1000) | M | 47±9.9 | 0.20±0.20 | 0.20±0.20 | 0±0 | 0.03±0.07 |
| | F | 55±17 | 0.20±0.28 | | 0.07±0.10 | |
| SKF-108566-J/HCTZ (1581) | M | 42±6.7 | 0.13±0.23 | 0.13±0.16 | 0.07±0.12 | 0.04±0.09 |
| | F | 51±13.8 | 0.13±0.12 | | 0±0 | |
| SKF-108566-J/HCTZ (2000) | M | 41±5.5 | 0.07±0.12 | 0.17±0.20 | 0.09±0.08 | 0.05±0.07 |
| | F | 52±9 | 0.27±0.23 | | 0±0 | |

*Main Study*

The mean frequency of micronucleated PCE for mice treated with the high dose combination of eprosartan mesylate/HCTZ was essentially identical to the negative control frequency (Table 44). The positive control, cyclophosphamide, elicited a substantial increase (>36-fold) above vehicle control frequency of micronucleated PCE. Thus, the eprosartan mesylate/HCTZ combination was judged to have tested negative in this genotoxicity assay. The exposure of bone marrow to eprosartan and/or HCTZ following administration of the high dose mixture in this study was not determined. Also, evidence of systemic toxicity or effect on the proportion of polychromatic erythrocytes relative to total erythrocytes with this (limit) dose of the combination was not detected. These is, however, indirect evidence that systemic exposure to eprosartan in excess of the human exposure was achieved in this mouse study. The same combination of doses of eprosartan mesylate and HCTZ administered to mice in a 3-month oral toxicity study (employing

the same mode of administration) yielded total eprosartan exposures (AUCs) of 3990 and 8370 ng.hr/ml in male and female mice, respectively, after a single dose. When adjusted for protein binding, the free (unbound) eprosartan exposure is about 5X and 11X (male and female mice, respectively) that achieved in humans given the maximum recommended dose of eprosartan mesylate/HCTZ (Table 45).

Table 44. Mouse Micronucleus Assay Results

| Treatment group (mg/kg/day) | Sex | Group mean %PCE | Group mean frequency %MNPCE (SD) | Group mean frequency %MNNCE (SD) |
|---|---|---|---|---|
| Positive control CPA (60) | M | 50±9.6 | 1.45±0.34 | 0.39±0.49 |
| Negative control (0) | M | 52±7 | 0.04±0.02 | 0.35±0.45 |
| SKF-108566-J/HCTZ (2000) | M | 50±9.4 | 0.04±0.03 | 0.33±0.41 |

Table 45. AUC in Mice vs Humans at the Maximum Recommended Dose.

| Species | Eprosartan/ HCTZ Dose | Sex | $AUC_{0-24}$, ng.hr/ml | | Multiple of Human Value |
|---|---|---|---|---|---|
| | | | Total | Free (Unbound) | |
| Human | 600/12.5 mg/day | Male (Normal subjects) | 9582[a] | 153[c] | N/A |
| Mouse | 2000/62.5 mg/kg/day | Male | 3990[b] | 806[d] | 5.3X |
| | | Female | 8370[b] | 1690[d] | 11.0X |

[a] AUC after single dose to male subjects; no gender pharmacokinetic differences detected for eprosartan (see NDA# 20,738).
[b] AUC value after single dose.
[c] Human protein binding of eprosartan (98.4% bound, 1.6% free).
[d] Mouse protein binding of eprosartan (79.8% bound, 20.2% free).

## SUMMARY AND EVALUATION

Eprosartan mesylate is an orally active angiotensin II receptor antagonist. The antihypertensive properties of eprosartan mesylate in experimental animal models of hypertension have been adequately demonstrated and have been previously described in our review of the TEVETEN™ NDA (Review and Evaluation of Pharmacology and Toxicology Data, NDA# 20,738; review dated July 29,1997). Hydrochlorothiazide (HCTZ) is presumed to exert its antihypertensive effects through its diuretic/natriuretic actions and consequent reduction in extracellular volume. A combination of eprosartan mesylate and HCTZ was effective in lowering the elevated blood pressure of spontaneously hypertensive rats whereas eprosartan mesylate and HCTZ alone (same doses) were without significant antihypertensive activity.

In an acute toxicity study of the effects of the eprosartan mesylate/HCTZ combination in dogs, the administration a single oral dose of 1000/0.3, 1000/1.0 or 1000/2.0 mg/kg of eprosartan /HCTZ caused no mortality. Emesis and mucoid feces were seen at each dose level of the drug combination.

Repeated dose toxicity studies (up to 3 months duration) with the eprosartan mesylate/HCTZ combination were conducted in mice and dogs. Toxicity was assessed in mice treated for 3

months with daily oral eprosartan/HCTZ doses of 300/9.375 and 2000/62.5 mg/kg (32:1 ratio). No adverse effects on survival, body weight or food consumption were seen at these dose levels. Slightly lower (~6%) than control erythrocyte parameters were noted at study termination for females receiving 2000 mg eprosartan/kg/day alone or in combination with 62.5 mg HCTZ/kg/day. This effect is common to agents that inhibit renin-angiotensin activity in the kidney which, in turn, leads to reduction of erythropoetin secretion. This effect is limited in degree and, based on previous studies with eprosartan mesylate, is reversible. Other adverse findings with the combination (higher than control serum urea, JG hyperplasia and renal tubular regeneration) also reflect drug-induced effects of angiotensin II receptor antagonism on the kidney, the primary organ of toxicity. The highest no-observed-adverse-effect level for eprosartan mesylate/HCTZ in mice in the 3-month study was 300 mg eprosartan/9.375 mg HCTZ/kg/day. In a companion toxicokinetic study in mice, systemic exposures (AUCs) to eprosartan were similar after 28 days of dosing with eprosartan mesylate/HCTZ or eprosartan mesylate alone (doses of 2000 mg eprosartan, 62.5 mg HCTZ/kg/day). Likewise, AUC values for HCTZ were similar following administration of eprosartan mesylate/HCTZ or HCTZ alone (same dose as above). Although the co-administration of eprosartan mesylate and HCTZ did not appear to influence the exposure to eprosartan or HCTZ, the administration of the mixture resulted in a higher incidence of renal histopathology (tubular regeneration in females and JG cell hyperplasia in males and females) than observed with either eprosartan mesylate or HCTZ given alone. The renal tubular lesions seen with eprosartan mesylate/HCTZ were also associated with higher than control levels of serum urea. Serum urea levels were slightly higher than control with HCTZ alone but were comparable to control after administration of eprosartan mesylate alone.

Toxicity was assessed in dogs treated with eprosartan mesylate and HCTZ for 1 month at daily oral eprosartan/HCTZ doses of 100/3.125 and 1000/31.25 mg/kg (32:1 ratio) and for 3 months at daily oral eprosartan/HCTZ doses of 1000/0.3 and 1000/1.0 mg/kg. Two of eight dogs (1M, 1F) treated with 1000/31.5 mg/kg/day were sacrificed in a moribund state prior to the end of the 1-month study, whereas, no drug-related deaths (excluding 2 gavage errors) or moribund sacrifices occurred in the 3-month study. Clinical signs of toxicity (emesis, loss of appetite and body weight loss) and serum chemistry findings (higher than control serum urea, ALT and ALP) were reported in dogs sacrificed in a moribund state. In addition to the clinical signs and serum chemistry findings, renal tubular degeneration was seen microscopically in these two dogs; such findings are associated with renal failure. Lower than control erythrocyte parameters (RBC counts, Hb and Hct) were seen mainly in females treated with eprosartan mesylate/HCTZ in the 1- and 3-month studies. The predominant treatment-related histopathology among surviving animals in the 1-month study consisted of higher than control incidences of renal tubular regeneration and GI ulceration. No drug-related histopathology was detected in the 3-month study, which employed much lower doses of HCTZ. AUCs for eprosartan and HCTZ were both increased after one month of dosing with eprosartan mesylate/HCTZ compared to the AUCs obtained with eprosartan mesylate or HCTZ given alone (doses of 1000 mg eprosartan, 31.25 mg HCTZ/kg/day). The renal toxicity observed with this dose level of the combination, and not with the same doses of eprosartan mesylate and HCTZ administered alone, is likely a reflection of the increased exposure to eprosartan. The highest no-observed adverse effect dose observed in the 1-month study was 100/3.125 mg/kg/day.

Two developmental toxicity studies were conducted in rabbits. In one study, the mated rabbits were treated on days 6 through day 17 of gestation (mid-gestation) with eprosartan mesylate (30 mg eprosartan/kg/day), HCTZ (10 mg/kg/day) or with a combination of the two drugs (eprosartan/HCTZ doses of 10/3 or 30/10 mg/kg/day). Maternal toxicity (mortality, lower than control body weight and food consumption) was seen with eprosartan mesylate whether administered alone or co-administered with HCTZ (both low and high dose combinations).

However, developmental toxicity was not observed in any of the drug-treated groups. When mated females were treated on day 18 through day 28 of gestation (late gestation) with eprosartan mesylate (10 mg eprosartan/kg/day), HCTZ (3 mg/kg/day) or a combination of the two drugs (eprosartan/HCTZ doses of 1/0.3, 3/1, or 10/3 mg/kg/day), no maternal or developmental toxicity was seen with HCTZ alone or with the lower dosage combinations (1/0.3 or 3/1 mg/kg/day); an increased incidence of late resorptions (indicative of embryotoxicity) was seen with the high dose of eprosartan mesylate (10 mg eprosartan/kg/day) whether administered alone or in combination with HCTZ. . No developmental toxicity studies were conducted in other species with the eprosartan mesylate/HCTZ combination. A comparison of the systemic exposure in rabbits with exposure in humans is summarized below (Table 46).

Table 46. Comparative Systemic Exposure to Eprosartan Following Oral Administration of Eprosartan Mesylate Alone or in Combination with HCTZ *

| | RABBIT<br>Eprosartan mesylate or Eprosartan mesylate/HCTZ<br>3/1 mg/kg/day | HUMAN<br>Eprosartan mesylate/HCTZ<br>600/12.5 mg/day |
|---|---|---|
| Eprosartan $AUC_{0-24}$ (ng.hr/ml) | 5968[a]<br>7090[b] | 9582 |
| Plasma Protein Binding [c] | 97.2% | 98.4% |
| Free (unbound) Eprosartan $AUC_{0-24}$ (ng.hr/ml) | 167[a]<br>198[b] | 153 |
| Animal/Human Exposure Multiple[d] | 1.1[a]<br>1.3[b] | |

*Mean values obtained from pregnant rabbits given eprosartan mesylate alone (3 mg/kg/day for 14 days), pregnant rabbits given eprosartan mesylate/HCTZ (3/1 mg eprosartan/HCTZ/kg/day for 6 days) and human male volunteers given a single dose of 600/12.5 mg eprosartan mesylate/HCTZ. (Note: Pharmacokinetics are similar for human males and females; see NDA # 20,738).

[a] Value from developmental toxicity study No.G98141 (3/1 mg/kg/day of eprosartan mesylate/HCTZ for 6 days).

[b] Value from developmental toxicity study No.G93063 (3 mg/kg/day of eprosartan mesylate for 14 days).

[c] Derived from in vitro experiments (see review of NDA # 20,738).

[d] Exposure multiple calculation based on amount of free eprosartan.

Three tests were conducted with eprosartan mesylate/HCTZ to evaluate the genotoxic potential of this drug combination (Table 46). A positive response was detected with eprosartan mesylate/HCTZ in the *in vitro* human lymphocyte chromosome aberration assay in the presence and absence of metabolic activation. No evidence of genotoxic potential was detected in the Ames bacterial mutagen test. The eprosartan mesylate/HCTZ combination tested negative in the *in vivo* mouse micronucleus test and although the presence of eprosartan and HCTZ in the bone marrow was not determined, this dose of the combination administered to mice in the 3-mo. repeat dose toxicity study yielded systemic exposures to eprosartan 5 and 12 times (males and females, respectively) that achieved in humans at the maximum recommended daily dose of the combination. *[As noted in the ICH Guidance for Genotoxicity Tests for Pharmaceuticals, April 1996, "The bone marrow is a well-perfused tissue and it can be deduced that drug-related materials in blood or plasma will be similar to those observed in bone marrow. Although drug levels are not always the same, there is sufficient correlation for measurements in blood or plasma to be adequate for validating bone marrow exposure"].*

## LABELING

Those statements in the proposed labeling that refer to non-clinical studies with eprosartan mesylate alone and that are contained in the approved labeling for TEVETEN are considered acceptable. Also, those statements in the proposed labeling that refer to non-clinical studies with hydrochlorothiazide and are identical to statements in the class labeling for this agent are considered acceptable. Statements in the sponsor's proposed labeling for the eprosartan mesylate/hydrochlorothiazide combination that are based on studies covered by this review should be modified as indicated below.

Under WARNINGS, Fetal/Neonatal Morbidity and Mortality, the sponsor provides a description of the results of embryofetal developmental toxicity studies. The sponsor's proposed text reads as follows:

The above paragraph provides no information on results from developmental toxicity studies conducted with eprosartan in rats. The following proposed revision of this section of the labeling corrects for this omission and also provides for an overall presentation consistent with that followed in the approved labeling for TEVETEN.

Eprosartan mesylate, alone or in combination with hydrochlorothiazide, has been shown to produce maternal and fetal toxicities (maternal and fetal mortality, low maternal body weight and food consumption, resorptions, abortions and litter loss) in pregnant rabbits given oral doses as low as 10 mg eprosartan and 3 mg hydrochlorothiazide/kg/day. No maternal or fetal adverse effects were observed in rabbits at 3 mg eprosartan/kg/day alone or in combination with 1 mg/kg/day of hydrochlorothiazide; this oral dose yielded a systemic exposure (AUC) to unbound eprosartan approximately equal to the human systemic exposure achieved with the dose of eprosartan mesylate contained in the maximum recommended human dose of TEVETEN [redacted] 600 mg eprosartan/day). No adverse effects on *in utero* or postnatal development and maturation of offspring were observed when eprosartan mesylate was administered to pregnant rats at oral doses up to 1000 mg eprosartan/kg/day (the 1000 mg eprosartan/kg/day dose in non-pregnant rats yielded systemic exposure to unbound eprosartan approximately 0.8 times the exposure achieved in humans given 600 mg/day).

Thiazides cross the placental barrier and appear in cord blood. There is a risk of fetal or neonatal jaundice, thrombocytopenia, and possibly other adverse reactions that have occurred in adults.

Under PRECAUTIONS, *Carcinogenesis, Mutagenesis, Impairment of Fertility,* the sponsor's proposed text includes the following:

In the above description of carcinogenesis, mutagenesis and fertility studies, the sponsor makes no mention of studies that were or were not conducted with the eprosartan mesylate/hydrochlorothiazide combination. In addition to inclusion of statements pertaining to the drug combination, the reviewer also recommends that the statements be rearranged to provide for reporting of findings by toxicity category rather than by drug. The paragraphs describing the carcinogenesis, mutagenesis and fertility studies should be revised to read as follows (note the absence of subheadings):

No carcinogenicity studies have been conducted with eprosartan mesylate in combination with hydrochlorothiazide. Eprosartan mesylate was not carcinogenic in dietary restricted rats or *ad libitum* fed mice dosed at 600 mg and 2000 mg eprosartan/kg/day, respectively, for up to 2 years. In male and female rats, the systemic exposure (AUC) to unbound eprosartan at the dose evaluated was only approximately 25% of the exposure achieved in humans given TEVETEN [ ]. In mice, the systemic exposure (AUC) to unbound eprosartan was approximately 35 times the exposure achieved in humans given TEVETEN [ ]. Two-year feeding studies in mice and rats conducted under the auspices of the National Toxicology Program (NTP) uncovered no evidence of a carcinogenic potential of hydrochlorothiazide in female mice (at doses of up to approximately 600 mg/kg/day) or in male and female rats (at doses of up to approximately 100 mg/kg/day). The NTP, however, found equivocal evidence for hepatocarcinogenicity in male mice.

Eprosartan mesylate was not mutagenic *in vitro* in mammalian cells (mouse lymphoma assay). Eprosartan mesylate alone or in combination with hydrochlorothiazide was not mutagenic *in vitro* in bacteria (Ames test) and did not cause structural chromosomal damage *in vivo* (mouse micronucleus assay). In human peripheral lymphocytes *in vitro*, eprosartan mesylate in combination with hydrochlorothiazide was positive for clastogenicity with and without metabolic activation. In the same assay, eprosartan mesylate alone was associated with polyploidy but there was only equivocal evidence of structural chromosomal damage. Hydrochlorothiazide was not genotoxic *in vitro* in the Ames test and in the Chinese Hamster Ovary (CHO) test for chromosomal aberrations, or *in vivo* in assays using mouse

germinal cell chromosomes, Chinese hamster bone marrow chromosomes, and the *Drosophila* sex-linked recessive lethal trait gene. Positive test results were obtained in the *in vitro* CHO Sister Chromatid Exchange (clastogenicity) and Mouse Lymphoma Cell (mutagenicity) assays and in the *Aspergillus nidulans* non-disjunction assay.

No fertility studies have been conducted with eprosartan mesylate in combination with hydrochlorothiazide. Eprosartan mesylate had no adverse effects on the reproductive performance of male or female rats at oral doses up to 1000 mg eprosartan/kg/day. Hydrochlorothiazide had no adverse effects on the fertility of mice and rats of either sex in studies wherein these species were exposed, via their diet, to doses of up to 100 and 4 mg/kg/day, respectively, prior to conception and throughout gestation.

Under OVERDOSAGE, Hydrochlorothiazide, the first sentence which describes animal toxicity data, should be moved to the end of the paragraph. This will make it clearer that the rest of the paragraph deals with humans. The revised text should read as follows:

The most common signs and symptoms observed are those caused by electrolyte depletion (hypokalemia, hypochloremia and hyponatremia) and dehydration resulting from excessive diuresis. If digitalis has also been administered, hypokalemia may accentuate cardiac arrhythmias. The degree to which hydrochlorothiazide is removed by hemodialysis has not been established. The oral $LD_{50}$ of hydrochlorothiazide is greater than 10 g/kg in both mice and rats.

## RECOMMENDATION

From a preclinical safety perspective, this new drug application is approvable with the above-recommended changes in labeling.

/S/ 4/19/01

Anthony G. Proakis, Ph.D.
Pharmacologist

NDA # 21,268
HFD-110
HFD-110/Project Mgr.
HFD-110/AProakis
HFD-110/CResnick 4/23/01
HFD-345/EButler
Accepted by _____ on _____

**Appendix A. Histopathology Inventory for NDA # 21,268**

| Study No. | G96129 | G69130 | G98015 | | | |
|---|---|---|---|---|---|---|
| Species | Mouse | Dog | Dog | | | |
| Adrenals | X | X | X | | | |
| Aorta | X | X | X | | | |
| Axillary lymph node | | | | | | |
| Brain | X | X | X | | | |
| Cecum | X | | | | | |
| Cervix | X | | | | | |
| Colon | X | X | X | | | |
| Duodenum | X | X | X | | | |
| Epididymis | X | X | X | | | |
| Esophagus | X | X | X | | | |
| Eye | X | X | X | | | |
| Fallopian Tubes | | | | | | |
| Gall Bladder | X | X | X | | | |
| Gross Lesions | | | | | | |
| Harderian Gland | X | | | | | |
| Head | | | | | | |
| Heart | X | X | X | | | |
| Ileum | X | X | X | | | |
| Injection Site | | | | | | |
| Jejunum | X | X | X | | | |
| Kidneys | X | X | X | | | |
| Lachrymal Gland | X | | | | | |
| Larynx | X | | | | | |
| Liver | X | X | X | | | |
| L nodes, cervical | | | | | | |
| L nodes, mandibular | X | | | | | |
| L nodes, mediastinal | X | | | | | |
| Lungs | X | X | X | | | |
| Mandibular Gland | | | | | | |
| Mammary Gland | X | X | X | | | |
| Nasal Turbinates | X | | | | | |
| Optic Nerves | X | | | | | |
| Ovaries | X | X | X | | | |
| Pancreas | X | X | X | | | |
| Parotid gland | | | | | | |
| Parathyroid | X | X | X | | | |
| Pituitary Gland | X | X | X | | | |
| Prostate | X | X | X | | | |
| Rectum | X | | | | | |
| Salivary Gland | X | X | X | | | |
| Sciatic Nerve | | X | X | | | |
| Seminal Vesicles | X | | | | | |
| Skeletal Muscle | | X | X | | | |
| Skin | X | X | X | | | |
| Spinal Cord | X | X | X | | | |
| Spleen | X | X | X | | | |
| Sternum with bone marrow | X | | | | | |
| Stomach | X | X | X | | | |
| Testes | X | X | X | | | |
| Thymus | X | X | X | | | |
| Thyroid | X | X | X | | | |
| Tongue | X | | | | | |
| Tonsil | | | | | | |
| Trachea | X | X | X | | | |
| Urinary Bladder | X | X | X | | | |
| Uterus | X | X | X | | | |
| Vagina | X | X | X | | | |
| Zymbal Gland | | | | | | |