Fertility Study in Female Rats (Segment I/B)

Study Facility: SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Study No.: G92108(Study Rpt. # TP-1010/SKF-108566/1)
Study Dates: Initiation of dosing: 9/14/92; Last day of necropsy: 5/15/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Sprague-Dawley rats (10 weeks of age; 200-300 gm)

Drug Administration: Eprosartan mesylate (Lot# BCT-L-03P) was suspended in 1 % aqueous carboxymethylcellulose and administered to female rats orally by gavage daily for 14 days prior to mating, during mating, gestation and lactation through postpartum day 21. Male rats were not dosed.

Dose Levels: 0 (vehicle), 30, 100 and 1000 mg eprosartan/kg/day (40F/dose group) and 0 (vehicle), 0.3, 3.0 and 300 mg eprosartan/kg/day (40F/dose group). The study was conducted in two separate series of doses. Doses of 0, 30, 100 and 1000 mg eprosartan/kg were used in the first series and ventricular septal defects were observed in all eprosartan-treated groups with no dose-response. A second series was conducted with doses of 0, 0.3, 3 and 300 mg eprosartan/kg to further evaluate this observation.

*Note: High dose based on findings from a 7-day dose-rangefinding toxicity study in male and non-pregnant female rats which showed that the 2000 mg eprosartan/kg dose yielded only a small increase in systemic exposure (AUC) from that seen with 1000 mg eprosartan/kg suggesting a saturation of drug absorption (see note on page 42 of this review).*

Observations/Measurements: After 14 days of treatment, female rats were cohabitated (1:1) with untreated males for up to 7 days and checked daily for the presence of sperm in a vaginal lavage. Females not inseminated after 7 days were paired with a different male for up to an additional 7 days. Inseminated females were separated from the males and the day of insemination was considered to be day 0 of gestation. All females were observed daily for mortality and clinical signs of toxicity. Body weights were measured daily during the treatment period. Food consumption was measured prior to mating, weekly throughout gestation and up through postpartum day 21. Approximately one-half of all females were killed on gestation day 21 and necropsied. The ovaries were removed and corpora lutea counted. The uterus was weighed and examined for implantation sites, resorptions and live and dead fetuses. Fetuses were weighed and examined for external abnormalities. One-half of the fetuses were examined for visceral malformations and the other one-half were examined for skeletal malformations. The other one-half of all $F_0$ females were allowed to proceed to natural delivery. Females not delivering by day 24 were killed and necropsied. The offspring were counted, examined externally and sex identified at delivery and individually weighed on postnatal days 0, 2, 4 and 7. On postnatal day 7, each litter was culled to 8 (4/sex if possible). Offspring were observed

for ages of pinna detachment, incisor eruption and eye opening as measures of physical development. Dams with viable litters were killed on postpartum day 21, necropsied and the number of uterine implantation sites counted. Surviving offspring were also killed on postnatal day 21 and necropsied. An internal examination of the heart was performed with the aid of a dissecting microscope.

Results:

*Mortality and Clinical Signs of Toxicity*

There were no drug-related deaths. One female in the 30 mg/kg/day group was found dead on gestation day (GD) 3. This death was considered to be non-drug-related since no deaths occurred at higher doses. No clinical signs of toxicity associated with eprosartan treatment were observed.

*Body Weights and Food Consumption*

No eprosartan-related effects on body weight (Table 43) or food consumption were observed.

Table 43. Maternal Body Weight ( mean gm)

| Treatment Day | Dose Group (mg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.3 | 3 | 300 | 0 | 30 | 100 | 1000 |
| Pre-Mating | | | | | | | | |
| Day 1 (n=40) | 260 | 263 | 262 | 263 | 272 | 273 | 273 | 276 |
| Day 14 (n=40) | 268 | 271 | 270 | 271 | 281 | 271 | 282 | 284 |
| Gestation | | | | | | | | |
| Day 0 (n=34-38) | 282 | 288 | 285 | 285 | 294 | 296 | 292 | 299 |
| Day 14 (n=34-38) | 348 | 353 | 350 | 351 | 367 | 371 | 364 | 374 |
| Day 21 (n=34-38) | 449 | 454 | 457 | 456 | 463 | 473 | 461 | 475 |
| Lactation | | | | | | | | |
| Day 2 (n=14-18) | 335 | 345 | 335 | 336 | 351 | 349 | 349 | 349 |
| Day 14 (n=13-18) | 362 | 368 | 361 | 362 | 376 | 382 | 379 | 375 |
| Day 21 (n=13-18) | 357 | 365 | 361 | 359 | 369 | 370 | 362 | 366 |

*Reproductive Performance*

No eprosartan-related effects on frequency of estrus (3.2 to 3.5 cycles/14 days of treatment), mating incidence, number of days required for mating and pregnancy incidence (Table 44) were observed.

Table 44. Reproductive Performance

| Parameter | Dose Group (mg/kg/day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| Mating Incidence<br># mated/# tested (%) | 76/80<br>(95%) | 40/40<br>(100 %) | 38/40<br>(95%) | 39/40<br>(98%) | 39/40<br>(98%) | 36/39<br>(92%) | 40/40<br>(100%) |
| # Days Needed<br>for Mating | 3.2 | 2.6 | 3.0 | 3.3 | 2.3 | 2.6 | 2.8 |
| Pregnancy Incidence<br># pregnant/# mated (%) | 73/76<br>(96%) | 37/40<br>(93%) | 36/38<br>(95%) | 35/38<br>(92%) | 38/39<br>(97%) | 34/36<br>(94%) | 39/40<br>(98%) |

* Data combined for 2 concurrent control groups in view of similar intergroup values (mating incidences= 39/40 & 37/40; # days for mating=3.15 & 3.27; pregnancy incidence= 37/39 & 36/37).

*Cesarean Section Results*

No eprosartan-related effects were observed in the numbers of corpora lutea, implantations, resorptions and dead or live fetuses (Table 45).

Table 45. Cesarean Section Results†

| Parameter | Dose Group (mg/kg/day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| Corpora Lutea, mean # | 17.5 | 17.0 | 18.1 | 17.5 | 17.3 | 18.0 | 17.9 |
| Implantations, mean #<br>Implantation loss, %[a,c] | 16.4<br>6.1 | 15.8<br>9.4 | 16.9<br>5.4 | 16.6<br>4.8 | 16.3<br>6.1 | 16.4<br>7.3 | 16.5<br>7.2 |
| Resorptions, mean #<br>Implants Resorbed, %[b,c] | 1.1<br>6.5 | 1.1<br>7.0 | 0.7<br>4.2 | 1.1<br>6.7 | 1.0<br>6.2 | 0.8<br>5.4 | 1.4<br>8.2 |
| Live Fetuses, total #<br>Male<br>Female<br>Dead Fetuses, total # | 15.3<br>7.8<br>7.4<br>0.0 | 14.7<br>7.6<br>7.2<br>0.0 | 16.2<br>8.2<br>8.0<br>0.0 | 15.5<br>8.0<br>7.5<br>0.0 | 15.3<br>7.3<br>7.9<br>0.0 | 15.6<br>7.5<br>8.1<br>0.0 | 15.1<br>7.8<br>7.3<br>0.0 |

† Results represent values per pregnant female.(see Table 44 for # of pregnant animals/group examined).
* Data combined for 2 concurrent control groups; Values were essentially similar for the 2 control groups.
[a] % Preimplantation loss= (# corpora lutea - # implants) X100/# corpora lutea
[b] % implants resorbed= (total # resorptions) X100/# implants
[c] Derived from individual % values and not computed using group means

Fetal weight did not differ statistically among groups (Table 46).

Table 46. Fetal Weights

| Parameter | Dose Group (mg/kg/day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| Fetus Weight, mean gm<br>Male<br>Female | <br>5.34<br>5.11 | <br>5.26<br>4.96 | <br>5.29<br>4.97 | <br>5.55<br>5.15 | <br>5.46<br>5.11 | <br>5.37<br>5.08 | <br>5.54<br>5.28 |

* Data combined for 2 concurrent control groups; values were essentially similar for the 2 control groups.
[a] Significantly different from control value ($p<0.05$).

Statistically significant increases were noted in the mean percent of fetuses/litter with any malformations at 0.3 and 1000 mg eprosartan/kg/day (Table 47a). This finding with the 0.3 mg/kg/day dose was primarily due to one litter that had 5/18 (28% incidence) fetuses with exencephaly. The result at 1000 mg/kg/day was primarily due to one litter that had 4/13 (31% incidence) fetuses with oligodactyly. Sponsor did not consider these to be eprosartan-related because they were each seen in only one litter. The fetal and litter incidences of ventricular septal defects (VSDs) in most of the drug-treated groups were elevated above concurrent control. However, the observed VSDs appear to be unrelated to eprosartan treatment in view of the absence of a dose-response (highest incidence in 0.3 mg/kg group with zero incidence in the 30 mg/kg group) and published data showing VSDs to be prevalent (~4%-5%) in control populations of Sprague-Dawley rats.

No statistically significant differences in the incidences of external variations were detected among eprosartan-treated and control groups (Table 47b). Statistically significant increases above control levels were noted in the mean % of fetuses per litter with visceral variations at 3 and 30 mg/kg/day. The sponsor considers these to be non-drug-related since similar increases were not detected at higher doses. (Actually, only the 300 mg/kg/day group showed levels comparable to control; the values seen in 100 and 1000 mg/kg/day groups were higher than control but did not achieve statistical significance.) No statistically significant differences in the overall incidence of skeletal variations were detected among eprosartan-treated and control groups, although a statistically significant trend was noted for the number of litters with morphological variations of sternebrae. The sponsor considered this observation to be unrelated to treatment since there were no significant differences (by pair-wise comparison) between the control and individual eprosartan-treated groups.

Table 47a. Incidences of Fetal Malformations

| Measurement | Dose Group (mg/kg/day | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| **Overall Malformations** | | | | | | | |
| Mean % malformed fetuses/litter | 0.7 | 4.1 ▪ | 2.6 | 0.6 | 0.3 | 1.5 | 3.7 ▪ |
| # Malformed fetuses/# fetuses examined | 4/580 | 12/280 | 8/292 | 1/264 | 1/290 | 4/312 | 10/301 |
| # Litters with ≥1 malformed fetus/total # litters | 3/38 | 7/18 ▪ | 5/18 | 1/17 | 1/19 | 4/20 | 6/20 |
| **Ventricular Septal Defects (VSDs)** | | | | | | | |
| Mean % fetuses with VSDs/litter | 0.6 | 3.5 | 4.0 | 0 | 0.6 | 2.1 | 3.1 |
| # Fetuses with VSDs/# fetuses examined | 2/292 | 5/139 | 6/146 | 0 | 1/147 | 3/159 | 4/146 |
| # Litters with ≥1 fetus with VSDs/total # litters | 1/38 | 5/18 ▪ | 3/18 | 0 | 1/19 | 3/20 | 4/20 |

* Data combined for 2 concurrent control groups.
▪ Significantly different from control value ($p<0.05$).

Table 47b. Incidences of Fetal Variations

| Measurement | Dose Group (mg/kg/day | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| External Variations | | | | | | | |
| Mean % fetuses with variations/litter | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| # Fetuses with variations/# fetuses examined | 0/580 | 0/280 | 0/292 | 0/264 | 0/290 | 0/312 | 0/301 |
| # Litters with ≥1 fetus w. variation/ # litters | 0/38 | 0/18 | 0/18 | 0/17 | 0/19 | 0/20 | 0/20 |
| Visceral Variations | | | | | | | |
| Mean % fetuses with variations/litter | 5.4 | 3.4 | 12.9 [a] | 12.3 [a] | 14.7 | 4.0 | 11.7 |
| # Fetuses with variations/# fetuses examined | 16/292 | 5/139 | 18/146 | 15/130 | 23/147 | 7/159 | 17/146 |
| # Litters with ≥1 fetus w. variations/# litters | 11/38 | 5/18 | 12/18 [a] | 11/17 | 9/19 | 6/20 | 8/20 |
| Skeletal Variations | | | | | | | |
| Mean % fetuses with variations/litter | 19.8 | 13.4 | 15.1 | 15.0 | 17.8 | 22.1 | 21.4 |
| # Fetuses with variations/# fetuses examined | 60/288 | 20/141 | 22/146 | 20/134 | 27/143 | 30/153 | 35/155 |
| # Litters with ≥1 fetus w. variations/# litters | 24/38 | 11/18 | 10/18 | 11/17 | 12/19 | 15/20 | 16/20 |

* Data combined for 2 concurrent control groups.
[a] Significantly different from control value ($p<0.05$).

*Natural Delivery Results*

There were no eprosartan-related effects on the mean duration of gestation, the number of live born(total, male and female), live birth index, % non-viable implants and offspring viability (Table 48).

Table 48. Maternal Reproductive Performance

| Parameter | Dose Group (mg/kg/day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| Gestation Period, days | 21.5 | 21.6 | 21.4 | 21.4 | 21.3 | 21.6 | 21.7 |
| Implants, mean # | 15.0 | 16.0 | 17.2 | 15.5 | 14.6 | 16.1 | 14.2 |
| Live Born, mean total | 14.4 | 15.2 | 16.1 | 15.3 | 14.2 | 15.4 | 13.5 |
| Male | 8.1 | 7.4 | 8.2 | 7.6 | 6.4 | 7.1 | 7.2 |
| Female | 6.3 | 7.6 | 7.9 | 7.7 | 7.8 | 8.3 | 6.4 |
| Nonviable Implants, %[a,d] | 3.7 | 5.2 | 6.5 | 3.2 | 7.8 | 4.6 | 4.7 |
| Dead Born, mean total | 0.1 | 0.0 | 0.1 | 0.2 | 0.0 | 0.1 | 0.1 |
| Live Birth Index[b,d] | 99.4 | 100 | 99.6 | 98.9 | 100 | 99.6 | 99.2 |
| Viability, %[c] | | | | | | | |
| Day 1-7 | 94.5 | 96.9 | 97.4 | 93.7 | 91.8 | 88.6 | 88.7 |
| Day 7-21 | 99.6 | 100 | 98.6 | 100 | 100 | 100 | 100 |

* Data combined for 2 concurrent control groups; values of the two control groups did not differ significantly.
[a] Nonviable implants= (# implants-# live pups) X100/# implants
[b] Live Birth Index= # live pups X 100/#live and dead pups
[c] %Viability= # pups alive on day 7 X 100/# pups alive on day 1
# pups alive on day 21 X 100/#pups alive on day 7
[d] Derived from individual % values and not computed using group means

No eprosartan-related effects were noted on pup body weight from birth through weaning. Physical development periods (pinna detachment, incisor eruption and eye opening) of eprosartan-treated pups did not significantly differ from those of control (Table 49).

Table 49. Body Weight and Physical Development of $F_1$ Offspring

| Parameter | Sex | Dose Group (mg/kg/day) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| Body Weight, gm | | | | | | | | |
| Day 1 | M | 6.40 | 6.36 | 6.27 | 6.,45 | 6.26 | 6.62 | 6.62 |
| | F | 6.03 | 5.98 | 6.00 | 6.13 | 6.01 | 6.26 | 6.36 |
| Day 7 | M | 12.33 | 12.52 | 11.62 | 12.32 | 12.24 | 12.35 | 13.05 |
| | F | 11.60 | 11.85 | 11.17 | 11.70 | 11.34 | 11.87 | 12.49 |
| Pinna Detachment, | | | | | | | | |
| median days Rt | M | 4 | 4 | 4 | 4 | 5 | 4 | 4 |
| | F | 4 | 4 | 4.5 | 4 | 4 | 4 | 4 |
| Lt | M | 4 | 4 | 4.5 | 4 | 5 | 4 | 4 |
| | F | 4 | 4 | 4.5 | 4 | 4 | 4 | 4 |
| Incisor Eruption, | | | | | | | | |
| median days, Upper | M | 12 | 11 | 12 | 12 | 12 | 12 | 11 |
| | F | 14 | 12 | 12 | 12 | 12 | 12 | 11 |
| Lower | M | 12 | 14 | 14 | 14 | 15 | 14 | 14 |
| | F | 14 | 14 | 14 | 15 | 15 | 14 | 14 |
| Eyelid Opening, | | | | | | | | |
| median days Rt | M | 16 | 16 | 16 | 16 | 16 | 16 | 15.5 |
| | F | 15.5 | 15 | 16 | 16 | 16 | 16 | 15.5 |
| Lt | M | 15 | 16 | 16 | 16 | 16 | 15 | 15.5 |
| | F | 15 | 15 | 16 | 16 | 16 | 16 | 15.5 |

* Data combined for 2 concurrent control groups; Values of the 2 control groups did not differ significantly.

Visceral examination of pups on day 7 or day 21 postpartum showed no eprosartan related increase in malformations or variations. Membranous ventricular septal defects (VSDs) were detected in control and treated pups necropsied on day 7 or 21 postpartum (Table 50). The incidence of VSDs in control was higher than with the 0.3, 100, 300 or 1000 mg/kg/day groups and lower than in the 3 or 30 mg/kg/day groups. When the incidences of VSDs are combined from fetuses and offspring, the overall incidence (litters affected/litters examined) was: 6/73 (control), 6/36 (0.3 mg/kg/day), 7/36 (3 mg/kg/day), 6/34 (30 mg/kg/day), 2/37 (100 mg/kg/day), 5/34 (300 mg/kg/day) and 5/39 (1000 mg/kg/day). This suggests that the observed VSDs are not related to eprosartan treatment.

Table 50. Incidence of Ventricular Septal Defects (VSDs) in $F_1$ Fetuses

| Measurement | Dose Group (mg/kg/day | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0* | 0.3 | 3 | 30 | 100 | 300 | 1000 |
| Visceral Malformations-VSD | | | | | | | |
| Mean % fetuses with VSD/litter | 1.4 | 0.7 | 1.8 | 3.3 | 0.3 | 0.9 | 1.0 |
| # Fetuses with VSD/# fetuses examined | 8/501 | 2/267 | 5/286 | 9/259 | 1/254 | 2/210 | 2/251 |
| # Litters with ≥1 fetus with VSD/total # litters | 5/35 | 1/18 | 4/18 | 6/17 | 1/18 | 2/14 | 1/19 |

* Data combined for 2 concurrent control groups.

Developmental Toxicity Study in Pregnant Rats (Segment II)

Study Facility: SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Study No: G93064, Rpt. # TP1008/SKF-108566/1
Study Dates: Initiation of treatment: 9/26/93; Last day of necropsy: 10/22/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Sprague-Dawley female rats (213-300 gm at time of mating)

Drug Administration: Eprosartan mesylate (Lot #BCT-L05C) was suspended in aqueous 1% carboxymethylcellulose and administered orally by gavage to pregnant rats on days 6 though 17 of gestation.

Dose Levels: 0 (vehicle), 30, 100 and 1000 mg eprosartan/kg/day (24/dose group).

*Note: High dose based on findings from a 7-day dose-rangefinding toxicity study in male and non-pregnant female rats which showed that the 2000 mg eprosartan/kg dose yielded only a small increase in systemic exposure (AUC) from that seen with 1000 mg eprosartan/kg suggesting a saturation of drug absorption (see note on page 41 of this review).*

Observations/Measurements: Female rats were mated (1:1) with male rats; the presence of sperm from a vaginal lavage was used to confirm insemination. Rats were observed daily for mortality and clinical signs of toxicity. Body weights were measured on day 0 and then daily from day 6 through day 21 of gestation. Food consumption by mated females was measured for the following gestation day intervals; 0-6, 6-9, 9-12, 12-15, 15-18 and 18-21. Mated females were killed on day 21 of gestation, the ovaries were removed and the corpora lutea counted. The uterus was weighed and examined for numbers of implantation sites, resorptions, live and dead fetuses and sex of each fetus. Fetuses were individually weighed and examined for external abnormalities. Fetuses were killed and half were examined for visceral abnormalities and half were examined for skeletal abnormalities.

Results

*Mortality and Clinical Signs of Toxicity*
No females died during the study; No clinical signs of toxicity were noted among control or eprosartan-treated groups.

*Body Weight and Food Consumption*
Eprosartan treatment had no effect on maternal body weight (Table 51). Food consumption among eprosartan-treated groups was comparable to control.

Table 51. Maternal Body Weight

| | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (Vehicle) | 30 | 100 | 1000 |
| Mean Body Weight, gm | | | | |
| GD 0 | 262 | 264 | 260 | 256 |
| GD 6 | 296 | 292 | 294 | 285 |
| GD 14 | 337 | 336 | 336 | 329 |
| GD 21 | 442 | 440 | 440 | 427 |

*Cesarean Section Results*

There were no eprosartan-related effects on numbers of corpora lutea, implantations, resorptions, sex ratio and dead or live fetuses per litter (Table 52).

Table 52. Cesarean Section Results

| Parameter | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (Vehicle) | 30 | 100 | 1000 |
| # Pregnant/# Mated | 23/24 | 23/24 | 24/24 | 22/24 |
| # Litters | 23 | 23 | 24 | 22 |
| Corpora Lutea, mean # | 17.3 | 17.3 | 16.8 | 17.0 |
| Implants, mean # | 16.9 | 16.4 | 16.3 | 16.3 |
| Pre-implantation Loss, mean %[a,c] | 1.9 | 5.0 | 2.8 | 4.2 |
| Resorptions, mean # | 1.0 | 1.0 | 0.8 | 1.1 |
| % Implants Resorbed [b,c] | 5.7 | 6.0 | 4.9 | 6.8 |
| Live. Fetuses, mean # | 16.0 | 15.5 | 15.5 | 15.2 |
| Male | 7.8 | 7.9 | 7.8 | 7.8 |
| Female | 8.2 | 7.6 | 7.6 | 7.5 |
| Dead Fetuses, mean # | 0.0 | 0.0 | 0.0 | 0.0 |

[a] Pre-implantation loss= (# corpora lutea- # implants) X 100/# corpora lutea

[b] Percent implants resorbed= # resorptions X 100/# implants

[c] Derived from individual % values and not computed using group means

*Fetal Body Weights*

Maternal treatment with eprosartan had no effect on fetal body weight (Table 53).

Table 53. Fetal Body Weights

| | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 30 | 100 | 1000 |
| Mean Body Weight, gm | | | | |
| Males | 5.47 | 5.51 | 5.52 | 5.31 |
| Females | 5.23 | 5.29 | 5.22 | 5.02 |

*Effects on Fetal Development*

Maternal treatment with eprosartan caused no significant increase in the overall incidence of fetal malformations (Table 54). Ventricular septal defects (VSDs) comprised the largest incidence of visceral (and overall) malformations; the incidences of VSDs in fetuses from eprosartan-treated females were comparable to control. No external malformations were detected among control and eprosartan-treated fetuses and the incidence of skeletal malformations was similar in control and eprosartan-treated groups.

Table 54. Fetal Development/ Malformations

| Measurement | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 30 | 100 | 1000 |
| Overall Malformations | | | | |
| Mean % malformed fetuses/litter | 5.4 | 4.2 | 4.2 | 5.4 |
| # Malformed fetuses/# fetuses examined | 19/367 | 15/356 | 15/371 | 15/335 |
| # Litters with ≥1 malformed fetus/total # litters | 10/23 | 10/23 | 9/24 | 9/22 |
| Ventricular Septal Defects (VSDs) | | | | |
| Mean % fetuses with VSDs/litter | 10.3 | 8.1 | 8.3 | 9.3 |
| # Fetuses with VSDs/# fetuses examined | 18/184 | 15/179 | 15/182 | 13/167 |
| # Litters with ≥1 fetus with VSDs/total # litters | 9/23 | 10/23 | 9/24 | 9/22 |

No increase above control incidences of external, visceral or skeletal variations was observed among eprosartan-treated groups (Table 55).

Table 55. Fetal Development/Variations

| Measurement | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 30 | 100 | 1000 |
| External Variations | | | | |
| Mean % fetuses with variations/litter | 0.0 | 0.5 | 0.2 | 0.0 |
| # Fetuses with variations/# fetuses examined | 0/367 | 2/356 | 1/371 | 0/335 |
| # Litters with ≥1 fetus w. variation/total # litters | 0/23 | 1/23 | 1/24 | 0/22 |
| Visceral Variations | | | | |
| Mean % fetuses with variations/litter | 1.7 | 1.0 | 2.0 | 2.4 |
| # Fetuses with variations/# fetuses examined | 3/184 | 2/179 | 4/182 | 4/167 |
| # Litters with ≥1 fetus w. variations/total # litters | 3/23 | 2/23 | 4/24 | 3/22 |
| Skeletal Variations | | | | |
| Mean % fetuses with variations/litter | 25.0 | 23.6 | 15.5 | 28.6 |
| # Fetuses with variations/# fetuses examined | 46/183 | 41/177 | 32/189 | 48/166 |
| # Litters with ≥1 fetus w. variations/total # litters | 19/23 | 21/23 | 13/24 | 18/22 |

Developmental Toxicity Study in Pregnant Rabbits (Segment II)

Study Facility: SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Study No: G93063, Rpt. # TP1007/SKF-108566/1
Study Dates: Initiation of treatment: 6/13/93; Last day of necropsy: 7/16/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: New Zealand White rabbits (9 months old; ~ 4.0 kg)

Drug Administration: Eprosartan mesylate (Lot #BCT-L05C) was suspended in aqueous 1% carboxymethylcellulose and administered orally by gavage to pregnant rabbits on days 6 though 28 of gestation.

Dose Levels: 0 (vehicle), 0.3, 3 and 30 mg eprosartan/kg/day (20/dose group, additional 5/dose group were used for toxicokinetic evaluation).

*Note: Dose selection was based on a dose-rangefinding study (Study # D 93022) in which maternal toxicity (including mortality, decreased body weight and food consumption and abortion) and fetal mortality were observed at doses ≥10 mg eprosartan/kg/day administered on days 6-28 of gestation. When eprosartan was given on days 6-18 of gestation, maternal toxicity, but no fetal toxicity, occurred at a dose of 100 mg eprosartan/kg/day; higher doses (500 and 1000 mg/kg/day) produced maternal and fetal toxicity and mortality. In view of the fact that fetal toxicity was elicited with lower doses of eprosartan at the later stage of fetal development, eprosartan dosing in the definitive Segment II study was extended to day 28 of gestation (the usual duration of treatment in a rabbit Segment II study is day 6 through day 18 of gestation).*

Observations/Measurements: Female rabbits were mated (1:1) with males; the presence of sperm from a vaginal lavage was used to confirm insemination. Mated females were observed daily from day 0 through day 29 of gestation for mortality and clinical signs of toxicity. Body weights were measured on day 0 and then daily from day 6 through day 29 of gestation. Food consumption by mated females was measured daily from day 0 through day 29 of gestation. Mated females were killed on day 29 of gestation, the ovaries were removed and the corpora lutea counted. The uterus was weighed and examined for numbers of implantation sites, resorptions, live and dead fetuses and sex of each fetus. Fetuses were individually weighed and examined for external, internal and skeletal abnormalities. Does employed only for toxicokinetic evaluation were dosed on days 6 through 20 of gestation. Venous blood was obtained from a marginal ear vein prior to treatment on gestation day 20 and at 0.5 1, 2, 4, 6, 8, 12 and 24 hours post-dosing. Plasma samples were analyzed for eprosartan levels by HPLC techniques.

Results:

*Mortality and Clinical Signs of Toxicity*

Four of 20 does in the 30 mg/kg/day group died (3 does) or were euthanized (1 doe) after receiving between 16 and 18 doses of eprosartan. In addition, one doe in the 30 mg/kg/day group died on gestation day 15 from an intubation injury and one doe in the same group died on gestation day 20 apparently due to a severe back injury. No deaths occurred in the 0.3 and 3.0 mg eprosartan/kg/day or control groups (Table 56).

A drug-related increase in abortions was observed among high dose does that survived to term (6 of 14 pregnant survivors aborted on either day 28 or 29 of gestation). No abortions occurred among 0.3 and 3 mg/kg/day treated females.

Table 56. Animal Fate

| Observation | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 0.3 | 3 | 30 |
| # Mated | 20 | 20 | 20 | 20 |
| # Pregnant | 19 | 19 | 20 | 20 |
| # Dead/# Euthanized | 0 | 0 | 0 | 6 |
| # Aborted | 0 | 0 | 0 | 6 |
| # Pregnant at Term | 19 | 19 | 20 | 8 |

*Body Weights and Food Consumption*

Lower than control body weights were observed on days 19 to 21 of gestation in does treated with 30 mg eprosartan/kg/day (Table 57). Food consumption in does treated with 30 mg eprosartan/kg/day was significantly lower than control on days 10 and 13 to 20 of gestation. A slight decrease (~ 9%) from control food consumption was also noted in does treated with 3 mg eprosartan/kg/day; this difference was statistically significant only on day 17 of gestation.

Table 57. Maternal Body Weights

| Gestation Day (GD) | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 0.3 | 3 | 30 |
| GD 0 | 3.69 | 3.75 | 3.74 | 3.66 |
| GD 7 | 3.90 | 3.91 | 3.89 | 3.81 |
| GD 14 | 4.00 | 4.02 | 3.98 | 3.78 |
| GD 21 | 4.11 | 4.14 | 4.12 | 3.77* |
| GD 29 | 4.24 | 4.25 | 4.31 | 3.96 |

* Significantly different from control ($p<0.05$)

*Necropsy of $F_0$ Females*

Macroscopic examination revealed no treatment-related effects on $F_0$ females.

*Cesarean Section Results*

Higher than control numbers of resorptions/litter and dead fetuses/litter, and a corresponding lower than control number of live fetuses/litter, were observed in the 30 mg/kg/day group (Table 58).

Table 58. Cesarean Section Results

| Parameter | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (Vehicle) | 0.3 | 3 | 30 |
| No. Litters | 19 | 19 | 20 | 8 |
| Corpora Lutea, mean # | 8.6 | 8.7 | 8.3 | 7.9 |
| Implants, mean #<br>Pre-implantation Loss, mean %[a,c] | 7.7<br>11.9 | 8.1<br>7.7 | 7.6<br>7.7 | 7.1<br>9.0 |
| Resorptions, mean #<br>% Implants Resorbed [b,c] | 0.5<br>5.6 | 0.7<br>8.6 | 0.2<br>1.8 | 4.6*<br>59.5* |
| Live. Fetuses, mean #<br>Male<br>Female | 7.2<br>3.5<br>3.7 | 7.4<br>3.8<br>3.6 | 7.5<br>4.4<br>3.1 | 2.1*<br>1.4*<br>0.8* |
| Dead Fetuses, mean # | 0.0 | 0.0 | 0.0 | 0.4* |

[a] Pre-implantation loss= (# corpora lutea- # implants) X 100/# corpora lutea

[b] Percent implants resorbed= # resorptions X 100/# implants

[c] Derived from individual % values and not computed using group means

* Significantly different from control ($p<0.05$)

*Fetal Body Weights*

Fetal body weight was similar for control and treated groups Table (59).

Table 59. Fetal Body Weights

| | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 0.3 | 3 | 30 |
| Mean Body Weight, gm<br>Males<br>Females | <br>45.77<br>44.10 | <br>43.79<br>42.91 | <br>44.70<br>43.48 | <br>45.84<br>45.92 |

*Effects on Fetal Development*

No significant increases above control incidences of external, visceral and skeletal malformations and variations were observed among eprosartan treated groups (Tables 60 & 61). Nonetheless, the mean percentage of fetuses/litter with incompletely ossified skull was notably higher for the 30 mg/kg/day group than for the control group (15.6% *vs* 1.2%). The sponsor states that this was due to the fact that the number of litters and mean litter size was reduced to a point where one or two affected fetuses inflated the mean % of affected fetuses/litter. The finding was regarded as unrelated to drug treatment.

Table 60. Fetal Development/ Malformations

| Measurement | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 0.3 | 3 | 30 |
| Overall Malformations | | | | |
| Mean % malformed fetuses/litter | 2.0 | 4.1 | 0.6 | 4.2 |
| # Malformed fetuses/# fetuses examined | 3/137 | 6/140 | 1/149 | 1/20 |
| # Litters with ≥ 1 malformed fetus/total # litters | 2/19 | 6/19 | 1/20 | 1/8 |

Table 61. Fetal Development/Variations

| Measurement | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 0.3 | 3 | 30 |
| External Variations | | | | |
| Mean % fetuses with variations/litter | 0 | 0 | 0 | 0 |
| # Fetuses with variations/# fetuses examined | 0/137 | 0/140 | 0/149 | 0/20 |
| # Litters with ≥ 1 fetus w. variation/total # litters | 0/19 | 0/19 | 0/20 | 0/8 |
| Visceral Variations | | | | |
| Mean % fetuses with variations/litter | 2.7 | 2.7 | 0.6 | 10.4 |
| # Fetuses with variations/# fetuses examined | 4/137 | 4/140 | 1/149 | 2/20 |
| # Litters with ≥ 1 fetus w. variations/total # litters | 3/19 | 4/19 | 1/20 | 2/8 |
| Skeletal Variations | | | | |
| Mean % fetuses with variations/litter | 48.3 | 39.2 | 39.8 | 69.8 |
| # Fetuses with variations/# fetuses examined | 63/137 | 57/140 | 58/149 | 13/20 |
| # Litters with ≥ 1 fetus w. variations/total # litters | 18/19 | 18/19 | 20/20 | 7/8 |

*Toxicokinetics*

Following oral administration of eprosartan, plasma concentrations of parent compound were detectable for 12 to 24 hours with the 0.3 mg/kg dose and for 24 hours with the 3 and 30 mg/kg doses. Cmax and AUC values increased, non-dose-proportionally, with increasing dose (Table 62). Plasma concentrations of eprosartan decreased with time and at 24 hours were approximately equal to the trough level of the previous daily dose.

Table 62. Toxicokinetic Results[a]

| Eprosartan Dose, mg/kg | Pharmacokinetic Parameter | | |
|---|---|---|---|
| | Cmax, ng/ml | Tmax, hr | $AUC_{0-24}$, µg.hr/ml |
| 0.3 (n=5) | 128 | 3.18 | 1.14 |
| 3 (n=4) | 859 | 3.03 | 7.09 |
| 30 (n=3) | 8369 | 2.05 | 82.4 |

[a] Values are the means from 3-5 pregnant rabbits

Pre- and Postnatal Reproductive Toxicity Study in Rats (Segment III)

Study Facility: SmithKline Beecham Pharmaceuticals, King of Prussia, PA
Study No: G94135, Rpt. # TP1012/SKF-108566/1
Study Dates: Initiation of treatment: 1/16/95; Last day of necropsy ($F_2$ pups): 5/17/95

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female Sprague-Dawley rats (10 weeks old)

Drug Administration: Eprosartan mesylate (Lot #BCT-K-12C) was suspended in aqueous 1% carboxymethylcellulose and administered orally by gavage once daily to mated females ($F_0$) on day 6 postcoitus (PC) through day 21 postpartum (PP). Male rats were not dosed.

Dose Levels: 0 (vehicle), 30, 100 and 1000 mg eprosartan/kg/day (24 females/dose group).

Measurements/Observations: Females were cohabitated (1:1) with male rats and the presence of sperm from a vaginal lavage was used to confirm insemination; day of insemination was designated day 0 PC. Dosing was initiated on day 6 PC and females were observed daily during the treatment period for mortality and clinical signs of toxicity. Body weight of mated females was measured on day 0 PC and on days 6 PC through day 21 PP. Food consumption was measured at varying intervals from day 6 PC to day 21 PP. Pregnant females were allowed to deliver naturally (day 1 PP). After completion of parturition, the pups were counted, examined externally and the sex identified. Parturient females ($F_0$) were killed on day 21 PP, necropsied and implantation sites counted. Females not completely delivering by day 24 were killed, necropsied and uterine contents examined. Offspring ($F_1$) were individually weighed on days 2, 7, 14 and 21 PP, weaned on day 21 PP and 1 pup/sex/litter was assigned randomly to one of 3 performance testing subsets (water maze and spontaneous locomotor activity, passive avoidance and startle reflex response, startle reflex response and reproductive performance). $F_1$ females were mated $F_1$ males and females were allowed to deliver naturally. The offspring ($F_2$) were counted, examined externally, sex identified and individually weighed on days 1, 2 and 7 PP. $F_1$ females were necropsied on day 7 PP and uterine implantation sites counted. $F_2$ offspring were killed on day 7 PP and discarded.

Results

*Mortality and Clinical Signs of Toxicity*

There were no unscheduled deaths among $F_0$ females during the study; no clinical signs of toxicity were observed with eprosartan treatment.

*Body Weights and Food Consumption*

No eprosartan-related effects on body weight in $F_0$ females during pregnancy or lactation were observed (Table 63). Also, eprosartan treatment had no effect on food consumption during pregnancy or lactation.

Table 63. Body Weights of $F_0$ Females

| Reproductive Period | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 30 | 100 | 1000 |
| Postcoital Day Body Wt., gm | | | | |
| Day 0 | 249 | 251 | 252 | 249 |
| Day 10 | 300 | 304 | 308 | 302 |
| Day 21 | 418 | 422 | 445 | 428 |
| Postpartum Day Body Wt., gm | | | | |
| Day 2 | 305 | 311 | 324 | 310 |
| Day 12 | 349 | 348 | 362 | 349 |
| Day 21 | 331 | 333 | 342 | 341 |

*Natural Delivery /Necropsy of $F_0$ Females*

The mean duration of gestation and average delivery time per pup were not significantly different among the groups. There were no eprosartan-related necropsy findings in the $F_0$ females. There were no significant differences among groups in the number of live pups, number of implants or percentage of non-viable implants. Live birth index was >95% and not significantly different among the groups. Offspring viability from day 1-7 PP (>95%) and from day 7 PP to weaning (>98%) was not significantly affected by eprosartan treatment (Table 64).

Table 64. $F_0$ Female Natural Delivery Results and Offspring Viability

| Parameter | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 30 | 100 | 1000 |
| # $F_0$ Treated Females | 24 | 24 | 24 | 24 |
| # $F_0$ Pregnant Females | 22 | 23 | 20 | 22 |
| Days of Pregnancy, mean # | 21.3 | 21.1 | 21.4 | 21.2 |
| Pup Delivery Time, mean # | 9.7 | 11.2 | 12.0 | 17.4 |
| # Viable Litters | | | | |
| At Birth | 22 | 22 | 20 | 22 |
| At Weaning | 22 | 21 | 20 | 22 |
| Live Pups, mean #/litter | 14.5 | 15.0 | 15.5 | 14.9 |
| Male | 6.9 | 7.6 | 8.1 | 8.0 |
| Female | 7.6 | 7.5 | 7.5 | 6.9 |
| Implants, mean # | 15.5 | 17.0 | 16.9 | 17.0 |
| Nonviable Implant, % [a,e] | 6.0 | 11.0 | 7.4 | 12.5 |
| Live Birth Index, % [b,e] | 99.4 | 96.3 | 98.6 | 95.5 |
| Pup Viability, % | | | | |
| Day 1-7 PP [c,e] | 96.1 | 95.2 | 99.2 | 98.4 |
| Day 7-21 PP [d,e] | 99.1 | 98.9 | 98.4 | 98.2 |

[a] Nonviable Implants= (# implants-#live pups) x 100/ # implants

[b] Live Birth Index= # live pups born x 100/# live and dead pups born

[c] Pup Viability= # pups alive on day 7 x 100/ # pups alive on day 1

[d] Pup Viability= # pups alive on day 21 X100/ # pups alive on day 7

[e] Derived from individual % values and not computed using group means

*$F_1$ Offspring Body Weight*

There were no significant differences among the groups for either male or female offspring body weights from birth through 98 days of age (Table 65).

Table 65. $F_1$ Offspring Body Weights[a]

| Postpartum Day | Sex | Maternal Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|---|
| | | 0 (vehicle) | 30 | 100 | 1000 |
| Body Wt.,gm Day 1 | M | 6.1 | 6.2 | 6.5 | 6.4 |
| Day 21 | | 36.9 | 35.1 | 38.4 | 37.6 |
| Day 56 | | 322 | 310 | 321 | 318 |
| Day 98 | | 539 | 516 | 537 | 534 |
| Body Wt.,gm Day 1 | F | 5.9 | 5.9 | 6.2 | 6.0 |
| Day 21 | | 36.5 | 34.4 | 36.6 | 36.6 |
| Day 56 | | 213 | 215 | 209 | 212 |
| Day 98 | | 306 | 316 | 303 | 322 |

[a]Values are the group means derived from 20-22 litters

*$F_1$ Offspring Physical Development*

Eprosartan treatment did not affect the age at which offspring attained physical signs of sexual maturation (Table 66).

Table 66. $F_1$ Offspring Physical Development (Sexual Maturation)[a]

| Developmental Stage (age in days) | Maternal Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 30 | 100 | 1000 |
| Male Balbano Preputial Separation | 46.8 | 47.4 | 46.2 | 46.6 |
| Female Vagina Opening | 34.4 | 34.4 | 33.2 | 33.9 |

[a] Values are the group means derived from 20-22 litters.

*$F_1$ Offspring Reflex Behavior*

Startle reflex following auditory stimulation, tactile stimulation and a pre-pulse condition (subthreshold auditory stimulus preceded the tactile stimulus), assessed at age 28 days and at age 70-74 days, showed no significant differences among control and treated groups (Table 67a & 67b).

Table 67a. $F_1$ Offspring Reflex Behavior[a]

| Startle Reflex Parameter | Sex | Age, days | Maternal Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|---|---|
| | | | 0 (vehicle) | 30 | 100 | 1000 |
| Auditory Stimulus, units | M | | | | | |
| Initial | | 28 | 40 | 51 | 59 | 56 |
| Subsequent | | | 25 | 27 | 31 | 33 |
| Initial | | 70-74 | 334 | 334 | 401 | 388 |
| Subsequent | | | 206 | 206 | 208 | 198 |
| Tactile Stimulation, units | M | 28 | 42 | 45 | 51 | 52 |
| | | 70-74 | 285 | 257 | 310 | 282 |
| Pre-Pulse Stimulus, units | M | 28 | 29 | 30 | 32 | 38 |
| | | 70-74 | 133 | 126 | 145 | 146 |

[a] See Table 67b for footnote

Table 67b. $F_1$ Offspring Reflex Behavior

| Startle Reflex Parameter | Sex | Age, days | Maternal Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|---|---|
| | | | 0 (vehicle) | 30 | 100 | 1000 |
| Auditory Stimulus, units | F | | | | | |
| Initial | | 28 | 56 | 47 | 77 | 51 |
| Subsequent | | | 27 | 26 | 30 | 30 |
| Initial | | 70-74 | 141 | 187 | 207 | 229 |
| Subsequent | | | 144 | 143 | 157 | 145 |
| Tactile Stimulation, units | F | 28 | 45 | 42 | 45 | 48 |
| | | 70-74 | 223 | 233 | 225 | 123 |
| Pre-Pulse Stimulus, units | F | 28 | 28 | 28 | 33 | 31 |
| | | 70-74 | 115 | 115 | 116 | 123 |

[a] Arbitrary units= Measured from animals placed in a sound-attenuated box with an accelerometer attached; this instrument transduced animal reflex movements into voltage changes occurring within a 100 msec recording interval. Auditory stimulus=111 dB sound; tactile stimulus=puff of compressed air; pre-pulse stimulus=subthreshold 88bB sound followed by air puff.

*$F_1$ Offspring Learning and Retention*

The swimming maze (escape time) and passive avoidance (latency of entry in dark compartment) tests were used for measurements of learning/retention development. Both tests were conducted in offspring at age 40-43 days. The test outcomes showed no significant differences among control and eprosartan-treated groups (Table 68).

Table 68. $F_1$ Offspring Learning/Retention

| Learning/ Retention Parameter | Sex | Maternal Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|---|
| | | 0 (vehicle) | 30 | 100 | 1000 |
| Swim Maze, escape time (sec) | | | | | |
| Trial 1 | M | 22.5 | 23.0 | 22.1 | 21.1 |
| Trial 3 | | 16.7 | 24.0 | 19.8 | 17.4 |
| Trial 1 | F | 27.9 | 25.8 | 23.6 | 28.0 |
| Trial 3 | | 22.7 | 21.3 | 22.6 | 18.5 |
| Passive Avoidance, time (sec) | | | | | |
| Approach latency | M | 10.5 | 8.5 | 14.0 | 10.0 |
| Avoidance latency | | 18.0 | 15.0 | 29.5 | 13.0 |
| Approach latency | F | 10.0 | 8.0 | 10.3 | 15.0 |
| Avoidance latency | | 25.5 | 38.5 | 15.0 | 29.0 |

*$F_1$ Offspring Motor Activity*

Locomotor activity which reflected different aspects of behavior (exploratory activity, circadian pattern of activity and activity in response to amphetamine) showed no significant differences among control and eprosartan-treated groups (Table 69).

Table 69. $F_1$ Offspring Motor Activity

| Locomotor Activity (# movements/period) | Sex | Maternal Dose Group (mg/kg/day | | | |
|---|---|---|---|---|---|
| | | 0 (vehicle) | 30 | 100 | 1000 |
| Exploratory (1100=1400 h) | M | 1751 | 1397 | 1642 | 1443 |
| | F | 2219 | 1782 | 1403 | 1526 |
| Dusk (1800-2100 h) | M | 1530 | 1381 | 1470 | 1159 |
| Night (2100-0400 h) | | 2555 | 1989 | 2252 | 1865 |
| Dawn (0400- 0700 h) | | 1027 | 999 | 1052 | 675 |
| Dusk (1800-2100 h) | F | 1172 | 1081 | 965 | 1129 |
| Night (2100-0400 h) | | 2023 | 1636 | 1718 | 1701 |
| Dawn (0400- 0700 h) | | 1027 | 568 | 496 | 475 |
| Amphetamine Treatment, | M | 1376 | 1257 | 1463 | 1130 |
| 0.5 mg/kg SC (0850-1000 h) | F | 1309 | 1606 | 1107 | 1373 |

*$F_1$ Offspring Reproductive Performance*

Maternal eprosartan treatment had no effect on the female ($F_1$) estrous cycle, mating incidence, mean number of days to mating , pregnancy incidence or numbers of $F_2$ offspring (Table 70). Also, other $F_1$ maternal parameters (gestation body weight, gestation length, parturition and $F_2$ offspring survival and growth to day 7 postpartum) did not significantly differ among groups.

Table 70. $F_1$ Offspring Reproductive Performance

| Reproductive Parameter | Maternal Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (vehicle) | 30 | 100 | 1000 |
| # $F_1$ Males | 21 | 21 | 20 | 19 |
| # $F_1$ Females | 21 | 21 | 20 | 19 |
| Estrus Frequency Prior to Mating (# cycles/10 days) | 2 | 2 | 2 | 2 |
| Mating Index, #females inseminated/# mated (%) | 19/21 (90) | 21/21 (100) | 20/20 (100) | 19/19 (100) |
| Average # Days to Mate | 4 | 3 | 4 | 3 |
| Fertility Index, #females pregnant/ # inseminated (%) | 18/19 (95) | 16/21 (75) | 19/20 (95) | 18/19 (95) |
| Live Pups/Litter, # | 15.1 | 15.3 | 13.9 | 15.2 |
| Male | 7.9 | 7.1 | 6.2 | 7.6 |
| Female | 7.2 | 8.3 | 7.6 | 7.5 |

## SUMMARY AND EVALUATION

Eprosartan is an orally active angiotensin II receptor antagonist that lowers arterial blood pressure in hypertensive animals.

Antihypertensive efficacy of eprosartan was assessed in conscious renin-dependent hypertensive rats and dogs. In renin-dependent hypertensive rats,1-10 mg eprosartan/kg ID caused a dose-dependent decrease in mean arterial pressure from a control value of 160 mmHg to 125 mm Hg. At the highest dose tested, the antihypertensive effect was maintained for at least 90 min. The reduction in arterial blood pressure was attributed to eprosartan-induced reduction of peripheral vascular resistance. In renin-dependent hypertensive dogs, 10 mg eprosartan/kg PO decreased mean arterial blood pressure approximately 35 mm Hg and the antihypertensive effect was sustained for at least 12 hours.

The renin-angiotensin system plays a minimal role in the control of blood pressure in conscious spontaneously hypertensive rats, and as such, ACE inhibitors and angiotensin II antagonists elicit minimal antihypertensive effects in these animals. However, treatment of this renin-independent animal model with a diuretic has been shown to activate the renin-angiotensin system. A bolus dose of eprosartan (10 mg eprosartan/kg) administered IV to rats that were previously treated with the diuretic, hydrochlorothiazide (10 mg/kg IV), significantly decreased mean arterial blood pressure by 37 mmHg.

The affinity and selectivity of eprosartan for the angiotensin II AT-1 receptor was demonstrated using radioligand binding studies in a number of tissues including membrane prepared from human liver and adrenal cortex. The eprosartan $IC_{50}$ values for displacement of radiolabeled angiotensin II from these membranes ranged from 1.5 to 9.2 nM. At concentrations up to 10 μM, eprosartan did not displace radiolabeled angiotensin II from cerebral AT-2 receptors. In isolated rabbit aortic rings, eprosartan produced parallel rightward shifts in the concentration-response curves to angiotensin II induced contractions. The ability of eprosartan to antagonize angiotensin II pressor responses was measured in conscious rats and dogs. In rats, the drug elicited dose-related inhibition of the pressor responses to exogenously administered angiotensin II; the dose of eprosartan needed to inhibit the response to angiotensin II by 50% ($ID_{50}$) was 80 μg/kg IV and 5.5 mg/kg intraduodenally. Intravenous (0.1 to 3 mg/kg) and oral (1 to 10 mg/kg) administration of eprosartan to conscious dogs resulted in dose-dependent reductions of angiotensin II-induced pressor responses which were maintained for 4 and 6 hours, respectively.

Eprosartan (10 mg/kg ID) did not interfere with cardiovascular reflexes in rats, such as the orthostatic blood pressure reflex induced by a $90^0$ vertical tilt for 60 seconds. Intragastric doses up to 1000 mg eprosartan/kg to rats caused no significant effects on cardiovascular (blood pressure, heart rate and dP/dt), renal (urine excretion rate, osmolarity and electrolyte excretion) and respiratory (ventilatory parameters and lung mechanics) functions.

Preclinical studies to assess the absorption, distribution, metabolism and excretion of eprosartan have

been carried out primarily in the rat and dog, the two main species used in the toxicological evaluation of eprosartan. Limited pharmacokinetic (toxicokinetic) information was obtained in the mouse and rabbit from satellite groups of animals used in selected toxicology (carcinogenicity, developmental toxicity) studies.

In male rats, it was estimated that about 8% of drug-related material was absorbed following oral administration of a solution of radiolabeled eprosartan mesylate at a dose of 100 mg eprosartan/kg. In another study which compared the pharmacokinetics of smaller oral and intravenous doses of the drug in solution (10 mg eprosartan/kg and 3 mg eprosartan/kg, respectively), the apparent bioavailability with the oral solution was estimated to be 1.7% . After a 3 mg eprosartan/kg radiolabelled dose to male rats, radioactivity was widely distributed throughout most organs and tissues, with negligible levels appearing in the CNS, bone and abdominal fat. The presence of radioactivity was greatly diminished in all tissues and organs by 1 hour after dosing; by 96 hours, notable radioactivity was detected only in the kidneys. In pregnant rats, the amount of drug-related material detected in placentae and amniotic fluid 3 hours after dosing was quite low and the concentrations of radioactivity in the fetuses were below the levels of detection. In lactating rats, secretion of radioactivity in milk occurred slowly and to a small extent; there was no evidence for accumulation of drug-related material in the milk. In the 30-day and 6-month repeat dose studies in male and female rats, oral (gavage) doses of 30, 100 and 1000 mg eprosartan/kg/day elicited dose-related (but not dose-proportional) increases in Cmax and AUC. There was no evidence of drug accumulation and no apparent gender-related differences in any of the pharmacokinetic parameters in either study. In the rat carcinogenicity study in which animals were fed a restricted diet, mean Cmax and AUC values (measured on days 1, 29 and 366 of dosing) increased with increasing doses. The increases were approximately dose-proportional between the 30 and 100 mg/kg/day doses but were less than dose-proportional between the 100 and 600 mg/kg/day doses. No apparent gender differences were detected in these parameters. In the dog, the toxicokinetic profile in the 1-year repeat dose study was similar to that observed in the 6-month repeat dose study. Following daily oral doses of 30, 100 and 1000 mg eprosartan/kg, T max was observed between 2 and 4 hours after dosing. Systemic exposure increased with increasing dose (approximately 6- to 14-fold increase in Cmax and AUC values for a 33-fold increase from low to high dose). In humans, the absolute oral bioavailability of eprosartan (as tablets) given as a dose of 300 mg was found to range from 6.4% to 28.8% with an average of 13.1%. In healthy male subjects, mean Cmax and AUC values increased with increasing dose over the 100-800 mg dose range; Cmax concentrations of 1.27 and 1.86 µg/ml and AUC values of 4.66 and 7.44 µg.hr/ml were observed for the 400 and 800 mg doses (recommended clinical doses), respectively. At a concentration range of 0.10 to 10 µg/ml, the observed free fraction of eprosartan remained constant in rat, dog and human plasma (1.9%, 11.1% and 1.6%, respectively). At concentrations of 100 µg/ml and above, the free fractions increased non-linearly.

Following both oral (100 mg) and IV (20 mg) administration of radiolabeled eprosartan mesylate to healthy male subjects, eprosartan was identified as the only radiolabeled component in plasma. One metabolite, characterized as the acyl glucuronide of eprosartan, was detected in the urine and represented 19% of the urinary radioactivity excreted; this metabolite corresponded to 6.8% of the

IV dose and 1.2% of the oral dose. Unchanged eprosartan was the primary drug-related material recovered in the feces. In intact male and female rats, the parent compound constituted >90% of the total radioactivity excreted in the feces after an oral dose of 100 mg/kg (>99% in bile cannulated rats). Similarly, all the radioactivity excreted in the feces (>90% of the dose) after oral administration of a 100 mg/kg dose to intact dogs was identified as the parent compound. Excretion of eprosartan-related material after an oral dose is predominately via the feces in rats (~92% after 100 mg/kg), dogs (~94% after 100 mg/kg) and humans (~90% after 100 mg); the remainder is excreted in the urine for all 3 species.

Eprosartan mesylate was not associated with adverse effects when given to rats or dogs as single oral doses ≤1000 mg/kg. Single intravenous doses up to 300 mg eprosartan/kg were not associated with toxicity in rats. Dogs given single intravenous doses ≥100 mg eprosartan/kg had emesis and mild intrahepatic cholangitis; a dose of 300 mg/kg IV was associated with 2- to 5-fold increases in serum enzymes (ALT, ALP and AST).

The sponsor chose a maximal dose of 1000 mg eprosartan/kg to be given to rats and dogs in studies ≥ 1 month based upon the highest attainable systemic (AUC) exposure in rats and the practical limits of administering 5 (0.5 oz) capsules daily to dogs for a prolonged period. In the repeat oral dose studies, no adverse drug-related effects were observed at doses ≤1000 mg/kg/day for up to 6 month in rats. A slight increase above control incidence of renal lesions (dilatation of renal pelvis, chronic progressive nephropathy and renal papilla or tubule mineralization) observed with the 1000 mg/kg/day dose in the 30-day repeat dose study in rats was not evident in the 6-month repeat dose study in rats receiving the same high dose. Mild decreases in hematologic parameters (RBC counts, hematocrit and hemoglobin concentrations) were observed in dogs given ≥30 mg/kg/day orally for up to 6 months. Although mild decreases in hematologic parameters were noted with 1000 mg eprosartan/kg/day in the 1-year study in dogs, they were transient since they were not observed at the conclusion of the study. Mild decreases in hematologic parameters have previously been observed in rats, dogs and other animals given ACE inhibitors and angiotensin II antagonists and have been ascribed to alteration of the renin-angiotensin system's regulation of erythropoietin production, a pharmacological class effect. The systemic exposure to 1000 mg eprosartan/kg during chronic toxicological evaluation was similar in rats and slightly greater in dogs than that observed in humans given single or 7-day repeated doses of 800 mg/day. At the doses compared, systemic exposure (eprosartan AUC) in rats was slightly less than that in humans, whereas in dogs it was 2-fold greater than that observed in humans. Due to differences in plasma protein binding, systemic exposure to free drug was much higher (~14-fold) in dogs than in humans. The extent of plasma protein binding in rats and humans was similar and unbound AUC values also remained similar.

Comparative Systemic Exposure Following Oral Administration of Eprosartan Mesylate

| | Rat 1000 mg*/kg/day | Rabbit 3 mg*/kg/day | Dog 1000 mg*/kg/day | Human 800 mg*/day |
|---|---|---|---|---|
| Eprosartan $AUC_{0-24}$ (ug.hr/ml)[a] | 6.58 | 7.09 | 18.96 | 9.52 |
| Animal/Human Exposure Multiple | 0.7 | 0.7 | 2.0 | |
| Drug Protein Binding[b] | 98.1% | 97.2%[c] | 88.9% | 98.4% |
| Free (unbound) Eprosartan $AUC_{0-24}$ (ug.hr/ml) | 0.13 | 0.20 | 2.11 | 0.15 |
| Animal/Human Exposure Multiple | 0.9 | 1.3 | 14 | |

[a] Mean values combined for male and female rats and dogs; 6-month (rat), 1-year (dog) and 7-day (human) repeat dose studies; data from pregnant rabbits on GD 20 following daily oral dosing from GD6..
[b] Based on in vitro data.
[c] Study results summarized in Appendix A
* Eprosartan

The reproductive toxicity profile of eprosartan mesylate was determined in Segment I (rat fertility and general reproductive performance), Segment II (rat and rabbit embryo-fetal development) and Segment III (rat prenatal/postnatal development) studies. In the fertility and general reproductive performance studies, oral doses ≤1000 mg eprosartan/kg/day given to male or female rats had no effect on mating or fertility. In the developmental toxicity study in pregnant rats given oral doses ≤1000 mg eprosartan/kg/day on days 6-17 of gestation, eprosartan produced no maternal toxicity, no fetal mortality and no developmental toxicity. A determination of the extent of systemic exposure of eprosartan in pregnant rats was not conducted. Systemic exposure following oral administration of eprosartan mesylate at doses up to 1000 mg eprosartan/kg was determined for non-pregnant rats, as shown in the table at the top of this page. Placental transfer of eprosartan-related material was studied in pregnant SD rats after a single oral dose of 100 mg eprosartan/kg administered as [$^{14}$C]-eprosartan mesylate on day 18 of gestation. Peak amniotic fluid levels of radioactivity were approximately 1/20 those of peak plasma levels. Fetuses showed no detectable levels of radioactivity up to 24 hours after this single dose of radiolabelled eprosartan mesylate to the dams. Thus, placental transfer of eprosartan-related material is low in the rat.

In the developmental toxicity study in pregnant rabbits treated orally on day 6 through day 28 of gestation, eprosartan produced maternal toxicity (decreased body weight gain, decreased food consumption and death) and fetal toxicity (higher than control levels of resorptions/litter and number of dead fetuses/litter) at 30 mg eprosartan/kg/day. Despite the maternal toxicity observed with the 30 mg/kg/day dose, eprosartan caused no significant increase above control incidence of external, visceral or skeletal malformations or variations. Based on the findings from the main and dose-rangefinding studies, the highest no-observed-toxic-effect-level in pregnant rabbits is 3 mg eprosartan/kg/day (10 mg eprosartan/kg/day in the rangefinding study produced effects similar to those seen with 30 mg/kg/day in the definitive study; next highest dose in the definitive study was 3 mg/kg/day). The 0.3, 3 and 30 mg eprosartan/kg/day oral doses to pregnant rabbits in this study yielded AUC values of 1.14, 7.09 and 82.4 ug eprosartan.hr/ml, respectively. Maternal toxicity and fetal toxicity observed in pregnant rabbits given eprosartan is consistent with the generally

recognized sensitivity of the rabbit to ACE inhibitors and angiotensin II antagonists.

In an oral prenatal and postnatal developmental toxicity study in pregnant rats treated from day 6 postcoitus to postpartum day 21, eprosartan mesylate did not produce maternal toxicity or affect pregnancy, parturition or lactation of dams and did not affect the survival, growth or postnatal development (including neurobehavioral and reproductive function) of the offspring at oral doses up to 1000 mg eprosartan/kg/day. The administration of single oral doses of 100 mg eprosartan/kg given as [$^{14}$C]-eprosartan mesylate to lactating rats showed that transfer of radioactivity into milk occurred to a small extent; peak levels of radioactivity in milk were approximately 1/4 those of peak plasma levels.

## LABELING

Those sections in the proposed labeling that refer to non-clinical studies that were covered by this review were reviewed and considered acceptable with the following exceptions:

Under WARNINGS, *Fetal/Neonatal Morbidity and Mortality*, the sponsor's proposed text summarizing the results of developmental and neonatal toxicity studies reads as follows:

> "Eprosartan mesylate has been shown to produce maternal and fetal mortality in pregnant rabbits dosed at 10 mg/kg/day during late pregnancy. Maternal toxicity but no fetal effects were observed at 3 mg/kg/day. Systemic exposure (AUCs) in pregnant rabbits was similar (3 mg/kg/day) or approximately 3 times greater (estimated at 10 mg/kg/day) than exposure achieved in humans given the maximum recommended human daily dose (800 mg)."

The above paragraph provides no information on results from the developmental toxicity studies conducted in rats. Also, information regarding the toxicity in rabbits needs to be expanded to describe maternal and fetotoxic effects in addition to mortality. Therefore, this section of labeling should be revised to read as follows:

> "Eprosartan mesylate 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/kg/day. No maternal or fetal adverse effects were observed at 3 mg eprosartan/kg/day; this oral dose yielded a systemic exposure to unbound eprosartan approximately 1.3 times the exposure (AUC) achieved in humans given the maximum recommended human daily dose of 800 mg. 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 a systemic exposure to unbound eprosartan approximately 0.9 times the exposure achieved in man at the maximum recommended human daily dose of 800 mg)."

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

"In general reproductive performance studies, there were no effects on mating, fertility or gonadal function in male or female rats given oral dosages up to 1000 mg/kg/day."

This statement does not provide information on the animal/human dose multiple and should be revised to read as follows:

"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. This dose provided systemic exposure to unbound eprosartan approximately 0.9 times the exposure (AUC) achieved in man at the maximum recommended human daily dose of 800 mg."

**RECOMMENDATION**

From a preclinical safety perspective, this new drug application is approvable with the recommended changes in labeling (see page 82).

Anthony G. Proakis, Ph.D.
Pharmacologist

NDA 20,738
HFD-110
HFD-110/CSO
HFD-110/AProakis
HFD-110/JKoerner
HFD-110/CResnick
HFD-110/TPapoian
HFD-345/EButler
Accepted by CAR on 7-30-97

D. Millazol

JUL 31 1997

NDA 20,738

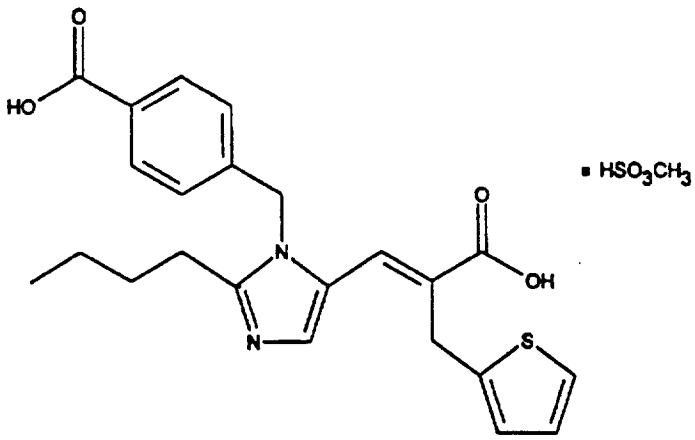
# REVIEW AND EVALUATION OF GENOTOXICITY AND CARCINOGENICITY DATA

**John E. Koerner, Ph.D.**
07/24/97

**ORIGINAL SUBMISSION DATE:** 10/11/96[1]
**CENTER RECEIPT DATE:** 10/11/96
**REVIEWER RECEIPT DATE:** 10/22/97

**SPONSOR:** SmithKline Beecham Pharmaceuticals

**DRUG:** Teveten
Generic Name: Eprosartan mesylate Code Name: SK&F 108566-J
Chemical Name: (E)-a-[[2-Butyl-1-[(4-carboxyphenyl)methyl]-1H-imidazol-5-yl] methylene]-2-thiophenepropanoic acid monomethanesulfonate
Chemical Structure:




Molecular Weight: 520.625

**FORMULATION AND ROUTE OF ADMINISTRATION:** Aqueous film-coated, scored Tiltab™ tablets for oral administration. Each tablet contains eprosartan mesylate equivalent to 300 or 400 mg of eprosartan zwitterion along with inactive ingredients (croscarmellose sodium, hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyethylene glycol, pregelatinized starch and titanium dioxide). Tablets may also contain one or more of the following inactive ingredients: iron oxide black, iron oxide red, iron oxide yellow and polysorbate 80.

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1. Also considered in this review and evaluation are amendments to the NDA dated: 3/10/97, 3/21/97, 4/21/97, 5/7/97, 5/20/97 and 7/23/97

**PHARMACOLOGICAL CLASS:** Angiotensin II ($AT_1$) receptor antagonist

**PROPOSED INDICATION:** Hypertension

**PROPOSED DOSAGE REGIMEN:** 400-800 mg eprosartan once daily

**IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:** IND # 39,721

## TABLE OF CONTENTS

## GENOTOXICITY

### Ames Bacterial Mutagen Test

Test Agent: Eprosartan Mesylate (Lot No. JF15943-164)

Study Facility:

Study Numbers: TP-0002/SKF-108566/1

Study Dates: 09/09/91 to 09/23/91

GLP Compliance: Statement indicates that these studies were conducted in compliance with GLP regulations.

Test System: *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100. *Escherichia coli* strain (WP2 uvrA).

Procedure: Eprosartan mesylate was dissolved in dimethylsulfoxide and added to culture plates containing the bacterial tester strains in the presence and absence of metabolic activation with rat liver S-9 from Aroclor 1254 treated rats. Dosing solutions were prepared daily and used within two hours of preparation. Samples of the testing solutions were provided to the sponsor for verification of eprosartan mesylate concentrations; the average variation from theoretical test article concentration was 5%.

Eprosartan mesylate was tested for toxicity at 50, 167, 500, 1670, and 5000 ug /plate in the presence and absence of S-9. Eprosartan mesylate was soluble at all doses evaluated. Toxicity was assessed (in duplicate) by evaluating the growth of the background lawn and the frequency of spontaneous revertants.

Eprosartan mesylate was evaluated for mutagenicity in all tester strains at 50, 167, 500, 1670, 3330 and 5000 µg /plate with and without S-9. Mutagenicity assays were performed in triplicate in initial and confirmatory assays. In the testing procedure, all tester strains were pre-incubated with test agent for 30 minutes at 30°C prior to addition of agar and then incubated with test agent in the dark for 48 hours at 37°C.

Positive controls in the absence of metabolic activation were sodium azide (10 ug/plate), 9-aminoacridine (150 ug/plate), 2-nitrofluorene (5.0 ug/plate) and ENNG (2.0 ug/plate). The positive control in the presence of metabolic activation with S-9 was 2-anthramine (2.5 µg/plate).

Mutagenicity was assessed by determining the mean number of revertant colonies for each concentration of eprosartan mesylate in the presence and absence of metabolic activation with S-9. Toxicity was assessed in the mutagenicity assays by evaluating background lawn growth compared to concurrent control.

To be considered positive for mutagenicity, the test agent had to increase the mean number of revertant colonies on the treated plates by more than twice that of the concurrent solvent negative control, and a dose-dependent effect had to be shown. Positive responses were defined as strong, moderate or weak based on a >10 fold, 5-10 fold or 2-5 fold increase, respectively, in revertant frequency compared to the concurrent negative control. Statistical analyses were not performed.

Results: Eprosartan mesylate was nontoxic at all doses in all tester strains evaluated. Eprosartan mesylate also did not increase revertant frequencies at any dose in any tester strain evaluated, with or without S-9 (Tables 1-3) Positive controls increased revertant frequencies, as expected.



APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

TABLE 1. AMES/SALMONELLA-E. COLI LIQUID PRE-INCUBATION ASSAY
TOXICITY PRESCREEN MEAN SUMMARY DATA

Sponsor: SMITHKLINE BEECHAM PHARMACEUTICALS
Test article: SK&F 108566-J
Description: WHITE POWDER
Other considerations: LOT# JF15943-164 PRETOX: OUT OF INCUBATOR 9/11

Date initiated: 09-09-1991
Date scored: 09-12-1991
Study ID: PH301-SK-007-91

CONTROLS

| | | AVERAGE REVERTANTS/PLATE AND BACKGROUND GROWTH | | |
|---|---|---|---|---|
| SOLVENT CONTROLS | S-9 | TA1538 | TA100 | UVR A |
| DMSO (100 UL) | (-) | 6 (1) + | 77 (12) + | 6 (1) + |
| DMSO (100 UL) | (+) | 17 (4) + | 90 (13) + | 10 (1) + |

TEST ARTICLE: SK&F 108566-J

| DOSE LEVEL UG/PL | S-9 | TA1538 | TA100 | UVR A |
|---|---|---|---|---|
| 50.0 | (-) | 5 (1) + | 94 (3) + | 8 (1) + |
| 167 | (-) | 6 (1) + | 83 (8) + | 8 (2) + |
| 500 | (-) | 4 (2) + | 70 (21) + | 10 (4) + |
| 1670 | (-) | 3 (3) + | 70 (4) + | 9 (2) + |
| 5000 | (-) | 8 (6) + | 83 (2) + | 6 (0) + |
| 50.0 | (+) | 11 (3) + | 93 (6) + | 15 (0) + |
| 167 | (+) | 10 (4) + | 85 (11) + | 8 (3) + |
| 500 | (+) | 19 (0) + | 90 (1) + | 9 (1) + |
| 1670 | (+) | 14 (0) + | 93 (16) + | 6 (4) + |
| 5000 | (+) | 11 (2) + | 94 (13) + | 9 (1) + |

Data Reported as: Mean, (Standard Deviation), Background Growth
Background Growth: + = Normal, ± = Inhibited, - = No Growth.
Normal Growth all Strains/Doses +/- 59
No Precipitate

TABLE 2. AMES/SALMONELLA-E. COLI LIQUID PRE-INCUBATION ASSAY
MEAN SUMMARY DATA

Sponsor: SMITHKLINE BEECHAM PHARMACEUTICALS
Test article: SK&F 108566-J
Description: WHITE POWDER
Other considerations: LOT# JF15943-164; OUT OF INCUBATOR 09-22-1991

Date initiated: 09-20-1991
Date scored: 09-23-1991
Study ID: PH301-SK-007-91

CONTROLS

| | | | AVERAGE REVERTANTS/PLATE | | | | | |
|---|---|---|---|---|---|---|---|---|
| NEGATIVE CONTROLS [a] | | S-9 | TA1535 | TA1537 | TA1538 | TA98 | TA100 | UVR A |
| DMSO | (100 UL; PRE) | (-) | 11 (5) | 10 (3) | 3 (2) | 17 (2) | 79 (17) | 4 (3) |
| DMSO | (100 UL; PRE) | (+) | 13 (1) | 9 (4) | 11 (5) | 30 (6) | 95 (17) | 5 (1) |
| DMSO | (100 UL; POST) | (-) | 9 (3) | 9 (3) | 4 (2) | 14 (2) | 87 (18) | 5 (1) |
| DMSO | (100 UL; POST) | (+) | 10 (4) | 14 (2) | 9 (5) | 26 (9) | 81 (13) | 5 (1) |
| POOLED | | (-) | 10 (4) | 9 (3) | 4 (1) | 16 (2) | 83 (16) | 5 (2) |
| POOLED | | (+) | 11 (3) | 12 (3) | 10 (4) | 28 (7) | 88 (15) | 5 (1) |
| POSITIVE CONTROLS | UG/PL | | | | | | | |
| SODIUM AZIDE | 10.0 | (-) | 1248*(45) | --- (---) | --- (---) | --- (---) | 1343*(79) | --- (---) |
| 9-AMINOACRIDINE | 150 | (-) | --- (---) | 1504*(144) | --- (---) | --- (---) | --- (---) | --- (---) |
| 2-NITROFLUORENE | 5.00 | (-) | --- (---) | --- (---) | 558*(101) | 489*(91) | --- (---) | --- (---) |
| 2-ANTHRAMINE | 2.50 | (+) | 232*(12) | 349*(40) | 1498*(143) | 1863*(81) | 2077*(122) | --- (---) |
| EMNG | 2.00 | (-) | --- (---) | --- (---) | --- (---) | --- (---) | --- (---) | 979*(42) |
| 2-ANTHRAMINE | 80.0 | (+) | --- (---) | --- (---) | --- (---) | --- (---) | --- (---) | 466*(68) |

TEST ARTICLE: SK&F 108566-J

| DOSE LEVEL UG/PL | S-9 | TA1535 | TA1537 | TA1538 | TA98 | TA100 | UVR A |
|---|---|---|---|---|---|---|---|
| 50.0 | (-) | 12 (4) | 10 (7) | 4 (3) | 17 (3) | 82 (4) | 4 (3) |
| 167 | (-) | 12 (5) | 14 (6) | 3 (1) | 16 (4) | 79 (9) | 4 (2) |
| 500 | (-) | 10 (3) | 9 (4) | 3 (1) | 15 (2) | 82 (12) | 5 (2) |
| 1670 | (-) | 16 (5) | 12 (2) | 2 (2) | 20 (7) | 80 (13) | 5 (1) |
| 3330 | (-) | 10 (2) | 8 (4) | 4 (2) | 15 (2) | 81 (8) | 3 (1) |
| 5000 | (-) | 11 (6) | 9 (5) | 4 (3) | 16 (5) | 73 (2) | 3 (4) |
| 50.0 | (+) | 16 (3) | 8 (1) | 15 (3) | 30 (6) | 89 (16) | 3 (1) |
| 167 | (+) | 11 (4) | 8 (4) | 10 (4) | 25 (6) | 82 (4) | 3 (2) |
| 500 | (+) | 14 (3) | 8 (1) | 12 (5) | 34 (5) | 88 (11) | 5 (5) |
| 1670 | (+) | 7 (2) | 7 (2) | 9 (4) | 33 (7) | 105 (10) | 4 (1) |
| 3330 | (+) | 12 (3) | 7 (4) | 12 (4) | 22 (6) | 91 (11) | 4 (2) |
| 5000 | (+) | 13 (3) | 10 (4) | 11 (6) | 27 (3) | 78 (11) | 2 (2) |

Data Reported as: Mean (Standard Deviation)
Normal Growth all Strains/Doses +/- 59
No Precipitate

[a] Concurrent controls; Pre and Post refer to concurrent control plates evaluated before and after each series of test agent treated cultures.

TABLE 3. AMES/SALMONELLA-E. COLI LIQUID PRE-INCUBATION ASSAY
MEAN SUMMARY DATA

Sponsor: SMITHKLINE BEECHAM PHARMACEUTICALS
Test article: SK&F 108566-J
Description: WHITE POWDER
Other considerations: LOT# JF15943-164; INDEPENDENT RETEST

Date initiated: 09-23-1991
Date scored: 09-25-1991
Study ID: PH301-SK-007-91

CONTROLS

| NEGATIVE CONTROLS [a] | S-9 | AVERAGE REVERTANTS/PLATE TA1535 | TA1537 | TA1538 | TA98 | TA100 | UVR A |
|---|---|---|---|---|---|---|---|
| DMSO (100 UL; PRE) | (-) | 8 (4) | 7 (3) | 6 (4) | 13 (6) | 73 (18) | 6 (2) |
| DMSO (100 UL; PRE) | (+) | 7 (2) | 7 (8) | 10 (2) | 26 (4) | 85 (3) | 5 (2) |
| DMSO (100 UL; POST) | (-) | 8 (4) | 5 (2) | 4 (3) | 15 (4) | 83 (8) | 4 (2) |
| DMSO (100 UL; POST) | (+) | 5 (2) | 7 (2) | 9 (1) | 23 (8) | 91 (10) | 5 (2) |
| POOLED | (-) | 8 (3) | 6 (2) | 5 (3) | 14 (4) | 78 (13) | 5 (2) |
| POOLED | (+) | 6 (2) | 7 (5) | 10 (1) | 24 (6) | 88 (7) | 5 (1) |

| POSITIVE CONTROLS | UG/PL | S-9 | TA1535 | TA1537 | TA1538 | TA98 | TA100 | UVR A |
|---|---|---|---|---|---|---|---|---|
| SODIUM AZIDE | 10.0 | (-) | 1189+(47) | --- (---) | --- (---) | --- (---) | 981+(164) | --- (---) |
| 9-AMINOACRIDINE | 150 | (-) | --- (---) | 1641+(240) | --- (---) | --- (---) | --- (---) | --- (---) |
| 2-NITROFLUORENE | 5.00 | (-) | --- (---) | --- (---) | 462+(30) | 379+(32) | --- (---) | --- (---) |
| 2-ANTHRAMINE | 2.50 | (+) | 238+(100) | 420+(114) | 1939+(62) | 2387+(103) | 2286+(228) | --- (---) |
| EMS | 2.00 | (-) | --- (---) | --- (---) | --- (---) | --- (---) | --- (---) | 1228+(51) |
| 2-ANTHRAMINE | 80.0 | (+) | --- (---) | --- (---) | --- (---) | --- (---) | --- (---) | 544+(127) |

TEST ARTICLE: SK&F 108566-J

| DOSE LEVEL UG/PL | S-9 | TA1535 | TA1537 | TA1538 | TA98 | TA100 | UVR A |
|---|---|---|---|---|---|---|---|
| DOSE LEVEL UG/PL | S-9 | TA1535 | TA1537 | TA1538 | TA98 | TA100 | UVR A |
| 50.0 | (-) | 8 (4) | 12 (3) | 5 (2) | 14 (2) | 87 (5) | 3 (1) |
| 167 | (-) | 6 (3) | 4 (1) | 2 (2) | 14 (7) | 83 (3) | 4 (3) |
| 500 | (-) | 6 (3) | 5 (2) | 3 (2) | 10 (2) | 66 (6) | 3 (2) |
| 1670 | (-) | 5 (1) | 3 (2) | 4 (2) | 17 (6) | 79 (11) | 5 (3) |
| 3330 | (-) | 11 (2) | 5 (1) | 4 (2) | 16 (2) | 73 (12) | 3 (2) |
| 5000 | (-) | 8 (4) | 6 (4) | 5 (1) | 13 (4) | 65 (3) | 4 (3) |
| 50.0 | (+) | 11 (3) | 9 (5) | 10 (2) | 27 (3) | 94 (15) | 4 (1) |
| 167 | (+) | 5 (2) | 9 (1) | 9 (6) | 24 (7) | 83 (7) | 4 (3) |
| 500 | (+) | 7 (2) | 7 (2) | 7 (3) | 28 (3) | 92 (19) | 4 (1) |
| 1670 | (+) | 6 (2) | 9 (3) | 6 (1) | 20 (5) | 91 (17) | 4 (1) |
| 3330 | (+) | 9 (6) | 9 (3) | 8 (5) | 26 (2) | 98 (1) | 4 (3) |
| 5000 | (+) | 7 (2) | 12 (3) | 6 (2) | 21 (10) | 94 (18) | 4 (1) |

Data Reported as: Mean (Standard Deviation)
Normal Growth all Strains/Doses +/- 5%
No Precipitate

[a] Concurrent controls; Pre and Post refer to concurrent control plates evaluated before and after each series of test agent treated cultures.

Chromosomal Aberrations in Human Lymphocytes, *In Vitro*

Test Agent: Eprosartan Mesylate
(Lot Nos. PL-20581-23 and BCT-L-02P)

Study Facility:

Study Number: SB Study No. TF-1003/SKF-108566/1

Study Dates: May 7, 1992 to September 7, 1993

GLP Compliance: Statement indicates that these studies were conducted in compliance with GLP regulations.

Test System: Human peripheral lymphocytes cultured *in vitro* in the presence of test agent.

Procedure: Human peripheral lymphocytes were incubated for 4 hours with eprosartan mesylate in the presence or absence of rat liver S-9 from rats treated with Arochlor 1254. Twenty four (24) or 48 hours after incubation with eprosartan mesylate, metaphase arrested lymphocytes (~200 metaphase cells/concentration) were evaluated for structural chromosomal aberrations (gaps, breaks, acentric fragments, exchange figures and multiple aberrations) and numerical chromosome aberrations (aneuploidy and endoreduplication). For polyploidy, ≥2000 metaphase cells/concentration were evaluated. Lymphocytes were arrested in metaphase prior to cell harvest by incubating with colcemid for 2-3 hours. Eprosartan mesylate concentrations used in main assays were determined in a preliminary toxicity test at 39, 78, 156, 313, 625, 1250, 2500 and 5000 µg /ml. Toxicity was evaluated on the basis of a decrease in mitotic index [(number of metaphase cells/total cells) X100], total cell number and trypan blue exclusion. Main assays were performed in duplicate, with the exception that the concurrent control for the second main assay was performed in quadruplicate (second main assay). Eprosartan mesylate was solubilized with dimethylsulfoxide (DMSO) and stored in the dark prior to use. Solvent control was DMSO (0.5% or 1%). Positive controls were cyclophosphamide (with S-9) and mitomycin C (without S-9). All test agent concentrations are expressed as µg eprosartan mesylate/ml.

The sponsor's criteria for a positive response are listed below.
1. There is a statistically significant difference from concurrent control using Fisher's Exact test at P<0.045 (one-sided) after Bonferonni correction for multiple comparisons.
2. There is a positive trend in the concentration-response relationship.
*(No statistical test for this analysis was provided).*
3. At least one response exceeds the 99% confidence limit of laboratory historical control data.
*(Laboratory historical control: structural chromosomal aberrations, 0-3%; polyploidy, 0-0.5%)*
4. At least one result is two-fold greater than the negative control.
5. At least two assays yield qualitatively and quantitatively similar results.

Results: In the preliminary toxicity assay, eprosartan mesylate was cytotoxic at 2500 and 5000 µg/ml. At 2500 µg/ml, mitotic index was reduced by 50% without S-9 and by 60% with S-9. At 5000 µg/ml, mitotic index was zero without S-9, and no surviving cells were evident with S-9.

In the first main assay, eprosartan mesylate at 2000 µg/ml with S-9 increased structural chromosomal aberrations significantly at 24 hour post treatment (Table 1). This concentration exhibited cytotoxicity, with mitotic index reduced by 44% from concurrent control; dead cells were also observed at this concentration (dead cells not seen in the concurrent control). Eprosartan mesylate without S-9 did not increase structural chromosomal aberrations at any concentration evaluated (Table 2). Numerical chromosomal aberrations were not scored with or without S-9 in this assay. Eprosartan mesylate at 2000 µg/ml reduced pH compared to concurrent control. The effects of eprosartan mesylate at 48 hours post-treatment are not shown since there were no eprosartan mesylate-related findings.

In the second main assay, eprosartan mesylate with and without S-9 did not increase structural chromosomal aberrations compared to concurrent control (Tables 1, 2). However eprosartan mesylate at 2500 µg/ml without S-9 tended to induce polyploidy. Mitotic index was reduced by 60% at 2500 µg/ml without S-9. Similar to the first main assay, eprosartan mesylate reduced the pH of the cell culture medium compared to the concurrent control in a concentration related manner, both with and without S-9. The influence of acidic pH on chromosomal aberrations and toxicity was evaluated by including additional negative controls with pH reduced by addition of 1 N HCl. With S-9, a pH of 6.57 was completely cytotoxic whereas a pH of 6.68 was nontoxic and did not produce chromosomal aberrations. Without S-9, a pH of 6.32 was nontoxic; a pH of 6.44 did not produce chromosomal aberrations compared to concurrent control but was slightly cytotoxic since it reduced mitotic index by 16%.

A third main assay was performed only with S-9 to resolve the difference in findings with S-9 seen in the first and second main assays. Results of this third assay were equivocal. Although there was a statistically significant increase in structural chromosomal aberrations with eprosartan mesylate at 2000 µg/ml compared to concurrent control, the percentage of cells with chromosomal aberrations was within the historical control range (Tables 1,3). Indeed, the

structural chromosomal aberration frequency seen with eprosartan mesylate in this assay was noted in about 6% of historical negative controls, and was similar to the concurrent control frequency in the second assay with eprosartan mesylate.

Eprosartan mesylate at 2500 µg/ml, with S-9, significantly increased the number of polyploid cells compared to concurrent control. Mitotic index was minimally (-8%) to moderately (-33%) reduced at 2000 and 2500 µg/ml, respectively. Cell debris was observed at both 2000 and 2500 µg/ml (not seen in the concurrent control). Eprosartan mesylate, with S-9, reduced pH at 2000 and 2500 µg/ml compared to the concurrent control.

The positive controls, mitomycin C and cyclophosphamide, increased structural chromosomal aberrations compared to concurrent control in all three main assays, as expected (Tables 1 and 2). Historical control data are summarized in Table 3.



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Table 1.
4-Hour Eprosartan Mesylate Exposure With S-9
(Twenty Four Hour Post-Treatment)

| Assay Number | Eprosartan mesylate (µg/ml) | pH | Mitotic Index (metaphase cells/total cells X 100) | | Chromosomal Aberrations | | | |
|---|---|---|---|---|---|---|---|---|
| | | | (% Cells) | (% Change from Concurrent Control) | Structural Aberrations | | Polyploidy | |
| | | | | | Aberrant/ Metaphase Cells | (% Cells) | Aberrant/ Metaphase Cells | (% Cells) |
| Assay 1 | 0 | ND | 3.03 | 0 | 1/200 | 0.50 | ND | ND |
| | 2000** | 6.50 | 1.69 | -44 | 11/200 | 5.50[abc] | | |
| | Cyclophosphamide (14 µg/ml) | ND | ND | ND | 17/200 | 8.50 | | |
| Assay 2 | 0 | 7.09 | 4.07 | 0 | 10/400 | 2.50 | 9/4019 | 0.23 |
| | 0 (pH Controls)* | 6.68 | 7.58 | +86 | 2/149 | 1.34 | ND | ND |
| | | 6.57 | Toxicity; chromosomal aberrations not scored | | | | | |
| | 1000 | 6.83 | 4.02 | -1 | 0/200 | 0 | 5/2055 | 0.24 |
| | 1750 | 6.74 | 4.53 | -11 | 3/200 | 1.50 | 1/2031 | 0.05 |
| | 2000 | 6.63 | 3.13 | -23 | 4/200 | 2.00 | 6/2006 | 0.30 |
| | Cyclophosphamide (14 µg/ml) | ND | ND | ND | 14/200 | 7.00 | ND | ND |
| Assay 3 | 0 | 7.25 | 9.34 | 0 | 0/200 | 0 | 11/4111 | 0.27 |
| | 1500 | 7.09 | 12.13 | +30 | ND | ND | 4/3115 | 0.12 |
| | 2000*** | 6.93 | 8.57 | -8 | 8/400 | 2.0[ac] | 23/4023 | 0.57 |
| | 2500*** | 6.92 | 6.24 | -33 | ND | ND | 33/3783 | 0.87[abc] |
| | Cyclophosphamide (7 µg/ml) | ND | ND | ND | 6/100 | 6.0 | ND | ND |
| | Cyclophosphamide (14 µg/ml) | ND | ND | ND | 11/100 | 11.0 | ND | ND |

ND, not determined.
* Negative controls with pH adjusted using 1N HCl.
** Dead cells were observed on the slide.
*** Cell debris observed on the slides.
[a] Significantly different than concurrent control by Fisher's exact test with Bonferonni correction for multiple comparisons (Assay 1, P≤0.006; Assay 2, P≤0.002; Assay 3, P≤0.01).
[b] Response exceeds the 99% confidence limit of laboratory historical control data.
*(Historical control: structural chromosomal aberrations, 0-3%; polyploidy, 0-0.5%)*
[c] Value more than two-fold greater than the concurrent negative control.

Table 2.
4-Hour Eprosartan Mesylate Exposure Without S-9
(Twenty Four Hour Post-Treatment)

| Assay Number | Eprosartan Mesylate (µg/ml) | pH | Mitotic Index (metaphase cells/total cells X 100) | | Chromosomal Aberrations | | | |
|---|---|---|---|---|---|---|---|---|
| | | | (% Cells) | (% Change from Concurrent Control) | Structural Aberrations | | Polyploidy | |
| | | | | | Aberrant/ Metaphase Cells | (% Cells) | Aberrant/ Metaphase Cells | (% Cells) |
| Assay 1 | 0 | 7.17 | 1.53 | 0 | 2/200 | 1.00 | ND | |
| | 2000 | 6.81 | 1.63 | +6 | 3/200 | 1.50 | | |
| | Mitomycin C (0.5 µg/ml) | ND | ND | ND | 28/175 | 16.00 | | |
| Assay 2 | 0 | 7.15 | 7.34 | 0 | 8/400 | 2.00 | 3/4113 | 0.07 |
| | 0 (pH Controls)* | 6.44 | 6.13 | -16 | 3/200 | 1.50 | 5/2025 | 0.25 |
| | | 6.32 | 8.13 | +10 | ND | | ND | |
| | 1000 | 6.86 | 5.65 | -23 | 2/200 | 1.00 | 5/2085 | 0.24 |
| | 1500 | 6.77 | 5.28 | -28 | 3/200 | 1.50 | 1/2061 | 0.05 |
| | 2000 | 6.62 | 3.75 | -50 | 6/200 | 3.00 | 8/2218 | 0.36 |
| | 2500** | 6.70 | 2.91 | -60 | 4/100 | 4.00 | 6/1000 | 0.60[abc] |
| | Mitomycin C (0.5 µg/ml) | ND | ND | ND | 30/200 | 15.00 | ND | |

ND, not determined
* Negative controls with pH adjusted using 1N HCl.
** Single plate evaluated since no metaphase cells were seen in duplicate plate.
[a] Tendency (P=0.003) for difference from concurrent control by Fisher's exact test with Bonferonni correction for multiple comparisons (P≤0.002 for statistical significance).
[b] Response exceeds the 99% confidence limit of laboratory historical control data. *(Historical control for polyploidy, 0-0.5%)*
[c] Value more than two-fold greater than the concurrent negative control.

Table 3.
Historical Control Data: Human Peripheral Lymphocyte Assay

Structural Chromosomal Aberrations

| Chromosomal Aberrations (% of Cells)[a] | With S-9 | | Without S-9 | |
|---|---|---|---|---|
| | Frequency of Studies | % of Studies | Frequency of Studies | % of Studies |
| 5 | 0/68 | 0 | 1/78 | 1 |
| 4 | 1/68 | 1.5 | 2/78 | 3 |
| 3 | 1/68 | 1.5 | 5//78 | 6 |
| 2 | 4/68 | 6 | 6/78 | 8 |
| 1 | 23/68 | 34 | 22/78 | 28 |
| 0 | 39/68 | 57 | 42/78 | 54 |
| Total Number of Control Studies | n=68 | | n=78 | |

[a] Percent of 100 metaphase cells evaluated.

Polyploidy

| Polyploidy (% of Cells)[b] | With S-9 | | Without S-9 | |
|---|---|---|---|---|
| | Frequency of Studies | % of Studies | Frequency of Studies | % of Studies |
| 0.5 | 1/11 | 9 | 1/11 | 9 |
| 0.4 | 0/11 | 0 | 0/11 | 0 |
| 0.3 | 0/11 | 0 | 0/11 | 0 |
| 0.2 | 1/11 | 9 | 3/11 | 27 |
| 0.1 | 2/11 | 18 | 3/11 | 27 |
| 0 | 7/11 | 64 | 4/11 | 36 |
| Total Number of Control Studies | n=11 | | n=11 | |

[b] Percent of 1000 metaphase cells evaluated.

Mouse Lymphoma Assay

Test Agent: Eprosartan Mesylate (Lot No. PL-20581-23)

Study Facility: SmithKline Beecham Pharmaceuticals
Research and Development
Toxicology Department
Genetic Toxicology Unit
The Frythe, Welwyn, UK

Study Numbers: TF-1001/SKF-108566/1

Study Dates: June 2, 1992 to June 9, 1992

GLP Compliance: Statement indicates that these studies were conducted in compliance with GLP regulations.

Test System: L5178Y 3.7.2C (TK+/-) mouse lymphoma cells

Procedure: This assay is based on selection of mouse lymphoma cells heterozygous at the thymidine kinase locus (TK+/-) that have undergone forward mutation to TK-/-. L5178Y cells in exponential growth are incubated for 4-hours at 37°C in the presence of eprosartan mesylate with or without liver S-9 from Arochlor 1254 treated rats. Following exposure to eprosartan mesylate, cells are washed and then grown for another 48 hours to allow for expression of TK-/- mutants. At the end of the expression period, cells are grown in the presence of trifluorothymidine at 4 µg/ml, which selects for mutants by allowing only TK-/- cells to grow as colonies. TK-/- colonies are counted and compared to concurrent control.

A preliminary toxicity assay was performed with eprosartan mesylate in ethanol at 5.2, 10.4, 20.8, 41.7, 83.3, 166.7, 333.3, and 666.7 µg/ml in the presence and absence of S-9. Eprosartan mesylate was evaluated for mutagenicity in the first two assays at concentrations (in ethanol) of 198, 296, 444 and 666 µg/ml with and without S-9. Solubility of eprosartan mesylate in ethanol was limited. However, it was later determined that eprosartan mesylate could be dissolved in DMSO at higher concentrations. Therefore, eprosartan mesylate was evaluated for mutagenicity in DMSO at 2500 and 2750 µg/ml in the presence of S-9 (assays 3 and 4), and at 2500, 2750, 3000 and 3250 µg/ml (assay 3) and at 2310, 2540, 2780 and 3000 (assay 4) without S-9. All concentrations are expressed as µg eprosartan mesylate/ml. Toxicity was evaluated in all mutagenicity assays as suppression of relative growth compared to concurrent control; relative growth was evaluated prior to selecting for TK-/- mutants by incubating with trifluorothymidine. Treatment and positive control groups were evaluated in duplicate. Negative controls were evaluated in quadruplicate.

The positive controls were ethyl methanesulphonate (EMS) at 600 µg/ml in the absence of S-9 and benzo(a)pyrene (B(a)P) at 1.25 µg/ml and 3-methylcholanthrene (3-MCA) at 2.5 µg/ml in the presence of S-9.

The sponsor provided three criteria for a positive response, which are listed below.

1. At least one test agent related result is significantly different than concurrent control at $P<0.005$.
2. Colony count in the treatment group must be greater than 2-fold that of the concurrent control.
3. Duplicate assays must be quantitatively and qualitatively similar.

Results: Eprosartan mesylate was nontoxic in the preliminary toxicity assay at concentrations up to 666.7 µg/ml with or without S-9. Eprosartan mesylate did not increase TK-/- colony count over concurrent control at concentrations ≤ 666 µg/ml with or without S-9 (Tables 1,2).

Eprosartan mesylate at 2500 and 2750 µg/ml with S-9 did not increase TK-/- colony count over concurrent control (Tables 3,4). Significant toxicity was noted at these concentrations with S-9, since relative suspension growth was suppressed by 60-70% at 2750 µg/ml compared to concurrent control. Eprosartan mesylate at concentrations up to 3250 µg/ml without S-9 also did not increase TK-/- colonies compared to concurrent control; significant concentration-related toxicity was noted at these concentrations without S-9, with relative growth suppressed by 40-80% at ≥ 3000 µg/ml. The positive controls increased TK-/- colony count compared to concurrent control, as expected.



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Table 1

FIRST MUTATION ASSAY - TREATMENT MEANS

METABOLIC ACTIVATION: YES

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| EtOH | 10 µl/ml | 100.0 | 9.5 | - |
| SK&F 108566-J | 666 | 108.4 | 6.8 | 0.7 |
| SK&F 108566-J | 444 | 106.7 | 8.3 | 0.9 |
| SK&F 108566-J | 296 | 96.3 | 7.6 | 0.8 |
| SK&F 108566-J | 198 | 110.7 | 4.8 | 0.5 |
| B(a)P | 1.25 | 114.6 | 21.6* | 2.3 |

METABOLIC ACTIVATION: NO

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| EtOH | 10 µl/ml | 100.0 | 4.8 | - |
| SK&F 108566-J | 666 | 99.8 | 6.6 | 1.4 |
| SK&F 108566-J | 444 | 84.7 | 8.6* | 1.8 |
| SK&F 108566-J | 296 | 99.9 | 8.7* | 1.8 |
| SK&F 108566-J | 198 | 105.0 | 6.3 | 1.3 |
| EMS | 600 | 43.7 | 82.9* | 17.3 |

* Significantly different from controls p <0.005.

Table 2

SECOND MUTATION ASSAY - TREATMENT MEANS

METABOLIC ACTIVATION: YES

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| EtOH | 10 µl/ml | 100.0 | 13.4 | - |
| SK&F 108566-J | 666 | 111.4 | 10.8 | 0.8 |
| SK&F 108566-J | 444 | 101.8 | 10.0 | 0.7 |
| SK&F 108566-J | 296 | 83.0 | 15.7 | 1.2 |
| SK&F 108566-J | 198 | 101.2 | 11.3 | 0.8 |
| B(a)P | 1.25 | 110.9 | 37.0* | 2.8 |

METABOLIC ACTIVATION: NO

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| EtOH | 10 µl/ml | 100.0 | 7.1 | - |
| SK&F 108566-J | 666 | 121.4 | 4.7 | 0.7 |
| SK&F 108566-J | 444 | 117.9 | 7.3 | 1.0 |
| SK&F 108566-J | 296 | 137.0 | 4.7 | 0.7 |
| SK&F 108566-J | 198 | 117.1 | 6.9 | 1.0 |
| EMS | 600 | 61.5 | 58.1* | 8.2 |

* Significantly different from controls p <0.005.

Table 3

THIRD MUTATION ASSAY - TREATMENT MEANS

METABOLIC ACTIVATION: YES

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| DMSO | 10 µl/ml | 100.0 | 4.4 | - |
| SK&F 108566-J | 2750 | 31.7 | 4.8 | 1.1 |
| SK&F 108566-J | 2500 | 75.1 | 5.2 | 1.2 |
| 3-MCA | 2.5 | 83.3 | 8.9* | 2.0(2.02) |

METABOLIC ACTIVATION: NO

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| DMSO | 10 µl/ml | 100.0 | 4.9 | - |
| SK&F 108566-J | 3250 | 57.3 | 4.6 | 0.9 |
| SK&F 108566-J | 3000 | 68.4 | 7.2 | 1.5 |
| SK&F 108566-J | 2750 | 80.3 | 4.7 | 0.9 |
| SK&F 108566-J | 2500 | 86.8 | 4.0 | 0.8 |
| EMS | 600 | 38.4 | 78.1* | 15.9 |

* Significantly different from controls p <0.005.

Table 4

FOURTH MUTATION ASSAY - TREATMENT MEANS

METABOLIC ACTIVATION: YES

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| DMSO | 10 µl/ml | 100.0 | 4.4 | - |
| SK&F 108566-J | 2750 | 40.9 | 5.3 | 1.2 |
| SK&F 108566-J | 2500 | 59.7 | 5.5 | 1.3 |
| 3-MCA | 2.5 | 88.7 | 11.2* | 2.5 |

METABOLIC ACTIVATION: NO

| TREATMENT | µg/ml | % RELATIVE TOTAL GROWTH | MUTATION FREQUENCY PER $10^5$ cfu | FOLD INCREASE IN MF OVER CONTROLS |
|---|---|---|---|---|
| DMSO | 10 µl/ml | 100.0 | 5.6 | - |
| SK&F 108566-J | 3000 | 18.7 | 8.2 | 1.7 |
| SK&F 108566-J | 2780 | 52.5 | 6.7 | 1.2 |
| SK&F 108566-J | 2540 | 82.7 | 6.0 | 1.1 |
| SK&F 108566-J | 2310 | 68.4 | 5.9 | 1.1 |
| EMS | 600 | 28.2 | 60.1* | 10.7 |

* Significantly different from controls $p < 0.005$.

**Chromosomal Aberrations (Micronucleus Test) *In Vivo* in Mouse Bone Marrow**

Test Agent: Eprosartan Mesylate (Lot No. PL20581-23)

Study Facilities: SmithKline Beecham Pharmaceuticals
Research and Development
Toxicology Department
Genetic Toxicology Unit
The Frythe, Welwyn, UK

Study No.: TF-1002/SKF-108566/1

Study Dates: Preliminary toxicity test: May 18, 1992 to May 20, 1992
Main Test: July 7, 1992 to July 9, 1992

GLP Compliance: Statement indicates that these studies were conducted in compliance with GLP regulations.

***Preliminary Toxicity Assay***

Animals: Crl:CD-1[ICR] BR mice. Males weighed 20.0-28.8 g; females weighed 20.4-26.7 g.

Procedure: The acute toxicity of eprosartan mesylate was determined. The drug was suspended in aqueous 1% w/v sodium carboxymethylcellulose, and administered by gavage at two daily doses of 156, 313, 625, 1250 and 2500 mg eprosartan mesylate/kg. Three mice/sex were included at each dose level. Observations for clinical signs and deaths were made at the beginning and the end of each dosing day. All surviving mice were sacrificed 24 hours after the final dose; bone marrow smears were prepared from femora from all surviving mice for determination of bone marrow toxicity.

Results: All mice survived to sacrifice. There were no clinical or bone marrow toxicities noted at any dose of eprosartan mesylate administered.

*Chromosomal Aberration Assay*

Animals: Crl:CD-1[ICR] BR mice. Males weighed 22.4-27.5 g; females weighed 19.9-25.3 g.

Procedure: Eprosartan mesylate was suspended in aqueous 1% w/v sodium carboxymethyl-cellulose, and administered by gavage at 1250 and 2500 mg eprosartan mesylate/kg/day for two days. Six mice/sex were included in each dose level. Clinical signs and deaths were noted at the beginning and the end of each dosing day. All surviving mice were sacrificed 24 hours after the final dose on day 3 (48 hours after the first dose); bone marrow smears were prepared from femora from all surviving mice for determination of bone marrow toxicity.

Aqueous 1% w/v sodium carboxymethylcellulose (20 ml/kg) was given orally by gavage as the negative control. The positive control, cyclophosphamide (75 mg/kg), was given orally by gavage to four mice/sex as a single dose; mice given cyclophosphamide were sacrificed 48 hours after dosing.

Bone marrow cells were prepared from femora of mice that survived to scheduled sacrifice, and slides prepared for morphological evaluation of chromosomal aberrations. Surviving animals were chosen for analysis in numerical order, using the animal identification numbers. Two thousand (2000) polychromatic erythrocytes were scored per animal given eprosartan mesylate. The primary endpoint is the number of micronucleated polychromatic erythrocytes.

The sponsor considered a response to be positive if the following criteria were met.
1. The number of micronucleated polychromatic erythrocytes (MNPCE's) was significantly increased over the combined (male plus female) control mean by Dunnet's test at $P \leq 0.05$.
2. The increase in MNPCE's over concurrent control was 1.5-fold the historic median negative control value.
3. The MNPCE's for test agent exceeded the 98% confidence limits of the historical control values.

Results: Bone marrow toxicity in the chromosomal aberration assay was absent after 2 days of treatment with 2500 mg eprosartan mesylate/kg/day (percentage of erythrocytes with polychromatic nuclei similar to concurrent control). The report states that genotoxicity was not evaluated in mice given 1250 mg/kg/day due to the lack of toxicity at 2500 mg/kg/day. Consequently, the only dose evaluated for genotoxicity was 2500 mg/kg/day.

| Treatment | Sex | No. of animals | Polychromatic Erythrocytes | |
|---|---|---|---|---|
| | | | Number Scored | % of Total Erythrocytes[c] |
| Negative Control[a] | M | 5 | 10,000 | 43±4 |
| | F | 5 | 10,000 | 42±4 |
| Eprosartan mesylate[a] (2500 mg/kg/day; two daily doses) | M | 5 | 10,000 | 41±3 |
| | F | 5 | 10,000 | 41±2 |
| Cyclophosphamide[b] (75 mg/kg; single dose) | M | 3 | 3,000 | 48±3 |
| | F | 3 | 3,000 | 48±4 |

[a] 2000 polychromatic erythrocytes were scored per animal
[b] 1000 polychromatic erythrocytes were scored per animal
[c] Mean ± SD

One female was found dead about 17 hours following the first 1250 mg/kg dose of eprosartan mesylate. Additionally, one female given 2500 mg/kg exhibited clinical signs of toxicity (abdominal distention, piloerection, hunched posture and gasping) about 22 hours after the first dose and was sacrificed in extremis. The sponsor considered these findings to be due to dosing error given the inconsistency with the preliminary toxicity assay.

In surviving male and female mice given eprosartan mesylate at 2500 mg/kg/day, there were no increases in the number of micronucleated polychromatic erythrocytes compared to concurrent control. The positive control, cyclophosphamide, increased the number of micronucleated polychromatic erythrocytes, as expected. Statistical analysis was not performed.

| Treatment | Sex | No. of animals | Polychromatic Erythrocytes | | |
|---|---|---|---|---|---|
| | | | Number of Cells Scored | No. of Cells with Micronuclei | % of Cells with Micronuclei[c] |
| Negative Control[a] | M | 5 | 10,000 | 5 | 0.05 ±0.05 |
| | F | 5 | 10,000 | 2 | 0.02 ±0.03 |
| Eprosartan mesylate[a] (2500 mg/kg/day; two daily doses) | M | 5 | 10,000 | 2 | 0.02 ±0.03 |
| | F | 5 | 10,000 | 4 | 0.04 ±0.07 |
| Cyclophosphamide[b] (75 mg/kg; single dose) | M | 3 | 3,000 | 88 | 2.93 ±0.65 |
| | F | 3 | 3,000 | 43 | 1.43 ±0.25 |

[a] 2000 polychromatic erythrocytes were scored per animal
[b] 1000 polychromatic erythrocytes were scored per animal
[c] Mean ± SD

## CARCINOGENICITY

### 24-Month Carcinogenicity Study in Rats

Study Facility: SmithKline Beecham Pharmaceuticals
Department of Toxicology
Department of Metabolism and Pharmacokinetics

Study Number: G93047

Study Dates: Initiation of Treatment: 11/14/93
Termination of Treatment and Necropsy: 11/16/95

GLP Compliance: Statement indicates that these studies were conducted in compliance with GLP regulations.

Animals: Male and female Sprague-Dawley rats (60/sex/dose group, were approximately 8 weeks of age at start of study. Body weights at start of study were 234-313 g for males and 153-200 g for females. Rats were housed individually in stainless steel cages.

Diet: Male and female rats were provided approximately 21 and 16 g, respectively, per day of 5002 This feeding regime was implemented approximately 2 weeks prior to the initiation of dosing. Feed was provided after the daily dosing during the two year dosing period; the time of feeding after dosing was not provided. Filtered tap water was allowed *ad libitum*.

Drug Administration: Eprosartan mesylate (Lot No's. BCT-K-07C, Kegs 1 and 2; BCT-K-12C, Keg 1) was given orally by gavage. Suspensions of drug were prepared weekly at concentrations of 3, 10 and 60 mg/ml in 1% (w/v) carboxymethylcellulose. Vehicle was given by gavage at an equivalent volume of 10 ml/kg/day. Dose was based on the most recent body weight.

Dose Levels: 0, 0, 30, 100 and 600 mg of eprosartan/kg/day

Observations/Measurements: All rats were observed daily for mortality and clinical signs. Detailed clinical examinations, including observations for palpable masses, were performed about every 4 weeks. Body weights were recorded weekly. Plasma drug concentrations were determined from blood samples taken from the tail veins of 5 rats/sex/group at 1, 3, 6, 9 and 24 hours after dosing on study days 29 and 366. Plasma drug concentrations were also determined from a separate group of rats with blood samples taken on study day 1 at the same time points; this group of rats was sacrificed after blood samples were taken. Rats that died or

were sacrificed *in extremis* were necropsied. All surviving rats were sacrificed and necropsied at the end of the 2 year dosing period (730 consecutive daily doses of test agent). The following tissues were collected at necropsy and examined macroscopically for all dose groups. Tissues marked with an asterisk were examined microscopically. Microscopic analysis of marked tissues was performed for all rats in the concurrent control and 600 mg/kg/day dose groups and for all rats that died or were sacrificed in extremis. Additionally, mammary glands of females and pituitary glands of males and females in all dose groups were examined microscopically.

**Macroscopic Observations**

The following tissues were collected at necropsy:

| | |
|---|---|
| Adrenal Glands* | Pancreas* |
| Animal Identification | Parathyroids* |
| Aorta, thoracic* | Pituitary* |
| Blood Smears** | Preputial/Clitoral Gland* |
| Brain* | Prostate* |
| Cecum* | Rectum |
| Cervix | Rib (CCJ) |
| Colon* | Salivary Glands (both sets)*§, mandibular, sublingual, parotid |
| Duodenum* | Seminal Vesicles*§ |
| Epididymides*√ | Skin* |
| Esophagus* | Skull, base, nose, ear canals and accessory tissues |
| Eyes*√√/Optic Nerves* | Spinal Cord, lumbar* |
| Harderian Gland | Spleen* |
| Heart* | Sternebrae* (includes skeletal muscle* and bone marrow*) |
| Hind limb, right | Stomach* |
| Ileum* | Testes*√ |
| Jejunum* | Thymus* |
| Kidneys* | Thyroid* |
| Larynx | Tongue |
| Liver* | Trachea* |
| Lung* | Urinary Bladder* |
| Lymph Nodes, mandibular* | Uterus* |
| Lymph Nodes, mesenteric* | Vagina* |
| Macroscopic Observations* | |
| Mammary Gland* | |
| Ovaries* | |

√ Testes and epididymides were fixed in Bouin's fixative.

** Prepared, but not examined, from each rat killed terminally, or when feasible, from animals killed *in extremis*.

√√ Eyes were fixed in Davidson's fixative.

§ One side examined.

Results

*Animal Survival:* The number of rats surviving to scheduled sacrifice at two years is shown.

| Dose (mg/kg/day) | Rats Surviving to Scheduled Sacrifice | |
|---|---|---|
| | Males | Females |
| 0 | 39 | 24 |
| 0 | 36 | 26 |
| 30 | 41 | 27 |
| 100 | 36 | 23 |
| 600 | 33 | 22 |

Several deaths were attributed to gavage related accidents.

| Dose (mg/kg/day) | Deaths Attributed to Dosing Errors | |
|---|---|---|
| | Males | Females |
| 0 | 4 | 1 |
| 0 | 2 | 0 |
| 30 | 2 | 0 |
| 100 | 5 | 1 |
| 600 | 9 | 2 |

The sponsor's statistical analysis (which excluded accidental deaths) showed no significant differences between survival of drug treated and concurrent control rats (Figures 1 and 2). The FDA statistical analysis also found no treatment related effect on survival when accidental deaths were excluded; however, when accidental deaths were not excluded, the FDA analysis indicated that male survival at 600 mg/kg/day was slightly less than concurrent control ($P<0.05$).

Figure 1
Kaplan-Meier Survival Curves for Male Rats
SK&F 108566-J: Two Year Carcinogenicity Study

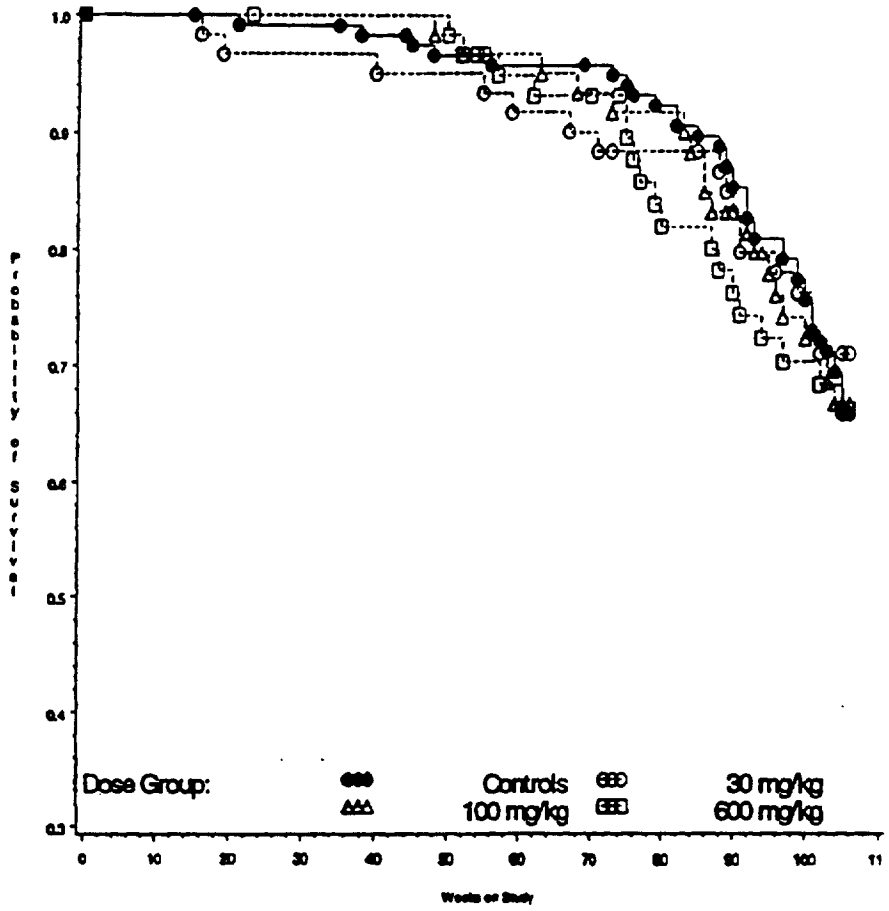
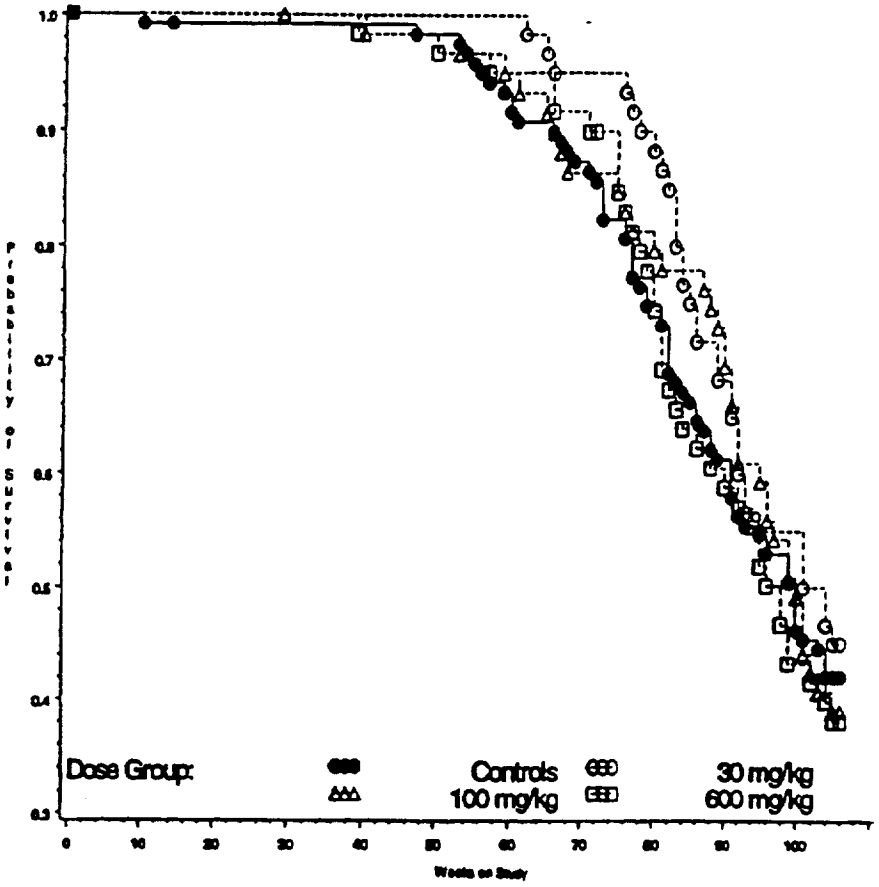


Figure 2
Kaplan-Meier Survival Curves for Female Rats
SK&F 108566-J: Two Year Carcinogenicity Study



The mean survival time for high dose male rats was slightly shorter than for concurrent controls when accidental deaths were not excluded; survival time was not affected by drug when accidental deaths were excluded. The mean survival time of females was not influenced by eprosartan, irrespective of whether accidental deaths were excluded.

| Dose (mg/kg/day) | Mean Survival Time (Weeks of Treatment) | | | |
|---|---|---|---|---|
| | All Rats | | Rats Excluding Accidental Deaths | |
| | Males | Females | Males | Females |
| 0 | 96.53 | 90.47 | 99.34 | 91.12 |
| 30 | 96.03 | 95.42 | 96.85 | 95.42 |
| 100 | 97.55 | 92.03 | 98.93 | 93.10 |
| 600 | 92.25 | 90.63 | 97.19 | 91.5 |

*Body Weight:* There was no consistent effect of test agent on body weight compared to concurrent controls (Figures 3 and 4).

**Figure 3 - SK&F 108566-J: 2 Year Oral Carcinogenicity Study in Rats**

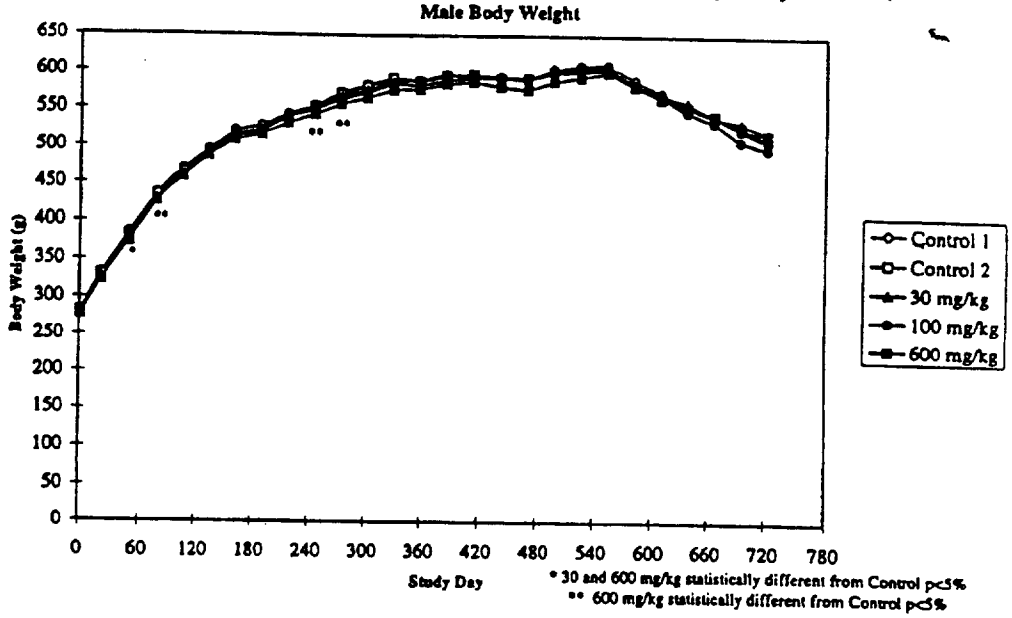
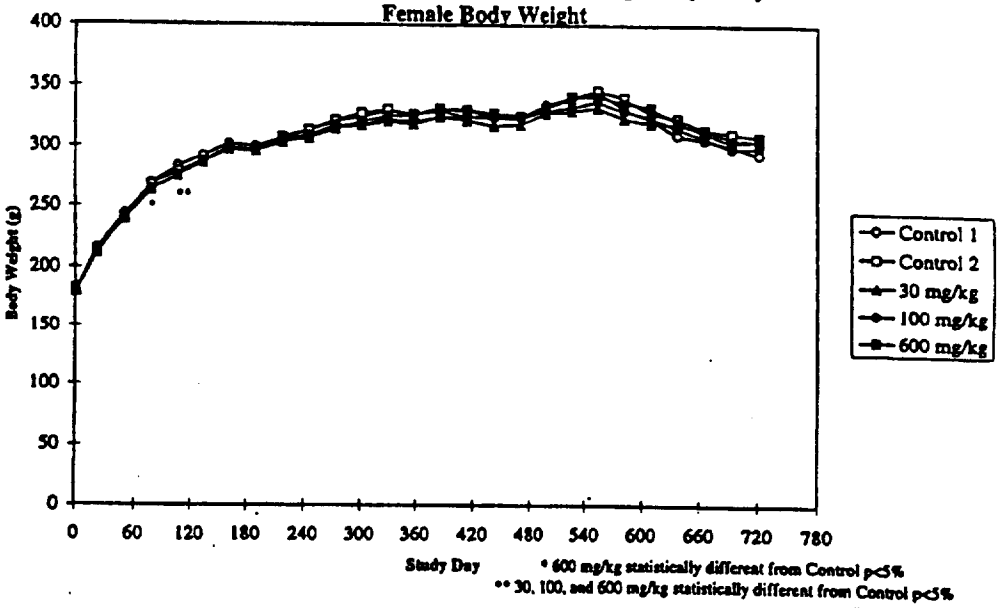



**Figure 4 - SK&F 108566-J: 2 Year Oral Carcinogenicity Study in Rats**



*Food Consumption:* The incidence of rats that did not eat their daily food allotment was not influenced by test agent.

| Dose (mg/kg/day) | Incidence of rats with greater than one food pellet (2.5-4.2 g) remaining | |
|---|---|---|
| | Male | Female |
| 0 | 7 | 7 |
| 0 | 5 | 7 |
| 30 | 5 | 8 |
| 100 | 7 | 12 |
| 600 | 10 | 5 |

*Clinical Signs:* There were no drug related clinical signs.

*Palpable Masses:* There were no significant differences in palpable masses in drug treated rats compared to concurrent controls. The incidence of palpable masses was calculated based upon the number of rats that were observed with a mass at any time during the course of the study (no data exclusion for masses not found at a later date).

| Dose (mg/kg/day) | Percentage of Rats with Palpable Masses | |
|---|---|---|
| | Males | Females |
| 0 | 18 | 80 |
| 0 | 32 | 78 |
| 30 | 35 | 82 |
| 100 | 30 | 92 |
| 600 | 23 | 75 |

*Postmortum Findings:* The incidences of lung necrosis, edema and hemorrhage were greater in drug treated males than in the combined male concurrent control groups. The sponsor attributed excess lung lesions in male rats to dosing errors. Other non-neoplastic lesions were similar in drug treated and concurrent control rats (Appendix 1a).

| Dose (mg/kg/day) | Incidence of Male Rats with Abnormal Lung Findings | | |
|---|---|---|---|
| | Necrosis | Edema | Hemorrhage |
| 0 | 0 | 2 | 1 |
| 0 | 0 | 2 | 2 |
| 30 | 0 | 4 | 3 |
| 100 | 0 | 6 | 4 |
| 600 | 9 | 9 | 6 |

The incidence of neoplastic lesions of any specific type was not significantly different in male and female rats given 600 mg/kg/day compared to their respective concurrent control groups (Appendix Ib, FDA Statistical Review and Evaluation).

The overall number of rats with tumors (from any site) was not affected by test agent.

| Sex | Dose (mg/kg/day) | Number of Rats Evaluated | Number of Rats with Tumors | Number of Rats with Benign Tumors | Number of Rats with Malignant Tumors |
|---|---|---|---|---|---|
| Male | 0 | 60 | 39 | 36 | 9 |
| | 0 | 60 | 45 | 36 | 16 |
| | 600 | 60 | 40 | 32 | 13 |
| Female | 0 | 60 | 57 | 53 | 33 |
| | 0 | 60 | 59 | 55 | 29 |
| | 600 | 60 | 58 | 56 | 21 |

*Toxicokinetics:* Systemic exposure to eprosartan was similar in male and female rats. AUC and $C_{max}$ were dose-proportional at 30 and 100 mg/kg/day. However, at 100-600 mg/kg/day, increases occurred in a less than dose-proportional manner. AUCs were more variable on day 366 than on days 1 and 29. At all doses, AUCs for eprosartan were lower than those reported for humans at the maximum recommended daily oral dose of 800 mg, qd. At the highest dose administered to rats, AUCs were approximately 20-50% (depending on when determined) of the average clinical AUC at 800 mg/day, qd given for 7 days (9521 ng.hr/ml). It should be noted that there is the possibility that the maximum recommended human daily dose may be divided into two daily doses for improved efficacy; in this case, relative exposure in rats would be even less than that with once daily dosing.

**Mean Toxicokinetic Data (S.D.)**

| | | DAY 1 | | DAY 29 | | DAY 366 | |
|---|---|---|---|---|---|---|---|
| DOSE mg/kg/day | SEX (n=5) | Cmax [ng/mL] | AUC[0-t] [ng.h/mL] | Cmax [ng/mL] | AUC[0-t] [ng.h/mL] | Cmax [ng/mL] | AUC[0-t] [ng.h/mL] |
| 30 | Male | 148 (62.4) | 433 (231) | 82.3* (23.1) | 168 (n=1) | 144 (62.9) | 477 (242) |
| 100 | Male | 529 (148) | 1044 (281) | 534 (145) | 1031 (303) | 621 (139) | 2282 (1250) |
| 600 | Male | 1317 (621) | 3396 (1533) | 1155 (632) | 2903 (1122) | 856 (573) | 3759 (1511) |
| 30 | Female | 93.9* (22.8) | 299 (48) | 77.5 (28.0) | 197** (54) | 193 (95.6) | 821* (385) |
| 100 | Female | 437 (345) | 930 (504) | 409 (67.2) | 1404 (524) | 464 (173) | 1586 (427) |
| 600 | Female | 696 (218) | 1885 (538) | 647 (186) | 2041 (551) | 1094 (291) | 5100 (2461) |

* n=4; ** n=3.

**24-Month Carcinogenicity Study in Mice**

Study Facility: Bio-Research Laboratories Ltd.
87 Senneville Road
Senneville, Quebec, Canada H9X 3R3

Study Number: G93095

Study Dates: Initiation of Treatment: 11/17/93
Termination of Treatment and Necropsy: 11/15/95-11/24/95

GLP Compliance: Statement indicates that these studies were conducted in compliance with GLP regulations.

Animals: Male and female CD-1 (ICR)BR mice (60/sex/dose group, ) were approximately 6 weeks of age at start of study. Body weights at start of study were 24.4-28.7 g for males and 22.8-23.1 g for females. Mice were housed individually in stainless steel cages and were provided food (Certified Rodent Chow #5002) and filtered tap water *ad libitum*.

Drug Administration: Eprosartan mesylate [Lot Nos. BCT-K-07C KEG 2, BCT-K-07C KEG 3(1)#1] was given orally by gavage. Suspensions of drug were prepared weekly at concentrations of 10 and 100 and 200 mg of eprosartan/ml in 1% (w/v) carboxymethylcellulose. Vehicle was given by gavage at an equivalent volume of 10 ml/kg/day. Dose was based on the most recent body weight.

Dose Levels: 0, 0, 100, 1000 and 2000 mg of eprosartan/kg/day

Observations/Measurements: All mice were observed twice daily for mortality and daily for clinical signs of overt toxicity. Detailed clinical examinations were performed weekly and, starting at week 27, animals were observed for palpable masses. Body weights were recorded for individual animals on a weekly basis. Food consumption (7 day measurements) were recorded individually weekly from weeks 0-13 and monthly thereafter. All surviving mice were sacrificed and necropsied at the end of the 2 year dosing period (736 consecutive daily doses of test agent). Mice that died or were sacrificed *in extremis* were also necropsied. Necropsy consisted of an external examination, including identification of all clinically recorded masses and lesions, as well as a detailed examination of internal organs.

The following tissues were collected at necropsy and examined macroscopically. Tissues were examined microscopically from all mice in both control groups and the high dose group (2000 mg/kg/day) and from all mice administered 100 or 1000 mg/kg/day that died or were sacrificed in extremis. Additionally, liver and lungs were examined microscopically from all male and female mice in all dose groups.

The following tissues were collected at necropsy:

| | |
|---|---|
| Abnormal Tissues | Nasal Cavity+ |
| Adrenal Glands | Optic Nerves#§ |
| Animal Identification+ | Ovaries |
| Aorta, thoracic | Pancreas |
| Bone and Marrow (sternum) | Parathyroids§ |
| Brain (cerebrum, midbrain, cerebellum and medulla oblongata) | Pituitary |
| | Preputial Gland(bilateral)+ |
| | Prostate |
| Cecum | Rectum+ |
| Cervix+ | Rib (Costochondral Junction)+ |
| Clitoral Glands (bilateral)+ | Salivary Gland, mandibular |
| Colon | Salivary Gland, sublingual |
| Duodenum | Salivary Gland, parotid (both sides) |
| Epididymides# | Sciatic Nerve+ |
| Esophagus | Seminal Vesicles |
| Eyes# | Skeletal Muscle (thigh) |
| Femur+ | Skin |
| Gall Bladder | Skull, base, nasal turbinates, ear and canals+ |
| Harderian Glands+ | |
| Heart | Spinal Cord, lumbar |
| Ileum | Spleen |
| Jejunum | Stomach |
| Kidneys | Testes# |
| Larynx+ | Thymus§ |
| Liver (left lateral and median lobes)* | Thyroid lobes |
| | Tongue+ |
| Lung (sample of 2 lobes)* | Trachea |
| Lymph Nodes, mandibular | Urinary Bladder |
| Lymph Nodes, mesenteric | Uterus (body and horn) |
| Mammary Gland (inguinal§) | Vagina+ |

\+ Retained, but not processed (unless associated with macroscopic abnormality).
* Lungs were infused with neutral buffered 10% formalin (sacrificed animals only).
\# Testes, eyes/optic nerves and epididymides were fixed in Zenker's fluid.
* Entire liver was collected, only samples of left lateral and median lobes were processed.
§ Only examined histopathologically when present in routine sections.

Results

*Animal Survival:* The number of mice surviving to scheduled sacrifice at two years is shown below.

| Dose (mg/kg/day) | Mice Surviving to Scheduled Sacrifice | |
|---|---|---|
| | Males | Females |
| 0 | 30 | 31 |
| 0 | 32 | 27 |
| 100 | 31 | 25 |
| 1000 | 36 | 30 |
| 2000 | 23 | 20 |

Several deaths were attributed to dosing errors. Deaths from dosing errors were greater for males at 2000 mg/kg/day compared to concurrent controls.

| Dose (mg/kg/day) | Deaths Attributed to Dosing Errors or Cage Accident | |
|---|---|---|
| | Males | Females |
| 0 | 0 | 2 |
| 0 | 0 | 4 |
| 100 | 1 | 2 |
| 1000 | 1 | 1 |
| 2000 | 8 | 2 |

Survival (excluding accidental deaths) was significantly decreased in male and female mice at 2000 mg/kg/day compared to concurrent controls (Figures 1 and 2 exclude accidental deaths). The sponsor's analysis, which excluded deaths attributed to accident, found that mortality in female mice at 2000 mg/kg/day was greater than that of concurrent control, but male mortality was not test agent related. In contrast, the FDA's statistical analysis found excess test agent related mortality at 2000 mg/kg/day for both male and female mice, irrespective of whether accidental deaths were censored.

**Figure 1**

**Kaplan-Meier Survival Curves for Males**

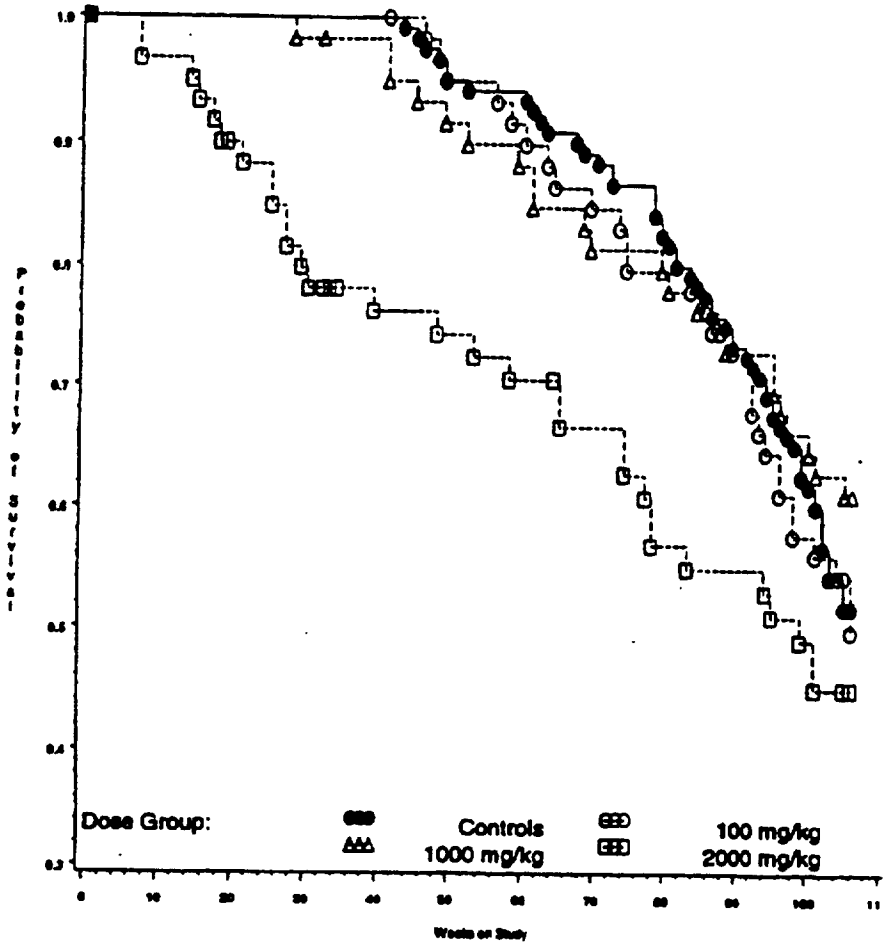
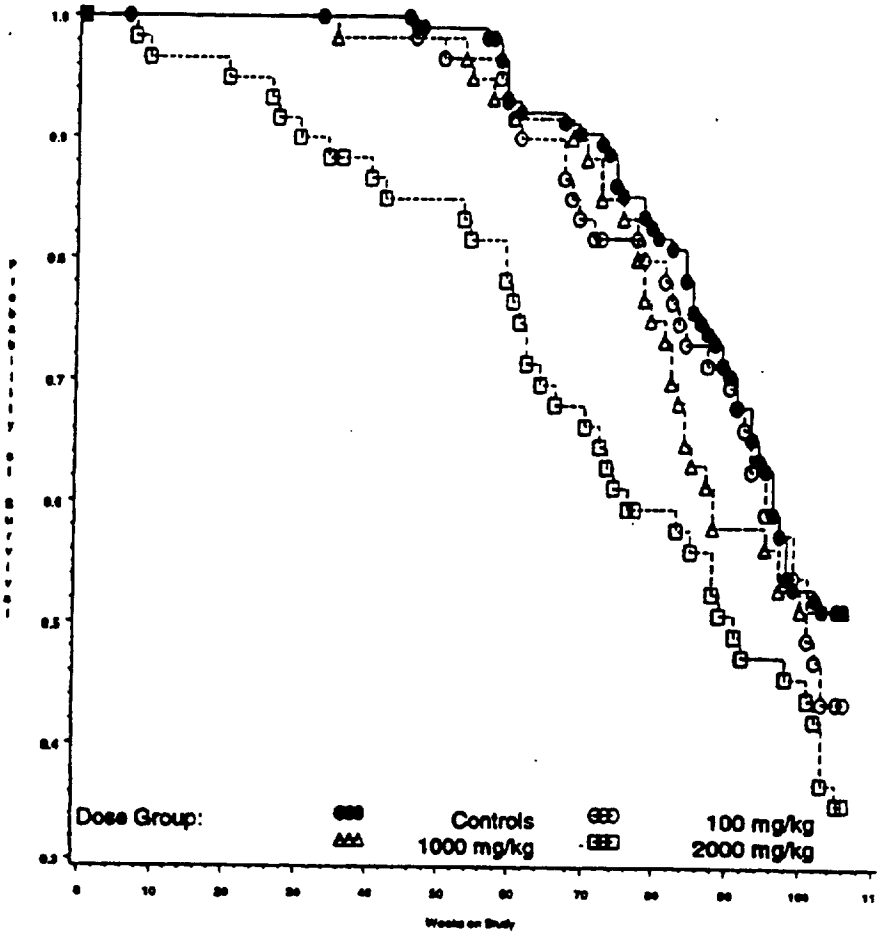


**Figure 2**

**Kaplan-Meier Survival Curves for Females**



The mean survival times of male and female mice at 2000 mg/kg/day were shorter than those for concurrent controls, irrespective of whether accidental deaths were excluded.

| Dose (mg/kg/day) | Mean Survival Time (Weeks of Treatment) | | | |
|---|---|---|---|---|
| | All Mice | | Mice Excluding Accidental Deaths | |
| | Males | Females | Males | Females |
| 0 | 94.68 | 91.88 | 94.68 | 94.33 |
| 100 | 92.43 | 91.75 | 93.31 | 92.59 |
| 1000 | 91.83 | 91.28 | 92.87 | 91.63 |
| 2000 | 70.17 | 79.08 | 76.77 | 80.35 |

*Body Weight:* At 2000 mg/kg/day, male and female body weights were decreased by eprosartan starting at about 12 months of study, at which time body weights at 2000 mg/kg/day were 10% and 6% less in males and females, respectively, than in concurrent controls. At 24 months, body weights at 2000 mg/kg/day were 7% and 14% less in males and females, respectively, than in concurrent controls. At 1000 mg/kg/day, female body weights were decreased sporadically by 5-6% starting at 70 weeks of study.

Body Weights (grams) in Mice Given Eprosartan Mesylate or Vehicle

| Sex | Treatment Week | Dose (mg/kg/day) | | | | |
|---|---|---|---|---|---|---|
| | | 0 (Control 1) | 0 (Control 2 | 100 | 1000 | 2000 |
| Males | 0 | 28.42 | 28.60 | 28.42 | 28.53 | 28.71 |
| | 26 | 38.22 | 28.03 | 38.14 | 37.85 | 37.01 |
| | 52 | 39.35 | 39.31 | 39.64 | 39.04 | 38.13 |
| | 78 | 39.44 | 39.48 | 40.02 | 38.54 | 36.90[a,b] |
| | 104 | 38.60 | 38.87 | 39.07 | 37.54 | 36.35[b] |
| Females | 0 | 23.14 | 22.95 | 23.04 | 22.80 | 22.87 |
| | 26 | 30.34 | 30.05 | 30.38 | 30.45 | 29.63 |
| | 52 | 33.36 | 33.32 | 33.27 | 32.03 | 31.57[a,b] |
| | 78 | 34.38 | 33.76 | 34.40 | 32.45[a] | 31.08[a,b] |
| | 104 | 34.41 | 31.59 | 34.13 | 32.09 | 29.72[a] |

[a] Significantly different from control group 1 at P<0.05.
[b] Significantly different from control group 2 at P<0.05.

*Food Consumption:* There were no drug-related findings.

*Clinical Signs:* There were no drug-related findings.

*Palpable Masses:* There were no drug-related findings.

| Dose (mg/kg/day) | Percentage of Mice with Palpable Masses | |
|---|---|---|
| | Males | Females |
| 0 | 17 | 12 |
| 0 | 27 | 5 |
| 100 | 27 | 7 |
| 1000 | 27 | 2 |
| 2000 | 10 | 3 |

*Postmortum Findings:* There were no drug-related findings (Appendices 2a, 2b and FDA Statistical Review and Evaluation).

The overall number of mice with tumors (from any site) was not affected by test agent.

| Sex | Dose (mg/kg/day) | Number of Mice Evaluated | Number of Mice with Tumors | Number of Mice with Benign Tumors | Number of Mice with Malignant Tumors |
|---|---|---|---|---|---|
| Male | 0 | 60 | 36 | 26 | 19 |
| | 0 | 60 | 39 | 25 | 24 |
| | 2000 | 60 | 22 | 17 | 7 |
| Female | 0 | 60 | 39 | 31 | 20 |
| | 0 | 60 | 36 | 24 | 24 |
| | 2000 | 60 | 30 | 14 | 19 |

## OVERALL SUMMARY AND EVALUATION:

*Genotoxicity Studies:*

Eprosartan mesylate was negative for genotoxicity in the Ames bacterial genotoxicity test at doses up to 5,000 µg per plate, both with and without metabolic activation; this concentration is recommended as the limit dose for nontoxic compounds by the ICH.[2] Bacterial tester strains evaluated were *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and *Escherichia coli* strain WP2, WP2uvrA. Eprosartan mesylate was also negative for genotoxicity in mouse lymphoma cells, *in vitro,* at concentrations up to 2750 µg /ml with metabolic activation and 3250 µg /ml without metabolic activation; toxicity at these concentrations was consistent with ICH guidelines.[2]

In human peripheral lymphocytes, *in vitro*, eprosartan mesylate in the absence of metabolic activation did not induce structural chromosomal aberrations. However, in the presence of metabolic activation, eprosartan mesylate at 2000 µg /ml was equivocal for clastogenicity, since it was positive, equivocal and negative for this finding in the three assays performed. The assay appeared to be adequate since cytotoxicity with eprosartan mesylate at the highest concentrations evaluated was similar to that recommended by the ICH (>50% inhibition of mitotic index).[2] Also in human peripheral lymphocytes, eprosartan mesylate at 2500 µg /ml with metabolic activation significantly increased polyploidy, and without metabolic activation tended to increase polyploidy (albeit nonsignificantly) compared to concurrent control; cytotoxicity at this concentration was not excessive (33% and 60% inhibition of mitotic index).

An orally administered dose of 2500 mg eprosartan mesylate/kg/day for two days did not induce chromosomal aberrations (micronuclei in polychromatic erythrocytes) in mouse bone marrow *in vivo* (micronucleus test). This dose is consistent with the oral limit dose of 2000 mg/kg proposed by the International Workshop on Standardization of Genotoxicity Test Procedures (1994) for evaluation of nontoxic compounds.[3] The current ICH guideline (1996) is silent on a limit dose for evaluation of nontoxic compounds in the mouse micronucleus assay.[2] A maximum tolerated dose was not reached in the mouse micronucleus assay, since clinical and bone marrow toxicities were not observed. Additionally, similar oral doses did not produce toxicity in mice in 10 day (3000 mg/kg/day) and 90 day (2000 mg/kg/day) toxicity studies. It seems likely that the dose given in the micronucleus test could have been increased above 2500 mg/kg/day, since systemic eprosartan exposure was dose-proportional with oral doses of 100, 300, 1000 and

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2. International Conference on Harmonization; Guidance on Specific aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. Federal Register; Vol. 61, No. 80; April 24, 1996.

3. Report of the International Workshop on Standardization of Genotoxicity Test Procedures. Mutation Research 1994; 312: 393-304.

2000 mg/kg/day in the 90 day mouse toxicity study. Although exposure was not monitored in the mouse micronucleus assay, total eprosartan exposure (AUC) in mice at 2000 mg/kg in the 90 day study (29,196 ng.hr/ml) was about three times that seen in normal volunteers and hypertensive patients at the maximum proposed human dose of 800 mg/day, qd (9000-10,000 ng.hr/ml); systemic exposure to unbound eprosartan in mice at 2000 µg/kg is approximately 30-times that achieved in humans at the maximum recommended human dose.[4] It should be noted that there is the possibility that the maximum recommended human daily dose may be divided into two daily doses for improved efficacy; in this case, relative exposure in mice would be less than that indicated above.

Summary of Genotoxicity Findings with Eprosartan Mesylate[a]

| Test | Genotoxic Effect | Eprosartan Mesylate Findings | |
|---|---|---|---|
| | | With Metabolic Activation | Without Metabolic Activation |
| Ames Bacterial Mutation | Point Mutation | Negative @ ≤5000 µg/ml | Negative @ ≤5000 µg/ml |
| Mouse Lymphoma, *In vitro* | Point Mutation/ Clastogenicity | Negative @ ≤2750 µg/ml | Negative @ ≤3250 µg/ml |
| Human Lymphocytes, *In vitro* | Clastogenicity | Equivocal @ 2000 µg/ml (1 positive, 1 equivocal, 1 negative assay) | Negative @ ≤2500 µg/ml (2 of 2 assays) |
| | Polyploidy | Positive @ 2500 µg/ml (1 of 1 assay) | Equivocal @ 2500 µg/ml (1 of 1 assay) |
| Mouse Bone Marrow Micronucleus Test, *In vivo* | Clastogenicity | Negative @ 2500 mg/kg/day, po, for two days | |

[a] All concentrations and the dose used in the micronucleus assay are expressed in terms of eprosartan mesylate.



4. Free fractions over concentration range of 0.01-10 µg/ml; human, 1.9%; mouse, 20.2%. (Preliminary results for mouse plasma protein binding submitted on 7/23/97).

*Rat Carcinogenicity Study:*

Eprosartan mesylate given orally at 30, 100 and 600 mg eprosartan/kg/day did not increase tumor incidence in dietary restricted male and female Sprague-Dawley rats.

Dose selection for the rat carcinogenicity study was based on saturation of exposure (AUC) at oral doses greater than 300 mg/kg/day in dietary restricted male rats in a single-dose toxicokinetic study (see attached IND review of G. Jagedeesh dated 11/10/93). Although it is likely that saturation of exposure also occurred in males and females in the two year rat study, this could not be directly demonstrated due to the absence of the 300 mg/kg/day dose.

Systemic exposures to eprosartan in dietary restricted rats were only about half of those seen in *ad libitum* fed rats given the same doses. Exposures in *ad libitum* fed rats appear to be limited by low absorption of test agent, since only about 8% of compound was absorbed in bile duct cannulated rats given radiolabeled test agent orally at 100 mg/kg (Study #BP-1006, see NDA review by A. Proakis). Metabolism is not important in limiting exposure in the rat since the majority of test agent is eliminated unchanged in the feces. It is suggested that dietary restriction further impedes absorption of test agent. The CAC-EC had previously recommended that the sponsor explore options to increase exposure in dietary restricted rats (see attached IND #39,721 review dated 11/10/93). Although the sponsor did not explore options to increase exposure, such as administration of test agent after (rather than prior to) feeding, or administration by dietary admixture, exposure nevertheless appeared to be saturated at the doses administered using the dosing methods employed by the sponsor.



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| Sex | Oral Dose (mg/kg/day) | AUC (ng.hr/ml) | | |
|---|---|---|---|---|
| | | Six Month Oral Rat Toxicity Study *(Ad Libitum Fed)* | Oral, Single Dose Male Rat Study *(Dietary Restriction)* | Two Year Rat Carcinogenicity Study *(Dietary Restriction)* |
| Male | 30 | 658 | ND | 433 |
| | 100 | 2632 | 1117 | 1044 |
| | 300 | ND | 2354 | ND |
| | 600 | ND | 2507 | 3396 |
| | 1000 | 5677 | 2460 | ND |
| Female | 30 | 638 | ND | 299 |
| | 100 | 2046 | ND | 930 |
| | 300 | ND | ND | ND |
| | 600 | ND | ND | 1885 |
| | 1000 | 5006 | ND | ND |

ND, Not determined

Survival of female rats was not drug-related. Survival of male rats at 600 mg/kg/day was slightly less than concurrent control, the excess mortality due to an increase in accidental deaths in this group. Body weights and signs of toxicity were not drug-related for male or female rats in this two year rat carcinogenicity study. These findings are consistent with the lack of toxicity observed in a six month oral toxicity study at doses up to 1000 mg/kg/day in the same (SD) rat strain (see NDA review by A. Proakis). A similar lack of toxicity was noted in previous studies in (SD) rats given test agent orally at doses up to 3000 mg/kg/day for 7 days or up to 1000 mg/kg/day for 30 days. These previous studies also showed non-proportional exposures with escalating doses (See attached IND reviews of G. Jagedeesh dated 7/20/92 and 11/10/93).

Male and female rats in the two year carcinogenicity study were dietary restricted by approximately 30-36% and 20-27%, respectively, compared to *ad libitum* food consumptions in the six month oral toxicity study. Consistent with dietary restriction, body weights in dietary restricted rats were approximately 25% and 20% lower than body weights of male and female *ad libitum* fed rats (at similar ages in the 6 month study), respectively. Food consumption and body weights were not drug-related.

| Sex | Food Intake (g/day) | | |
|---|---|---|---|
| | *Ad Libitum* Fed | Dietary Restriction | |
| | Six Month Oral Rat Toxicity Study | Single Dose, Oral Rat Study *(%Dietary Restriction)* | Two Year Rat Carcinogenicity Study *(%Dietary Restriction)* |
| Male | 30-33 | 23 (23-30% Restricted) | 21 (30-36% Restricted) |
| Female | 20-22 | ND | 16 (20-27%) |

*ND, Not determined*

Eprosartan exposure (AUC) and $C_{max}$ at the highest dose evaluated in the two year rat carcinogenicity study (600 mg/kg/day) are considerably less than levels reported for human patients orally administered 800 mg eprosartan/day, qd, the maximal recommended therapeutic dose. At 600 mg/kg/day, exposure of male and female rats to unbound eprosartan was only 18% and 26%, respectively, of that seen in humans with once a day dosing at 800 mg/day. In both rat and man, eprosartan is essentially nonmetabolized. In man, unchanged eprosartan was the only drug-related compound found in plasma and feces following both intravenous and oral administration of radiolabeled compound (HH-1001/SKF-108566). The majority (~90%) of orally administered drug is eliminated unchanged in the feces. Although an acyl glucuronide conjugate of eprosartan is found in human urine, this accounts for only about 2% of orally administered, and 7% of intravenously administered eprosartan. In rats, parent compound was the predominant drug-related compound observed in plasma (% not provided), bile (>97%) and feces (>99%) with oral administration of eprosartan; urinary elimination was negligible (0.4%) (BP-1006/SKF-108566/l; reviewed by A. Proakis). As indicated earlier, there is the possibility that the maximum recommended human daily dose may be divided into two daily doses for improved efficacy; in this case, relative exposure in rats would be less than that with once daily dosing.

AUC in Rats vs Humans at the Maximum Recommended Dose

| Species | Dose | Sex | AUC $_{(0-t)}$ | | | |
|---|---|---|---|---|---|---|
| | | | Total | | Free (Unbound) | |
| | | | (ng.hr/ml) | % Human | (ng.hr/ml) | % Human |
| Human Hypertensive Subjects | 800 mg/day, qd | Male | 9521 | 100% | 180.90 | 100% |
| Rat | 600 mg/kg/day | Male | 2903 | 30% | 46.44 | 26% |
| | | Female | 2041 | 21% | 32.65 | 18% |

Human and rat values from days 7 and 29 of treatment, respectively.
Human values from protocol 048 (pg 42, 70, vol 1.050)
Rat values from carcinogenicity study #G93047 reviewed on pgs 10-17.
Free fractions over concentration range of 0.01-10 µg/ml; human, 1.9%; rat 1.6%

$C_{max}$ in Rats vs Humans at the Maximum Recommended Dose

| Species | Dose | Sex | $C_{max}$ | | | |
|---|---|---|---|---|---|---|
| | | | Total | | Free (Unbound) | |
| | | | (ng/ml) | % Human | (ng/ml) | % Human |
| Human Hypertensive Subjects | 800 mg/day, qd | Male | 2103 | 100% | 39.96 | 100% |
| Rat | 600 mg/kg/day | Male | 1155 | 55% | 18.48 | 46% |
| | | Female | 647 | 21% | 10.35 | 26% |

Human and rat values from days 7 and 29 of treatment, respectively.
Human values from protocol 048 (pg 42, 70, vol 1.050)
Rat values from carcinogenicity study #G93047 reviewed on pgs 10-17.
Free fractions over concentration range of 0.01-10 µg/ml; human, 1.9%; rat 1.6%

*Mouse Carcinogenicity Study:*

Eprosartan mesylate given orally at 100, 1000 and 2000 mg eprosartan/kg/day did not increase tumor incidence in male and female CD-1 mice. The highest dose of 2000 mg/kg/day significantly reduced survival and decreased body weights in male and female mice, and therefore is considered the maximum tolerated dose in this two year study. This dose was suggested by the CAC-EC in response to the sponsor's initial protocol (see IND #39,721; review dated 11/10/93).

At 2000 mg/kg/day, total eprosartan exposure (AUC) in male and female mice is about three (3) times that calculated for humans given 800 mg eprosartan/day, once daily, the maximum recommended dose (MRHD). However, free eprosartan exposure is about 30-times that achieved in humans given the maximum recommended dose. As mentioned previously, there is the possibility that the maximum recommended human dose may be split into two daily doses for improved efficacy; in this case, relative exposure in mice would be less than that indicated above.

AUC in Mice vs Humans at the Maximum Recommended Dose

| Species | Dose | Sex | AUC $_{(0\text{-}t)}$ | | | |
|---|---|---|---|---|---|---|
| | | | Total | | Free (Unbound) | |
| | | | (ng.hr/ml) | Multiple of Human Value | (ng.hr/ml) | Multiple of Human Value |
| Human Hypertensive Subjects | 800 mg/day, qd | Male | 9521 | na | 180.90 | na |
| Mouse | 1000 mg/kg/day | Male | 8927 | 0.9 | 1803 | 10 |
| | | Female | 11638 | 1.2 | 2351 | 13 |
| | 2000 mg/kg/day | Male | 27415 | 2.9 | 5538 | 30 |
| | | Female | 30978 | 3.2 | 6258 | 35 |

na, not applicable.
Human and mouse values from days 7 and 30 of treatment, respectively.
Human values from protocol 048 (pg 42, 70, vol 1.050)
Mouse values from 3 month dose range-finding study #85569; (see IND #39,721; review dated 11/10/93).
Free fractions over concentration range of 0.01-10 µg/ml; human, 1.9%; mouse, 20.2%.[5]

5. Preliminary results for mouse plasma protein binding submitted on 7/23/97.

$C_{max}$ in Mice vs Humans at the Maximum Recommended Dose

| Species | Dose | Sex | $C_{max}$ | | | |
|---|---|---|---|---|---|---|
| | | | Total | | Free (Unbound) | |
| | | | (ng/ml) | Multiple of Human Value | (ng/ml) | Multiple of Human Value |
| Human Hypertensive Subjects | 800 mg/day, qd | Male | 2103 | na | 39.96 | na |
| Mouse | 1000 mg/kg/day | Male | 3019 | 1.4 | 610 | 15 |
| | | Female | 3608 | 1.7 | 729 | 18 |
| | 2000 mg/kg/day | Male | 16439 | 7.8 | 3320 | 83 |
| | | Female | 14011 | 6.7 | 2830 | 70 |

na, not applicable.
Human and mouse values from days 7 and 30 of treatment, respectively.
Human values from protocol 048 (pg 42, 70, vol 1.050)
Mouse values from 3 month dose range-finding study #85569; (see IND #39,721; review dated 11/10/93).
Free fractions over concentration range of 0.01-10 µg/ml; human, 1.9%; mouse, 20.2%.

## APPROVABILITY RECOMMENDATION

Based on consideration of carcinogenicity and mutagenicity studies, the application is approvable. Although the two year rat carcinogenicity study was carried out at systemic exposures below the human exposure level, it is unlikely that exposure in rats at any dose could have been increased above that seen in humans at the maximum recommended daily dose. The two year mouse carcinogenicity study, in which a maximally tolerated dose achieved plasma eprosartan exposures above the levels of human plasma exposure, gave no indication that eprosartan mesylate possesses the potential for increased tumor incidence.

Regarding mutagenicity, although eprosartan mesylate was equivocal for clastogenicity, and induced polyploidy in human peripheral lymphocytes *in vitro*, eprosartan mesylate was negative in the Ames bacterial assay, in the mouse lymphoma assay *in vitro*, and in the mouse micronucleus assay *in vivo*. Consequently, the weight of the evidence indicates that eprosartan mesylate in not genotoxic. The genotoxicity assays performed fulfill the draft ICH guidelines for evaluation of genotoxicity.[6]

## LABELING CONSIDERATIONS

The proposed mutagenesis labeling statement for eprosartan mesylate should be changed.

Presently reads (Vol 1.002, page 000490, paragraph 3, lines 1-3):
*"Eprosartan was not mutagenic in bacteria or mammalian cells in the Ames or mouse lymphoma in vitro tests or in vivo (mouse micronucleus test) and did not cause chromosomal aberrations in human lymphocytes in an in vitro assay."*

Change to read:
**"Eprosartan mesylate was not mutagenic *in vitro* in bacteria (Ames Test) or mammalian cells (mouse lymphoma assay). Eprosartan mesylate also did not cause structural chromosomal damage *in vivo* (mouse micronucleus assay). In human peripheral lymphocytes *in vitro*, eprosartan mesylate was equivocal for clastogenicity with metabolic activation, but was negative for clastogenicity without metabolic activation. In the same assay, eprosartan mesylate was positive for polyploidy with metabolic activation and equivocal for polyploidy without metabolic activation."**

6. International Conference on Harmonisation; Draft Guideline on Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals; Notice. Federal Register; Vol. 62, No. 64; April 3, 1997.

The proposed carcinogenicity labeling statement for eprosartan mesylate should be changed.

Presently reads (Vol 1.002, page 000490, paragraph 2):
*"Eprosartan was not carcinogenic in rats or mice dosed at 600 mg/kg/day and 2,000 mg/kg/day, respectively, for up to 2 years; the systemic exposure (AUC's) at these doses was approximately similar to that or 3 times greater, respectively than exposure achieved in humans given the maximum recommended human dose (800 mg/day)."*

Change to read:
**"Eprosartan mesylate was not carcinogenic in dietary restricted rats or *ad libitum* fed mice dosed at 600 mg eprosartan/kg/day and 2,000 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 20% of the exposure achieved in humans given the maximum recommended human dose (800 mg/day, once daily). In mice, the systemic exposure (AUC) to unbound eprosartan was approximately 30-times the exposure achieved in humans given the maximum recommended human dose."**

John E Koerner

John E. Koerner, Ph.D.
Pharmacologist

CC:
Original NDA
HFD-110
HFD-110/CSO
HFD-110/JKoerner
HFD-345
Accepted by CAR on 7-31-97

Attachments: Statistical Review and Evaluation
Appendices 1a, 1b, 2a, 2b
IND #39721 Reviews dated 07/20/92 and 11/10/93
C. Resnick comments re: CAC-EC recommendations to sponsor.