Results:

At high dose some animals (both sexes) experience subdued behavior, low posture, labored breathing and budging eyes. One female from control group and 2 males and 6 females from high dose group were killed or died during study period. Cause of deaths considered not to be treatment related. Body weight gains and food intakes were not affected by treatment in males. However, body weight gains in high dose treated females were reduced by 6% during gestation and by 34% during lactation when compared to respective control values. The estrous cycle of the female rats revealed no differences between the control and treated groups. Precoital intervals, mating rates and pregnancy rates were comparable in all groups.

Parameters	Control	Low Dose	Mid Dose	High Dose
# of Female Pairs	29	30	30	29
# of Females Mated	29	27	28	29
Mating Rate (%)	100	90	93	100
# of Pregnant	22	26	26	26
Pregnancy Rate (%)	76	87	87	90

Dams Sacrificed at Day 20:

No treatment related gross lesions were seen in female rats of F_0 generation. There were no significant changes in pregnancy parameters (numbers of implantations, pre-implantation loss, litter size, sex ratios, and mean fetal weights). A dose related increase in the incidence of post-implantation loss were seen (control = 3.6%, low dose = 2.6%, mid dose = 4.7% and high dose = 7.7%). A total of 666 fetuses were examined for external and visceral abnormalities and 346 fetuses were examined for skeletal abnormalities. No treatment related major malformation was seen in fetuses. However, increased incidences of renal pelvic cavitation (control = 8.8%, low dose = 4.3%, mid dose = 11.7% and high dose = 11.5%, historical mean: 5.3% [range) and delayed metacarpal and nasal or hyoid bone ossification were seen in mid and high dose treated groups.

Dams Sacrificed on Day 20 of Pregnancy				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
# of Pregnant Dams	12	13	12	13
# of Corpora Lutea	171	198	179	186
Mean # of Corpora Lutea/Dam	14.3	15.2	14.9	14.3
# of Implants	166	193	170	169
Mean # of Implants/Dam	13.8	14.8	14.2	13.0
Total # of Fetuses	160	188	162	156
# of Live Fetuses	160	188	162	156
Mean # of Live Fetuses/Dam	13.3	14.5	13.5	13.3
# of Dead Fetuses	0	0	0	0
Mean # of Dead Fetuses/Dam	0	0	0	0
Pre-implantation Loss (%)	2.9	2.5	5.0	9.1
Post-implantation Loss (%)	3.6	2.6	4.7	7.7
Mean Fetal Weight (g)	3.8	3.8	3.8	3.7
Sex Ratio (% females)	53.1	47.9	53.1	48.1

Dams Allowed to Deliver: No significant differences in the gestation period between the groups were noted. The number of implantation sites, post-implantation survival, litter size, sex ratios, viability and pups weights throughout lactation period were not affected by the treatment. Postnatal development and differentiation were comparable in all groups except testes descent were delayed in high dose group males and vaginal opening was also delayed in

females of mid and high dose groups. There was no significant effect on fertility test and mating performance test of F_1-generation rats. No treatment related gross lesions were seen in rats of F_1 generation. Physical development was comparable in all groups, and no drug related gross lesion were seen in the F_2 pups at necropsy.

Dams Allowed to Deliver				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
# of Pregnant Dams	9	12	14	12
Gestation Length (days)	21	21	21	21
# of Live Pups at day 2	114	162	178	161
# of Dead Pups at day 2	2	5	1	1
Birth Index (median)	0.87	0.90	1.0	0.94
Viability Index (median)	1	1	1	1
Lactation Index (median)	1	1	1	1

Birth index = # of live offspring born/# of implantations.
Viability Index = # of live offspring at day 4/# of live offspring born.
Lactation index = # of live offspring at day 20/# of live offspring at day 4.

In conclusion, there were no abnormal effects on the fertility and mating performance of the treated male and female rats at oral doses up to and including 40 mg/kg/day of GR68755.

Addendum: Approximately half the females were sacrificed on day 21 of pregnancy and the other half were allowed to litter and sacrificed on litter day 22.

Segment II. Teratology Study in Rats (Study # R12151)

Testing Laboratories: Pathology and Toxicology Division
Glaxo Group Research Ltd.,
Hertfordshire, U.K.

Study Started: January 29, 1989

Study Completed: May 30, 1990

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Animals: Pregnant AHA rats (Wistar/SD derived with Wistar characteristics).

No. of Animals: 36 pregnant rats/group

Drug Batch No.: C1034/96/1 and C1017/133/1

Methods: Pregnant rats were given oral (gavage) doses of 0 (water), 1.0, 6.5 and 40 mg/kg/day from day 7 to 16 day of gestation. The volume of administration was fixed at 10 ml/kg. The selection of the doses were based on preliminary study (# 11996: see above). Body weights were recorded on days 1 (day 1 of the pregnancy), 4, 7-16 and 20 of gestation. Twenty-four dams were sacrificed on day 21 of gestation, and were examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed. Approximately one-half of the fetuses eviscerated and examined for skeletal major/ minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining of the dams (about 12 /group) were allowed to deliver spontaneously. The numbers of live/dead pups were recorded, and the live pups were weighed and sexed. Culling was carried out to make 8 offspring (4 male and 4 female) per dam. Pups were also weighed on days 2, 4, 8, 12, 16 and 20 of post partum. The offspring were reared by the dams until day 21 of post partum. On day 21 of post partum all dams were sacrificed and necropsied, and examined as mentioned above. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pupil reflex, pinnae detachment, upper incisor eruption,

eye opening, testes descent, vaginal opening learning ability test, open field test). On litter day 24, a minimum of one male and one female pup were selected from each litter for F_1 generation study. At 9 weeks of age they were continuously mated and females were killed on day 21 of gestation and their uterine contents were examined as mentioned above.

Results:

Dams Sacrificed at Day 21: During the first 5 days of treatment, clinical signs such as piloerection, subdued behavior, labored breathing, half closed eyes and croaking were seen in high dose treated rats. No significant effect on body weight or food consumptions were seen in low and mid dose treated rats. However, during treatment period, significant reductions in body weight gains (about 25%) and food intakes (about 18%) were seen in high dose treated dams. No treatment related macroscopic abnormalities were seen in dams. The number of corpora lutea, the number of implants, pre-implant losses, numbers of live fetuses, weights of fetuses and sex ratio did not show any significant difference between the treated groups and the control group. However, post-implantation loss (%) were increased dose dependently (control: 2.5%, low dose: 4.5%, mid dose: 6.5% and high dose: 6.0% [historical control: mean 4.8%]). No treatment related abnormalities were observed on external, skeletal and visceral examination in any group, except increased incidence of supernumerary ribs were seen in high dose treated group (control: 7.2%, low dose: 5.2%, mid dose: 6.6% and high dose: 13.9% [historical control: 0-12%]).

Effect of GR 68755C on Maternal and Fetal Parameters in Rats				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
Total Mated	24	24	24	24
# of Pregnant	22	22	23	21
% Pregnant	92	92	95.8	87.5
# of Dams with Live Fetuses	22	22	23	21
# of Corpora Lutea	320	302	296	295
# of Implants	275	269	277	284
Post-implantation Loss/Dam (%)	2.5	4.5	4.7	6.0
# of Live Fetuses	268	257	264	267
Mean Fetal Wt. (g)	3.8 ± 0.3	3.8 ± 0.4	3.9 ± 0.4	3.8 ± 0.3
Sex Ratio (% female)	44.8	50.6	49.2	54.3
Pre-implantation loss/dam (%)	14.1	10.9	6.5	3.7
Fetal Malformations				
# of Fetuses Examined	268/139	257/134	264/137	267/137
External	0/268	0/257	0/264	0/267
Skeleton	0/139	0/134	0/137	0/137
Visceral	0/268	0257	0/264	0/267

Dams Allowed to Deliver: No significant differences in the gestation period between the groups were noted. There were no significant effects on postnatal development and differentiation. There was no significant effect on fertility test and mating performance test of F_1-generation rats. No drug related abnormalities were seen in F_2 pups at necropsy.

Plasma Levels of GR 68755X (WBP/90/063): Plasma drug level increased with increasing doses. Mean plasma levels of GR 68755X at 15 min after drug administration on day 7 of gestation were 171.5, 783 and 9245 ng/ml and on day 16 of gestation were 179.5, 855.0 and 12900 ng/ml in low, mid and high dose groups respectively. Plasma levels of GR 68755X at 24hr after drug administration on days 7 and 16 of gestation was below detection limit, indicating that there was no accumulation after repeat dosing.

In this study, no teratogenic effect at dosage up to 40 mg/kg/day was seen in rats. However, the highest tested dose was maternotoxic (decreased body weight gains and food intakes, increased post-implantation loss) and fetotoxic (increased incidence of supernumerary ribs). The postnatal development and the fertility of the offspring were comparable in all groups.

Oral Segment II. Teratology Study in Rabbits (Study # L11998)

Testing Laboratories: Pathology and Toxicology Division
Glaxo Group Research Ltd.,
Hertfordshire, U.K.

Study Started: September 8, 1989

Study Completed: March 9, 1990

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Test Species: At least 16 weeks old female Dutch Rabbits.

No. of Animals: 25-33 pregnant females/group

Route of Administration: Oral (gavage)

Dose Levels: 1, 6.5 and 40 mg/kg/day

Drug Batch No.: C1017/133/1 and 1028/98/1

Methods: The selection of the doses was based on the preliminary Segment II. teratology study (study # L11961) in rabbits in which oral doses of 0, 20, 30 and 40 mg/kg/day were used. Pregnant rabbits were treated from day 8-20 of gestation. In this preliminary study, drug did not produce any maternal toxicity, embryotoxicity or

teratogenic effects. However, treatment did produce clinical signs (dilated pupils, increased respiration, subdued behavior and a stepping action) in low dose (1/5), mid dose (1/5) and high dose (3/5) treated rabbits. Based on these results sponsor selected 40 mg/kg/day as the highest dose for the main study. In the main study, pregnant rabbits were given oral doses of 0 (vehicle: water), 1, 6.5 and 40 mg/kg/ day from day 8 to 20 day of gestation. The volume of administration was 2.0 ml/kg. Pregnant dams were observed daily for mortality and clinical signs. Body weights were recorded during days 1, 4, 6, 8-20 and every second day thereafter until day 30 of the pregnancy. Food intake were recorded daily. All surviving dams were sacrificed at day 30 of gestation, and were examined for the number of corpora lutea, the number of implants, number of early/late resorptions, number of live/dead fetuses and identification of any malformed fetuses or uterine abnormalities. Live fetuses were weighted, sexed and examined for external abnormalities. All fetuses were eviscerated and were examined for skeletal malformations and variations, and visceral abnormalities.

Results:

Plasma Levels of GR 68755X (WBP/90/057): Plasma drug levels were highly variable (in high dose group it ranged from less than ng/ml to ng/ml). Hence no comment can be made. Eight high dose treated females showed clinical signs (see above). A total of 6 dams (which included one dam from control group) were killed due to poor condition or found dead during study period. Among these 6 dams, 3 dams (2 in low dose group and 1 in high dose group) had abortion. During the treatment period, body weight gains were reduced by 94%, 86% and 66% in low, mid and high dose treated dams. This reduction in body weight gain persisted till the end of the study period and was associated with sever decrease (25-31%) in food intakes. No treatment related macroscopic abnormalities were seen in dams. The number of corpora lutea, the number of implants, pre-implantation and post-implantation losses, numbers of live fetuses, and sex ratio did not show any significant difference between the treated groups and the control group. Weights of fetuses were significantly lower (13%) than that seen in control group. Skeletal abnormalities such as fusion between vertebral arches were seen in 2 fetuses (from 2 litter) of mid dose group and 2 fetuses from 1 litter of high dose group. Sponsor considered this finding as incidental and not treatment related. Minor skeletal findings such as increased

incidences of supernumerary rib (bilateral), abnormal positioning of pelvic girdle, additional lumber vertebra, spinous process present on 4th lumber centrum were seen in treated groups. Additionally, generalized reduction of ossification was seen in fetuses of high dose. With respect to visceral abnormalities, the incidence of abnormal positioning of the right common carotid artery (minor abnormality) was increased in treated groups (control = 0.0%, low dose = 13.9%, mid dose = 4.8% and high dose = 7.9%; historical incidence rate: mean = 5.7% [range = 0.0 - 15.8%]). However, no treatment related major abnormalities were observed on external, skeletal and visceral examination in any group.

Effect of GR 68755C on Maternal and Fetal Parameters in Rabbits

Parameters	Control	Low Dose	Mid Dose	High Dose
Total Mated	33	25	25	29
# of Pregnant	16	17	19	17
% Pregnant	48.5	68.0	76.0	58.6
# of Dams with Live Fetuses	14	15	18	14
Mean # of Corpora Lutea/dam	7.2 ± 1.3	6.3 ± 2.5	7.1 ± 1.7	7.9 ± 1.9
Mean # of Implants/dam	5.4 ± 1.9	5.1 ± 2.9	4.9 ± 2.1	5.9 ± 2.9
Post-implantation Loss/Dam (%)	8.0	6.5	5.7	8.4
# of Live Fetuses	69	72	83	76
Mean Fetal Wt. (g)	37.9 ± 4.8	36.0 ± 5.3	37.4 ± 4.5	32.0 ± 6.5
Sex Ratio (% male)	55.1	58.3	63.9	59.2
Pre-implantation Loss/dam (%)	25.7	18.1	31.3	25.2
Fetal Major Malformations				
# of Fetuses Examined	69	72	83	76
External	0	0	0	0
Skeleton (fusion between vertebral arches)	0	0	2 (2)	2 (1)
Visceral	0	0	0	0

Number in () indicates # of litters affected.

Thus no teratogenic effect at dosage up to 40 mg/kg/day was seen in rabbits.

Segment III Perinatal and Postnatal Reproductive Toxicity Study in Rats (R20827)

Testing Laboratory: Glaxo Wellcome Research and Development, Hertfordshire, UK

Study Start and Completion Dates: February 6, 1995 and July 17, 1998

GLP and QAU Compliance Statement: A statement of compliance with GLP regulation and a Quality Assurance statement were included.

Animals: Females (197-273 g on first day of pregnant, ~8 weeks old), AHA (Wistar/Sprague Dawley with Wistar characteristics) rats.

Methods: To study the potential effects of GR 86755 on perinatal and postnatal development in rats, GR 68755 was given oral gavage to pregnant female rats (15/group, 17 in high dose group) at 0, 1, 6.5, and 40 (days 1-3)/30 (day 4 onward) mg/kg/day during gestation day 17 through day 22 after delivery. The dose selection was based on the results of the Segment II study in female rats reviewed above. All animals were observed for clinical signs of toxicity and mortality. Body weights and food consumptions were also determined. The pregnant females were allowed to deliver naturally and sacrificed on 22 day after delivery. Sex, body weight and external alteration of fetus were determined. The offsprings were examined for physical development markers (body weight, incisor eruption, eye opening, testes descent, vaginal opening, locomotor coordination), and rotor rod test). F_1 animals were allowed to mate and the pregnant females were sacrificed on gestation day 13 and their uterine contents were examined. Plasma levels of GR 68755 were determined at 15 minutes after dosing on day 12 after delivery.

Results: High dose was lethal (2 high dose animals were found dead). Another high dose female had severe clinical signs of toxicity including low posture, cold to touch, piloerection, subdued behavior, convulsion, and labored breathing observed on day 3. These clinical signs disappeared after the high dose was reduced to 30 mg/kg/day. Decreased body weight gain (~24%) was noted in the high dose group during gestation days 17-21. Food consumption during gestation days 17-21 were also decreased in the high dose group (high dose: ~23.23 g/animals/day and control: 29.53 g/animal/day). No treatment related macroscopic changes were observed at terminal examination. The reproductive performance was not adversely affected and the data were summarized on a table on page 37 in volume 39. This table is attached below.

Summary Table of Reproductive Performance Data (F0-F1)

Parameter	Dosage (mg/kg)			
	0	1.0	6.5	30.0
Number of dams with litters	15	15	13	14
Gestation length (median)	21.0	21.0	21.0	21.0
Gestation index (Proportion)	1.000	1.000	0.867	0.933
Median number of offspring born	14.0	15.0	14.0	14.5
Proportion of male offspring (median)	0.538	0.500	0.500	0.550
Birth index (median)	0.947	1.000	0.941	0.909
Viability index (median)	1.000	1.000	1.000	1.000
Lactation/weaning index (median)	1.000	1.000	1.000	1.000

Following clinical signs of toxicity were observed in the offsprings in the mid and high dose groups: tip toe gait, hyperactivity, piloerection, and vocalization. The birth weight was lower in the high dose group (g) as compared to the control (g). The body weight of offspring on day 20 in high dose group was ~92% of the control. The body weight gain from day 28 through day 64

was reduced by 8.4% and 11.5% in the mid and high dose F_1 males. The testes descent and vaginal opening were slightly delayed in the high dose group. These results were summarized in tables on page 42 in volume 39 and these tables are attached below.

Testes descent

Marker	Dosage (mg/kg)			
	0	1.0	6.5	30.0
Median day	29.0	29.5	30.0	31.5
Range				
ZBAR	-	-0.023	1.783	3.135
p-value	-	ns	p<0.05	p<0.01

Vaginal opening

Marker	Dosage (mg/kg)			
	0	1.0	6.5	30.0
Median day	36.0	36.0	38.0	38.5
Range				
ZBAR	-	0.315	3.019	3.610
p-value	-	ns	p<0.01	p<0.01

No other significant treatment related effect on physical development were observed.

The reproductive performance of the F_1 generation was not adversely affected. The results of F_1 uterine examination were presented in a table on page 53 in volume 39 and this table is attached below.

Summary table of group mean F1 uterine examination data

Parameter	Dosage (mg/kg)			
	0	1.0	6.5	30.0
Number of pregnancies	13	11	12	12
Median number of corpora lutea	14.0	15.0	15.0	13.0
Total number of implantations Mean	190 14.6	139 12.6	171 14.3	154 12.8
Pre-implantation loss (%) Proportion of litters affected	1.6 3/13	17.8 7/11	5.0 5/12	6.1 3/12
Total number of foetuses Mean	180 13.8	135 12.3	167 13.9	152 12.7
Total early intra-uterine deaths Mean	10 0.8	4 0.4	4 0.3	2 0.2
Total late intra-uterine deaths	0	0	0	0
Post implantation loss (%) Proportion of litters affected	5.3 5/13	2.9 4/11	2.3 3/12	1.3 2/12

No significant treatment related changes were observed except for the high incidence of pre-implantation loss in the low dose group. The terminal examination of F_1 generation revealed no treatment related changes.

In conclusion, GR 68755 was given by oral gavage to pregnant female rats (15/group, 17 in high dose group) at 0, 1, 6.5, and 40 (days 1-3)/30 (day 4 onward) mg/kg/day during gestation day 17 through day 22 after delivery. GR 68755 at high dose produced maternal toxicity including death, decreased body weight gain (~24%) and food consumption (~21%). At this maternal toxic dose, following were observed in the offspring: clinical signs of toxicity (tip toe gait, hyperactivity, piloerection, and vocalization), decreased birth weight (g, control = g), decreased body weight gain during days 28-64 (12%), and slightly delayed testes descent and vaginal opening. The clinical signs of toxicity (tip toe gait, hyperactivity, piloerection, and vocalization), and decreased body weight gain of F_1 generation during days 28-64 (8%) were also observed in the mid dose group. Reproductive performance of F_1 generation was not adversely affected at doses up to high dose (40/30 mg/kg/day).

GENETIC TOXICITY:

Ames Test
(Study # V12622)

Testing Laboratories: Glaxo Group Research Ltd., Genetic & Reproductive Toxicology Dept., Hertfordshire, UK.

Dates Studies Started and Completed: December 4, 1990 and December 21, 1990

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537; and E. coli strains WP2 pKM101 and WP2 UVrA pKM101.

Concentration Employed: 40-1000 mcg/plate (50-1000 mcg/ml in fluctuation test).

Solvent Control: Water and dimethyl sulfoxide (DMSO)

Positive Control: NaN_3 (2.0 mcg/plate), ICR-191 (1.0 mcg/plate), hycanthone (10.0 mcg/plate), 2-aminoanthracene (2.0-5.0 mcg/plate or 5 mcg/ml), neutral red (10 mcg/plate),N-ethyl- N-nitro- N-nitrosoguanidine (0.1-0.25 mcg/plate), cyclophosphamide (100 mcg/ml).

Source of Metabolic Activation: Phenobarbitone & beta-Naptholflavone induced rat liver microsomal enzymes (S-9 mix).

Drug Batch No.: C 1026/133/1

Criteria of Positivity: When Dunnett's test gave a significant response ($p \leq 0.01$) and the data set showed a significant correlation then the test substance is considered positive provided results were reproducible.

Methods: Ames Ames test and test were conducted to assess the mutagenic potential of the drug by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium [TA 98, TA 1537 and TA 1538 (frame shift); TA 100 and TA 1535 (baser pair substitution)] and E. coli WP2 UVrA in the presence and absence of S-9 activation.

Results: Drug was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

In Vitro Chromosome Aberration Test (Study # V1192)

Testing Laboratories: Glaxo Group Research Ltd., Genetic & Reproductive Toxicology Dept., Hertfordshire, UK.

Dates Studies Started and Completed: July 4, 1989 and December 7, 1989.

Strain Employed: Cultured human lymphocytes cells.

Concentration Employed: 10-300 mcg/ml in the absence of S9-mix and 100-1000 mcg/ml in the presence of S9-mix.

Solvent Control: Water

Positive Control: Daunomycin (0.05 mcg/ml) and cyclophosphamide (5 mcg/ml).

Source of Metabolic Activation: Rat liver microsomal enzymes (S-9 mix).

Drug Batch No.: C1017/133/1

Methods: Human lymphocytes cultured cells were treated with the drug in the presence and absence of metabolic activator (S-9 mix). Cells were harvested at 24 hours after the start of treatment (cells in the presence of S-9 mix were treated only for 3 hours then washed and incubated for additional 69 hours). At 24 hr, in the absence of S-9 mix, the effect of three concentrations (10, 30 and 100 mcg/ml) were assessed and at this time highest concentration produced about 49% and 55% mitotic inhibition in experiment 1 and 2 respectively. In the presence of S-9 mix, the chromosomal aberrations were analyzed in cell sampled at 72 hr at three dose levels (100, 300 and 1000 mcg/ml). The highest concentration selected for analysis at this time produced about 57% and 35% mitotic inhibition in experiment 1 and 2 respectively. At the end of experiment 200 metaphases were examined per treatment