FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) RODENT CARCINOGENICITY FACTSHEET

NDA: IND: DRUG CODE #:
CAS #: DATE:
DIVISION(s): HFD-180
DRUG NAME(s): Alosetron (GR 68755)

SPONSOR: Glaxo Wellcome Inc.

LABORATORY:

P/T REVIEWER(s): Tanveer Ahmad, Ph.D.
P/T REVIEW DATE: 3/21/96
CARCINOGENICITY STUDY REPORT DATE:
THERAPEUTIC CATEGORY:

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: 5-HT_3 receptor antagonist

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No

MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): No

RAT CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc):

RAT STUDY DURATION (weeks): 104
STUDY STARTING DATE: 3/4/91
STUDY ENDING DATE: 6/30/95
RAT STRAIN: Wistar
ROUTE: Via diet
DOSING COMMENTS:

No. Rats in Control1 (C1): 60 Control2 (C2):
Low Dose (LD): 60 Middle Dose (MD): 60
High Dose (HD): 60 High Dose2 HD2):

RAT DOSE LEVELS (mg/kg/day)

Rat Low Dose: 1.0 Rat Middle Dose: 6.5
Rat High Dose: 40 Rat High Dose2:

*Dose adjusted during study.

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): AUC

RAT CARCINOGENICITY (negative; positive; MF; M; F): Negative

RAT TUMOR FINDINGS: None

RAT STUDY COMMENTS:

In 104-week oral (via diet) carcinogenicity study in Wistar rats doses of 0, 1, 6.5 and 40 mg/kg/day were used. In this study, highest tested dose is the maximum tolerated dose since at this dose level body weight in males and females were 6% and 9% lower than the control body weights respectively. Furthermore, based on AUC values, high dose treated rats (both sexes) were exposed to 123-141 fold higher levels of GR 68755 than human [$AUC_{0\text{-}24\,hr}$ = 396.4 ng.hr/ml; 8 mg b.i.d. = 0.32 mg/kg/ day, 50 kg body weight assumed]. Hence, dose selection was appropriate. Treatment had no significant effect of intercurrent mortality rates. Survival rates at the end of treatment period were comparable in all groups. Increased incidences of basophilic foci in the liver of high dose treated females and increased incidences of clear cell foci in liver of high dose treated males were seen. No treatment related neoplastic findings were evident in this study. Hence, GR 68755 has no carcinogenic potential in Wistar rats.

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS

1. Study No.: R12458
2. Name of Laboratory:
3. Strain: Wistar
4. No./sex/group: 60
5. Doses (O, L, M, H): 0, 1, 6.5 and 40 mg/kg/day
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 104
9. Week/site for first tumor:

	Male	Female
O	52/Malignant Astrocytoma (brain)	49/Malignant Leiomyo Sarcoma (vagina)
L	67/Benign Adenoma (Pituitary)	51/Malignant Carcinoma (Mammary)
M	50/Malignant Schwannoma (miscellaneous)	49/Malignant Carcinoma (mammary)
H	42/Malignant Lymphoma (hemopoietic tumor)	56/Malignant Histocytic Sarcoma (skin)

10. No. alive at termination:

	Male	% Survival	Female	% Survival
O	64/120	53	76/120	63
L	36/60	60	29/60	48
M	36/60	60	38/60	63
H	39/60	65	41/60	68

11. Statistical Methods Used: Peto, etal (IARC, 1980) for the analysis of tumor incidence.

12. Attach tumor and non-tumor data for each tissue (i.e., benign; malignant; hyperplastic): See Appendix 1

104-Week Oral Dietary Carcinogenicity Study in Rats
(Study # R12458)

Testing Laboratories: [redacted]

Study Started: March 26, 1991

Study Completed: June 30, 1995

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Test Species and Strain: Wistar rats

Route of Administration: Via diet

Dose Levels: 0, 1, 6.5 and 40 mg/kg/day

Drug Batch No.: C1026/120/1, C1026/123/1 and C1757/106/1

Methods: Groups of rats (60/sex/group) were given GR 68755 via diet at daily doses of 1, 6.5 and 40 mg/kg/day for 104 weeks. A control group of 120 rats/sex was included which received unmedicated diet. Additionally, 20 rats/sex/group were included as satellite animals for monitoring plasma levels of GR 68755. All animals were observed for clinical signs and mortality twice daily. Body weights were recorded weekly during the first 14 weeks of the study then twice monthly. Food intakes were recorded weekly. Auditory function tests were performed on 20 rats/sex from control and high dose groups during weeks 44, 73 and 102 of the study. During week 103 of the study, blood samples were collected from tail vein for hematology tests. Blood samples were also collected from satellite animals at 4 hour interval after 4 and 101 weeks of treatment (5 rats/sex/time point were used) for monitoring drug levels in the plasma. At T_{max} blood samples were also collected during weeks 26, 52 and 78 of the study from 5 rats/sex/group for measuring drug levels in plasma. At the end of study period all surviving rats were sacrificed and subjected to histopathological examinations. Sponsor used the approach of Peto, etal., (IARC, 1980) for the analysis of tumor incidence.

Results:

1. Achieved Doses: The mean intakes of GR 68755 were within 1% of the intended doses (low dose: mean = 1.0 mg/kg/day for males and females [range: mg/kg/day for both sexes], mid dose: mean = 6.5 mg/kg/day for males and females [range: mg/kg/day for males and mg/kg/day for females] and high dose: mean = 39.9 mg/kg/day for males and 40.0 mg/kg/day for females [range: mg/kg/day for males and mg/kg/day for females]).

2. Observed Effects: No treatment related effects were seen.

3. Mortality: Treatment had no significant effect on intercurrent mortality rates (see below). At termination survival rates were comparable in all groups (53-65% in males and 48-68% in females).

Intercurrent Mortality Rates

Male Rats

Weeks	Control	%	Low Dose	%	Mid Dose	%	High Dose	%
0-52	5/120		0/60		2/60		3/60	
53-78	10/115		4/60		4/58		5/57	
79-104	41/105		20/56		18/54		13/52	
Terminal	64	--	36	--	36	--	39	--
Survival Rate	--	53	--	60	--	60	--	65

Female Rats

Weeks	Control	%	Low Dose	%	Mid Dose	%	High Dose	%
0-52	2/120		1/60		2/60		0/60	
53-78	8/118		4/59		4/58		3/60	
79-104	34/110		26/55		16/54		16/57	
Terminal	76	--	29	--	38	--	41	--
Survival Rate	--	63	--	48	--	63	--	68

4. Body Weight/Food Consumption/Water Consumption: At the end of treatment period, absolute body weights of high dose treated males and females were 6% and 9% lower than the control values respectively. Treatment had no significant effect on food consumptions.

Body Weight (g) of Male Rats				
Weeks	Control	Low Dose	Mid Dose	High Dose
0	151 ± 15.2	150 ± 15.0	147 ± 13.8	153 ± 14.2
104	703 ± 101.2	720 ± 108.9	701 ± 98.5	660 ± 101.6
Body Weight (g) of Female Rats				
Weeks	Control	Low Dose	Mid Dose	High Dose
0	136 ± 11.9	135 ± 12.1	134 ± 11.3	137 ± 10.6
104	459 ± 67.7	503 ± 94.9	509 ± 94.9	419 ± 69.5

FIGURE 3A
Group mean bodyweight versus period of treatment - males

Group	:	1	2	3	4
Compound	:	Control	— GR68755C —		
Dosage (mg GR68755X/kg/day):		0	1.0	6.5	40.0

—— Group 1M — - Group 2M —— Group 3M ------ Group 4M

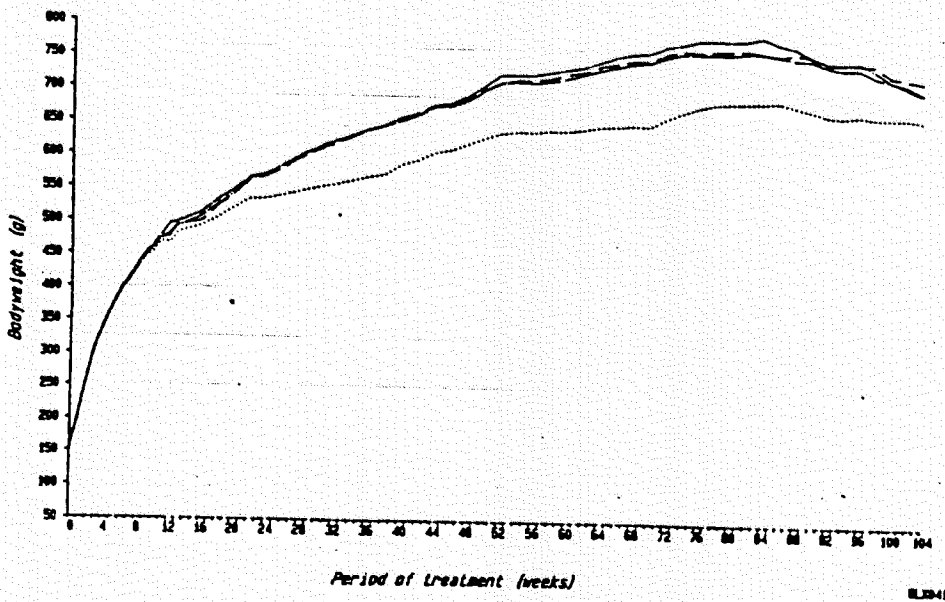

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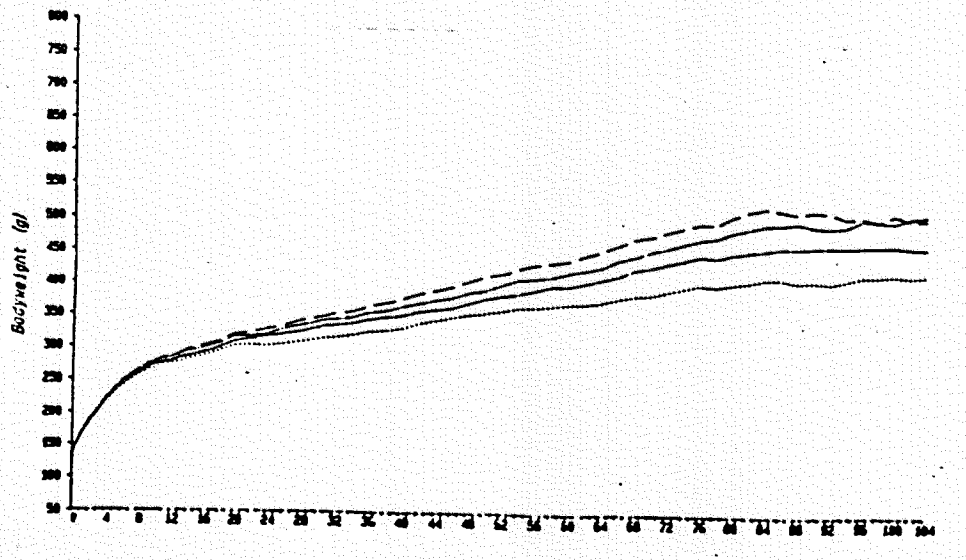
FIGURE 3B
Group mean bodyweight versus period of treatment - females

Group	:	1	2	3	4
Compound	:	Control	— GR68755C —		
Dosage (mg GR68755X/kg/day):		0	1.0	6.5	40.0

—— Group 1F — - Group 2F —— Group 3F ------ Group 4F



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Food Consumption (g/Animal/Week) in Male Rats				
Weeks	Control	Low Dose	Mid Dose	High Dose
1	220 ± 9.4	222 ± 13.1	223 ± 11.1	226 ± 11.4
13	207 ± 8.6	211 ± 9.0	214 ± 11.0	205 ± 8.5
52	205 ± 11.2	207 ± 7.2	212 ± 7.5	198 ± 12.7
104	176 ± 16.0	179 ± 13.3	177 ± 18.5	185 ± 19.1
Food Consumption (g/Animal/Week) in Female Rats				
Weeks	Control	Low Dose	Mid Dose	High Dose
1	190 ± 12.2	185 ± 13.9	190 ± 13.8	190 ± 12.0
13	163 ± 8.6	169 ± 8.4	166 ± 7.8	163 ± 6.7
52	158 ± 9.1	164 ± 8.4	162 ± 9.0	155 ± 8.5
104	167 ± 21.3	171 ± 12.3	171 ± 14.3	166 ± 18.3

5. Hearing Test: No treatment related effects were seen.

6. Hematology: No treatment related effects were seen.

7. Gross Pathology: No treatment related effects were seen.

8. Histopathology:

Non-neoplastic Findings: Increased incidences of basophilic foci in the liver were seen in high dose treated females and increased incidences of clear cell foci were seen in the liver of high dose treated males. The incidences of above abnormalities were as follows:

Non-neoplastic Findings					
Site/Type	Sex (M/F)	Control	Low Dose	Mid Dose	High Dose
Liver:					
Basophilic foci	M	1/120	0/60	3/60	3/60
	F	34/120	13/60	16/60	38/60
Clear cell foci	M	42/120	25/60	19/60	33/60
	F	16/120	6/60	7/60	8/60

Neoplastic Findings: No treatment related effects were seen.

9. Levels of GR 68755 in Plasma (WBP/94/037): Plasma levels of GR 68755 increased with increasing dosages. There were no sex differences.

AUC_{0-24} (ng.hr/ml) During Week 101			
	Low Dose	Mid Dose	High Dose
Male	608	3120	56000
Female	784	4890	48800

In this study, highest tested dose is the maximum tolerated dose since at this dose level body weight in males and females were 6% and 9% lower than the control body weights respectively. Furthermore, based on AUC values, high dose treated rats (both sexes) were exposed to 123-141 fold higher levels of GR 68755 than human [$AUC_{0-24\,hr}$ = 396.4 ng.hr/ml; 8 mg b.i.d. = 0.32 mg/kg/day, 50 kg body weight assumed]. Hence, dose selection was appropriate. Treatment had no significant effect of intercurrent mortality rates. Survival rates at the end of treatment period were comparable in all groups. Increased incidences of basophilic foci in the liver of high dose treated females and increased incidences of clear cell foci in liver of high dose treated males were seen. No treatment related neoplastic findings were evident in this study. Hence, GR 68755 has no carcinogenic potential in Wistar rats.

REPRODUCTIVE TOXICITY:

Oral Segment I. Fertility and General Reproductive Performance Study in Rats
(Study # R12036)

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

Study Started: October 30, 1989

Study Completed: July 3, 1990

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

Animals: 7-9 weeks old AHA rats (Wistar/SD derived with Wistar Characteristics).

Drug Batch No.: C1028/98/1

Methods: The dose selection was based on preliminary oral organogenesis study in pregnant rats (study # R11996), in which doses of 0, 20, 30 and 40 mg/kg/day were used. At high dose, body weight gains were reduced by 21% compared to control value and this effect persisted throughout pregnancy period. In view of these findings the highest dose selected for the present study was 40 mg/kg/day.

In the main study, groups of 15 male and 30 female rats were given orally (gavage) 0 (water), 1, 6.5 and 40 mg/kg/day of GR68755. The volume of administration was fixed at 10 ml/kg. The male rats were treated from 71 days prior to mating and throughout the mating phase and until they were sacrificed. Females were treated for 22 days prior to mating and throughout mating, gestation, lactation and till they were sacrificed (approximately 22 days after postpartum). Parents were observed daily for mortality and toxic signs. Body weights and food/water consumptions were recorded weekly. Additionally, dams were weighed daily throughout pregnancy until day 22 or termination. The mating performance and fertility of both sexes were evaluated. Blood samples were also collected from tail vein of 3 males and 5 females of each group during week prior to pairing at 15 minutes after drug administration for monitoring drug levels in plasma. About one-half of pregnant rats were sacrificed on day 20 of gestation, and was 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. Fetuses were eviscerated and one-half of fetuses were examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining dams were allowed to deliver spontaneously. The number of live/dead pups were recorded, and the live pups were weighed and sexed. The offspring were reared by the dams until weaning. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pinna detachment, tooth-eruption, eyelid separation, visual and auditory function tests, testes descent, vaginal opening, learning ability test and open field test). On day 24 of post partum all dams were sacrificed and necropsied, and examined as mentioned above. Postnatal body weight changes of the pups were recorded until the age of 24 days. At day 24 of post partum, a minimum of one male

and one female pup were selected from each litter for F_1/F_2 generation study. At 9 weeks of age they were continuously mated and study was repeated as mentioned above except animals were not treated and all females were allowed to litter. F_2 generation were examined for abnormalities and then killed on day 20 of post partum.

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 0.0-14.7%]) 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 were 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.

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 number 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.