Mortality rate was comparable in control and treatment groups. The terminal body weight in the high dose female was 91.4% of the control. Higher incidences of Harderian gland adenoma and liver cell tumors were found in the treated males and females, respectively. These increased incidences were not dose related and not statistically significant (both trend and pairwise tests). They are within the background incidence. Treatment with GR 68755 produced benign interstitial cell tumor of the testes in a dose dependent manner and a single incidence of malignant interstitial cell tumor of the testes in a mid dose male (none in the controls). The increased incidences were not statistically significant by the trend test. The increased incidences in each of the treatment groups were not significantly (pairwise test) different from the incidences in the vehicle control group.

3-Month Oral (via diet) Dose-Ranging Study in Wistar Rats (Study # R12457)

Testing Laboratories:

Study Started and Completed: October 25, 1990 and March 24, 1992

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

Animals: Male and female Wistar rats (29-36 days old; 82-112 g).

Drug Batch No.: C1026/120/1

Methods: In this study dose selection was based on the results of 21-day palatability study (# R12495) in which 100 mg/kg/day caused lethality and reductions in body weight gains (males: 12% and females: 4%) were seen at 30 mg/kg/day dose levels. Based on these findings, sponsor selected 10, 20 and 40 mg/kg/day for the present study. Groups of rats (10/sex/group) were given GR 68755 via diet at daily doses of 10, 20 and 40 mg/kg/day for 3 months. The control group animals were given unmedicated diet. Additionally, 6 rats/sex/group were included as satellite animals for toxicokinetics study. All animals were observed for clinical signs twice daily. Body weights and food intakes were recorded weekly. Blood samples were

collected from tail vein on day 8/9 from satellite rats and on days 90/91 from main study rats. The blood collection time points were 0800, 1200, 1600, 2000, 0400 and 0800 hours and 3 rats/sex/group/time points were used. All surviving animals were sacrificed at the end of study period and subjected to complete necropsy. Only tissues from control and high dose groups were examined histopathologically. Liver from low and mid dose groups, pituitary from low and mid dose treated females and gross abnormal tissues from all animals were also examined histopathologically.

Results: The intakes of the drug were within 2% of the intended doses (low dose = mg/kg/day, mid dose = mg/kg/ day and high dose = mg/kg/day). There were no deaths and no treatment related signs were seen. Treatment had no significant effect on food consumptions and body weights. Only in high dose treated females, absolute as well as relative, weights of pituitary were reduced by 27-28% compared to control values. No treatment related histopathological abnormalities were evident in this study.

Levels of GR 68755 in plasma were measured at (WBP/91/045). Levels of GR 68755 increased with increasing dosages. Levels seen on day 90/91 of the study were higher than that seen on days 8/9 of the study. Throughout the study period, plasma concentrations in males were lower than that seen in females.

$AUC_{0-24\ hr}$ (ng.hr/ml)				
Dose (mg/kg/day)	**Day 8/9**		**Day 90/91**	
	Male	**Female**	**Male**	**Female**
10	1660	2520	2410	4090
20	2260	3790	7510	11600
40	9460	19500	21400	35500

In this study only in high dose treated females, absolute as well as relative, weights of pituitary were reduced by 27-28% compared to control values. No treatment related histopathological abnormalities were evident in this study. The systemic exposure of GR 68755 at 40 mg/kg/day dose level was about 24-89 fold higher than the anticipated human exposure ($AUC_{0-24\ hr}$ = 396.4 ng.hr/ml, 0.32 mg/kg/day [8 mg b.i.d.], 50 kg body wt. assumed). Based on multiple of human exposure, sponsor selected 40 mg/kg/day as the top dose for the carcinogenicity study in rat and the mid and low doses were set at 6.5 and 1.0 mg/kg/day respectively.

3-Month Oral (gavage) Dose-Ranging Study in Wistar Rats (Study # 12569)

Testing Laboratories:

Study Started and Completed: October 29, 1990 and June 29, 1995

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

Animals: Male and Female Wistar Rats (33-40 days old, 82-113 g)

Drug Batch No.: C1026/120/1

Methods: Groups of rats (10/sex/group) were given orally (gavage) GR 68755 at daily doses of 10, 20 and 20/40 (20 mg/kg/day for the first seven days) mg/kg/day for 3 months. The volume of administration was fixed at 10 ml/kg. Control group animals received vehicle (water) in similar fashion. Additionally, 7 rats/sex/group were included as satellite animals for toxicokinetic study. All animals were observed for clinical signs and mortality twice daily. Body weights and food consumptions were recorded weekly. Blood samples from tail vein were collected on days 1/2 from low and mid dose groups, on days 8/9 from control and high dose group and on days 92/93 from all groups. The collection time points were 10, 20 and 30 min, 1, 2, 4 and 24 hr after drug administration. All surviving rats were killed at the end of study period and subjected to complete necropsy. Only tissues from control and high dose groups were examined microscopically. Liver from low and mid dose groups and all gross abnormal tissues from all groups were also examined histopathologically.

Results: Transient noisy respiration was seen in mid and high dose treated rats. One mid dose treated female died on day 28 of the study and the cause of death was not treatment related. Treatment had no significant effect on body weight gains and food consumptions. In high dose treated females, liver weights were increased by 28% while a 10-13% increase in liver weights were seen in all treated males. Histopathological examinations revealed periacinar hepatocytic hypertrophy in 6/10 high dose treated females (and none in the control group) and foci of "pre-basophilic" hepatocytes in 3/10 high dose treated females (and none in the control).

Levels of GR 68755 in plasma were measured at (WBP/91/046). Levels of GR 68755 increased with increasing dosages. In low and mid dose groups, levels seen on day 92 were higher than that seen on day 1 of the study. Furthermore, there is an indication that a given dose level of GR 68755 in males were less than that seen in females (may be due to higher clearance of GR 68755 in male rats). In all treated rats, GR 68755 levels at 24 hr after drug administration were ≤3.1% of C_{max} values.

$AUC_{0-4\ hr}$ (ng.hr/ml)				
Dose (mg/kg/day)	**Day 1**		**Day 92**	
	Male	**Female**	**Male**	**Female**
10	2970	5130	6340	10500
20	6520	12100	13700	17600
20*/40	13600**	18900**	20700	24400

* = given during the first 7 days of the study
** = levels were measured on day 8 of the study

In this study, increase in liver weights were seen in all treated males (10-13%) and in high dose treated females (28%). Histopathological examinations revealed periacinar hepatocytic hypertrophy and foci of "pre-basophilic" hepatocytes in some of the high dose treated females (and none in the control).

In both dietary and gavage 3-month dose ranging study in rats, the systemic exposure of GR 68755 at 40 mg/kg/day dose level was at least 24 fold higher than the anticipated human exposure ($AUC_{0-24\ hr} \approx 396.4$ ng.hr/ ml, 0.32 mg/kg/day [8 mg b.i.d.], 50 kg body wt. assumed). Based on multiple of human exposure, sponsor selected 40 mg/kg/day as the top dose for the carcinogenicity study in rat and the mid and low doses were set at 6.5 and 1.0 mg/kg/day respectively. The selection of top dose is appropriate (see the result of main carcinogenicity study in rat).

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

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

SPONSOR: Glaxo Wellcome Inc.

LABORATORY: [redacted]

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 [$AUC_{0-24\ hr}$ = 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: [redacted]
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

Addendum: The tumor and non-tumor data for each tissue are included in the Appendix IV in this NDA review.

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 inter-current 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

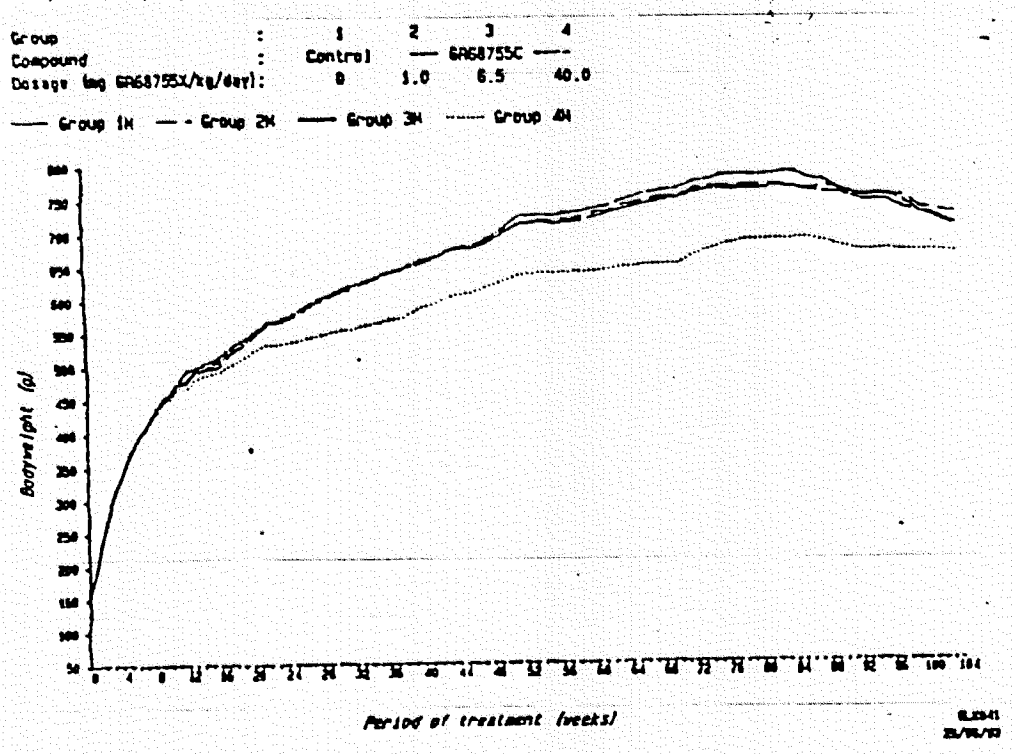
Female Rats

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

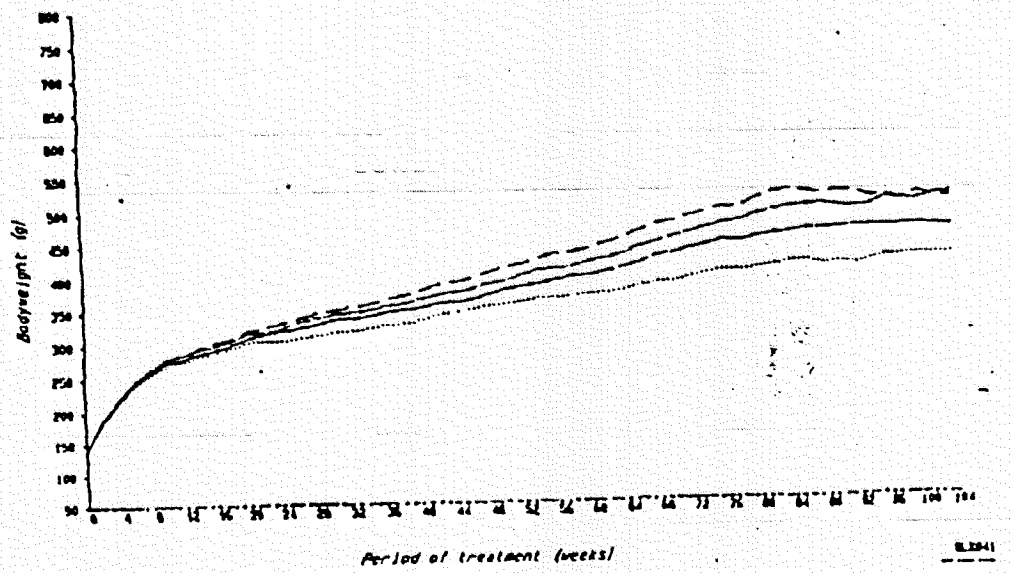


APPEARS THIS WAY
ON ORIGINAL

FIGURE 38
Group mean bodyweight versus period of treatment - females

Group	:	1	2	3	4
Compound	:	Control	--- GR68755C ---		
Dosage (mg GR68755X/kg/day):		0	1.0	8.5	40.0

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



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	43800

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.