

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 were 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 produced 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 volumes 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 =           ]). 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
<b>Fetal Major Malformations</b>				
# 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.

APPEARS THIS WAY  
 ON ORIGINAL

**GENETIC TOXICOLOGY:**

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<sub>3</sub> (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-  
Naphthoflavone 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 ( $p < \text{or} = 0.01$ ) 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 (base 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 (S9 mix). Cells were harvested at 24 hours after the start of treatment (cells in the presence of S9-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 S9-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 group. If a statistically significant increases in proportion of structurally aberrant cells (without gaps) occurred at one or more concentrations compared to control and proportion of aberrant cells at such data points exceed the normal range then the compound is genotoxic.

**Results:** Irrespective of the presence or absence of metabolic activation, treatment with GR 68755C did not produce any significant reproducible increase in chromosomal aberration over the value obtained for the control group. The positive control gave expected results. Thus GR 68755C had no clastogenic activity in this in vitro cytogenetic test.

Unscheduled DNA Synthesis in Primary Hepatocytes of  
Male Rat (Ex-Vivo)  
(Study # R12689)

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

**Dates Studies Started and Completed:** January 14, 1991 and  
August 29, 1991.

**Animals:** Male and female AHA rats (189.7-257.2 g).

**Doses Used:** 25, 50, 75 and 100 mg/kg in males and 10, 25 and 40 mg/kg in females.

**Drug Batch No.:** C10261120/1 & C1026/123/1

**Solvent Control:** Water

**Positive Control:** 2-Acetylaminofluorene (2-AAF: 75 mg/kg) and 1, 2-dimethylhydrazine HCl (DMH: 20 mg/kg).

**Methods:** Groups (3/group) of rats were given a single dose of GR 68755C via gavage (25, 50, 75 and 100 mg/kg in males and 10, 25 and 40 mg/kg in females). The positive control group animals received 2-AAF or DMN and the negative control group rats received vehicle (water). The volume of administration was 10 ml/kg. Groups of animals were sacrificed at 2-4 hours after drug administration, and another groups were sacrificed at 12-14 hours after the drug administration. Hepatocyte primary cultures from the liver of the rats were obtained, and [<sup>3</sup>H] thymidine incorporation was measured. Incorporation was followed by <sup>3</sup>H of the hepatocytes and grains were counted in 100 nuclei other than S-phase. Net increases in nuclear grains

induced by each compound were determined. The test compound is considered positive when the mean nuclear grain count is  $\geq 0$  and 20% or more of the cells responding (a cell is regarded as being "in repair" when net grain is  $\geq 5$ ).

**Results:** No net increase in nuclear grain counts was observed in hepatocytes when rats were treated with GR 68755C at oral (gavage) doses up to and including 100 mg/kg in males and 40 mg/kg in females. The positive controls were genotoxic, while no repair was induced by vehicle (negative control). Thus results suggest that BRL 43694A was not genotoxic in this test.

Thymidine Kinase Mutation Test in Mouse Lymphoma L5178Y Cells  
(Study # V13036)

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

Dates Studies Started and Completed: September 2, 1991 and  
December 29, 1991.

Strain Employed: L5178Y TK<sup>+</sup> mouse lymphoma cells.

Concentration Employed: 200-400 mcg/ml in the absence of S9-mix  
and 100-300 mcg/ml in the presence of S9-mix (close to solubility  
limit in \_\_\_\_\_).

Solvent Control: Water

Positive Control: Methyl methanesulphate (MMS: 10 mcg/ml) and  
dimethylbenzanthracene (DMBA: 2.5 mcg/ml).

Source of Metabolic Activation: Rat liver microsomal enzymes.

Drug Batch No.: C1026/133/1

Methods: In the range-finding study, cells were incubated with various concentrations of GR 68755C in the absence (10-350 mcg/ml) and presence (10-400 mcg/ml) of S9-mix. The data indicated that the drug was cytotoxic at 300 mcg/ml in the presence of S9-mix (relative survival rate = 42.3%) and heavy precipitate was seen at 400 mcg/ml. In the absence of S9-mix, highest tested dose (350 mcg/ml) had no effect on survival rate, however, 2 days after the treatment relative survival rate at the highest dose was decreased by 34%. Based on the above findings,

GR 68755C concentrations selected for the main study were 200 - 400 mcg/ml in the absence of S9-mix and 100 - 300 mcg/ml in the presence of S9-mix. A total of 5 assays were performed (3 in the absence of S9-mix and 2 in the presence of S-9 mix. Cells were incubated for 3 hours, then cells were washed, resuspended in media and viability rates and mutation frequency were determined at appropriate time. The test substance was considered to be mutagenic if the mutation frequency at 1 or more dose was significantly greater than that of the negative control and it was dose related.

**Results:** Irrespective of the treatment with metabolic activation system (S-9 Mix), no increase in mutant colonies was seen in this study. Thus, the drug is not mutagenic at the tk locus of L5178Y mouse lymphoma cells.

**Rat Micronucleus Test**  
(Report # 1152/92)

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

**Dates Studies Started and Completed:** March 1, 1989 and  
July 27, 1989.

**Test Species:** Male PVG rats

**No. of Animals:** 5 - 7/group

**Route of Administration:** Orally (gavage) or I.V.

**Dose Levels:** Oral: 50, 125 and 250 mg/kg (10 ml/kg), I.V.: 6.25 or 12.5 mg/kg (5 ml/kg).

**Drug Batch No.:** C1017/133/1

**Basis of Dose Selection:** Dose levels are based on the preliminary toxicity study.

**Solvent Control:** 0.9% saline (10 ml/kg).

**Positive Control:** Cyclophosphamide (25 mg/kg, 10 ml/kg).

**Methods:** Animals were given a single dose at 24 or 48 hours prior to sacrifice and preparation of the bone marrow (positive control animals were sacrificed at 24 hr after cyclophosphamide administration). On the \_\_\_\_\_ slides, 2000 polychromatic erythrocytes per animal were examined for the presence

of micronuclei. The compound is considered positive if the number of micronucleated polychromatic erythrocytes at one or more doses are significantly greater than the negative control value and it is dose related.

Results: GR 68755C did not induce an increase of micronucleated polychromatic erythrocytes in rat bone marrow. In contrast, the % of micronucleated polychromatic erythrocytes in cyclophosphamide treated group was markedly higher than the negative control. These findings suggest that GR 68755C is not mutagenic in this test system.

Ames Test  
(Study # V12468)

This test was conducted to assess the mutagenic potential of GR 62202X which is an intermediate in the synthesis of GR 68755C).

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

Dates Studies Started and Completed: Not given (report date: December 14, 1990).

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537.

Concentration Employed: 50 - 5000 mcg/plate.

Solvent Control: Dimethyl sulfoxide (DMSO).

Positive Control: NaN<sub>3</sub> (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) and neutral red (10 mcg/plate).

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

Drug Batch No.: C 1017/321/1

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



#### SUMMARY AND EVALUATION:

GR 68755 is a potent and selective 5-HT<sub>3</sub> receptor antagonist. It is about 5-10 times more potent 5HT-3 receptor antagonist than ondansetron. It inhibits 2-methyl-5-HT (5-HT<sub>3</sub> receptor agonist) induced Bezold-Jarisch reflex in anesthetized cats. It also dose dependently inhibited cisplatin- and cyclophosphamide induced emesis in conscious ferret. Irritable Bowel Syndrome (IBS) is a functional disorder of gastrointestinal (GI) tract characterized by alterations in bowel function and abdominal pain or discomfort. Sponsor has not provided any rationale for the use of GR 68755 (5-HT<sub>3</sub> antagonist) for the treatment of IBS. In anesthetized rat, one of the main symptoms of IBS i.e. pain or discomfort to rectal distention was produced by "step-wise inflation of latex balloon inserted in the rectum. In this model, GR 68755 (1-100 mcg/kg, i.v.) dose dependently inhibited the hypotensive response to colorectal distention (ID<sub>50</sub> = 3 mcg/kg). At 30 mcg/kg (i.v.) the hypotensive response to 1.5 ml distention was decreased from 26.7 to 1.7 mmHg. Clinical studies performed in Europe and Canada indicated that GR 68755 may be beneficial in "diarrhea-predominant IBS patients". In cynomolgus monkeys, alosetron (0.01-1 mg/kg, i.v.) had no effect on heart rate or arterial blood pressure. However, in one monkey a single ventricular ectopic beat was seen at 5 min after administering of 0.1 mg/kg of alosetron and in second monkey similar finding was seen at 6 min after the administration of 1 mg/kg (i.v.) of alosetron along with a small increase in Q-T interval. According to sponsor, these are isolated findings and considered not to be treatment related. Furthermore, in anesthetized cat, alosetron (0.1-1 mg/kg, i.v.) had no significant effect on cardiovascular system.

Sponsor proposed to conduct a phase II, randomized, dose-ranging, double-blind, placebo-controlled, parallel group, multicenter study to assess the safety and efficacy of alosetron (0, 1, 2, 4 or 8 mg b.i.d. = 0, 0.02, 0.04, 0.16 or 0.32 mg/kg/day; 50 kg body weight assumed) in 350 (70/group) irritable bowel syndrome (IBS) patients. The duration of treatment will be 12 weeks.

In support of the above protocol, sponsor has submitted preclinical pharmacology studies; absorption, distribution, metabolism and excretion (ADME) studies in mouse, rats, rabbits and dogs; oral and i.v. acute toxicity studies in mice and rats; 35 days and 6-month oral toxicity studies in rats and dogs; 12-month oral toxicity study in dogs; 13-week oral (via drinking water) in mice, 3-month oral (via diet and gavage) toxicity studies in rats, 2-year carcinogenicity study in rat; Segment I. oral fertility and general reproductive performance study in rats; Segment II. oral teratology studies in rats and rabbits;

genotoxicity studies: Ames Test, in vitro chromosomal aberration test in human lymphocytes, L5178 Y/TK mouse lymphoma mutation assay, ex-vivo UDS assay in rat hepatocytes and in vivo rat micronucleus test.

Absorption, distribution, metabolism, and excretion studies were conducted in mice, rats, rabbits and dogs. In mice, rats, rabbits and dogs, GR 68755 absorbed rapidly ( $T_{max}$ : mice, rats and rabbits  $\leq 0.5$  hr and dogs = 0.75 hr) and completely (bioavailability: rats = 100% and dogs = 96%). In mice the  $t_{1/2}$  value was  $< 0.25$  hr while  $t_{1/2}$  values in rats, rabbits and dogs were about 1 hr. In human, depending upon dosages (1-16 mg b.i.d.) the  $t_{1/2}$  values were \_\_\_\_\_ hr,  $C_{max}$  and AUC are not proportional to increasing dosages, suggesting that GR68755 may be a "mild inducer" of its own metabolism (report # UCP/91/014, page 50, volume 1.35). Irrespective of strains of rats, the systemic exposure of GR 68755 and/or its metabolites in females were significantly greater than in males (based on AUC values).

In rats, administered radioactivity was widely distributed throughout the body, and concentrations in liver, kidneys and adrenals were significantly higher than blood. Radioactivity was also seen in the eyes of pigmented rats. In pregnant rats and rabbits, radioactivity crosses placental barrier and is widely distributed in fetuses.

Irrespective of species, the drug is rapidly metabolized following oral or i.v. administration. More than 10 radioactive peaks were seen in urine and fecal samples and one of the peak was identified as GR 87620 (N-desmethyl analogue of GR 68755, it represented only 4% of the dose in rat urine sample). A bis-oxidized metabolite of GR 68755 (GR 153732) which is present in human plasma and urine following oral administration of GR 68755 was also identified in rat's and dog's urine samples.

Less than 10% of dose was excreted as unchanged drug. Irrespective of species, about 42-59% and 33-48% of administered radioactivity were excreted in urine and fecal respectively, (most of the fecal excretion represented biliary excretion).

Acute oral and i.v. toxicity of GR 68755 was studied in mice and rats. In mice and rats, irrespective of route of administration, the clinical signs were subdued behavior, occasional croaking (rat), half-closed eyes (rat), decreased activity, labored respiration, tremor, ataxia and convulsions. The minimum oral lethal doses were 25 and 15 mg/kg in male and female mice respectively and 8.0 mg/kg was the minimum lethal i.v. dose in male mice (minimum lethal i.v. dose in female mouse was not determined). The minimum oral lethal dose in female rat was

60 mg/kg (minimum lethal oral dose in male rat was not determined). The minimum lethal i.v. dose in male rat was 20 mg/kg/day (minimum lethal i.v. dose in female rat was not determined). The highest non-lethal oral doses were 15 and 10 mg/kg in male and female mice respectively, and 4.11 mg/kg was the highest non-lethal i.v. dose for mice of both sexes. In rats, the highest non-lethal oral doses were 120 and 40 mg/kg in males and females respectively, and the corresponding i.v. doses were <20 and 20 mg/kg/day.

In the 35-day oral (gavage) toxicity study in rats, doses of 1, 8 and 40 (day 1-5)/64 mg/kg/day were used. In this study target organ of toxicity was thymus. Highest tested dose (40/64 mg/kg/day) also produced CNS toxicities, decreased platelet counts in females, increases in serum alkaline phosphatase and alanine aminotransferase activities (in both sexes) without accompanying histopathological changes in liver and deaths. Mid dose level (8 mg/kg/day) can be considered as no effect dose since it only produced clinical sign (bulging eyes) in some of the mid dose treated rats.

In the 6-month oral (gavage) toxicity study in rats, doses of 1, 8 and 20 (days 1-4)/40 (days 5-7)/64 (days 8-54/55)/40 (days 55/56 onward) mg/kg/day were used. In this study CNS (salivation, tense behavior, moist eyes, croaking, tiptoe gait, pushing at cage floor with forepaws and tremor) and liver (basophilic foci of cellular alteration) are the target organs of toxicities. Findings in the liver were still present at the end of 40-day recovery period in some of the rats. Mid dose level (8 mg/kg/day) can be considered as no effect dose since it only produced some of the clinical sign occasionally in some of the mid dose treated rats.

In the 35-day oral (gavage) toxicity study in dogs, doses of 1, 5.5 and 30 (in females dosage was reduced to 20 mg/kg on day 6 then increased to 25 mg/kg on day 10 and 30 mg/kg from day 12 onwards) mg/kg/day were used. In this study target organ of

toxicity was thymus, and highest tested dose also produced CNS toxicities and death. Mid dose level (5.5 mg/kg/day) could be considered as well tolerated dose since it only produced minimal involution of the thymus in 1 out of 3 male dogs.

In the 6-month oral (gavage) toxicity study in dogs, doses of 1, 5.5 and 20 (days 1-3)/30 (days 4-8)/25 (day 9 onwards) mg/kg/day were used. In this study, highest tested dose (20/30/25 mg/kg/day) produced CNS toxicities, increases in serum alkaline phosphatase (both sexes) and alanine aminotransferase activities (in males) without accompanying histopathological changes in liver and deaths. Mid dose level (5.5 mg/kg/day) could be considered as well tolerated dose since it only produced increases in plasma alkaline phosphatase levels (males: 68% and females: 34%).

In the 12-month oral (capsules) toxicity study in dogs, doses of 1, 5 and 20 (day 1-3)/25 (day 4 onward) mg/kg/day were used. In this study, highest tested dose (20/25 mg/kg/day) produced CNS toxicities, "increased hearing threshold" (which at the end of recovery period returned to normal) and deaths. Mid dose level (5 mg/kg/day) could be considered as well tolerated dose since it only produced increase urinary volumes (158%) in females without any significant changes in specific gravity.

In 13-week oral (via drinking water) toxicity study in B6C3F<sub>1</sub> mice doses of 20, 30 and 40 mg/kg/day were used. In this study, data indicated that GR68755 was unpalatable in females when given via drinking water. In females, water intakes were 12%, 11% and 22% lower than the control values in low, mid and high dose groups respectively. During the study period, GR 68755 concentration in water was adjusted twice weekly, hence animal did received the intended dosages. Based on this finding, sponsor selected 30 mg/kg (via drinking water) as the top dose for mouse carcinogenicity study in mouse and the mid and low doses were set at 5.5 and 1 mg/kg/day respectively (for appropriateness of dose selection, see below).