

**RABBIT:**

Pharmacokinetics of GR 68755 in The Rabbit  
Following A Single Oral Dose  
(Study # MET779, Report # WBP/90/099)

**Methods:** Pregnant Dutch rabbits were given a single oral (gavage) dose of  $^{14}\text{C}$ -GR 68755 (40 mg/kg) on day 20 of pregnancy. Volume of administration was fixed at 1 ml/kg. Blood samples were collected at pre-test, 0.08, 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0 and 24 hr after drug administration (4 rabbits/time point were used). Various pharmacokinetic parameters were calculated. Additionally, urine and feces samples were collected over 24 hr period for 144 hours after drug administration. Levels of GR 68755 in samples were measured by \_\_\_\_\_ methods and total radioactivity was determined by \_\_\_\_\_ methods. One pregnant rabbit were killed at 1, 3 and 24 hr and three rabbits were killed at 144 hr after drug administration, and \_\_\_\_\_ was \_\_\_\_\_ Radioactivity in fetus were measured by \_\_\_\_\_

**Results:** GR 68755 absorbed rapidly ( $T_{\max} = 0.5$  hr) and plasma levels of GR 68755 decreased rapidly ( $t_{1/2} = 0.8$  hr). Based on AUC values about 31% of the total radioactivity represented parent drug (even at  $C_{\max}$ , only about 47% of the total radioactivity accounted for parent drug). Thus, indicating that GR 68755 undergoes first-pass metabolism in rabbits.

Pharmacokinetic Parameters in Rabbit After A Single Oral Dose		
Parameters	GR 68755	Radioactivity
$C_{\max}$ ( $\mu\text{g/ml}$ )	$7.8 \pm 2.8$	$16.7 \pm 3.7$
$T_{\max}$ (hr)	$0.5 \pm 0.3$	$0.5 \pm 0.3$
$\text{AUC}_{0-\infty}$ ( $\mu\text{g}\cdot\text{hr/ml}$ )	$13.4 \pm 9.0$	$42.9 \pm 3.0$
$t_{1/2}$ (hr)	$0.8 \pm 0.2$	$1.2 \pm 0.1$

Radioactivity crosses placental barrier and is widely distributed in fetuses. In fetuses, radioactivity declined very rapidly and at 24 hr after drug administration only fetal eye had significant amount of radioactivity (1150 ng/g; limit of detection = \_\_\_\_\_ ng/g). The radioactivity in eye of the fetus at 1 hr post-dose was about 4 times lower than that seen in maternal blood.

About  $47.7 \pm 3.3\%$  and  $44.3 \pm 2.2\%$  of the administered radioactivity were excreted in urine and feces during 0-144 hr period respectively, and most of the radioactivity was cleared during the first 24 hr period (urine =  $38.1 \pm 9.0\%$  and feces =  $25.5 \pm 16.3\%$ ). In both urine and feces, more than 9 radioactive peaks were seen. Only 2% and 3% of the dose represented unchanged drug in urine and feces respectively. Metabolite "U<sub>1</sub>" was the major urinary metabolite which accounted for 23% of the dose. Metabolite "F<sub>1</sub>" was the major fecal metabolite which accounted for 28% of the dose.

DOG:

Pharmacokinetics of GR 68755 in Dogs Following  
A Single Oral or I.V. Dose  
 (Study # MET535/566, Report # WBP/89/062)

Methods: Beagle dogs (3/sex) were given a single oral (gavage) or i.v. dose of <sup>14</sup>C-GR 68755 (2 mg/kg). Blood samples were collected from jugular vein at 0.08 (i.v. only), 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 24, 30, 48 and 72 hours after drug administration. Urine and feces were collected at 24 hr intervals up to 96 hours (urine) and 144 hours (feces) after drug administration. Levels of GR 68755 in samples were measured by methods and total radioactivity was determined by methods. Various pharmacokinetic parameters were calculated.

Results:

Mean Pharmacokinetic Parameters in Dogs After A Single Oral or I.V. Dose				
Parameters	GR 68755		Radioactivity	
	Oral	I.V.	Oral	I.V.
C <sub>max</sub> (ng/ml)	900 ± 156	1820 ± 450	1270 ± 129	2048 ± 452
T <sub>max</sub> (hr)	0.75 ± 0.39	0.08	0.8 ± 0.5	0.08
AUC <sub>0-∞</sub> (µg.h/ml)	2070 ± 500	2270 ± 550	4820 ± 850	5280 ± 735
Cl <sub>p</sub> (ml/min/kg)	---	16.0 ± 5.0	---	---
Vd (L/kg)	---	1.2 ± 0.4	---	---
t <sub>½</sub> (hr)	1.3 ± 0.3	0.9 ± 0.2	2.3 ± 0.4*	1.8 ± 0.2*
Bioavailability	96 ± 24	---	---	---

Cl<sub>p</sub> = plasma clearance

Vd = volume of distribution

\* = calculated for the first 8 hr, t<sub>½</sub> from 8-24 hr was approx. 4-5.5 hr

The data indicated that GR 68755 rapidly absorbed when given orally ( $T_{max}$  = 0.75 hr, bioavailability is almost 100%). Irrespective of route of administration, basis of AUC values, unchanged drug represented about 43% of total plasma radioactivity and  $t_{1/2}$  of the parent drug was about 1 hr. The plasma half-life of radioactivity was about 4-5.5 hr (oral and i.v.). Irrespective of route of administration, about 52-59% and 33-39% of the administered radioactivity were excreted in urine and feces, and most of the radioactivity was cleared during the first 24 hr period. Urinary excretion of radioactivity after oral and i.v. dose were comparable which suggest that oral dose is completely absorbed and fecal elimination mainly represents biliary excretion. Unlike rat, there were no sex related differences with respect of pharmacokinetic parameters. About 8 radiolabelled peaks were detected in urine and 11 in feces. N-desmethyl GR 68755 (GR 87620) was the major metabolite in urine (18-19% of the dose) and feces (10-11% of radioactivity in feces). Irrespective of route, unchanged drug levels were less than 3% of the dose in urine and feces.

In Vitro Binding to Plasma Proteins  
(Study # MET788, Report # WBP/91/008)

Rat, rabbit, dog and human plasma samples were incubated with \_\_\_\_\_ ng/ml of drug and mouse plasma samples were incubated with \_\_\_\_\_ mg/ml of drug. About 78.2%, 88.0%, 71.5%, 77% and 81.7% of the drug were bound to mouse, rat, rabbit, dog and human plasma.

In Vitro Distribution in Whole Blood  
(Study # MET787, Report # WBP/91/047)

Methods: Whole blood from pigmented (RH) rat, beagle dog and human were incubated with  $^{14}C$ -GR 68755 (20, 100, 600, 1200, 2000 or 4000 ng/ml) for 1 hr at 37°C. Radioactivity was measured in whole blood, red blood cells and plasma by \_\_\_\_\_ methods.

Results: About 35%, 37% and 31% of the radioactivity were bound to red cells of rat, dog and human respectively, and the whole blood plasma concentration ratio was about 0.9 for all levels tested and in all three species. Furthermore, when  $^{14}C$ -bound red cells were washed with control plasma, approximately one-half to two-thirds of the bound radioactivity was removed after each washing. Hence, binding of radioactivity with red cells was reversible.

Detection of A Bis-Oxidized Metabolite of  
GR 68755 in Rat, Dog and Man  
(Study # MET/594, Report # WBP/91/105)

In report WBP/91/108, sponsor reported bis-oxidized metabolite of GR 68755 as the major urinary metabolite in human urine after administration of 4 mg GR 68755 (base) orally. Rat (0-24 hr, Report # WBP/89/060) and dog (0-24 hr, Report # WBP/89/062) urine samples collected after oral administration of 3.5 mg/kg and 2 mg/kg of GR 68755 respectively, were analyzed for the presence of bis-oxidized metabolite of GR 68755. Like in human urine, rat and dog urine also contained bis-oxidized metabolite of GR 68755 (data presented as a peak and no quantitation was provided).

Interaction of GR 68755 With Rat, Dog and Human  
Hepatic Cytochrome(s) P-450  
(Study # MET877, Report # WBP/91/041)

**Methods:** Microsomal suspension prepared from male RH rat, beagle dog and human liver were incubated with <sup>14</sup>C-testosterone in the presence of GR 68755 (\_\_\_\_\_  $\mu$ m) and NADPH regenerating system. Metabolites of testosterone were analyzed by

It should be noted that 7 $\alpha$ -hydroxytestosterone production reflects the activity of CYP2A1. 6 $\beta$ -hydroxytestosterone production reflects the activity of CYP3A and 2 $\alpha$  and 16 $\alpha$ -hydroxytestosterone production reflects the activity of CYP2C11.

**Results:** GR 68755 at 100  $\mu$ m inhibited the formation of 6 $\beta$ -hydroxytestosterone from testosterone by rat, dog and human cytochrome P-450 indicating the interaction with CYP3A2 (rat) and CYP3A4 (dog and human) isozymes of cytochrome P-450.

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. 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

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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).

## TOXICOLOGY:

### Acute Toxicity:

Methods: Acute oral and i.v. toxicity of GR 68755 was studied in mice and rats. Control animals received the vehicle (water or 0.9% saline) in similar fashion. All animals were observed for clinical signs and mortality for various length of time (4-15 days). At the end of observation period, animals were sacrificed and necropsied.

### Results:

Acute Oral and I.V. Toxicity of GR 68755								
Species	Strain	Route	No/Dose/ Sex	Dose Levels (mg/kg)	Highest Non-Lethal Dose (mg/kg)		Minimum Lethal Dose (mg/kg)	
					Males	Females	Males	Females
Mouse	CRH	Oral	3	10, 15, 25* & 50	15	10	25	15
Mouse	CRH	Oral	4-10	0, 10	10	10	ND	ND
Mouse	CRH	I.V.	2	4, 8, 12*, 16* & 20*	4	ND	8	ND
Mouse	CRH	I.V.	5-10	0, 4.11	4.11	4.11	ND	ND
Rat	RH	Oral	2	40, 60, 80, 100 & 120	120	60	ND	80
Rat	RH	Oral	5-10	0, 60** & 100	100	ND	ND	60
Rat	RH	I.V.	2	20, 24* & 28*	20	20	24	ND
Rat	RH	I.V.		0 & 20	ND	20	20	ND

\* = only males

\*\* = only females

ND = not determined

CRH = Charles River Harefied random-bred albino

RH = random-bred hooded pigmented rat

In mice, irrespective of route of administration, the clinical signs were subdued behavior, decreased activity, labored respiration, tremor, ataxia and convulsions. The highest non-lethal oral doses were 15 and 10 mg/kg in males and females respectively, and 4.11 mg/kg was the highest non-lethal i.v. dose for mice of both sexes.

In rats, irrespective of route of administration, the clinical signs were subdued behavior, occasional croaking, tremor, ataxia, half-closed eyes, labored respiration and convulsions. The highest non-lethal oral doses were 120 and 60 mg/kg in males and females respectively, and the corresponding i.v. doses were <20 and 20 mg/kg/day.

**Subacute/Subchronic/Chronic Toxicity:**

**34/35 Days Oral Toxicity Study in Rats**  
(Study # R11832)

**Testing Laboratories:** Pathology and Toxicology Division  
Glaxo Group Research Ltd.,  
Hertfordshire, UK

**Study Started:** April 20, 1989

**Study Completed:** May 25, 1989

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

**Animals:** Random Hooded (RH) rats (7-10 weeks old, males = 170.2-211.5 g and females = 142.2-174.2 g).

**Drug Batch No.:** DR11363

**Methods:** In this study dose selection was based on dose-ranging study (Study # R11618) in which RH rats were given 5, 10, 20, 40, 60 and 80 mg/kg/day in dose escalating fashion (each dose level was given for 2-4 days before administering the next higher dose level). Only clinical signs (ataxia, bulging eyes, cold to touch and abnormal gait) were seen in rats treated with 40 mg/kg/day and higher dose levels, provided dosages were increased gradually. Additionally, there were evidence of severe retardation on body weight gains when dosages were escalated to 60 mg/kg/day. However, when a dose of 60 mg/kg/day was given to previously untreated rats it produced lethality. Based on these

findings, sponsor selected 64 mg/kg/day as the top dose for the present study and this dose will be given after 5 daily doses of 40 mg/kg/day. The other two remaining doses were 8 and 10 mg/kg/day.

Groups of rats (15/sex/group) were given orally (gavage) GR 68755 at daily doses of 0 (vehicle: water), 1, 8 and 40 (day 1-5)/64 mg/kg/day for 34/35 days. The volume of administration was fixed at 10 ml/kg. Five rats/sex/group were used for absorption study. All animals were observed daily for clinical signs and mortality. Body weights and food consumptions were recorded weekly. Ophthalmoscopic examinations and hearing test were performed at pretest and on day 26 of the study. EKG recordings were obtained from 4-5 rats/sex of control and high dose treated groups during pretest and on days 9 and 27 of the study. Overnight urine samples were also collected on day 14, 28 and 34 of the study. Blood samples were collected from tail vein or abdominal aorta at pretest, days 15, 20, 29 and 33 of the study for hematological and serum chemistry tests. All surviving rats were sacrificed at the end of treatment period and subjected to complete necropsy. Only control and high dose group rats were examined histologically. The cecum, rectum, seminal vesicles, harderian glands, peripheral nerve, skeletal muscle, femur and thymus were also examined microscopically from low and mid dose groups.

### Results:

- 1. Observed Effects:** Bulging eyes, ataxia, labored respiration, piloerection and reduction of body temperature were seen in most of the high dose treated rats. Bulging eyes were also seen on day 28/29 of the study in 7/10 females and 2/10 males of mid dose group.
- 2. Mortality:** Two females and one male from high dose group (main study animals) died or killed in extremis during the study period. The cause of deaths were CNS toxicities (bulging eyes, ataxia, labored respiration, piloerection, prostration, tremor and reduction of body temperature).
- 3. Body Weight/Food Consumption/Water Consumption:** The initial and final mean body weights of control males were 196.5 g and 235.7 g and the corresponding mean weights of control females were 162.3 g and 162.9 g respectively. Food consumptions in control males and females were \_\_\_\_\_ g/100 g/day and \_\_\_\_\_ g/100 g/day respectively. Effect on body weight gain in treated males were highly erratic. The final body weight of high dose

treated males were about 5% less than control values. During first week of treatment, only in high dose treated females, food consumptions were decreased significantly compared to control group rats.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen except platelet count in high dose treated females were decreased by 41% compared to control values (control = 638,000/ml and high dose = 374,000/ml).
5. Blood Chemistry/Urinalysis: Serum alkaline phosphatase and serum alanine aminotransferase activities were increased by 71% and 67% in high dose treated males, and the corresponding increases in high dose treated females were 183% and 61% respectively, when compared to control values. Water consumption and urine output were increased proportionally in high dose treated males on day 28 of the study.
6. Vital Signs/Physical Examination/Ophthalmic Examination/EKG/Hearing Tests: No treatment related effects were seen.
7. Organ Weights: Absolute as well as relative weights of liver were increased by 31-32% in high dose treated males. In high dose treated females, thymus weights were reduced by 64% (relative wt.: 62%) when compared to control values.
8. Gross Pathology: No treatment related effects were seen.
9. Histopathology: Partial thymic involution was seen in 4/10 and 7/10 high dose treated males and females respectively (no such finding was seen in control, low and mid dose group rats).
10. Plasma Levels of GR 68755 (# WBP/89/097): From satellite animals, blood samples were collected at 30 min after drug administration on days 1 and 30 of the study. At 30 min post dose, plasma levels of GR 68755 increased with increasing dosages (\_\_\_ mg/kg/day). Plasma levels in females were generally higher than that seen in males. Twenty-four hours after drug administration levels were negligible in plasma samples (sensitivity of assay = \_\_\_ ng/ml). It should be noted that when dose was increased from 40 mg/kg/day to 64 mg/kg/day, then there was an indication of accumulation upon repeat dosing.

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Mean Plasma GR 68755 Base Concentrations (ng/ml) Following  
 Repeat Oral Administration of GR 68755C (R11832)

Group	Dose mg/kg/day	Sex	Day 1		Day 6		Day 30	
			0.5HR	24HR	0.5HR	24HR	0.5HR	24HR
6	1	Male	147 (37.6)	<25	-	-	274 (55.5)	<25
		Female	317 (58.3)	<25	-	-	483 (71.6)	<25
7	8	Male	2950 (310)	<25	-	-	2470*(860)	<25
		Female	4310*(762)	<25	-	-	4370*(487)	<25
8	40	Male	5800 (1080)	<25	-	-	-	-
		Female	12900 (2630)	<25	-	-	-	-
8	64	Male	-	-	9190 (2090)	169-184	12700 (2480)	276 (113)
		Female	-	-	15000*(3870)	NS	13900*(2550)	1970*(870)

SD in brackets  
 \* - Mean and SD value calculated using less than 5 values.  
 NS - No sample.

Table 1, Pg. 417, Vol. 1.12

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.

6-Month Oral Toxicity Study in Rats  
 (Study # R11867)

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

Study Started: July 24, 1989

Study Completed: February 6, 1990

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

Animals: Random Hooded (RH) rats (9-10 weeks old).

Drug Batch No.: C1034/102/1

Methods: In this study, dose selection was based on 34/35-day oral toxicity study in rats (see above). Groups of rats (12/sex/group) were given orally (gavage) GR 68755 at daily doses of 0 (vehicle: water), 1, 8 and 20 (days 1-4)/40 (days 5-7)/64 (days 8-54/55)/40 (days 55/56 onwards) mg/kg/day for 196 days. The volume of administration was fixed at 10 ml/kg. Additionally, 8 rats/sex were included in control and high dose groups for 40-day recovery study. Four rats/sex/group (satellite groups) were observed daily for clinical signs and mortality. Body weights and food intakes were recorded weekly. Ophthalmoscopic examinations were performed at pre-test, days 101 and 193 of the study. Hearing tests were conducted at pre-test, days 96 and 184 of the study. Blood samples were collected from caudal veins/abdominal aorta at pre-test, on days 37, 92 and 183 of the study and at the end of recovery period for hematology and serum chemistry tests. Overnight urine samples were also collected during days 39/40, 94/95 and 185/186 of the study for urinalysis. All surviving rats were sacrificed at end of treatment/recovery period and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: Highest tested dose (20/40/64/40 mg/kg/day) produced CNS toxicities (salivation, tense behavior, moist eyes, croaking, tiptoe gait, pushing at cage floor with forepaws and tremor). Severity of clinical signs were marked, therefore dosage was reduced from 64 mg/kg/day to 40 mg/kg/day on day 55/56 of the study. Occasionally, some of these findings of lessor magnitude were also seen in mid dose treated rats.
2. Mortality: There was no treatment related deaths.
3. Body Weight/Food Consumption/Water Consumption: The initial and final mean body weights of control males were 205.3 g and 323.4 g and the corresponding mean weights of control females were 133.6 g and 175.0 g respectively. Food consumptions in control males and females were \_\_\_\_\_ g/100 g/day and \_\_\_\_\_ g/100 g/day respectively. Only in high dose treated males, body weight gains were reduced by 3.2% compared to control values. At the end of recovery period, body weights of high dose

treated rats were about 3% lower than that seen in control rats. Food intakes were not affected by the treatment (there was an indication of increase food intakes in high dose treated rats of both sexes).

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry/Urinalysis: No treatment related effects were seen in blood chemistry tests. Water consumptions and urine output were increased in high dose treated rats. However, no consistent effects were seen on urine specific gravity.

6. Organ Weights: Absolute as well as relative weights of liver were increased by 12-13% in high dose treated females. Additionally, prostate weights were decreased by 14-23% in all treated males, when compared to control values.

7. Gross Pathology: Not reported.

8. Histopathology: Basophilic foci of cellular alteration were seen in high dose treated females (control = 1/12, low dose = 1/12, Mid dose = 0/12 and high dose = 6/12). This finding was still present at the end of recovery period (control = 0/8 and high dose = 4/8).

9. Plasma Levels of GR 68755 (Report # WBP/90/052): Blood samples were collected on day 1 (day 8 for the high dose group), 55, 99 and 190 at 15 min and 24 hours after drug administration. Drug levels in plasma were determined by \_\_\_\_\_ methods. GR 68755 was detected in plasma at first sampling point (15 min). Levels of GR 68755 increased with increasing dosages, however, no comment can be made regarding linearity because the way drug was given in high dose group animals (see methods). Furthermore, drug levels at 24 hr after drug administration was very low (median: <42 ng/ml; limit of detection = \_\_\_\_\_ ng/ml) which indicates that GR 68755 does not accumulate following repeat oral dosing (this statement is contradictory to the findings reported in 34/35 days oral toxicity study; see above). Therefore, these data should be viewed with caution (for complete assessment see ADME section).

In this study CNS (salivation, tense behavior, moist eyes, croaking, tiptoe gait, pushing at cage floor with forepaws and tremor) and liver are the target organ of toxicities, and the mid dose level (8 mg/kg/day) is the no effect dose.

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35-Days Oral Toxicity Study in Dogs  
(Study # D 11825)

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

Study Started: April 17, 1989

Study Completed: November 27, 1989

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

Animals: Beagle dogs (4-5 months old; males = 7.20-9.95 kg and females = 6.90-8.55 kg).

Drug Batch No.: C1017/69/1 and C1017/77/1

Methods: In this study dose selection was based on dose-ranging study (# D 11616) in which escalating doses of 5 to 35 mg/kg/day over 32 day period were used in 1 male and 1 female dog. A dose level of 35 mg/kg/day was lethal. A dose level of 30 mg/kg/day was well tolerated dose, however, when given on empty stomach, dogs experience severe reaction such as salivation, inactivity, tremor, lying down, eye half closed, labored respiration and ataxia. The male dog died and the female dog was killed in extremis at 1.5 hr after the drug administration. Based on these findings sponsor selected 30 mg/kg/day as the top dose for the present study and dogs were allowed to eat at least 2 hr prior to drug administration. Groups of dogs (3/sex/group) were given orally (gavage) GR 68755 at daily doses of 0 (vehicle: water), 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. The volume of administration in control, low dose, mid dose and high dose males was fixed at 1 ml/kg while in high dose females volumes were 2 ml/kg on days 1-5, 1.33 ml/kg on days 6-9, 1.67 ml/kg on days 10 and 11 and 2 ml/kg from day 12 onwards. All dogs were observed for clinical signs twice daily. Body weights were recorded twice weekly and food intakes were recorded weekly. Ophthalmic examinations and ECG recordings were performed on all dogs once pre-test and at the end of study period. Blood samples were collected from jugular vein for hematology and serum chemistry tests at pre-test and on day 16 and 29 of the study. Blood samples were also collected from 2 dogs/sex/group at 0 (before drug administration), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hr after drug

administration on days 1/2 and 33/34 of the study for measuring drug levels in plasma according to \_\_\_\_\_ methods. At the end of study period all surviving dogs were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: Salivation, subdued behavior, vomiting, half-closed eyes and trembling were seen in high dose treated dogs. Subdued behavior was also seen in low and mid dose treated dogs.
2. Mortality: One high dose treated male died during study period. Prior to death this dog had severe reaction (lack of coordination, staggering, paleness of the ears and gums, falling, salivation and collapse) to the treatment. Microscopic examination of this dog failed to establish the cause of death.
3. Body Weights/Food Consumption/Water Consumption: The initial and final mean body weights of control males were 8.67 kg and 10.28 kg and the corresponding mean weights of control females were 7.50 kg and 8.34 kg respectively. Food consumptions in control males and females were \_\_\_\_\_ g/kg/day and \_\_\_\_\_ g/kg/day respectively. In high dose treated males, body weight gains were reduced by 13.7% compared to control values while high dose treated females lost 8% of their initial weights. Mean food consumptions were decreased by 16% and 22% in high dose treated males and females respectively, when compared to the mean control values.
4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.
5. Blood Chemistry/Urinalysis: Serum creatinine levels were increased by 16% in high dose treated females and 1 out of 2 high dose treated males the cholesterol level was increased by 71% over pre-test value at day 29 of the study.
6. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment related effects were seen.
7. Organ Weights: There were only 2 dogs/sex in high dose at the termination of the study, therefore, a meaningful statistical comparison is not possible. Nevertheless, thymus relative weights in high dose group were decreased by 21% and 34% in males and females respectively. Relative liver weights were increased by 22% and 15% in high dose treated males and females respectively. Relative weights of kidneys were increased by 11%, 23% and 31% in low, mid and high dose treated male dogs, when compared to control values.

8. Gross Pathology: No treatment related effects were seen.
9. Histopathology: Minimal involution of the thymus was seen in 2 out of 3 each high dose treated males and females and 1 out of 3 mid dose treated males.
- 10 Plasma Level of GR 68755 (Report # WPT/89/247): Plasma levels of GR 68755 increased with increasing dosages in a non-linear fashion. There were no sex differences. This non-linearity resulted in significant increases in  $t_{1/2}$  at high dose. Non-linearity in  $C_{max}$  and AUC values could be related to saturation of drug metabolizing enzymes. However, at 24 hr after drug administration on days 1 and 33 of the study the levels of GR 68755 were close to detection limit (detection limit:  $\text{ng/ml}$ ), hence there is no indication of accumulation of the drug after repeat administration.

Mean GR 68755 Pharmacokinetic Parameters Following a Repeat Oral Dose of GR 68755C to the Dog (Study D11825)

Parameter	Dose Administered					
	1mg/kg		5.5mg/kg		30mg/kg	
	Day 1	Day 33	Day 1	Day 33	Day 1	Day 33
$C_{max}$ (ng/ml)	307 (67)	217 (21)	1420 (277)	1100 (141)	9530 (1310)	7260 (1615)
$T_{max}$ (hour)	0.75 (0.2)	0.69-0.75 (0.2-0.3)	0.56 (0.1)	1.4 (0.5)	0.56 (0.3)	1.8 (0.9)
$t_{1/2}$ (hour)	0.8	0.8	1.2	0.9	2.6	2.6
AUC <sub>(0-t)</sub> (ng/ml.h)	498 (175)	255 (76)	3330 (724)	2930 (499)	26500 (4520)	46000 (11100)
AUC <sub>(0-∞)</sub> (ng/ml.h)	554 (173)	296 (93)	3500 (684)	3040 (474)	27100 (4410)	46500 (10900)

Standard deviation in brackets

Table 2, Fg. 312, Vol. 1.22