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Interaction of GR 68755 and Cimetidine With Rat Hepatic Microsomes In Vitro (Report # WBP/90/069)

Methods: To determine the inhibitory effects of GR 68755 and cimetidine on the human hepatic microsomal cytochrome P450 enzymes, human hepatic microsomes were incubated with specific substrate probes for the cytochromes P450 in the presence of GR 68755 and cimetidine (0-100 μ M) and the cytochrome P450 activities were then determined.

Results: The results indicated that GR 68755 specifically inhibited the formation of 6 β -hydroxytestosterone from testosterone, indicating that GR 68755 interacts with IIIA2 isozyme of cytochrome P450. Cimetidine, however, specifically inhibited the formation of 2 α and 16 α -hydroxytestosterone and 4-androstene 3,17-dione from testosterone.

The Concentration of GR 62202 in Human Plasma Following Repeated Oral Dose of GR 68755 Hydrochloride at 4 or 16 mg Base (Report #WBP/91/099)

Methods: GR 62202, an intermediate in the synthesis of GR 68755, was mutagenic in an Ames test. To determine the plasma level of this intermediate, plasma samples were obtained from healthy volunteers following repeated oral dose at 4 or 16 mg b.i.d. for 9 or 4 days, respectively. The plasma level of GR 62202 was determined using _____ assay and _____

Results: In a total of 68 samples from 7 volunteers, the level of GR 62202 was less than 1 ng/ml.

To compare the similarities and differences between mice, rats, dogs, and humans, the pharmacokinetic parameters of GR 68755 are summarized in the following table.

Pharmacokinetics of GR 68755 in mice, rats, dogs, and humans

| Species & dose | C _{max} , ng/ml | T _{max} , hours | AUC, ng.hr/ml | T _{1/2} , hours | F (%) |
|-----------------------------|------------------------------|--------------------------|------------------------------|----------------------------|-------|
| Mouse, Oral, 5.5 mg/kg | 770 \pm 536 | 0.08 | <672 | <0.25 | ---- |
| Rat, Oral, 3.5 mg/kg | 2450 (male) 3240 (female) | 0.25 0.25 | 1980 (male) 4880 (female) | 0.6 (male) 1.0 (female) | ---- |
| Dog, Oral, 2 mg/kg | 900 \pm 156 | 0.75 \pm 0.39 | 2070 \pm 500 | 1.3 | 96 |
| Human, Oral tablet, 2 mg | 11.38 | 1.04 | 36.62 | 1.52 | ---- |

GR 68755 was absorbed rapidly in mice, rats, rabbits and dogs, (T_{max} : _____ hr) and nearly completely in dogs (bioavailability: 96%). Half life ($t_{1/2}$) was <0.25 hr in mice and ~1-1.5 hour in rats, rabbits, dogs and humans. Irrespective of strains of rats, the systemic exposure of GR 68755 and/or its metabolites in females were significantly greater than in males. This difference was not seen in mice and dogs. 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. The radioactivity was also detected in the milk of the lactating rats. About 72-88% of GR 68755 were bound to the plasma proteins in mice, rats, rabbits, dogs, and humans. 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 3-6% of the dose in rat feces and urine and ~10-19% in dog feces and urine. 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. This metabolite (GR 153732) has very low affinity for human 5-HT₃ receptors (pK_i <6) as compared to GR 68755 (pK_i ~9). 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 feces, respectively. In rats, about 27.6% of administered radio-activity were excreted in the bile.

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.

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Results:

| Acute Oral and I.V. Toxicity of GR 68755 | | | | | | | | | |
|--|--------|-------|-----------------|-----------------------|--------|------------------------------------|---------|--------------------------------|---------|
| Species | Strain | Route | No/Dose/ Sex | Dose (mg/kg) | Levels | 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 | 50 |
| Rat | RH | Oral | 5-10 | 0, 60** & 100 | | 100 | ND | ND | 50 |
| 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:

One-Month I.V. Toxicity Study in Random Hooded Rats
(R11655)

Testing Laboratories: Glaxo Group Research Ltd.,
Hertfordshire, UK.

Study Started: September 13, 1988

Study Completed: October 5, 1998

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

Animals: Random Hooded rats (9-11 weeks old; males = 157.3-200.6 g and females = 128.7-160.5 g).

Drug Batch No.: C1281/16/1

Methods: In this study dose selection was based on dose-ranging study (# R11587) in which escalating doses of 1 to 16 mg/kg/day were used in Random Hooded rats. At doses of 12 mg/kg/day or higher severe clinical signs of toxicity were observed (bulging eye, nodding of head, ataxia, subdued behavior, irregular breathing, and tremor). In a separate group without previous exposure to the drug, a dose of 12 mg/kg/day was given for 5 days. In this group, the above mentioned clinical signs of toxicity were observed but much less severe. Therefore, the dose 12.25 mg/kg/day was considered as tolerated dose and selected as the high dose in the present study.

In the current study, GR 68755 was given intravenously to rats (10/sex/group) at 0 (vehicle: water), 1, 3.5, and 12.25 mg/kg for up to 36 days. Five additional rats in the control and high dose groups were included for recovery period. All rats were observed for clinical signs daily. Body weights and food consumption were recorded weekly. Ophthalmic examination was performed once pre-test and on day 29. Blood samples were collected for hematology and serum chemistry tests at pre-test and on days 14, 28, and 64 (recovery, clinical chemistry only). Urinalysis was conducted on days 16/17 and 30/31. At the end of study period all surviving rats were sacrificed and subjected to complete necropsy. The histopathological examination was conducted in all animals in the control and high dose groups, tissues from any animals in the low and mid dose groups with macroscopic lesions and all tissues from intercurrent deaths.

Results:

1. **Observed Effects:** The treatment related clinical signs of toxicity were mainly in the high dose group and these included subdued behavior, low posture, moist eyes, bulging eyes, and "croaking". Moist eyes were also noted in the mid dose group.

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2. Mortality: One high dose female was sacrificed on day 27 due to moribund condition. This animal had weight loss, a hunched posture, half closed eyes, and red discharge around mouth and forepaws noted on day 21 and cold to touch, piloerect coat, subdued behavior, unsteady gait, and shallow breathing on day 27. Necropsy revealed marked bilateral pyelonephritis and epithelial hyperplasia in the urinary bladder with minimal cystitis. Sponsor considered the death as incidental.
3. Body Weights: There were no treatment related changes in the mean terminal body weight gain.
4. Food Consumption: There were no treatment related changes.
5. Hematology: There were no treatment related changes.
6. Clinical Chemistry: Alanine aminotransferase activity was slightly increased by ~17-44% in the mid and high dose groups.
7. Urinalysis: There were no treatment related changes.
8. Ophthalmic Examination: No treatment related effects were seen.
9. Organ Weights: Thymus weight was slightly lower in the high dose group (14-15.6%) as compared to the control.
10. Gross Pathology: No treatment related effects were seen.
11. Histopathology: There were no treatment related changes.

In summary, rats were treated intravenously with GR 68755 at 0, 1, 3.5, and 12.25 mg/kg/day for up to 36 days. The major treatment related changes were clinical signs of toxicity including subdued behavior, low posture, moist eyes, bulging eyes, and "croaking" mainly in the high dose group. The central nervous system was the target organ of toxicity based on the clinical signs of toxicity.

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34/35 Days Oral Toxicity Study in Rats
(Study # R11332)

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 = 170.2-211.3 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 6.6-8.1 g/100g/day and 6.8-8.0 g/100g/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.

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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 (1-40 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 = μ g/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.

Mean Plasma GR 68755 Base Concentrations (ng/ml) Following Repeat Oral Administration of GR 68755G (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) | - | 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.

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, male: 205.3 g, female: 133.6 g).

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 lesser magnitude were also seen in mid dose treated rats.
2. **Mortality:** There were 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.

Addendum:

The high dose of 64 mg/kg was achieved by increasing dose of 20 mg/kg on days 1-4 to 40 mg/kg on days 5-7 and to 64 mg/kg on day 8. However, the high dose of 64 mg/kg was reduced to 40 mg/kg on day 55 due to excessive toxicity observed.

53/54-Week Oral Toxicity Study in Random Bred Hooded Rats
(R12486)

Testing Laboratories: Glaxo Research and Development Ltd.,
Hertfordshire, UK.

Study Started: August 24, 1990

Study Completed: October 23, 1997

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

Animals: Random Hooded (RH) rats (8-9 weeks old)
Males: 145.3-222.4 g, females: 112.4-177.1 g

Drug Batch No.: C1757/106/1

Methods: In this study, dose selection was based on 6-month 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, 6.5 and 20 (days 1-7)/40 (day 8 onward) mg/kg/day for 377/380 days. Additionally, 12 rats/sex were included in control and high dose groups for 71-day recovery study. Two rats/sex/group were sacrificed on day 378 to study hepatic metabolism. Clinical signs and mortality were observed daily. Body weights and food intakes were recorded weekly. Ophthalmoscopic examinations were performed at pre-test, days 189/190 and 367/368. Hearing tests were conducted at pre-test, days 189 and 366 of the study. Blood samples were collected at pre-test, on days 31-32, 91-92, 185-186, and 364-365 of the study and at the end of recovery period for hematology and serum chemistry tests. Overnight urine samples were also collected during days 28-30, 93-95, 170-172, 275-277, and 366-368 of the study for urinalysis. All surviving rats were sacrificed at end of treatment/recovery period and subjected to complete necropsy and histopathological examinations. Toxicokinetic parameters were determined on days 1-2, 8-9, 38-39, 116, 192, 283, and 371-382.

Results:

- 1. Observed Effects:** Salivation, noisy breathing and/or "croaking" were noted mainly in the high dose group. Thin appearance, hunched posture, and chin rubbing against the cage floor were seen in all treatment groups.
- 2. Mortality:** Two high dose animals (a male and a female) were sacrificed due to a general deterioration in health condition. The sacrificed male had ulceration of the esophagus suggestive of misdosing. Microscopic examination revealed abscess of the skin on the left scapula and focal pneumonia in the other sacrificed animals. These are not considered treatment related.
- 3. Body Weight:** The initial and final mean body weights of control males were 183.3 g and 343.4 g and the corresponding mean weights of control females were 138.4 g and 177.8 g respectively. Terminal body weight gain was not clearly affected.
- 4. Food Consumption:** Food consumptions in control males and females were _____ g/kg/day and _____ g/kg/day respectively. Food intakes were not clearly affected by the treatment (there was an indication of increase food intakes in high dose treated rats).

5. Ophthalmoscopy: There were no treatment related changes.
6. Hearing Test: A dose related reduction in hearing acuity was found on day 366/367 in GR 68755 treated animals with loss of hearing in 2 high dose animals. This change was not associated with histopathological changes.
7. Hematology: Slight decrease in mean cell hemoglobin, cell volume, and platelet count and increase in the white blood cells were found occasionally in the high dose group.
8. Blood Chemistry: Alkaline phosphatase activity was increased up to 49% in males and 89% in females in the high dose group. Alanine aminotransferase activity was also increased in high dose group by 65% in males and 91% in females. The activities of these enzymes were also slightly increased in the low and mid dose groups. Slight decreases in serum glucose and cholesterol were noted in the high dose group.
9. Urinalysis: Water consumption, urine output, and urine specific gravity were increased in high dose treated rats mainly in the high dose group.
10. Organ Weights: Absolute and relative weights of the liver were increased by ~13% (males) or 21-24% (females) in the high dose group as compared to the control. Absolute and relative weights of the adrenal (males and females) and prostate (males) were decreased by ~11-16% and 14-15% in the high dose group, respectively, as compared to the control.
11. Gross Pathology: Discoloration of the liver and hair loss were noted in the high dose group.
12. Histopathology: Multiple basophilic foci of hepatocellular alteration were seen in high dose treated females (control = 0, high dose females = 16, none in males). The incidence of fine, minimal fatty vacuolation of peri-acinar hepatocytes was increased in the treated males as compared to the control (control = 0/22, low dose = 1/22, Mid dose = 2/22 and high dose = 6/22).
13. Plasma Level of GR 68755: The plasma levels of GR 68755 were summarized in a table on page 29 in volume 18 and this table is attached below.

Summary of toxicokinetic results

| Dosage ¹ | Measurement ² | Occasion (Day) | | | | | |
|---------------------|--|----------------------|--------|--------|--------|---------|--------|
| | | Day 1/8 ³ | | Day 38 | | Day 371 | |
| | | Male | Female | Male | Female | Male | Female |
| 1.0 | T _{max} (h) ² | 0.17 | 0.50 | 0.75 | 0.50 | 0.75 | 1.00 |
| | C _{max} (µg/mL) ³ | 0.19 | 0.39 | 0.19 | 0.38 | 0.36 | 0.32 |
| | AUC _{0-7h} (µg.h/mL) ⁴ | 0.32 | 0.79 | 0.38 | 0.68 | 0.51 | 0.74 |
| 6.5 | T _{max} (h) ² | 0.33 | 0.33 | 0.33 | 1.00 | 0.33 | 1.00 |
| | C _{max} (µg/mL) ³ | 1.88 | 4.45 | 2.09 | 2.59 | 2.55 | 2.70 |
| | AUC _{0-7h} (µg.h/mL) ⁴ | 3.73 | 7.61 | 3.72 | 6.05 | 4.85 | 6.75 |
| 40.0 | T _{max} (h) ² | 0.5 | 0.5 | 0.5 | 0.75 | 0.5 | 1.00 |
| | C _{max} (µg/mL) ³ | 8.93 | 9.11 | 9.31 | 12.52 | 10.14 | 12.80 |
| | AUC _{0-7h} (µg.h/mL) ⁴ | 25.70 | 35.20 | 34.91 | 53.70 | 41.86 | 50.95 |

- 1 Expressed in terms of mg GR68755X/kg
 - 2 The time taken to achieve C_{max}
 - 3 The maximum concentration achieved
 - 4 Area under the plasma level time curve (up to 7 hours after dosing)
 - 5 Mean of two samples
- Samples were collected on Day 1 for animals in the 1.0 and 6.5mg/kg groups and on Day 8 for animals treated with 40mg/kg. Animals in the 40mg/kg group received this dose from Day 8 following a build up period.

Levels of GR 68755 increased with increasing dosages. GR 68755 does not accumulate following repeat oral dosing but was higher in females than in males. GR 68755 induced hepatic microsomal CYP1A1 and CYP1A2 activities but inhibited the activities of CYP3A and CYP2E1.

In summary, GR 68755 was given to rats by oral gavage at 0, 1, 6.5, and 20/40 mg/kg/day for 377/380 days. CNS and liver were the target organs of toxicity as evidenced by clinical signs of toxicity (salivation, noisy breathing, "croaking", and hunched posture) and histopathological changes in the liver (multiple basophilic foci of hepatocellular alteration and fine, minimal fatty vacuolation of periportal hepatocytes). Treatment with GR 68755 decreased hearing acuity in the treated animals (both males and females) and resulted in loss of hearing in 2 high dose males.

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DOG:

One-Month I.V. Toxicity Study in Beagle Dogs
(D 11654)

Testing Laboratories: Glaxo Group Research Ltd.,
Hertfordshire, UK.

Study Started: December 6, 1988

Study Completed: September 15, 1998

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

Animals: Beagle dogs (4-7 months old; males = 6-7.9 kg and
females = 4.9-7.4 kg).

Drug Batch No.: C1281/58/60/2, C1281/58/1, C1297/52/2 and
C1281/54/1.

Methods: In this study dose selection was based on dose-ranging study (# D 11588) in which escalating doses of 2 to 24 mg/kg/day over 24 day period were used in Beagle dogs. At doses of 16 mg/kg/day or higher severe clinical signs of toxicity were observed (tremor, ataxia, arched back, and subdued behavior). Dose of 12 mg/kg was considered tolerated dose and at this dose level, following clinical signs of toxicity were observed: lip licking, salivation, partly closed eyes, open mouth, ataxia, and restless behavior. Based on these findings sponsor selected 12.25 mg/kg/day as the top dose for the present study. Groups of dogs (3/sex/group) were given intravenously GR 68755 at daily doses of 0 (vehicle: water), 1, 3.5, and 12.25 mg/kg for 35 or 36 days. Two additional dogs in the control and high dose groups were included for recovery period. All dogs were observed for clinical signs daily. Body weights and food consumption were recorded. Ophthalmic examinations and ECG recordings were performed once pre-test and at termination (ECG were also recorded on day 15/16). Blood samples were collected from jugular vein for hematology and serum chemistry tests at pre-test and on days 14, 30 and 70 (recovery). Blood samples were also collected from 2 dogs/sex/group before dosing and at 5, 10, 15, and 30 minutes after dosing for measuring drug levels in plasma. At the end of study period all surviving dogs were sacrificed and subjected to complete necropsy and histopathological examinations.