**ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME) STUDIES:**

**MOUSE:**

**Pharmacokinetics of GR 68755 in the Mouse Following a Single Oral Dose (Study # MET862, Report # WBP/92/021)**

**Methods**: $B6C3F_1$ male mouse were given a single oral (gavage) dose of $^{14}C$-GR 68755 (5.5 mg/kg) with specific activity of 6.97 MBq/mg. Volume of administration was fixed at 10 ml/kg. Blood samples were collected from abdominal vena cava at pre-test, 0.08, 0.17, 0.33, 0.5, 0.75, 1, 2, 3, 4, 6 and 8 hours after drug administration (5 mice/sampling time points were used). Levels of GR 68755 in plasma were measured by methods and total radioactivity was determined by methods. Various pharmacokinetic parameters were calculated.

**Results**: GR 68755 absorbed rapidly ($T_{max}$ = 0.08 hr [first sampling point]). Plasma levels of GR 68755 decreased rapidly ($t_{1/2}$ = <0.25 hr) and at 2 hr post-dosing the levels were below detection limit (limit of detection = ng/ml). Based on AUC values about 25-34% of the total radioactivity represented parent drug.

| Pharmacokinetic Parameters in Mouse After A Single Oral Dose | | |
|---|---|---|
| **Parameters** | **GR 68755** | **Radioactivity** |
| $C_{max}$ (ng/ml) | 770 ± 536 | 1643 ± 271 |
| $T_{max}$ (hr) | 0.08 | 0.33 |
| $AUC_{0-1}$ (ng.hr/ml) | 481 | 1375 |
| $AUC_{0-\infty}$ (ng.hr/ml) | <672* | 2757 |
| $T_{1/2}$ (hr) | <0.25* | 0.63 |

* = Estimated because at 2 hr post-dose plasma levels were below detection limit.

**Metabolism and Excretion in the Mouse After A Single Oral Dose of $^{14}$C-GR 68755 (Study # BPW191, Report # WBP/91/019)**

**Methods**: Male and female mice ($B6C3F_1$) were given a single oral (gavage) dose of $^{14}$C-GR 68755 (5.5 mg/kg, 10 ml/kg) with specific activity of 6.97 MBq/mg. Urine and feces were collected over 24 hr period for up to 144 hours after drug administration. In all samples, radioactivity was measured by

**Results**: About 47.4% and 47.5% 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. There were no sex differences in excretion pattern. About 13 and >15 radioactive peaks were seen in urine and feces samples. Only about 11% and 4% of the dose was excreted in urine and feces respectively as unchanged drug. In urine, about 3 - 7% of the dose was glucuronide conjugate of GR 68755.

**RAT**:

**Pharmacokinetics of GR 68755 in Pigmented (RH) Rats Following A Single Oral or I.V. Dose (Study # 541/579, Report # WBP/89/071)**

**Methods**: Male and female Random-bred Hooded rats were given a single oral (gavage) or i.v. dose of $^{14}$C-GR 68755 (3.5 mg/kg) with specific activity of 6.07 MBq/mg. The volumes of administration were 4 ml/kg for oral dose and 2 ml/kg for i.v. dose. Blood samples were collected from abdominal vena cava at 0, 0.08 (only post i.v. dose), 0.25, 0.5, 0.75 (only post oral dose), 1, 1.5, 2, 3 (only post oral), 4, 6, 8 and 24 hr after drug administration (2 rats/sex/time point were used). Levels of GR 68755 in plasma were measured by methods and total radioactivity was determined by methods. Various pharmacokinetic parameters were also calculated.

**Results**: The data indicated that GR 68755 rapidly absorbed when given orally (bioavailability is almost 100%). In the calculation of bioavailability it is generally assumed that plasma clearance is the same after i.v. or oral dose. Oral bioavailability of >100% may be due to the differences in $Cl_p$ of the drug depending on route of administration. Irrespective of route of administration, based on AUC values, unchanged drug represented 57 - 59% and 76 - 78% of total plasma radioactivity in males and females respectively. Furthermore, systemic exposure to GR 68755 in females were about 2.5 fold higher than

males. This conclusion was further supported by the lower $Cl_p$ values in females than males (16.4 vs 44.7 ml/min/kg). Since Vd is similar in both sexes, the difference in $Cl_p$ may be due to shorter $t_{1/2}$ in males than in females (0.4 vs 1.1 hr). Both parent drug as well as total radioactivity levels reached to negligible levels at 24 hr after dosing (limit of detection: ng/ml). Plasma radioactivity declined in a biphasic manner ($t_{1/2\beta}$: i.v.: males = 2 hr and females = 9 hr; oral: males = 2 hr and females = 5 hr).

**Mean Pharmacokinetic Parameters in Rats After A Single Oral or I.V. Dose (n=2)**

| | Male | | Female | |
|---|---|---|---|---|
| | **Oral** | **I.V.** | **Oral** | **I.V.** |
| $C_{max}$ (ng/ml) | 2450 | 2240 | 3240 | 3400 |
| $T_{max}$ (hr) | 0.25 | 0.08 | 0.25 | 0.08 |
| $AUC_{0-\infty}$ (ng.hr/ml) | 1980 | 1360 | 4880 | 3620 |
| $T_{1/2}$ (hr) | 0.6** | 0.4* | 1.0** | 1.1* |
| $Cl_p$ (ml/min/kg) | --- | 44.7 | --- | 16.4 |
| Bioavailability | 139 | --- | 123 | --- |
| Vd (L/kg) | --- | 1.6 | --- | 1.6 |

$Cl_p$ = plasma clearance
Vd = volume of distribution
* = $t_{1/2}$ calculation from 15 min-2 hr for males and 15-6 hr for female rats
** = $t_{1/2}$ calculation from 15 min-2 hr for both sexes

## Pharmacokinetics of GR 68755 in AHA and Wistar Rats Following A Single Oral Dose (Study # MET703, Report # WBP/90/073 and Study # BPW335, Report # WBP/94/012)

**Methods**: AHA rats were used in reproductive toxicity studies and Wistar rats were used in rat carcinogenicity study. Therefore, sponsor used these strains of rats for the present pharmacokinetic studies. Rats were given a single oral (gavage) dose of $^{14}C$-GR 68755 (1 mg/kg) with specific activity of 6.07 MBq/mg. The volume of administration was fixed at 4 ml/kg. Blood samples were collected from dorsal aorta at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 24 hr after drug administration (2 rats/sex/time point were used). Levels of GR 68755 in plasma were measured by methods and total radioactivity was determined by methods. Various pharmacokinetic parameters were calculated.

**Results**: GR 68755 absorbed rapidly in AHA and Wistar rats ($T_{max}$: AHA rats = 0.08-0.25 hr and Wistar rats = 0.3-0.5 hr). The decline of GR 68755 and/or total radioactivity in plasma was linear with $t_{1/2}$ ranging from hr depending upon strain and sex of rats. Irrespective of strain and sex, based on AUC values, unchanged drug in plasma represented about 33-53% of the total radioactivity. Furthermore, systemic exposure to GR 68755 in female AHA and Wistar rats were about 79% and 43% greater than corresponding males respectively. This trend of greater exposure of GR 68755 and/or radioactivity in females than males after a given dose is in line with the earlier findings (see above: report # WBP/89/071). At 4/6 hr after drug administration plasma levels of GR 68755 were close to detection limit ( ng/ml) and at 24 hr post-dose radioactivity levels were negligible.

**Mean Pharmacokinetic Parameters in Rats (AHA and Wistar) After A Single Oral Dose (n=2)**

| | AHA Rats | | Wistar Rats | |
|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** |
| $C_{max}$ (ng/ml) | 198 | 328 | 320 | 450 |
| $T_{max}$ (hr) | 0.25 | 0.08 | 0.3 | 0.5 |
| $AUC_{0-\infty}$ (ng.hr/ml) | 295 | 528 | 510 | 730 |
| $T_{1/2}$ (hr) | 1.6 | 1.1 | 0.9 | 0.9 |
| $T_{1/2}$(hr) [radioactivity] | 1.6 | 1.3 | 1.4 | 1.5 |

In study BPW335 (Wistar rats), sponsor also collected urine and feces samples over 24 hr period for up to 144 hr after drug administration. About 52% and 47% of 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. There was no sex difference in excretion pattern. During the first 24 hr <10% of dose was excreted in the urine as unchanged drug. Ten unidentified radioactive peaks in urine and 8 unidentified peaks in feces were seen in 0 - 24 hr samples. Thus, indicating drug is extensively metabolized before elimination.

**Distribution:**

**Distribution of Radioactivity Following A Single Oral (3.5 mg/kg) or I.V. (1 mg/kg) Administration of $^{14}$C-GR 68755 to Male Albino (AHA) and Pigmented (RH) Rats (Study # MET536/578, Report # WBP/89/101)**

**Methods:** Male albino (AHA) and pigmented (RH) rats were given a single oral (gavage; 3.5 mg/kg) or i.v. (1 mg/kg) dose of $^{14}$C-GR 68755 with specific activity of 6.07 MBq/mg. One pigmented rat per time point was sacrificed at 5, 15, 30 min., 1, 2, 4, 6, 24, 48, 72 and 168 hours after drug administration via oral route. Following oral administration, single albino rats were sacrificed at 1, 6, 24 and 168 hr. Following i.v. dose, one albino rat was sacrificed at 5, 15, 30 min., 1, 2, 4, 6, 24, 48, 72 and 168 hours and one pigmented rat was killed at 1, 24 and 168 hr. Whole body was Additionally, sagittal sections of 20 µm thick were obtained at various levels and radioactivity in various samples were measured by

**Results:** Irrespective of route of administration and strain, radioactivity was widely distributed throughout the body. Radioactivity levels in liver, kidneys and adrenals were significantly higher than the blood. Significant level of radioactivity was also seen in the eye of pigmented rats. Radioactivity from all tissues was cleared within 24 hr after drug administration except from adrenals, which was cleared by the end of 168 hr. Radioactivity in the eye of pigmented rat was still present at end of 168 hr after drug administration, indicating binding of drug and/or metabolites to melanin of the uveal tract.

| Tissue | Concentrations (µg base equivalents/g of tissue) RH (po)* | AHA (po)† | RH (iv)† | AHA (iv)* |
|---|---|---|---|---|
| Blood | 1.44 (0.25) | 0.56 (1.0) | 0.11 (1.0) | 0.42 (0.08) |
| Adrenal | 8.27 (1.0) | 4.81 (1.0) | 2.19 (1.0) | 6.39 (0.08) |
| Bone | 1.40 (1.0) | 0.49 (1.0) | <LS (1.0) | 1.07 (0.08) |
| Bone marrow | 3.44 (0.25) | 1.29 (1.0) | 0.22 (1.0) | 1.34 (0.08) |
| Testes | 0.31 (1.0) | 0.63 (1.0) | 0.12 (1.0) | 0.35 (1.0) |
| Heart | 3.39 (0.25) | 1.42 (1.0) | 0.36 (1.0) | 1.20 (0.08) |
| Kidney | 12.67 (1.0) | 5.64 (1.0) | 1.23 (1.0) | 4.93 (0.08) |
| Liver | 9.59 (1.0) | 3.61 (1.0) | 1.39 (1.0) | 4.65 (0.08) |
| Lung | 4.44 (0.25) | 2.30 (1.0) | 0.40 (1.0) | 1.32 (0.5) |
| Small intestine | 6.44 (0.25) | 2.23 (1.0) | 0.65 (1.0) | 1.05 (0.08) |
| Large intestine | 3.49 (0.25) | 3.57 (1.0) | 0.21 (1.0) | 0.79 (1.0) |
| Thyroid | 2.75 (0.08) | 0.94 (1.0) | 0.30 (1.0) | 1.32 (0.08) |
| Salivary gland | 4.27 (0.25) | 1.75 (1.0) | 0.95 (1.0) | 1.97 (0.5) |
| Pituitary | 5.11 (0.25) | 1.75 (1.0) | 0.97 (1.0) | 1.31 (0.25) |
| CNS | 0.29 (0.25) | <LS (1.0) | <LS (1.0) | <LS (0.08) |
| Brown fat | 3.60 (0.08) | 1.42 (1.0) | - | - |
| Spleen | - | - | 0.36 (1.0) | 0.73 (0.25) |
| Eye | 6.46 (4.0) | 0.48 (1.0) | 1.07 (1.0) | 0.17 (0.08) |
| Eye | 2.30 (168) | 0.003 (168) | 0.35 (168) | BKG (168) |

Key:
RH = Random-bred Hooded rat.
AHA = Allen & Hanbury Albino
GR68755D = $^{14}$C-GR68755 hydrochloride.
GR68755C = Non-radiolabelled GR68755 hydrochloride.

* = Values given are for peak concentrations
Values in parentheses are the sample time in hours.
† = Values given are concentrations observed at the first sampling time.
Values in parentheses are the sample time in hours.
<LS = Concentration lower than value of lowest visible standard on
BKG = Concentration not distinguishable from background.

Sponsor's Table, Page 198, Vol. 1.31

## Placental Transfer of Radioactivity in Pregnant Albino (AHA) Rat (Study # MET698, Report # WBP/90/055)

Methods: Pregnant albino (AHA) rats were given a single oral dose of $^{14}$C-GR 68755 (1 mg/kg) with specific activity of 6.07 MBq/mg on day 19 of gestation. At 1, 6 and 24 hr after drug administration, rats (1/time point) were sacrificed and whole body was Radioactivity levels in maternal blood, placenta and fetus were measured by

**Results**: Radioactivity crosses placental barrier and is widely distributed in fetuses. The levels of radioactivity in fetus at 1 hr post-dose were lower than that seen in maternal blood or placenta and the placental radioactivity was about 2-fold higher than that in maternal blood. Twenty-four hours after drug administration levels of radioactivity in maternal blood, placenta and fetus were below detection limit.

The Concentrations of Radioactive Drug-Related Material in Selected Tissues of Pregnant Albino Rats Following Oral Administration of $^{14}$C-GR 68755 at 1-c Base/kg

| Animal No. | 805 | 807 | 808 |
|---|---|---|---|
| Time (h) | 1 | 6 | 24 |
| | (Concentration Expressed as ng GR 68755 base/g tissue) | | |
| Tissues | | | |
| Maternal blood | 117 | 28.9 | <13.0 |
| Placenta | 236 | 48.6 | <13.0 |
| Foetus | 31.2 | <13.0 | <13.0 |

the value of the lowest visible standard measurable on the

Sponsor's Table 1, Page 346, Vol. 1.32

## Metabolism and Excretion of $^{14}$C-GR 68755 in Rats After A Single Oral or I.V. Dose (Study # MET564/594, Report # WBP/89/060 and Study # MET533/711, Report # WBP/90/056)

**Methods**: Male and female rats (AHA and RH) were given a single oral (gavage) or i.v. dose of $^{14}$C-GR 68755 (3.5 mg/kg or 1 ng/kg) with specific activity of 6.07 MBq/mg. The volumes of administration were 4 ml/kg for oral dose and 2 ml/kg for i.v. dose. Urine and feces samples were collected over 24 hr period for 144 hours after drug administration. In all samples radioactivity was measured by methods. Drug and its metabolites were identified by methods.

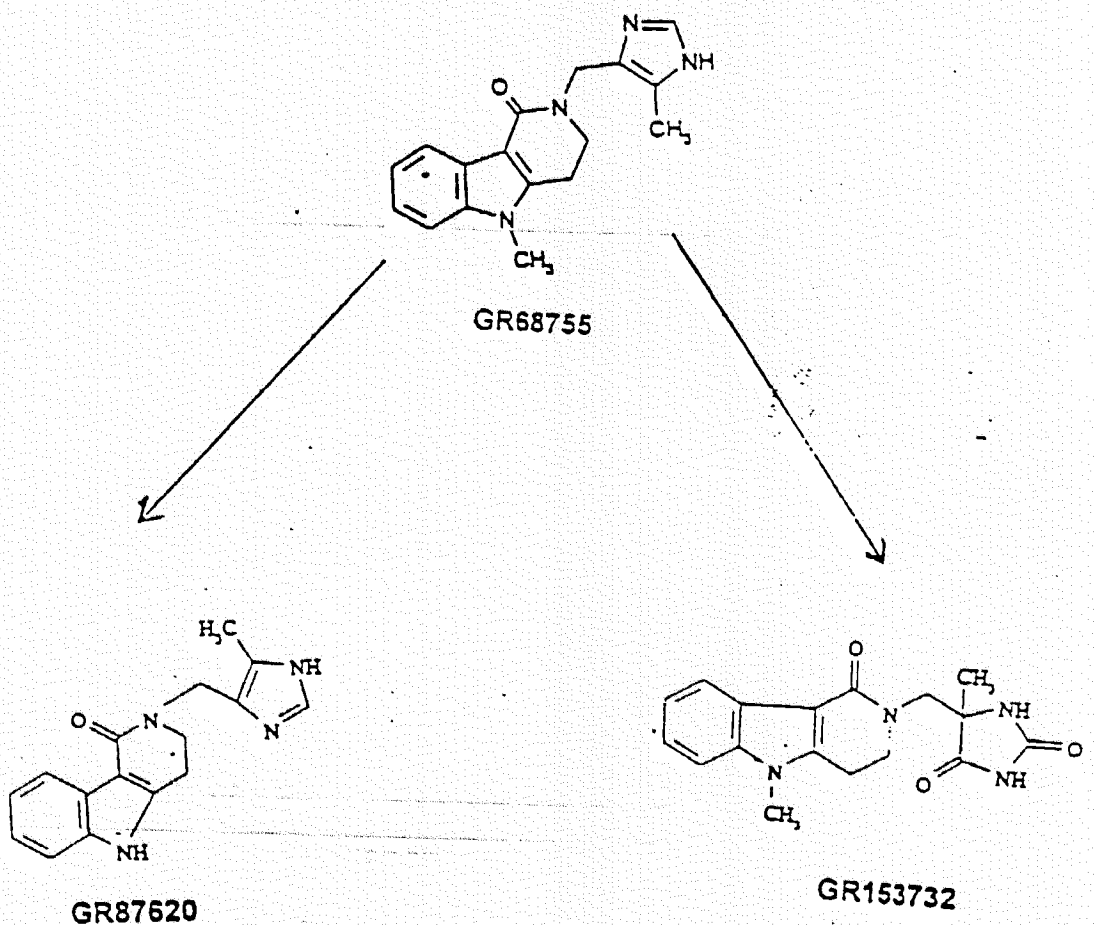
**Results**: Irrespective of route of administration, strain and sex, about 42-47% and 43-47% of the administered radioactivity were excreted in urine and feces during 0-144 hr period respectively, and most of the radioactivity was cleared during the first 24 hr period. Urinary excretion of radioactivity after oral and i.v. dose were comparable which suggest that oral dose is completely absorbed and fecal excretion mainly

represents biliary excretion. In both urine and feces samples (0-24 hr), about >10 radioactive peaks were seen and one of the peaks is identified as GR 87620 (N-desmethyl analogue of GR 68755). GR 87620 represented about 4% of the dose in urine. Furthermore, unchanged drug levels were less than 7% of the dose in urine and feces. Like in human urine, rat urine also contained bis-oxidized metabolite of GR 68755 (data presented as a peak and no quantitation was provided) (see fig 1.). Hence, the drug is metabolized rapidly in rats.

| Percent of Dose Recovered (n-8) | | | | |
|---|---|---|---|---|
| | RH Rats (3.5 mg/kg) | | AHA Rats (1 mg/kg) | |
| | Oral | I.V. | Oral | I.V. |
| **Urine** | | | | |
| 0-24 hr | 39.3 ± 9.6 | 38.9 ± 7.5 | 41.5 ± 8.1 | 38.2 ± 6.7 |
| 0-144 hr | 42.9 ± 9.0 | 42.6 ± 7.6 | 47.4 ± 10.1 | 41.5 ± 7.3 |
| **Feces** | | | | |
| 0-24 hr | 37.5 ± 5.7 | 38.6 ± 7.2 | 33.4 ± 19.1 | 38.9 ± 10.0 |
| 0-144 hr | 47.5 ± 6.1 | 43.9 ± 8.2 | 45.1 ± 9.7 | 46.8 ± 7.2 |

## Proposed Metabolic Pathway

Fig. 1



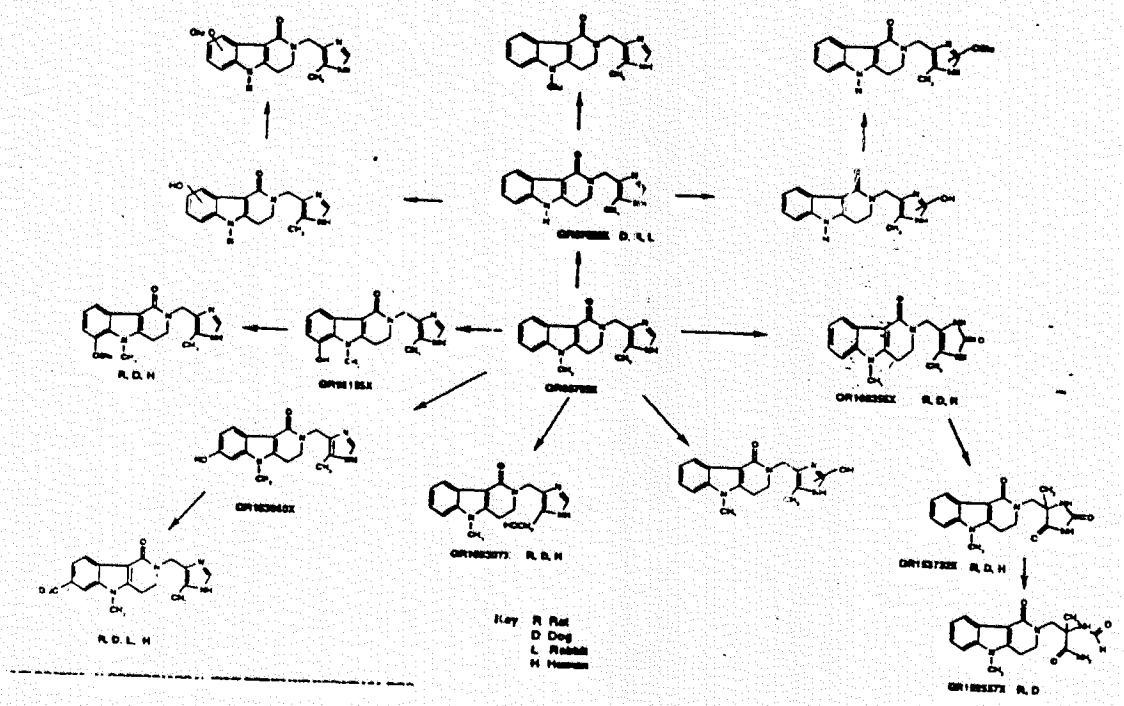

(N-desmethyl GR68755)
(Rat and Dog)

(bis-Oxidation of GR6855)
(Rat, Dog and Man)

Note: On page 38, Vol. 1.1 (Investigator's Brochure) sponsor indicated the presence of GR 96105 (6-hydroxy-alosetron), a metabolite of GR 68755, in rat and dog urine sample. However, the presence of this metabolite (GR 96105) was not documented in the study reports.

The metabolic pathways of alosetron in rats, dogs, and humans were depicted in Figure 7 on page 361 in volume 1.6 in this NDA and this figure is attached below.

**Figure 7. Metabolic Pathways of Alosetron in Rat, Dog, Rabbit and Human**



## Pharmacokinetic Studies of GR 68755: Absorption, Distribution, and Excretion in Rats

**(Study # AE-1585, Report # NME/94/030)**

Methods: Wistar SPF rats (male and female) were given a single oral (gavage) dose of $^{14}$C-GR 68755 (0.5 mg/kg) with specific activity of 6.14 MBq/mg. Pregnant and lactating female rats were also given a single oral (gavage) dose of $^{14}$C-GR 68755 (0.5 mg/kg) with specific activity of 6.14 MBq/mg. Additionally, male rats were treated with $^{14}$C-GR 68755 (0.5 mg/kg) with specific activity of 6.14 MBq/mg for 21 days. The volume of administration was fixed at 4 ml/kg. The radioactivity was determined in the plasma, tissues, urine, feces, and milk using . Plasma samples were collected up to 168 hours after dosing on days 1 and 21 and up to 24 hours after dosing on days 7 and 14 in the repeated dose study. was also performed.

Results: The radioactivity level in the plasma after the 1st, 7th, 14th, and 21st doses reached the peak levels of 65-89 ng eq./ml at 1 hour and declined with a half life of 3 hours. The radioactivity in the plasma after 21st dose was detectable at 24 hours and declined slowly with a half life of 5.2 days. The highest radioactivity was detected in the adrenal gland followed by the kidney and liver at 30 minutes after dosing which were ~22, 8, and 7 times higher than that in the plasma, respectively. The radioactivity levels in all tissues were less than 1% at 168 hours after dosing. The radioactivity levels in all tissues were similar after 1st and 21st dose except for the thyroid gland and skin after 21st dose in which the radioactivity declined slowly to 11.6 and 9.6%, respectively on day 28. The distribution ratios of radioactivity into blood cells were 25.7% and 19.3% at 30 minutes and 4 hours after dosing, respectively. The radio-activity in fetus was ~17-18% of that in the maternal plasma at 30 minutes after dosing and declined quickly to below the detection limit at 24 hours. The radioactivity in milk reached the peak level at 1 hour after dosing and declined with a half life of 5.2 hours in the lactating rats. The radioactivity levels in the milk were 3-18 folds higher than those in the plasma during 1-24 hours after dosing. The excretion of radioactivity in the urine and feces was ~45% and 50%, respectively.

## The Fate of GR 68755 in the Bile Duct Cannulated Male Pigmented rat Following Single Oral Dose of $^{14}$C-GR 68755 Hydrochloride at a Nominal Dose-Level of 3.5 mg base/kg Body Weight

**(BPW 198)**

Methods: A single dose of $^{14}$C-GR 68755 (specific activity = 6.97 MBq/mg) was given to bile duct cannulated male pigmented rats (HR) by oral gavage at 3.5 mg base/kg. The bile, urine, and feces samples were collected up to 24 hours after dosing and the radioactivity was determined using

Results: The results indicated that the total recovery of the radioactivity was 93.8 ± 2.5% at 24 hours after dosing. The major route of elimination was in the urine (48.8±4.4%) followed by the bile (27.6±4.2%) and feces (12.8±3.5%).

**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 $^{14}$C-GR 68755 (40 mg/kg) with specific activity of 6.97 MBq/mg 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** |
| $C_{max}$ (µg/ml) | 7.8 ± 2.8 | 16.7 ± 3.7 |
| $T_{max}$ (hr) | 0.5 ± 0.3 | 0.5 ± 0.3 |
| $AUC_{0-\infty}$ (ug.hr/ml) | 13.4 ± 9.0 | 42.9 ± 3.0 |
| $T_{1/2}$ (hr) | 0.8 ± 0.2 | 1.2 ± 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 ± 3.3% and 44.3 ± 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 ± 9.0% and feces = 25.5 ± 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 "$U_1$" was the major urinary metabolite which accounted for 23% of the dose. Metabolite "$F_1$" 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 $^{14}$C-GR 68755 (2 mg/kg) with specific activity of 6.07 MBq/mg. 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.** |
| $C_{max}$ (ng/ml) | 900 ± 156 | 1820 ± 450 | 1270 ± 129 | 2048 ± 452 |
| $T_{max}$ (hr) | 0.75 ± 0.39 | 0.08 | 0.8 ± 0.5 | 0.08 |
| $AUC_{0-\infty}$ (µg.h/ml) | 2070 ± 500 | 2270 ± 550 | 4820 ± 850 | 5280 ± 735 |
| $Cl_p$ (ml/min/kg) | --- | 16.0 ± 5.0 | --- | --- |
| Vd (L/kg) | --- | 1.2 ± 0.4 | --- | --- |
| $T_{1/2}$ (hr) | 1.3 ± 0.3 | 0.9 ± 0.2 | 2.3 ± 0.4* | 1.8 ± 0.2* |
| Bioavailability | 96 ± 24 | --- | --- | --- |

$Cl_p$ = plasma clearance
Vd = volume of distribution
* = calculated for the first 8 hr, t1/2 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, based on 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 20 - 4000 ng/ml of drug and mouse plasma samples were incubated with 100-600 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) with specific activity of 6.97 MBq/mg 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.

## Concentrations of GR 68755 Base and its N-desmethyl Metabolite (GR 87620) in Rat, Dog, and Human Plasma After Oral Dose (Report #WBP/91/002)

In this report, sponsor reported the N-desmethyl metabolite of GR 68755 (GR 87620) detected in the rat and dog plasma at 10-20% and 30-50% of the GR 6755 levels, respectively, following oral administrations of GR 68755. The dog plasma samples were obtained from the 6-month oral toxicity study in dogs dosed at 0, 5.5, and 20 mg/kg/day and a single oral dose at 2 mg/kg. The rat plasma samples were obtained following a single oral dose at 20 mg/kg. The N-desmethyl metabolite was not found in the human plasma following oral dose of GR 68755 at 4 mg b.i.d. in healthy volunteers.

**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 $^{14}$C-testosterone in the presence of GR 68755 (0-100 µm) and NADPH regenerating system. Metabolites of testosterone were analyzed by It should be noted that 7α-hydroxytestosterone production reflects the activity of CYP2A1. 6ß-hydroxytestosterone production reflects the activity of CYP3A and 2α and 16α-hydroxytestosterone production reflects the activity of CYP2C11.

**Results**: GR 68755 at 100 µm inhibited the formation of 6ß-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.

**Interaction of GR 68755 With Human Hepatic Microsomes In Vitro (Report # WD1998/00330,00)**

**Methods**: To determine which human hepatic microsomal cytochrome P450 forms are involved in the metabolic conversion of GR 68755, microsomal suspension prepared from the human liver were incubated with inhibitors for cytochromes P450 and $^{14}$C-GR 68755 (5 µM) with specific activity of 188 µCi/mg was added to each incubate at ~37° C for 2 hours. The metabolic profiles of $^{14}$C-GR 68755 were determined using The second part of this study is to determine the inhibitory effects of GR 68755 on the human hepatic microsomal cytochrome P450 enzymes. In this study, human hepatic microsomes were incubated with specific substrate probes for the cytochromes P450 in the presence of GR 68755 (0.2 and 2 µM) and the cytochrome P450 activities were then determined.

**Results**: The results indicated that CYP2C9 and CYP3A4 were the major contributors (~30% and ~18%) for metabolic conversion of GR 68755. CYP1A2 was also involved to less extent (~10%). Others including CYP2C8, 2C19, 2D6, and 2E1 appeared to contribute <5%. Non-CYP450 mediated phase I metabolic conversion of GR 68755 also contributed to ~11%. GR 68755 did not markedly inhibit the activities of CYP2D6 (<11%), CYP3A4 (<19%), and CYP2C9 (<12%). GR 68755 had no inhibitory effect on CYP2C19 but inhibited the activities of CYP1A2 (60%) and CYP2E1 (50%).