7. **Organ Weights**: No treatment related effects were seen.

8. **Histopathology**: No treatment related effects were seen.

9. **Levels of GR 68755 in Plasma (Report # WBP/91/044)**: Levels of GR 68755 in plasma samples were measured at [redacted] At the end of treatment period, plasma concentrations of GR 68755 were 21.4 ± 9.1, 30.1 ± 8.5 and 47.1 ± 34.7 ng/ml in low, mid and high dose treated males and the corresponding levels in females were 9.9 ± 1.3, 14.2 ± 11.4 and 22.3 ± 4.4 ng/ml. Thus, levels of GR 68755 in plasma increased with increasing dosage and levels in males were generally higher than that seen in females.

In this study, data indicated that GR 68755 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 receive the intended dosages. Based on this finding, sponsor selected 30 mg/kg (via drinking water) as the maximum tolerated dose in mouse carcinogenicity study.

Oral (drinking water) Oncogenicity Study in B6C3F1 Mice

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

P/T REVIEWER(s): Ke Zhang, Ph.D.
DATE: October 1, 1999

IND/NDA: NDA 21,107
DRUG CODE#:
CAS#:
DIVISION(s): HFD-180
DRUG NAME(s): Alosetron / GR 68755

SPONSOR: GlaxoWellcome Inc.
LABORATORY: GlaxoWellcome, UK
CARCINOGENICITY STUDY REPORT DATE: November 30, 1998

THERAPEUTIC CATEGORY:
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: 5-HT_3 Antagonist
MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay): No

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc.):

MOUSE STUDY DURATION (weeks): 94/95 weeks (males) and 104/105 weeks (females).
STUDY STARTING DATE: December 21, 1990
STUDY ENDING DATE: November 30, 1998
MOUSE STRAIN: B6C3F1
ROUTE: Drinking water
DOSING COMMENTS: Dose selection was considered adequate and the study was acceptable by Executive CAC on April 23, 1996 (See Appendix I).

NUMBER OF MICE:
- Control-1 (water): 60
- Control-2 (Acidic water, pH = 5.5): 60
- Low Dose (LD): 60
- Middle Dose (MD): 60
- High Dose (HD): 60

MOUSE DOSE LEVELS (mg/kg/day):
- Low Dose: 1
- Middle Dose: 5.5
- High Dose: 30

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): Maximum feasible (palatable) dose via drinking water.

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): Dose selection was considered adequate and the study was acceptable by Executive CAC on April 23, 1996.

MOUSE CARCINOGENICITY (conclusion: negative; positive; MF; M;F): Negative (MF).

MOUSE TUMOR FINDINGS (details):

The incidence of Harderian gland adenoma was higher in the treated males (8, 7, 6 in low, mid, and high dose groups) than in control (2) but it was not statistically significant (both trend and pairwise tests) and the increased incidence was not dose related. A single incidence of Harderian gland carcinoma was found in the low and high dose female groups (none in the mid dose female group and in males). The incidence of liver cell tumors was higher in the treated females (13, 15, and 13) than in controls (3, 8) but it was not statistically significant (both trend and pairwise tests) and was not dose related. The incidences of both Harderian gland adenoma and liver cell tumors are within the background incidence. Treatment with GR 68755 produced benign interstitial cell tumor of the testes in a dose dependent manner (1, 1, and 2 in the low, mid, and high dose groups, trend test $p = 0.047$) and a single incidence of malignant interstitial cell tumor of the testes in a mid dose male (none in the controls). The increased incidences of the benign interstitial cell tumor in each of the treatment groups were not significantly (pairwise test) different from the incidences in the vehicle control group.

MOUSE STUDY COMMENTS:

In the oral carcinogenicity study in mice, mice (60/sex/group) were treated with GR 68755 via drinking water at 0, 0, 1, 5.5, and 30 mg/kg/day for 94/95 weeks in males or 104/105 weeks in females. There were no treatment related clinical signs of toxicity. Mortality rate was comparable in control and treatment groups. The terminal body weight in the high dose female was 91.4% of the control. Higher incidences of Harderian gland adenoma and liver cell tumors were found in the treated males and females, respectively. These increased incidences were not dose related and not statistically significant (both trend and pairwise tests) when compared to the vehicle control group. They are within the background incidence. Treatment with GR 68755 produced benign interstitial cell tumor of the testes in a dose dependent manner (trend test $p = 0.047$) and a single incidence of malignant interstitial cell tumor of the testes in a mid dose male (none in the controls). The increased incidences of the benign interstitial cell tumor in each of the treatment groups were not significantly (pairwise test) different from the incidences in the vehicle control group. It was concluded in the Executive CAC meeting held on October 12, 1999 that alosetron did not have tumorigenic potential in this study (Appendix II).

COVERSHEET FOR CARCINOGENICITY STUDY IN MOUSE

1. Study No.: M12401
2. Name of Laboratory: GlaxoWellcome, UK
3. Strain: B6C3F1
4. No./sex/group: 60
5. Dose (O,L,M,H): 0, 1, 5.5, and 30 mg/kg/day
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 94/95 for males, 104/105 for females.
9. Week/site for first tumor:

	Male	**Female**
Control (water)	42/M. adenoma in liver	57/M., cystic adenocarcinoma in mammary gland and M. lymphoma in lymphoreticular tissue
Control 2 (acidic water, pH=5.5)	59/M. lymphoma & histiocytic sarcoma in lymphoreticular tissue	57/M. hepatocellular carcinoma
Low dose	63/M. hepatocellular carcinoma in liver	59/M. lymphoma in lymphoreticular tissue
Mid dose	50/M. osteosarcoma in skeleton	65/B. adenoma in liver and M. fibrosarcoma in skin
High dose	57/M. hepatocellular carcinoma & adenoma	65/M. lymphoma in lymphoreticular tissue

B = Benign, M = malignant

10. No. alive at termination:

	Male (%)	**Female (%)**
Control 1	32/60 (53.3)	34/60 (56.7)
Control 2	25/60 (41.7	29/60 (48.3)
Low dose	21/60 (35.0)	19/60 (31.7)
Mid dose	29/60 (48.3)	27/60 (45.0)
High dose	29/60 (48.3)	23/60 (38.3)

11. Statistical Methods used: The prevalence method of Peto (Peto, R. et.al., Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiment in Long-term and short term screening assays for carcinogens: a critical appraisal. IARC, pp 311-426, 1980).

12. Attach tumor data for each tissue: See Appendix III.

Oral (drinking water) Carcinogencity Study in Mice (Study # M12401)

Testing Laboratory: Sponsor's Lab, UK

Study Start and Completion Dates: December 21, 1990 and November 30, 1998.

GLP and QAU Compliance Statement: Statements of compliance with GLP regulations and the quality assurance unit were included.

Animals: Male (20.1-32.2 g, 5-6 weeks old)
Female (15.6-27 g, 5-6 weeks old)
B6C3F1 mice

Drug Batch No.: C1026/120/1

Methods: To determine the carcinogenic potential of GR 68755, mice (60/sex/group) were treated with GR 68755 via drinking water at 0 (tap water), 0 (acidified water, pH=5.5), 1, 5.5, and 30 mg/kg/day for 94/95 weeks in males or 104/105 weeks in females. The dose selected was considered adequate in Executive CAC meeting held on April 23, 1996. The dosages achieved were calculated by multiplying the mean water consumption for each group (g/kg/day, 1 ml solution = 1 g) by the solution concentration in mg/ml. The achieved dosages were summarized in a table on page 219 in Volume 1.10 and this table is attached below.

Achieved dosages

Target dosage (mg/kg)	Sex	Achieved dosage (mg/kg/day)	
		Mean	Range
1.0	M	1.01	
	F	1.01	
5.5	M	5.51	
	F	5.53	
30.0	M	29.86	
	F	29.84	

In the current study, clinical signs of toxicity and mortality were observed daily. Body weights were determined weekly. Food consumption was determined weekly during the first 13 weeks and then at ~3 month interval throughout the study. Water consumption was determined

over each 3 or 4 day period throughout the study except for the 1 mg/kg/day group for which it was determined daily from day 113. Hematology was conducted at termination. All dead or terminal sacrificed animals were subjected to gross and histopathological examinations. The tissues examined histopathologically were listed in a table on page 223 in volume 1.10 and this table is attached below.

Adrenals	Lachrymal gland (exorbital)	Rectum
Animal ID (tattoo) #	Lachrymal gland (Harderian)	Salivary glands
Aorta	Larynx (& oropharynx) $	Seminal vesicles
Bone marrow	Liver	Skeletal muscle
Brain	Lungs	Skin
Caecum	Lymph nodes	Spinal cord
Colon	Macroscopic abnormalities	Spleen
Duodenum	Mammary glands	Stomach
Epididymis	Nasal cavity	Testes
Eyes *	Oesophagus	Thymus (or thymic area)
Gall bladder	Ovaries	Thyroid
Heart	Pancreas	Tongue
Joint (femur)	Parathyroids	Trachea
Ileum	Peripheral nerve	Urinary bladder
Jejunum	Pituitary	Uterus
kidneys	Prostate	Vagina

* With optic nerve # From Day 379 only. $ From Day 450 only

Plasma levels of GR 68755 were determined during weeks 6, 26, 52, 78, 94 (males), and 104 (females) weeks in the satellite animals. The tumor data were analyzed using the prevalence method of Peto (Peto, R. et.al., Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiment in Long-term and short term screening assays for carcinogens: a critical appraisal. IARC, pp 311-426, 1980).

Results:

1. **Clinical Signs**: There were no treatment related clinical signs of toxicity.

2. **Mortality**: The survival rate at the end of the treatment period was 53.3%, 41.7%, 35%, 48.3%, and 48.3% for males or 56.7%, 48.3%, 31.7%, 45%, and 38.3% for females treated at 0, 0, 1, 5.5, and 30 mg/kg, respectively.

The intercurrent mortality (unscheduled deaths) was summarized in the following tables.

Mortality (Unscheduled Death) in Males

Weeks	Control 1	Control 2	1 mg/kg	5.5 mg/kg	30 mg/kg
0-58	6	5	1	1	2
58-77	7	10	13	5	12
77-94	15	20	25	25	17
Total	28	35	39	31	31

Mortality (Unscheduled Death) in Females

Weeks	Control 1	Control 2	1 mg/kg	5.5 mg/kg	30 mg/kg
0-58	4	2	2	0	0
58-77	2	2	3	4	2
77-90	10	6	11	3	12
90-104	10	21	25	26	23
Total	26	31	41	33	37

animals in each group: 60/sex/group,

3. **Body Weight**: The initial and final body weights in the control groups were 25.9-26.2 and 51.2-51.4 g for males or 20.2-20.3 and 47-47.3 g for females. The terminal body weights in males treated at 1, 5.5, and 30 mg/kg were 100.8, 101, and 100.6% of the control, respectively. The terminal body weights in females treated at 1, 5.5, and 30 mg/kg were 104.8, 104, and 91.4% of the control, respectively. The body weight information is summarized in the following tables.

Mean body weights (g) in Males

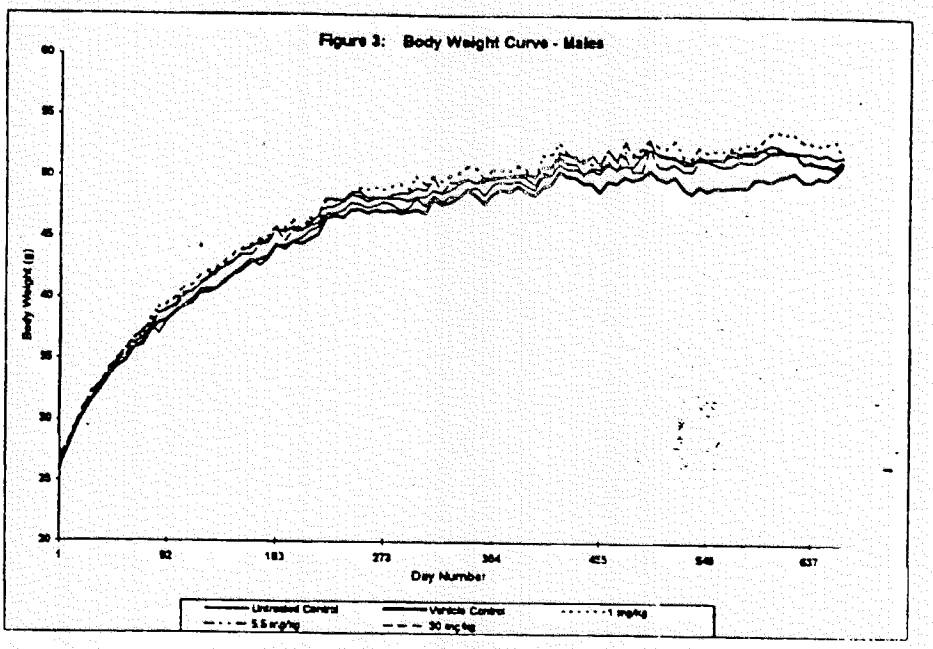
Days	Control 1	Control 2	1 mg/kg	5.5 mg/kg	30 mg/kg
1	25.9	26.2	26.5	26.9	27
92	38.1	38.1	39.4	39	38.9
364	49.4	48.6	50.6	49.8	50
659	51.4	51.2	51.7(100.8%)	51.8 (101%)	51.6 (100.6%)

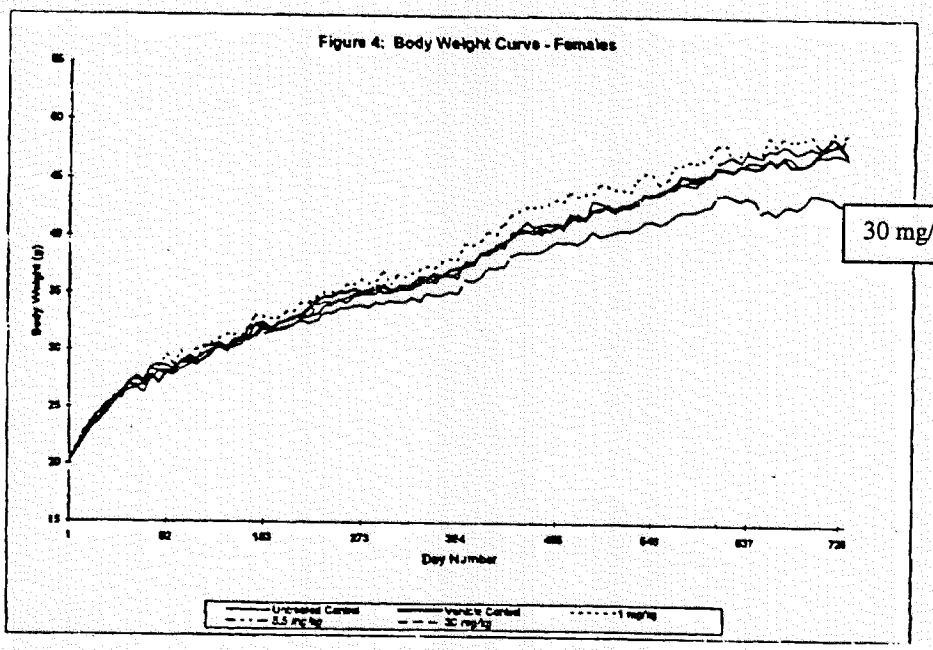
Mean body weights (g) in Females

Days	Control 1	Control 2	1 mg/kg	5.5 mg/kg	30 mg/kg
1	20.2	20.3	20.8	20.4	20.4
92	27.9	28	29.5	28.6	28.2
364	36.4	37.3	38.1	36.9	35.1
546	43.9	43.7	45.5	43.7	41.5
729	47	47.3	49.4(104.8%)	49 (104%)	43.1 (91.4%)

The numbers in parenthesis are % of the combined control.

The growth curves were depicted in Figures 3 and 4 on pages 246 and 247 in volume 1.10. These figures are attached below.





30 mg/kg

4. **Food and Water Consumption**: Average food consumption in the control group was (males) or females) g/animal/day. There were no treatment related effects on food consumption. Water consumption was not consistent during the study.

5. **Hematology**: In the unscheduled dead females, lymphoma/ lymphoma-like cells and histiocytes were present in the treatment groups with a slightly higher incidence than in the control groups. These changes were not found at termination. These data were presented in tables on pages 231 and 232 in volume 10. These tables are attached below.

Incidence of lymphoma / lymphoma-like cells with and without azurophilic granulation in female intercurrent deaths

Observation	Day Nos.	Untreated control	Vehicle control	1 mg/kg	5.5 mg/kg	30 mg/kg
Lymphoma/ Lymphoma-like cells	300 - 399	-	0/1	-	-	-
	400 - 499	0/3	0/0	1/1	0/1	1/2
	500 - 599	1/6	2/5	1/5	0/3	3/6
	600 - 699	6/12	6/13	6/12	7/15	11/14
	700 - end	1/1	3/5	2/4	2/3	2/4
	Total	8/22	11/24	11/22	9/22	17/26
Lymphoma with Azurophilic granulation	500 - 599	0/6	0/5	0/5	0/3	2/6
	600 - 699	1/12	4/13	1/12	2/15	5/14
	Total #	1/22	4/24	1/22	2/22	7/26

- No deaths occurred in this group during this period.
\# Only those periods where the finding was noted are shown (therefore totals of animals examined may not add up)

Incidence of circulating histiocytes in female intercurrent deaths

Observation	Day Nos.	Untreated control	Vehicle control	1 mg/kg	5.5 mg/kg	30 mg/kg
Histiocytes	400 – 499	0/3	-	1/1	0/1	0/2
	500 – 599	1/6	1/5	1/5	0/4	1/6
	600 – 699	2/12	2/13	1/12	4/15	5/14
	700 – end	0/1	0/5	1/4	0/3	0/4
	Total #	3/22	3/24	4/22	4/22	6/26

- No deaths occurred in this group during this period.
\# Only those periods where the finding was noted are shown (therefore totals of animals examined may not add up)

Incidence of lymphoma / lymphoma-like cells with and without azurophilic granulation and histiocytes in females killed at term

Observation	Untreated control	Vehicle control	1 mg/kg	5.5 mg/kg	30 mg/kg
Lymphoma/ lymphoma-like cells	7/32	4/34	2/29	4/37	4/32
Lymphoma with azurophilic granulation	1/32	0/34	0/29	1/37	0/32
Histiocytes	1/32	1/34	0/29	0/37	0/32

These changes are not considered treatment related.

6. **Gross Pathology**: There were no clear treatment related effects.

7. **Histopathology**:

Non-neoplastic Changes: The higher incidences of tooth anomalies in males and angiectasis in the lymph nodes in both male and females were noted. This information was summarized in tables on pages 237 and 238 in volume 1.10. These tables are attached below.

Incidence of tooth anomalies

	MALES Dosage (mg/kg/day)					FEMALES Dosage (mg/kg/day)				
	0 (a)	0(b)	1	5.5	30	0 (a)	0(b)	1	5.5	30
No. examined *	60	60	60	60	60	59	60	60	60	60
enamel organ convolution	4	7	2	15	15	1	5	2	3	5
tooth deformation	0	3	2	1	0	0	0	1	0	0
rudimentary tooth in pulp	7	9	3	6	4	1	1	1	2	0

(a) Untreated control (b) Vehicle control * Tooth section present in sections of nasal cavity

Increased incidence of angiectasis of the lymph node in GR68755C-treated animals

	MALES Dosage (mg/kg/day)					FEMALES Dosage (mg/kg/day)				
	0 (a)	0(b)	1	5.5	30	0 (a)	0(b)	1	5.5	30
No. examined	57	60	60	59	60	60	60	59	59	60
angiectasis	8	11	19	20	19	2	2	2	7	4

(a) Untreated control (b) Vehicle control

Neoplastic Changes: The increased incidences of Harderian gland adenoma and testicular interstitial cell tumors in males and liver cell tumors in females were noted. The incidences of these tumors were summarized in tables on pages 236 and 237 in Volume 1.10. These tables are attached below.

Incidence of Lachrymal (Harderian) gland tumours

	MALES Dosage (mg/kg/day)					FEMALES Dosage (mg/kg/day)				
	0 (a)	0(b)	1	5.5	30	0 (a)	0(b)	1	5.5	30
No. examined	60	60	60	60	60	60	59	59	60	60
adenoma	2	2	8	7	6	5	7	4	8	4
adenocarcinoma	0	0	0	0	0	0	0	1	0	1

(a) Untreated control (b) Vehicle control

	Males Dosage (mg/kg/day)					Females Dosage (mg/kg/day)				
	0	0	1	5.5	30	0	0	1	5.5	30
Combined incidence of adenoma and adenocarcinoma	2	2	8	7	6	5	7	5	8	5

Incidence of interstitial cell tumours of the testes

	MALES Dosage (mg/kg/day)				
	0 (a)	0(b)	1	5.5	30
No. examined	60	60	60	60	60
benign	0	0	1	1	2
malignant	0	0	0	1	0

(a) Untreated control (b) Vehicle control

	Males Dosage (mg/kg/day)			
	0	0	1	5.5	30
Combined incidence of benign and malignant	0	0	1	2	2

Incidence of liver cell tumours

	MALES Dosage (mg/kg/day)					FEMALES Dosage (mg/kg/day)				
	0 (a)	0 (b)	1	5.5	30	0 (a)	0 (b)	1	5.5	30
No. examined	60	60	60	60	60	60	60	60	60	60
adenoma	28	29	29	21	24	2	7	11	11	10
carcinoma	17	13	21	16	17	1	1	6	5	4

(a) Untreated control (b) Vehicle control

	Males Dosage (mg/kg/day)					Females Dosage (mg/kg/day)				
	0	0	1	5.5	30	0	0	1	5.5	30
Combined incidence of adenoma and carcinoma	41	35	42	31	37	3	8	13	15	13

If adenoma and carcinoma were found in the same animal, count only one.

Sponsor did not provide the historical control data from the testing laboratory and stated that no historical tumor incidence data for the B6C3F1 mouse are available from the testing facility. Instead, sponsor included historical control data for these tumors from literature in a table on page 240 in Volume 1.10. This table is attached below.

Reported incidences of specific tumour types in B6C3F1 mice (as range or average %)

Reference	Liver cell adenoma		Hepatocellular - carcinoma		Harderian gland adenoma		Interstitial cell tumour testes
	Male	Female	Male	Female	Male	Female	Male
Maronpot *et al*, 1987	0-44	0-18	8-32	0-15			
Sher *et al*, 1982	0-42	0-8	0-37	0-10			
Chandra & Frith, 1992	15	2.5	9.5	4.5	1	15.5	0.5
Ward *et al*, 1979	7.9	1.6	13.7	2.3	0.8	0.5	0.27
Yamano *et al*, 1988			23.8	4.5	2.5	2.8	
Haseman *et al*, 1994					0-20	0-16	
Lang, 1989	0-41	0-17	4-25	0-6	0-11	0-9	0-3.3

In the absence of historical control data from the testing laboratory, additional data on the background incidence of these tumors in B6C3F1 mice are obtained from National Toxicology Program (NTP). These data are summarized in the following table.

	Hepatocellular Adenoma	Hepatocellular Carcinoma	Harderian Gland Adenoma	Harderian Gland Carcinoma	Testes Adenoma
Male					
Mean±SD	61 ± 8.2%	27.3 ± 10.7%	7.7 ± 3.9%	2.1 ± 1.4%	0.6 ± 1%
Range	47-70%	10-42%	2-13%	0-4%	0-2%
Female					
Mean±SD	55 ± 21.2%	19.7 ± 12.8%	2.5 ± 2.5%	1.6 ± 1.5%	
Range	26-80%	8-42%	0-6%	0-4%	

The incidence of Harderian gland adenoma was higher in the treated males (not in females) but it was not statistically significant and the increased incidence was not dose related (trend test, p = 0.27). A single incidence of Harderian gland carcinoma was found in the low and high dose female groups (none in the mid dose female group and in males). These are within the background incidence in the above tables. The increased incidence of liver cell tumors in females was not statistically significant and was not dose related (trend test, p = 0.28 for carcinoma, p = 0.16 for adenoma). These are also within the background incidence. The increased incidences of Harderian gland adenoma and liver cell tumors in each of the treatment groups were not significantly (pairwise test) different from the incidences in the vehicle control group. Treatment with GR 68755 produced benign interstitial cell tumor of the testes in a dose dependent manner and a single incidence of malignant interstitial cell tumor in a mid dose male (none in the controls). The increased incidences were not statistically significant by the trend test. The increased incidences in each of the treatment groups were not significantly (pairwise test) different from the incidences in the vehicle control group.

The incidence of neoplastic findings extracted from sponsor's table 7 on pages 279 to 303 in Volume 1.10 is attached in Appendix III.

8. **Drug Plasma Levels**: Mean plasma levels of GR 68755 were summarized in tables on pages 225 and 226 in Volume 1.10. These tables are attached below.

Mean plasma GR68755X concentrations (ng/mL) determined at midnight (middle of dark cycle)

Day number	Dosage (mg/kg/day)		
	1	5.5	30
43 [a]	3 [b]	5 [b]	14 [b]
183	4.0	16.9	137
365	1.8	9.2	117
547	<0.3	<5	14.7
Termination	3.8	15.0	104

[a] Values reported on this occasion were actually samples taken at 02.00h on Day 44

[b] Values reported include data below the lowest calibration limit, reported to 1 significant figure below 10 ng/mL and 2 significant figures above.

Mean plasma GR68755X concentrations (ng/mL) determined during terminal 24 hours*

	Dosage (mg/kg/day)		
	1	5.5	30
18.00 h	1.7	4.1 [a]	30.2
24.00 h	3.6	15.0	104
06.00 h	1.6	9.2	84.8
12.00 h	5.8	12.0	24.9
18.00 h	4.1	6.4	138 [b]
Cmax (ng/mL)	5.8	15.0	104
Cmean (0-24h) (ng/mL)	3.4	9.3	76.0
AUC (0-24h) (ng.h/mL)	82	246	1743

* Data for males and females combined. Male data obtained on Days 858/859 and female data on Days 728/729

[a] Female value <5ng/mL. For purpose of calculation of mean data, the value corresponding to half the limit of quantification was used

[b] This value depends heavily on an unusually high male value of 234 ng/mL which appears anomalous. The value at 24.00h is the more likely to represent Cmax and so this is quoted.

In general, the plasma concentrations were increased with the doses and there was no clear accumulation of the drug over time. Further examination on the original data revealed no difference in the plasma level of GR 68755 between males and females.

In summary, in the oral carcinogenicity study in mice, mice (60/sex/group) were treated with GR 68755 via drinking water at 0, 0, 1, 5.5, and 30 mg/kg/day for 94/95 weeks in males or 104/105 weeks in females. The dose selection was considered adequate in the Executive CAC meeting held on April 23, 1996. The high dose was maximum feasible dose. There were no treatment related clinical signs of toxicity.