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

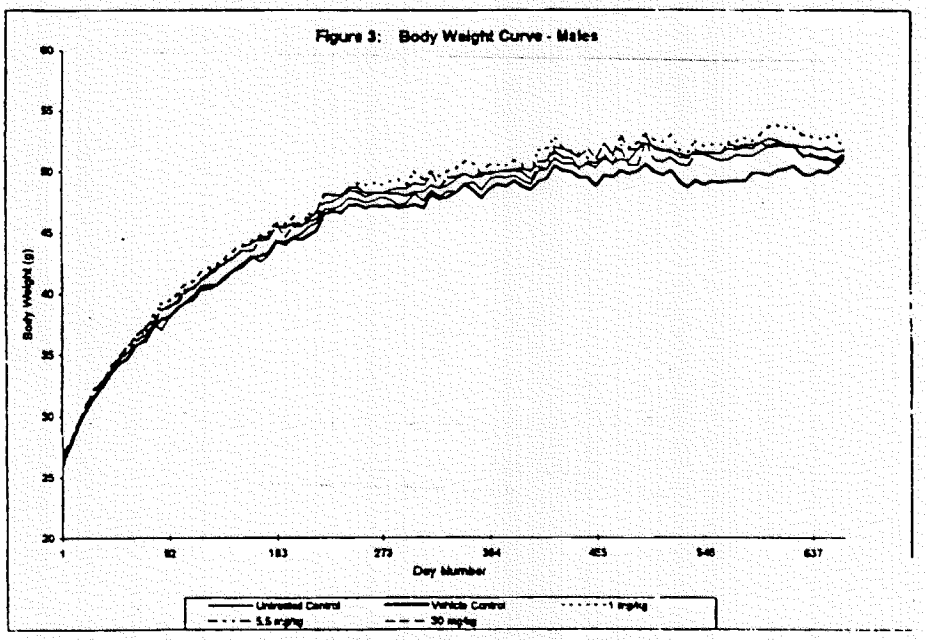
Days	Control 1	Control 2	1 mg/kg	5.5 mg/kg	30 mg/kg
1	25.9	26.2	26.5	26.9	27
92	38.1	38.1	39.4	39	38.9
364	49.4	48.6	50.6	49.8	50
659	51.4	51.2	51.7 (100.8%)	51.8 (101%)	51.6 (100.6%)

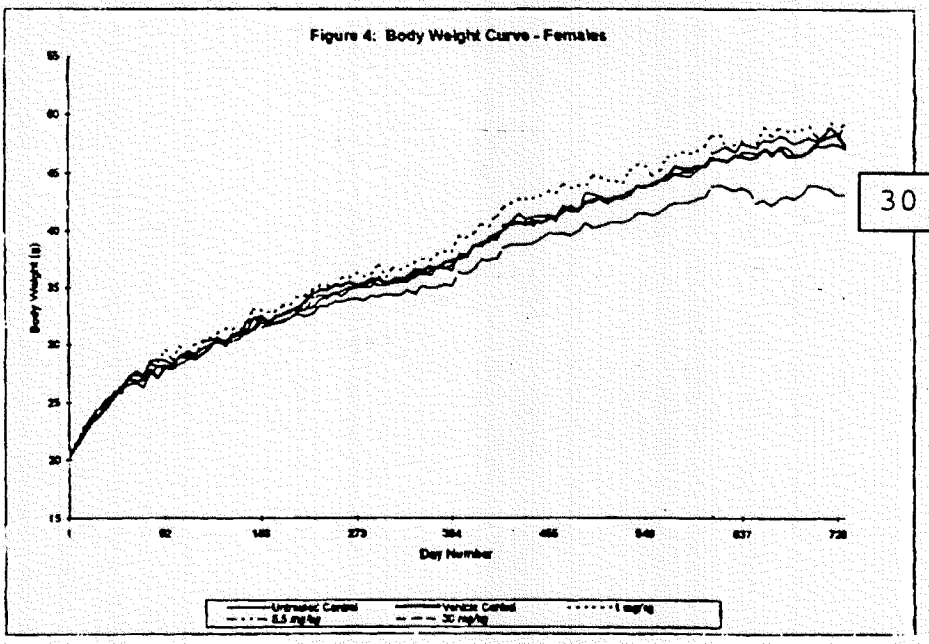
Mean body weights (g) in females

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	0-6	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
Tamano *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 backgound 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. 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). No significant dose-tumor positive trend was detected in all reported tumors by FDA statisticians. Combined tumor incidences were not tested in the trend tests. The pairwise test on these tumors is pending.

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

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	136 [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 658/659 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 ng/mL) 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.

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. 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. They are within the background incidence. 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 of the testes in a mid dose male (none in the controls).

SUMMARY AND EVALUATION:

Sponsor submitted the preliminary report of the carcinogenicity study in mice and the final report of the carcinogenicity study in rats in the initial submission of IND These studies were reviewed on April 16, 1996 (Pharmacology review) and discussed at the Executive CAC meeting on April 23, 1996. The dose selections of both studies were considered adequate and both studies were acceptable. Alosetron did not have tumorigenic potential in the rat carcinogenicity study. It was not conclusive for the mouse study at that time since the final report of this study was not available. Sponsor submitted the final report of the carcinogenicity study in mice in this NDA 21,107. In this 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. They are within the background incidence. 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 of the testes in a mid dose male (none in the controls).

RECOMMENDATION: None.

/S/ 10/1/99
Ke Zhang, Ph.D.

Appendix I: Executive CAC Report on April 23, 1996

Appendix II: Tumor Data

/S/ 10/1/99

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

R/D Init.: J. Choudary 9/14/99

KZ/hw/9/14/99 & 9/30/99

020

/S/

IND [redacted]

REVIEW # 1

Reviewer: Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

APR 16 1996

Sponsor and Address: Glaxo Wellcome Inc.
Research Triangle Park, NC

Rat Carcinogenicity Study Review Begins on Page 60

Date of Review: April 16, 1996

Date of Submission: Initial Submission: August
Amendment: January 22, 199(

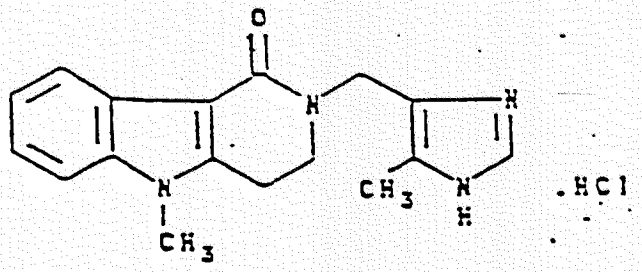
Date of HFD-180 Receipt: Initial Submission: August 8, 1995
Amendment: January 23, 1996

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
(Original Summary)

Drug: GR 68755 (Alosetron Hydrochloride) Tablets

Chemical Name: 2,3,4,5-Tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-1H-pyrido[4,3-b]indol-one hydrochloride

Chemical Structure:



$C_{17}H_{18}N_4O.HCl$

M.W. 330.9

Formulation: Each tablet contains 1, 2, 4 or 8 mg of alosetron (GR 68755X, the base) as alosetron hydrochloride along with lactose, microcrystalline cellulose, pregelatinized starch, magnesium stearate, hydroxy-propyl methylcellulose USP, titanium dioxide USP and

Category: 5-HT_3 receptor antagonist

Related INDs: IND [redacted] GR 68755 (tablets), Glaxo, Inc.
IND [redacted] GR 68755 (tablets), Glaxo, Inc.
IND [redacted] GR 68755 (tablets),

PROPOSED CLINICAL STUDY:

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

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study Report #	Drug Batch #	Testing Laboratories
Pharmacology			
Absorption:			
Mouse (oral)			
Rat (oral & I.V.)			
Rabbit (oral)			
Dog (oral & I.V.)			
Distribution:			
Rat (oral & I.V.)			
Metabolism:			
Mouse, rat, rabbit & dog			
Excretion:			
Mouse, rat, dog			
Acute Toxicity:			
Mouse			
Oral	M11909/M11910	C1281/83/1, C1017/133/1	GGR
I.V	M11903/M11904	C1281/83/1, C1017/133/1	GGR
Rat			
Oral	R11898/R11917	C128/83/1, C1017/133/1	GGR
I.V.	R11899/R11900	C128/83/1, C1017/133/1	GGR
Subacute/Subchronic/Chronic Toxicity:			
Rat			
34/35-Days (oral)	R11832	DR11363	GGR
6-month (oral)	R11867	C1034/102/1	GGR
		---	---

Dog			
35-day (oral)	D11825	C1017/69/1, C1017/77/1	GGR
6-month (oral)	D11865	C1034/104/1, C1034/98/1, C1017/189/1, C1034/1021	GGR
12-month (oral)	D12561	M91/025, M91/026, M91/027	GGR
Carcinogenicity Studies:			
Mouse			
13-week (drinking water)	M12419	C1026/120/1	GGR
		---	---
Rat			
3-month (diet)	R12457	C1026/120/1	
3-month (gavage)	R12569	C1026/120/1	
2-years (diet)	R12458	C1026/120/1, C1026/123/1, C1757/106/1	
Reproductive Toxicity:			
Fertility and Reproductive Performance (Segment I)			
Rat (oral)	R12036	C1028/98/1	GGR
Teratology (Segment II)			
Rat (oral)	R11996/R12151	C1017/133/1, C1034/96/1	GGR
Rabbit (oral)	L11961/L11998	C1028/98/1, C1017/133/1	GGR
Mutagenicity:			
Ames test	V12622/V12468	C1026/133/1, C1017/231/1	GGR
Chromosomal aberration test in human lymphocytes	V11912	C1017/133/1	GGR
L5178Y/TK mouse lymphomas assay	V13036	C1026/133/1	GGR
Micronucleus test in rat	R11921/R11823	C1017/133/1, C1297/69/1	GGR
UDS assay in rat liver (in vivo)	R12689	C1026/120/1, C1026/123/1	GGR

GGR = Glaxo Group Research Ltd., Hertfordshire, UK

PHARMACOLOGY:

Irritable Bowel Syndrome (IBS) is a functional disorder of gastrointestinal (GI) tract characterized by alterations in bowel function and abdominal pain or discomfort. Sponsor has not provided any rational for the use of GR 68755 (5-HT3 antagonist) for the treatment of IBS. In anesthetized rat, one of the main symptoms of IBS i.e. pain or discomfort to rectal distention was produced by "step-wise" inflation of latex balloon inserted in the rectum. In this model, GR 68755 (1-100 mcg/kg, i.v.) dose dependently inhibited the hypotensive response to colorectal distention. At 30 mcg/kg (i.v.) the hypotensive response to 1.5 ml distention was decreased from mmHg. Clinical studies performed in Europe and Canada indicated that GR 68755 may be beneficial in "diarrhea-predominant IBS patients".

All of the pharmacology studies were previously submitted under INDs and These INDs were reviewed by Dr. Rosloff (HFD-120) (date reviewed: 5/9/90 and 4/9/92). His review of pharmacology is reproduced below: