CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-006

PHARMACOLOGY REVIEW(S)

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET
Review of Carcinogenicity Study Results

P/T REVIEWER(s):	Aisar Atrakchi, Ph.D.
DATE:	Oct 2nd 2001
IND/NDA:	N21006
DIVISION(s):	HFD-120/Neuropharmacological Drug Products
DRUG NAME(s):	Frovelan (Frovatriptan succinate) tablets
SPONSOR:	Elan Pharmaceuticals S. San francisco, CA

CARCINOGENICITY STUDY REPORT DATE: Aug 2000-May 2001

THERAPEUTIC CATEGORY: Migraine

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: $5HT_{1B/1D}$ partial agonist

MUTAGENIC/GENOTOXIC (y/n/equivocal/ na; assay): Yes.

P53 Mouse study:
STUDY DURATION (weeks): 26wks
STRAIN: C57BL/6TacfBR-[KO]p53 N5 heterozygous mice
ROUTE: oral gavage
DOSING COMMENTS: NA
NUMBER OF MICE: 15/sex/group; additional 3/sex in high dose during wk3.
DOSE LEVELS: 0, 20, 62.6, 200, 400mg/kg/d. vehicle control was 1% w/v MC
POSITIVE CONTROLS: MNU 90mg/kg and p-cresidine 400mg/kg.
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): No

CARCINOGENICITY (conclusion: negative; positive; MF; M; F): Positive

TUMOR FINDINGS (details): transponder site skin subcutis sarcoma in both sexes (statistically significant in females).

STUDY COMMENTS: this is the 2nd p53 study conducted by this sponsor with this drug. The 1st study was deemed by the Exec CAC and the Division to be invalid due to failure of the positive control. No transponder site sarcomas in either positive controls, the sarcomas in drug groups exceeded the concurrent as well as historical control values (negative control within the historical range).

Review of Pharmacology & Toxicology Data
Major Amendment - Complete Response to Approvable Letter

IND, NDA# ———N21-006
Drug: Frovelan™ (Frovatriptan succinate) tablets
VML 251
Sponsor: Elan Pharmaceuticals
S. San Francisco, CA 94080
Indication: Migraine
Sub. Date: May 7th 2001
Rec. Date: May 8th 2001
Rev. Date: Sep 24th 2001
Reviewer: Aisar Atrakchi, Ph.D.
Team Leader: Barry Rosloff, Ph.D.
Related IND/NDAs: N21,001 (Almotriptan).

This submission included the 1st of 2 part submission, it provided final study report of the 2nd p53 mouse alternative model of carcinogenicity entitled: "26 week oral gavage oncogenicity study with VML 251 in p53(+/-) C57BL/6 mice". In addition to this study, this submission included summary for previously conducted (and reviewed) genotoxicity, carcinogenicity studies (1 each in mouse and rat and the 1st p53 mouse study), transponder related information, and relevant bibliography. The following is a review of the final report of the 2nd p53 mouse carcinogenicity study together with interpretation of the results and labeling.

Background:
Frovatriptan NDA 21-006 was filed with the FDA on Jan 29th 1999. Additional preclinical studies were recommended, they were then conducted and completed final study reports were submitted as major amendment to the NDA on Jan 21st 2000. Approvable letter was issued to the sponsor on Apr 28th, 2000 with several CMC, clinical, and preclinical issues. The preclinical issues included the results for then, the ongoing p53 26 week mouse carcinogenicity study. The study results were submitted to the Agency on Oct 3rd 2000 and the Executive CAC concluded that the study was invalid due to the failure of the positive control to produce the appropriate response (Exec CAC minutes Oct 31st 2000). Therefore, the carcinogenic risk assessment of frovatriptan was inadequate. The sponsor consequently initiated a 2nd p53 mouse 26 week carcinogenicity study and withdrew the labeling text of the Complete Response on Feb 20th 2001.

Study Objective:
To determine the effects of VML251 on the incidence and morphology of tumors following oral daily gavage administration to p53 heterozygous C57BL/6 mice for 26wks.

Study# 1165/238; [redacted] study# 1165/238-D6154
Conducting lab: [redacted]
Study initiation/Termination Dates: Aug 2000/May 2001
GLP: Yes [redacted]
QA report: Yes
Drug lot#/purity: 60811-44/99.9%
Strain: C57BL/6TacfBR-[KO]p53 N5 heterozygous obtained from [redacted]

No./sex/gr: 15/sex/group; additional 3/sex were added to HD during wk3. There were **3 mice/sex housed per cage.**
Doses: **20, 62.5, 200, 400mg/kg/d**
Positive Controls: N-nitrosomethylurea (MNU), 90mg/kg* and p-cresidine, 400mg/kg/d. The vehicle for the former was citrate-buffered saline and the latter, corn oil.
Negative/vehicle control: 1% w/v methylcellulose
Administration volume: 10ml/kg
Route/Duration: daily oral gavage for 26wks
B.wt/age at initiation: 21-27g m; 16-22g f; 8wks old at start of dosing.

* note that mice in this group were administered a single dose of MNU on d1 and kept untreated for the 26wk study period. Mice in the p-cresidine were dosed daily for 26wks.

Mice in all groups were identified by subcutaneous implanted [redacted] transponders. This transponder type has not been tested in p53 mice prior to this study.

Parameters assessed:
Mortality & Clinical Signs: daily and weekly for detailed exam including palpation for tissue masses. Postdose exam was done on drug groups immediately postdose and 2hr postdose.
B.wt: predose, d1 of dosing, weekly, and on day of necropsy.
Food Intake: weekly, consumption calculated as g/mouse/wk.
Hematology: blood was collected from abdominal aorta of all mice at terminal necropsy and when possible, from mice that died during the study. Standard parameters were determined. Bone marrow and blood smears were prepared from all animals at end of dosing and stored for possible analysis.
Organ wt.: mice were weighed prior to necropsy and the following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes/epididymides, and thymus.
Necropsy: non-fasted mice from all groups including the additional 3/sex mice in HD had standard full exam including internal inspection of carcass, body orifices, abdominal, thoracic, and cranial cavities. Standard list of tissues/organs were preserved and processed.

Histopathology: the following tissues were preserved in the appropriate fixative and processed for LM H&E stained (table from sponsor):

adrenals (†) (š)
animal identification (site of transponder) (š)
aorta
bone marrow smear (femur) (a)
blood film (a)
brain (†) (š)
caecum (š)
colon (š)
duodenum (š)
eyes (b) (š)
femur with bone marrow and articular surface (š)
gall bladder (š)
gross lesions (š)
Harderian glands (š)
head
heart (†) (š)
ileum (š)
jejunum (š)
kidney (†) (š)
lacrimal glands (d)
larynx
liver (†) (š)
lungs with mainstem bronchi (š)
mammary (f) (š)
mandibular lymph nodes (š)
mesenteric lymph nodes (š)
muscle (quadriceps)
nasal turbinates (d)
nasopharynx (d)
oesophagus (š)
optic nerves
ovaries (†) (š)
pancreas (š)
pituitary (š)
prostate (š)
rectum (š)
salivary glands (š)
sciatic nerves (š)
seminal vesicles (š)
skin (š)
spinal cord cervical (š)
spinal cord lumbar (š)
spinal cord thoracic (š)
spleen (†) (š)
sternum with bone marrow
stomach (š)
testes + epididymides (†) (š)
thymus (†) (š)
thyroids + parathyroids (š)
tissue masses (š)
tongue
trachea (š)
urinary bladder (š)
uterus / cervix (š)
vagina (š)
zymbal glands (d)

fixative = 10% neutral buffered formalin except where indicated by: a – methanol b - Davidson's fluid

d - preserved with the head *in situ*
f - female only
Bone designated for histopathological examination was decalcified using Kristenson's fluid.

Histopathology was done on the following:
- gross lesions & masses from all animals,
- transponder site from all animals at end of dosing including dead ones and additional HD mice,
- tissues from HD and control denoted by (S) in the above list including additional HD mice,
- thymus from MNU group and urinary bladder from p-cresidine, and
- vagina, uterus, and cervix from drug groups 20, 62.5, and 200mg/kg/d.

Note that although kidney findings were observed in HD, kidneys from low and mid doses were not examined in this study because as the sponsor stated these findings in HD mice have been previously reported (no reference provided).

TK: blood was collected on d11 and at necropsy wk26 from all drug groups (3/sex/group), at 1, 2, 4, 8, and 24hr postdose and from 3/sex control group at 2hr postdose. There were several timepoints during wk26 collection from 3/sex/gr that could not be done therefore, mean values for these times were based on n=2. Blood was collected from orbital sinus under halothane anesthesia, VML 251 concentrations were measured using ——————— TK analysis of frovatriptan blood concentrations was done using ——————— The sponsor (vol.2 page 35 of report), reported the presence of a small interfering peak. The mobile phase was changed in order to resolve the interference of this peak with VML 251 peak and the method was re-validated.

^{14}C-VML 251 dosing: labeled drug was orally administered as a single dose (10ml/kg), to 3/sex mice in 20mg/kg/d group and the surviving additional mice (3m/2f), in the 400mg/kg/d group on day 4 of wk26. Batch# for cold VML 251 was 60811-44 and the drug formulated in 1% w/v MC. Urine and feces were collected in metabolic cages from 0-8hr and 8-24hr postdose. Urine, feces, and cagewash samples were shipped to [redacted] for analysis. Samples at each time point were pooled from the 2 or 3 animals per sex. Radioactivity was quantified using [redacted] and metabolic profile in excreta was determined using [redacted]

Methods:
On day4 during wk26, 3/sex from LD, 3m/2f from HD (those are the additional mice that were added to HD group on wk3 of study), were administered ^{14}C-VML 251. The addition of 3/sex mice to HD group was due to "early concerns" about group size being reduced due to non-tumor-related deaths in this group.

Comment:
Protocol stated that pH of MNU be tested prior to dosing, but this was not done. The sponsor indicated that the pH was assured by validation studies done under study# 1165-239. Stability and concentrations of all cpds were checked at specified times and were within accepted values.

During the 1st 2wks of the study, several mice were replaced and 2 more mice were replaced prior to B.wt measurement at the start of wk3. One female mouse in HD was replaced on d4 of wk3.

Results:
Mortality: survival was analyzed by life table methods: Kaplan-Meier, Cox-Tarone binary regression, and Gehan-Breslow non-parametric methods according to NCI life table package (Thomas, Breslow, and Gart 1977). Trend analysis evaluation was done at the 5% significance level. The following tables from the sponsor show mortality incidences and their statistical significance:

Text Table 1a
Results of Statistical Analyses of Survival for the Males

Group	1 Control	2 Low	3 Mid	4 Mid-High	5 High	6 Positive	7 Positive
Unadjusted Mortality	2/15 (0.133)	0/15 (0.000)	0/15 (0.000)	0/15 (0.000)	1/18 (0.056)	11/15 (0.733)	1/15 (0.067)
Kaplan-Meier Estimate	0.133	0.000	0.000	0.000	0.056	0.733	0.083
Standard Error	0.088	0.000	0.000	0.000	0.054	0.114	0.080
Cox-Tarone Test	0.2372 -	0.2322 -	0.2322 -	0.2474 -	0.4131 -	0.0007+**	0.3274 -
Gehan-Breslow Test	0.1749 -	0.0786 -	0.0786 -	0.0862 -	0.2066 -	0.0003+**	0.3200 -

** = Significant at $p \leq 0.01$.
+ = Effect in the positive (increasing) direction.
- = Effect in the negative (decreasing) direction.

Text Table 1b
Results of Statistical Analyses of Survival for the Females

Group	1 Control	2 Low	3 Mid	4 Mid-High	5 High	6 Positive	7 Positive
Unadjusted Mortality	1/15 (0.067)	2/15 (0.133)	0/15 (0.000)	1/15 (0.067)	3/18 (0.167)	13/15 (0.867)	1/15 (0.067)
Kaplan-Meier Estimate	0.067	0.133	0.000	0.067	0.167	0.867	0.071
Standard Error	0.064	0.088	0.000	0.064	0.088	0.088	0.069
Cox-Tarone Test	0.2940 +	0.4756 +	0.1760 -	0.4903 -	0.3417 +	0.0000+**	0.4701 +
Gehan-Breslow Test	0.2495 +	0.2555 +	0.1854 -	0.4905 -	0.1743 +	0.0000+**	0.4703 +

** = Significant at $p \leq 0.01$
+ = Effect in the positive (increasing) direction.
- = Effect in the negative (decreasing) direction.

These data show that there was no drug related mortalities or a trend in increased incidence of mortality compared to the control in either sex. However, MNU had a significant increase in mortality, 13/15f (start on wk14 and up), and 11/15m (start on wk11 and up). These deaths were contributed to malignant lymphoblastic lymphomas; macroscopic exam showed enlarged thymus or thymic mass. P-cresidine group did not show any effect on survival.

Note1 to sponsor: table 3.1 page 82 of report vol. 1 shows 1/15m death in frovatriptan 200mg/kg/d group whereas table1a above from stat reports 0/15m. A clarification is requested.

Note 2 to sponsor: table 3.1 page 88 of report vol. 1 shows 2/15f dosed p-cresidine found dead wks 9&10 of dosing whereas, table 1b above from stat reports 1/15f. A clarification is requested.

Note 3 to sponsor: table 3.1 page 84 of report vol. 1 shows 4/15m dosed p-cresidine dead (3 found dead 1 killed moribund wks 5, 9, 19, and 27 of dosing), so does table 3.2 page 90. However, Table 1a above from stat reports 1/15m dead. A clarification is requested.

There were 10 mice that were replaced during 1st 3wks of dosing. Cause of death was technical/gavage error or accident in 6 mice, 3 cause unknown, and 1 was a p-cresidine male mouse that was moribund and therefore, replaced with another mouse; it did survive and was returned to stock (table from sponsor).

Table of Replaced Animals – Probable Cause of Deaths (Study No. 1165/238)[1]

Day of death	Study Day	Study Week	Group and Sex Number	Mouse Number	Probable Cause of Death Necropsy Findings
8/24/00	4	1	3F	149	Gavage error No obvious perforation but blood and fluid in the thoracic cavity.
8/24/00	4	1	7F	201	Gavage error Oesophageal perforation, blood and corn oil in thoracic cavity.
9/1/00	12	2	1M	2	Cause of death unknown Fluid around brain
8/30/00	10	2	7M	104	Gavage error Abnormal oily thoracic contents
8/30/00	10	2	4F	162	Gavage error Dark lungs, abnormal thoracic contents
8/25/00	5	1[2]	5F	176	Gavage error Fluid in thoracic cavity
8/28/00	8	2	5F	176	Replacement mouse found dead Accidental death, trapped in automatic watering system. Necropsy not done
9/4/00	15	3	2M	22	Cause of morbidity unknown Killed moribund Necropsy not done
9/4/00	15	3	7M	94	Did not die[3]
9/6/00	17	3	5F	179	Cause of death unknown Cannibalised, not necropsied

[1] This Table supplements Appendix 4 of the Final Report for Study 1165/238, page 265 of the report; or Volume 2, page 97 of the NDA submission; and is cross-referenced to footnote 1 in Table 2, Summary of Mortality During the In-Life Phase of the 26-week Study in p53(+/-) Mice, Volume 1, page 19 of the NDA submission.

[2] This animal died on 8/25/00, Day 5, Week 1. It was incorrectly listed as dying in Week 2 (see Table in Appendix 4).

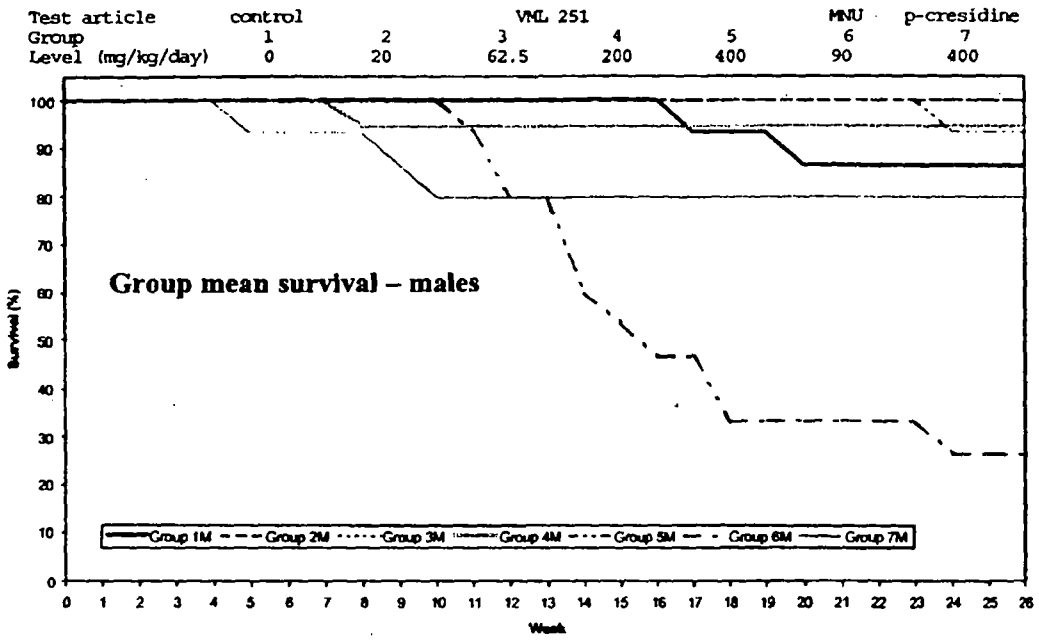
[3] This animal did not die. It was observed to be moribund, therefore it was replaced. However, the animal recovered and returned to stock without a necropsy. The cause of the morbidity was unknown.

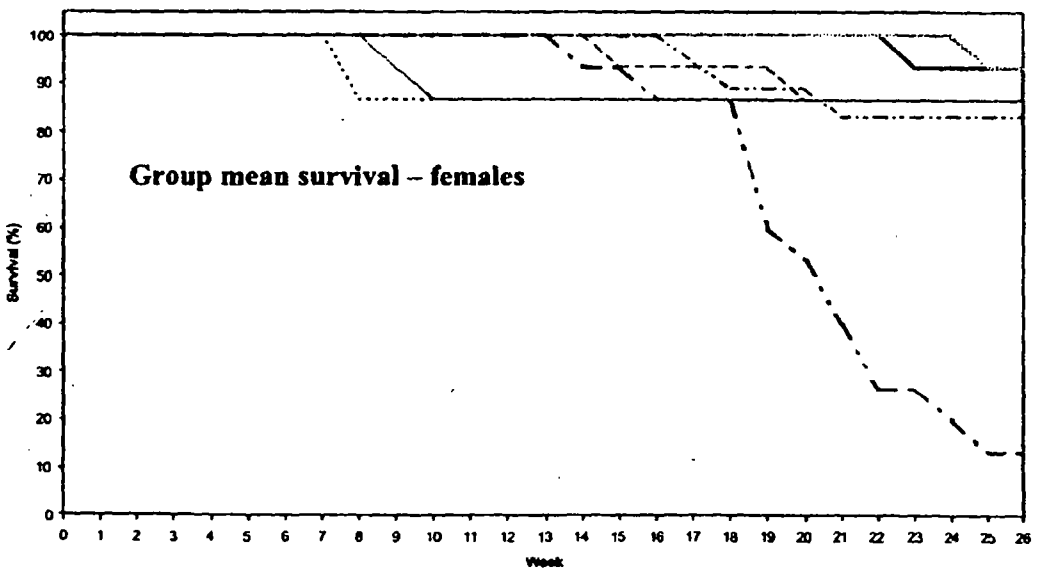
The following table from sponsor shows mortality in control and frovatriptan groups due to neoplasia. Nine mice died due to neoplasia, 6 of those as a result of skin sarcomas at site of transponder and, the remaining 2 due to tumors in bone and abdomin.

Group incidence of morbidity and mortality – vehicle control and VML 251 groups

		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
	Level (mg/kg/day)	0	20	62.5	200	400	0	20	62.5	200	400
Cause of demise	No. examined:	2	0	0	1	1	1	2	2	1	3
not determined		0	0	0	0	0	0	0	0	0	1
study procedure		0	0	0	1	0	0	0	2	0	0
skin + subcutis tumour		1	0	0	0	1	0	1	0	1	2
bone tumour		0	0	0	0	0	1	1	0	0	0
abdominal cavity tumour		1	0	0	0	0	0	0	0	0	0

Below are survival graphs in both sexes from the sponsor:

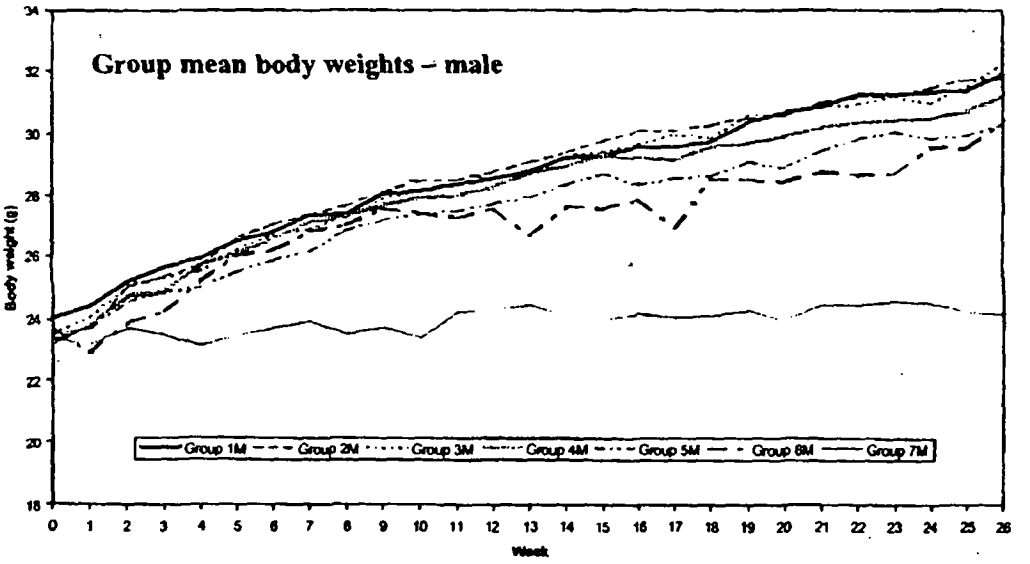


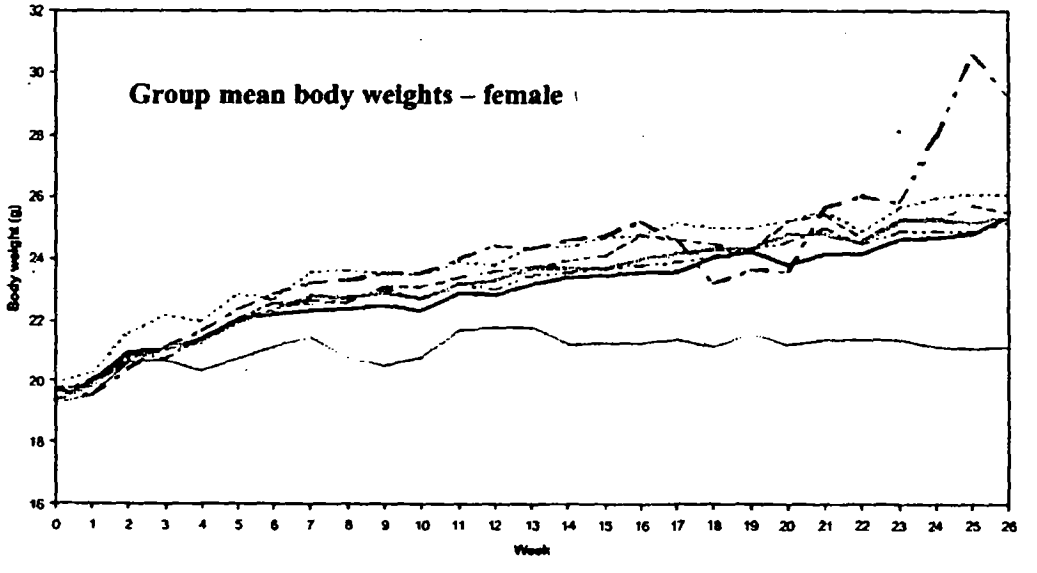


Clinical signs: there were no drug related clinical signs in frovatriptan groups. Mice dosed with MNU appeared thin and hunched from wk11 onwards, laboured/rapid breathing was also observed in some of these mice that resulted in death or early kill in moribund. Similar signs were observed in cresidine group from start of dosing in males and from wk6 in females. Clinical sign incidence was maximum between wks 11 & 19 of dosing; these signs did not contribute to death in p-cresidine group. There was no effect on palpable masses in any group.

B.wt: there were no frovatriptan-related effect on B.wt or wt gain. Mean wt gain was markedly reduced in mice dosed with p-cresidine throughout dosing with 17-24% lower wt than the negative controls at end of study. In general, there was no body wt change noted in MNU group, an increase noted in females during the 1st 16wks of dosing and small decrease seen in males. Body wt loss was observed in individual mice dosed MNU prior to deterioration in condition and resultant death or moribund termination. The following figures show B.wt in males and females, (higher quality copies could not be obtained).

Test article	control	VML 251			MNU	p-cresidine
Group	1	2	3	4	5	6	7
Level (mg/kg/day)	0	20	62.5	200	400	90	400





Food Intake: no drug related effect on food consumption in any frovatriptan group. Food intake was reduced in parallel to decreased B.wt in the p-cresidine group throughout study period in both sexes. The sponsor stated that food intake was increased in p-cresidine group during wks10-12 due to "diet moistening" that was attempted to improve intake. There was no effect in MNU group, however, food intake was increased during wks 23-26 perhaps due to single housing of mice as a result of death in this group (mice competing less for food).

Hematology: results are not conclusive due to loss of blood samples as a result of clotting or insufficient volume, also high degree of inter-animal variability across groups. Overall, there seem to be no drug related findings in frovatriptan groups. MNU and p-cresidine mice showed marked elevation in retics (absolute and relative) compared to control specially in females (6 fold higher in MNU over the control and 2 fold in p-cresidine)(males values increased between 1.75-2.3x control). Also, small decrease noted in positive controls in Hb and RBC count relative to corresponding negative/vehicle control.

Organ wts: there was a dose dependent increase ($p<0.05$) in absolute and relative spleen wts in frovatriptan female groups, no other organ wt effects in either sex. Only absolute wt of thymus and spleen were increased in MNU group relative to the control. The sponsor correlated these wt changes in MNU mice to the neoplastic findings observed in these mice. P-cresidine group in general, had lower organ wts relative to corresponding controls but that was correlated to lower B.wt in these mice.

Macroscopic Findings: no macroscopic findings in frovatriptan groups except for skin/subcutis in females where "a mass" was observed in **0, 3, 1, 6, 6*, 2, and 0** each out of 15f and **1, 1, 1, 1, 3*, 1, and 1** each out of 15m (* out of 18m and 18f), in control, 20, 62.5, 200, 400, MNU, and p-cresidine respectively. Pale and large liver and spleen were seen in few MNU mice and 400mg/kg/d female frovatriptan group (2 & 3 out of 18 each for liver and spleen respectively). Mandibular lymph node was enlarged in 3/15m dosed MNU vs. none in all other groups and large lymph node (not specified), was seen in 4/15f MNU and none in any other group of either sex. Thymus was enlarged in 6/15m and 4/15f dosed MNU with no findings in other groups.

Histopathology:

NON NEOPLASTIC FINDINGS: table from sponsor showing drug related findings in kidney and urinary bladder:

Group incidence: microscopic data - all animals - non-neoplastic

Test article	control		VML 251			MNU	p-cresidine	
Group	1	2	3	4	5	6	7	.
Level (mg/kg/day)	0	20	62.5	200	400	90	400	.

STUDY NUMBER: 1165238

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;FIND=P;SUBSET=T

--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---

SEX: ----------MALE----------

ORGAN AND FINDING DESCRIPTION	GROUP:	-1-	-2-	-3-	-4-	-5-	-6-	-7-
	NUMBER:	15	15	15	15	18	15	15
KIDNEY	NUMBER EXAMINED:	15	0	0	1	18	11	8
--HYDRONEPHROSIS		0	0	0	0	0	0	1
--INFLAMMATORY CELL FOCI		7	0	0	0	11	1	4
--CORTICAL MINERALISATION		0	0	0	1	0	0	0
--PAPILLARY MINERALISATION		6	0	0	0	8	4	5
--CASTS		1	0	0	0	1	0	0
--TUBULAR DILATION		0	0	0	0	0	1	1
--GLOMERULONEPHROPATHY		0	0	0	0	0	1	0
--BASOPHILIC TUBULES/FOCAL NEPHROPATHY		2	0	0	0	10	0	2
--SEGMENTAL CORTICAL ATROPHY		0	0	0	0	0	0	4
--PYELITIS		0	0	0	0	0	1	1
--PAPILLARY NECROSIS		0	0	0	0	0	0	3
URINARY BLADDER	NUMBER EXAMINED:	15	0	0	2	18	11	15
--INFLAMMATORY CELL FOCI		0	0	0	0	1	0	0
--DIFFUSE TRANSITIONAL CELL HYPERPLASIA		0	0	0	0	0	0	13
--SQUAMOUS CELL METAPLASIA		0	0	0	0	0	0	10

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;FIND=P;SUBSET=T

SEX: ----------FEMALE----------

ORGAN AND FINDING DESCRIPTION	GROUP:	-1-	-2-	-3-	-4-	-5-	-6-	-7-
	NUMBER:	15	15	15	15	18	15	15
KIDNEY	NUMBER EXAMINED:	15	2	2	1	18	13	3
--HYDRONEPHROSIS		0	0	0	0	0	0	1
--INFLAMMATORY CELL FOCI		4	0	0	0	3	1	1
--PAPILLARY MINERALISATION		1	0	1	1	2	3	0
--CASTS		3	0	0	0	3	3	0
--BASOPHILIC TUBULES/FOCAL NEPHROPATHY		0	0	0	0	3	0	1
--SEGMENTAL CORTICAL ATROPHY		1	0	0	0	0	0	1
URINARY BLADDER	NUMBER EXAMINED:	15	2	3	2	18	9	15
--INFLAMMATORY CELL FOCI		3	0	0	0	6	1	0
--ARTERITIS		0	0	0	0	1	0	0
--DIFFUSE TRANSITIONAL CELL HYPERPLASIA		0	0	0	0	0	0	15
--SQUAMOUS CELL METAPLASIA		0	0	0	0	0	0	10
UTERUS	NUMBER EXAMINED:	15	15	15	15	18	13	2
--CYSTIC GLANDS		5	1	5	9	5	1	0
--SQUAMOUS CELL METAPLASIA		1	0	2	0	3	0	0
VAGINA	NUMBER EXAMINED:	15	15	15	15	18	12	2
--PRO-OESTRUS		2	2	0	0	1	0	0
--OESTRUS		6	4	7	8	16	0	2
--METOESTRUS		5	6	5	4	1	4	0
--DIOESTRUS		2	3	3	3	0	7	0

Also observed were stomach hyperkeratosis in 4/12m & 5/11f, squamouse cell hyperplasia in 4/12m & 6/11f dosed MNU vs. 0 in all male frovatriptan and p-cresidine groups and all female groups except for hyperkeratosis in 1/2f dosed 20mg/kg/d frovatriptan. Harderian gland inflammatory cell foci/adenitis was increased significantly in HDm relative to control. The sponsor did not suggest this is drug related since it could be a results of orbital sinus bleeding. The kidney and female reproductive/estrous findings reached statistical significance. At the transponder site, table below from sponsor presents the data for both sexes:

Group incidence: microscopic data - all animals - non-neoplastic

SEX: ----------MALE----------	GROUP:	-1-	-2-	-3-	-4-	-5-	-6-	-7-
ORGAN AND FINDING DESCRIPTION	NUMBER:	15	15	15	15	18	15	15
TRANSPONDER SITE	NUMBER EXAMINED:	12	15	14	14	15	14	14
--FIBROSIS		9	15	13	13	14	13	14
--PIGMENT		1	1	2	0	2	2	2
--INFLAMMATORY CELL INFILTRATION		1	2	0	1	0	0	0
--MESENCHYMAL DYSPLASIA		1	2	3	8	3	4	1

SEX: ----------FEMALE----------	GROUP:	-1-	-2-	-3-	-4-	-5-	-6-	-7-
ORGAN AND FINDING DESCRIPTION	NUMBER:	15	15	15	15	18	15	15
TRANSPONDER SITE	NUMBER EXAMINED:	15	14	14	14	17	8	14
--FIBROSIS		14	13	14	13	15	8	14
--PIGMENT		2	5	3	3	2	3	3
--INFLAMMATORY CELL INFILTRATION		1	1	0	1	1	0	0
--MESENCHYMAL DYSPLASIA		5	2	2	2	3	0	0

The transponder site findings were comparable among all groups compared to the control except for 8/14m dosed 200mg/kg/d had mesenchymal dysplasia compared with 1/12m control. Also, the incidence of mesenchymal dysplasia in control females was 5/15 compared with 2-3 incidences in frovatriptan and 0 in positive control groups. The sponsor suggested that the higher incidence of skin dysplasia in control female is related to higher incidence of sarcoma noted in frovatriptan females, because dysplasia is a precursor of sarcoma.

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NEOPLASTIC LESIONS:

The only tumor findings in frovatriptan groups were those at the transponder site with skin sarcomas in both sexes (table from sponsor). Both positive controls produced their expected tumors, MNU caused lymphoblastic lymphoma in all males and females and p-cresidine induced bladder lesions of transitional cell carcinoma in both sexes. There were no other tumors in any group. The sponsor stated that **additional 8 sarcomas were ONLY discovered following microscopic exam of the transponder site.**

Group incidence: microscopic data - all animals - neoplastic

Test article	control		VML 251			MNU	p-cresidine	
Group	1	2	3	4	5	6	7	.
Level (mg/kg/day)	0	20	62.5	200	400	90	400	.

STUDY NUMBER: 1165238

SEX=ALL;GROUP=ALL;WEEKS=ALL — GROUP: -1- -2- -3- -4- -5- -6- -7-

DEATH=ALL;FIND=B,M;SUBSET=T — SEX: ----------MALE----------

ORGAN AND FINDING DESCRIPTION	NUMBER:	15	15	15	15	18	15	15
** TOP OF LIST **								
SKIN + SUBCUTIS	NUMBER EXAMINED:	15	3	1	2	18	12	5
--M-SARCOMA - NOS		3	2	1	1	5	1	0
URINARY BLADDER	NUMBER EXAMINED:	15	0	0	2	18	11	15
--B-TRANSITIONAL CELL PAPILLOMA		0	0	0	0	0	0	1
--M-TRANSITIONAL CELL CARCINOMA		0	0	0	0	0	0	8
--M-SQUAMOUS CELL CARCINOMA		0	0	0	0	0	0	2
--M-SARCOMA		0	0	0	0	0	0	1
HAEM/LYMPH/RETIC	NUMBER EXAMINED:	15	0	0	1	18	12	4
--M-MALIGNANT LYMPHOMA-LYMPHOBLASTIC		0	0	0	0	0	12	0
ABDOMINAL CAVITY	NUMBER EXAMINED:	1	0	0	0	0	0	0
--M-SARCOMA - NOS		1	0	0	0	0	0	0

GROUP: -1- -2- -3- -4- -5- -6- -7-

SEX: ----------FEMALE----------

ORGAN AND FINDING DESCRIPTION	NUMBER:	15	15	15	15	18	15	15
** TOP OF LIST **								
SKIN + SUBCUTIS	NUMBER EXAMINED:	15	5	6	6	18	13	2
--M-SARCOMA - NOS		0	4	3	6	6	1	0
URINARY BLADDER	NUMBER EXAMINED:	15	2	3	2	18	9	15
--B-TRANSITIONAL CELL PAPILLOMA		0	0	0	0	0	0	1
--B-SQUAMOUS CELL PAPILLOMA		0	0	0	0	0	0	2
--M-TRANSITIONAL CELL CARCINOMA		0	0	0	0	0	0	4
--M-SQUAMOUS CELL CARCINOMA		0	0	0	0	0	0	1
HAEM/LYMPH/RETIC	NUMBER EXAMINED:	15	2	2	1	18	14	2
--M-MALIGNANT LYMPHOMA-LYMPHOBLASTIC		0	0	0	0	0	13	0
BONE	NUMBER EXAMINED:	1	1	0	0	0	1	0
--M-OSTEOSARCOMA		1	1	0	0	0	1	0

TK: VML 251 concentrations increased with increase in dose with females having slightly lower concentrations and exposure than males at the same doses. The increase was linear between the 2 lowest doses but non-linear and less than proportional at higher doses. There seemed to be small drug accumulation with repeated dosing as reflected in the accumulation index (AUC wk26/AUC d11), values that ranged between ——— (table from sponsor for combined sexes). Mean T_{max} for both sexes were 1-2hrs. Tables below from sponsor presents the C_{max} and AUC values in each sexes.

combined male and female $AUC_{(0\text{-}24h)}$ values

Group number	Dose (mg/kg/day)	Dose ratio	Day 11		Week 26		Accumulation index AUC Week 26/ AUC Day11
			$AUC_{(0\text{-}24h)}$	AUC ratio@	$AUC_{(0\text{-}24h)}$	AUC ratio$	
2	20	1	14636	-	16766	-	1.15
3	62.5	3.1	41099	2.8	50369	3.0	1.23
4	200	10	87545	6.0	109296	6.5	1.25
5	400	20	166454	11.4	176957	10.6	1.06

@ AUC ratio calculated using AUC value from the low dose on Day 11
$ AUC ratio calculated using AUC value from the low dose on Week 26

Group number	Dose (mg/kg/day)	Dose ratio	Male					
			$AUC_{(0\text{-}24h)}$ (ng.h/mL) Day 11	AUC ratio Day 11	AUC normalised Day 11$	$AUC_{(0\text{-}24h)}$ (ng.h/mL) Week 26	AUC ratio Week 26	AUC normalised Week 26$
2	20	1	17508	-	875.4	18711	-	935.6
3	62.5	3.1	45662	2.6	730.6	52215	2.8	835.4
4	200	10	92185	5.3	460.9	111239	5.9	556.2
5	400	20	186717	10.7	466.8	186472	10.0	466.2

$ normalised AUC calculated by dividing the AUC by the dose

Group number	Dose (mg/kg/day)	Dose ratio	Male					
			C_{max} (ng/mL) Day 11	C_{max} ratio Day 11	C_{max} normalised Day 11$	C_{max} (ng/mL) Week 26	C_{max} ratio Week 26	C_{max} normalised Week 26$
2	20	1	1430	-	71.5	1325	-	66.3
3	62.5	3.1	3691	2.6	59.0	3392	2.6	54.3
4	200	10	8509	6.0	42.5	10671	8.0	53.4
5	400	20	20425	14.3	51.0	15164	11.4	37.9

$ normalised C_{max} calculated by dividing the C_{max} by the dose

Group number	Dose (mg/kg/day)	Dose ratio	Female					
			$AUC_{(0\text{-}24h)}$ (ng.h/mL) Day 11	AUC ratio Day 11	AUC normalised Day 11$	$AUC_{(0\text{-}24h)}$ (ng.h/mL) Week 26	AUC ratio Week 26	AUC normalised Week 26$
2	20	1	11764	-	588.2	14820	-	741.0
3	62.5	3.1	36536	3.1	584.6	48522	3.3	776.4
4	200	10	82905	7.0	414.5	107352	7.2	536.8
5	400	20	146190	12.4	365.5	167441	11.3	418.6

$ normalised AUC calculated by dividing the AUC by the dose

Group number	Dose (mg/kg/day)	Dose ratio	Female					
			C_{max} (ng/mL) Day 11	C_{max} ratio Day 11	C_{max} normalised Day 11$	C_{max} (ng/mL) Week 26	C_{max} ratio Week 26	C_{max} normalised Week 26$
2	20	1	1117	-	55.9	1459	-	73.0
3	62.5	3.1	2731	2.4	43.7	2817	1.9	45.1
4	200	10	6257	5.6	31.3	8416	5.8	42.1
5	400	20	10594	9.5	26.5	8951	6.1	22.4

$ normalised C_{max} calculated by dividing the C_{max} by the dose

The following table from the sponsor compares exposure in this study and exposure in humans. The sponsor selected clinical study# VML/96/12 which tested young women using combined oral contraceptive drugs. The VML 251 $AUC_{0\text{-}inf}$ was 0.94ug.hr/ml following single dose of 2.5mg/d. Because exposure at 5mg/d has not been determined, the sponsor doubled the exposure value at 2.5mg since they stated this is a conservative value as the kinetics of VML 251 is less than linear between 1-100mg dose. Also, oral contraceptive drugs can have an inhibitory effect on metabolism which may explain the higher VML 251 blood levels in these women compared to males or the population mean.

Study (report no)	Sampling period	Daily dose (mg/kg)	AUC** (0-24h) (µg·h/mL)	Ratios rodent:corresponding human* AUC		
				Human Dose	2.5 mg	5 mg
				AUC (0-inf) (µg.h/mL)	0.094	0.188
p53 mouse study 1165/238	Day 11	20	14.6		155	78
		62.5	41.1		437	219
		200	87.5		931	465
		400	166.0		1770	883
	Week 26	20	16.8		179	89
		62.5	50.4		536	268
		200	109.0		1160	580
		400	177.0		1880	941

* Human AUC based on mean AUC for 2.5 mg dose in healthy young females in Study VML 251/96/12 (value for 5 mg is a simple extrapolation from the 2.5 mg value)

** p53 mouse AUC based on combined male and female data

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Radioactivity in Urine and Feces: Most of radioactivity was eliminated in feces (52-94% of dose), with relatively small amount excreted in 24hr urine (3-8% of dose). There was no difference in excretion profile with respect to dose or sex and the pattern was similar to that observed previously in CD-1 mice. The main urinary and fecal radioactivity was the unchanged drug in both sexes at 20 & 400mg/kg/d doses which accounted for 85&100% of total radioactivity. Other identified radioactivity was desmethyl VML 251 and the N-acetyl desmethyl VML 251 each accounted for 2-6% of urinary radioactivity and 0.1-1.5% of fecal radioactivity. There were 2 unidentified peaks, M1 & M2, in some urine and feces samples that accounted for 0.11-1.63% of total radioactivity. The metabolic profile in C57BL/6 mice is not only similar to that in CD-1 mice but also to the profile seen in human with mice having less metabolism (figures below from sponsor).

The following tables from the sponsor presents the 20&400mg/kg/d group recovery of radioactivity in each sex in urine and feces as well as total recovery. It is clear that most radioactivity was recovered in feces in both sexes: 3-8% in urine and 51-94% in feces at 20mg/kg/d and 4.7-6.8% in urine and 70-76.5% in feces at 400mg/kg/d. Total recovery ranged between 71-109%.

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^{14}C-VML 251 (20 mg/kg)

Sample	Time	Male	Female	Mean ± SD
Urine	8 h	2.95	1.13	2.04 ± 1.29
	24 h	5.16	2.13	3.65 ± 2.14
	Subtotal	8.11	3.26	5.69 ± 3.43
Feces	8 h	45.88	84.14	65.51 ± 26.36
	24 h	4.86	10.13	7.50 ± 3.78
	Subtotal	51.74	94.27	73.01 ± 30.07
Water cagewash	24 h	9.19	7.42	8.31 ± 1.25
Methanol cagewash	24 h	1.89	3.16	2.53 ± 0.90
Cage debris	24 h	0.02	1.35	0.69 ± 0.94
	Subtotal	11.10	11.93	11.52 ± 0.59
Total recovery		70.95	109.46	90.21 ± 27.23

Results expressed as % dose administered

^{14}C-VML 251 (400 mg/kg)

Sample	Time	Male	Female	Mean ± SD
Urine	8 h	3.42	5.67	4.55 ± 1.59
	24 h	1.31	1.14	1.23 ± 0.12
	Subtotal	4.73	6.81	5.77 ± 1.47
Feces	8 h	63.67	58.59	61.63 ± 3.82
	24 h	6.46	14.85	11.66 ± 4.62
	Subtotal	70.13	70.44	73.49 ± 4.31
Water cagewash	24 h	6.61	11.24	8.93 ± 3.27
Methanol cagewash	24 h	3.14	1.05	2.10 ± 1.48
Cage debris	24 h	0.24	BLD	0.24
	Subtotal	9.99	12.29	11.14 ± 1.63
Total recovery		91.25	89.54	90.40 ± 1.21

BLD Below the limit of detection

SUMMARY:

Oral gavage administration of frovatriptan (VML 251), to male and female p53 heterozygous C57BL/6 mice for 26wks at 20, 62.5, 200, and 400mg/kg/d had no effect on survival, body wt, food intake, organ wts, or hematology (for the latter, accurate conclusion could not be made due to large variability in the data). There were no drug related **macroscopic findings** except for skin/subcutis in females where a "mass" was observed in **0, 3, 1, 6, 6** each out of 15f and **1, 1, 1, 1, 3***, each out of 15m (* out of 18m and 18f), in control, 20, 62.5, 200, and 400mg/kg/d groups respectively. **Non-neoplastic** findings in frovatriptan groups were mild observed only in 400mg/kg/d group and included basophilic tubular nephropathy in 10/18m and 3/18f and higher number of females in this group were in estrus compared to controls (16/18 vs. 6/15), and there were uterine squamous cell metaplasia in 3/18f vs. 1/15f controls. Transponder site findings were comparable among all groups compared to the control except for **mesenchymal dysplasia** in 8/14m dosed 200mg/kg/d compared with 1/12m control. The incidence of mesenchymal dysplasia in control females was 5/15 compared with 2-3 incidences in frovatriptan and 0 in positive control groups. The sponsor considered this higher incidence of skin dysplasia in control females to be related to the higher incidence of sarcoma in frovatriptan females, since dysplasia is a precursor of sarcoma. There **were no neoplastic lesions** in any frovatriptan group relative to the control except for **skin sarcomas** in females attributed to transponder implants. The incidence of transponder site sarcomas was high in females (**0, 4, 3, 6, 6** out of 15, 14, 14, 14, and 18 in cont., 20, 62.5, 200, and 400mg/kg/d respectively), and reached statistical significance (table from sponsor). Also, the incidence in the 2 higher dose groups (44&33% for sarcoma respectively), exceeded the historical range for p53 mice **(0-12% for macroscopic incidence and 5.5-21% for microscopic sarcomas** as reported for Biomedic transponders; no data for

—— transponders. The sponsor stated that the zero incidence out of 15 in female controls was low compared to the historical range. It should be noted that transponder-site sarcoma incidence in the 1st p53 study in the negative control and frovatriptan groups was 7/150 or **4.7% using BioMedic transponders**.

Group incidence: selected microscopic findings, skin + subcutis and transponder site

		Males							Females						
		VML 251					MNU	p-c	VML 251					MNU	p-c
		1M	2M	3M	4M	5M	6M	7M	1F	2F	3F	4F	5F	6F	7F
Tissue and finding	Level VML-251(mg/kg/day)	0	20	62.5	200	400	0	0	0	20	62.5	200	400	0	0
Transponder site	No. examined:	12	15	14	14	15	14	14	15	14	14	14	17	8	14
fibrosis #	Grade -	3	0	1	1	1	1	0	1	1	0	1	2	0	0
	1	8	9	7	5	7	7	5	8	5	5	3	6	1	4
	2	1	3	4	7	4	6	9	6	4	9	8	8	7	10
	3	0	2	2	1	3	0	0	0	4	0	1	1	0	0
	4	0	1	0	0	0	0	0	0	0	0	1	0	0	0
mesenchymal dysplasia †	Grade -	11	13	11	6	12	10	13	10	12	12	12	14	8	4
	1	0	2	0	4	1	4	1	2	2	1	1	2	0	0
	2	0	0	1	2	1	0	0	1	0	0	0	0	0	0
	3	1	0	2	1	1	0	0	0	0	1	1	1	0	0
	4	0	0	0	1	0	0	0	1	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	Total	*1*	*2*	*3*	*8*	*3*	*4*	*1*	*5*	*2*	*2*	*2*	*3*	*0*	*0*
Skin + subcutis/transponder site*	No. examined:	15	15	14	14	18	14	15	15	14	14	14	18	15	15
M-sarcoma - NOS		3	2	1	1	5	1	0	0	4	3	6	6	1	0

"-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe, 5 = severe.
Total = total incidence, grades 1 – 5 combined.
p-c = p-cresidine

Fibrosis was not recorded for some sites due to size of the sarcoma.
† Mesenchymal dysplasia not recorded for animals with sarcoma
*This is the combined totals for skin + subcutis and transponder site.
Sarcomas from the transponder site samples were all recorded under skin + subcutis.

The transponder-site sarcomas in the current study were described as compressive masses of pleomorphic, spindle-shaped cells with many mitotic figures and occasional giant cells and some masses had areas of necrosis, hemorrhage, and cystic spaces. Also noted that no transponder site sarcomas were seen in p-cresidine and found in only 2/29 mice dosed with MNU; both positive controls are known genotoxic compounds. Therefore, the sponsor concluded that these lesions occurred via non-genotoxic mechanisms. The positive controls did however, induce the expected tumors, thymic lymphoblastic lymphoma for MNU and transitional cell carcinoma and squamous cell carcinoma of urinary bladder for p-cresidine. Findings with the positive controls were as follows, marked mortality in MNU group of both sexes during the study with only 4/15m and 2/15f remaining by end of 26wks of study; there was no p-cresidine related mortality in either sex. Clinical signs in p-cresidine group included hunched appearance, rapid or labored breathing,

and/or thin appearance. MNU mice were thin, with hunched and/or sluggish appearance, and rapid/labored breathing, these signs were severe and led to death or early kill of these mice. Mean B.wt gain was reduced in p-cresidine mice whereas, MNU females slightly gained wt but males gained less wt than the control. Also noted in MNU mice that were found dead or killed in moribund, had shown marked loss in wt prior to termination or death. Food intake was slightly reduced in p-cresidine but unaffected in MNU group.

Sponsor's Conclusions:

- transponder-site sarcomas were increased in the 2 highest dosed female mice (43&33% respectively), but the increase was not significant (Fischer Exact test), compared to historical data of p53 female mice as reported in 2 studies where no microscopic exam of transponder sites was done (20%)(page 32 vol. 1),
- no increase in any other tumor type in any drug group,
- no increase in any human-relevant neoplastic or pre-neoplastic lesions,
- transponder-site sarcomas in rodents are considered irrelevant to humans and,
- humans do not develop sarcomas when implanted with foreign bodies such as s.c. depot injections, pacemaker wires, or prosthetic devices.

REVIEWER EVALUATION AND SUMMARY:

Two p53 studies were conducted for frovatriptan (VML 251). Both studies were *almost* identical in design including duration, batch of frovatriptan, doses, animal number per group, and the same histopathologist examined the data. As indicated above, there were 4 times higher incidence of transponder-related s.c. sarcomas in this study in frovatriptan and negative control (31/156; 19.9%), compared to the 1st one (7/150; 4.7%). The following describe the differences in the conduct of the two p53 mouse carcinogenicity studies:

1. Different study locations: 1st study was conducted by [redacted] (#7070-102); the 2nd study by [redacted] (# 1165/238).

2. 1st study used BioMedic transponder type and the 2nd study used [redacted] Note that [redacted] has never been tested previously in p53 mice however, BioMedic has, with low transponder-related sarcoma incidence reported (4.7%)*. The 2 types of transponders are similar in dimensions, 11x2.2mm but [redacted] lacks the polyethylene sleeve that covers one end of the transponder and the anti-migration anchorage barb which is characteristic of BioMedic type. Differences in transponder surface properties and/or material can influence the incidence of transponder-related sarcomas. Studies have shown that surface properties of transponder can affect the degree of fibrosis and tumor incidence as well as latency in rodents. [redacted] has a smooth glass surface that was proposed by the sponsor to be more indusive of transponder-site sarcomas. According to Bates & Klein 1966, plastic film implants with sand-papered surfaces produce more cellularity, less fibrosis, and less tumors than untreated smooth films. Also when the same material is perforated or roughened at the surface, tumors were markedly reduced (Brand et al., 1975; Brand 1994; Engel et al., 1995). Based on the increased incidences of skin sarcomas in the current study, the sponsor recommended not to use transponders in p53 mice irrespective of the manufacturer.

*** It is unclear to this reviewer why ——— used ——transponders in the 2nd p53 study when they have had the knowledge and experience of such devices producing 3 fold higher incidence of transponder related sarcomas in NON-p53 mice compared to mice implanted with BioMedic (page 7 vol.1 of preclinical update section & p37 vol. 1)(see #6 below for detail).**

3. 1st study used one positive control, MNU (90mg/kg), the 2nd study used MNU (90mg/kg) and additional positive control, p-cresidine (400mg/kg/d).

4. 1st study housed mice individually and sexes were placed on separate racks. Mice in the 2nd study were housed 3 per cage and male and female cages were put on the same rack.

5. The manufacture and composition of the diet differed between the 2 studies as did the water supply and air quality.

6. The sponsor also stated that the level of stress in mice may have been altered because of 1 or more of the following: 2nd study mice were transported from the USA to UK across the Atlantic; mice in 2nd study were dosed using rigid, stainless steel cannula but flexible plastic one was used in the 1st study; and in the 1st study, mice in high dose were administered frovatriptan at 625mg/kg/d for the 1st 45d then dose reduced to 400mg/kg/d, however, mice in 2nd study were dosed 400mg/kg/d throughout the 26wk.

The sponsor continued to explain the 4 fold higher incidence of transponder-related sarcoma incidences in the 2nd study compared to the 1st in spite of using the same doses and comparable level of exposure. The effects of transponder type on transponder-induced sarcomas in p53 mice has not yet been examined systemically. All previously conducted studies with p53 used BioMedic transponder type. The sponsor indicated that their historical data from carcinogenicity studies with "CONVENTIONAL" mice support the contention that —— transponders are more sarcomagenic than BioMedic type. Table below from sponsor presents these data with almost 3x higher incidence using —— than BioMedic transponders:

Percentage and Incidence of Subcutaneous Sarcoma in p53(+/-) Mice without Implanted Transponders

Males	Females	Both	Reference
1.9[1] 2/108[2]	3.7 4/109	2.8 6/217	Mahler *et al.*, 1998
0.4 1/268	1.5 4/269	0.9 5/537	Storer, 2000 (Data from control groups of 19 different studies)
7 1/15	0 0/15	3.3 1/30	Long *et al.*, 2000.

[1] Percent of mice with sarcoma
[2] Incidence (number with sarcoma/total in the group)

Incidence of Suspect Transponder-related Sarcoma in Control CD1 or B6 Mice from Six Different 104-Week Studies Using —— or BioMedic Transponders (Unaudited Data)[1]

—— transponder		BioMedic transponder	
Males	**Females**	**Males**	**Females**
2.2[2]	2.6	0.7	0.8
6/270[3]	7/273	4/594	4/503

[1] ——— personal communication)
[2] Percent of mice with sarcoma
[3] Incidence (number with sarcoma/total in the group)

Historical Data on Spontaneous Incidences of Sarcoma:

Tables from sponsor presents historical findings in p53 male and female mice with and without transponders. The data show that females are more sensitive than males with **2x higher** incidence than the incidence in males. Only 1 study showed higher incidence of s.c.sarcomas in male mice than in females (Long et.al., 2000) and no difference between sexes noted in Morton et al., 2000 when the incidence was determined for the control group only (BioMedic)(table below). The sponsor used these data to support the high incidence in female p53 mice dosed with frovatriptan in the 2nd study compared to the low incidence in male mice.

Percentage and Incidence of Subcutaneous Sarcoma in p53(+/-) Mice Implanted Subcutaneously with an Identification Transponder

Subcutaneous Implant	Males	Females	Both	Reference
BioMedic transponder	2.0[1] 3/150[2]	6.6 10/150	4.3 13/300	Storer, 2000 (data from control groups of 10 different studies)
BioMedic transponder	3.3 3/90	5.5 5/90	4.4 8/180	Masson *et al.*, 2000[3]
BioMedic transponder	20 3/15	20 3/15	20 6/30	Morton *et al.*, 2000 Control Groups Only
BioMedic transponder	6.7 5/75	12 9/75	9.3 14/150	Morton *et al.*, 2000 All Groups Combined[3]
BioMedic transponder	0 0/20	20 4/20	10 4/40	Ciaravino *et al.*, 2001 Control Groups Only
BioMedic transponder	0 0/115	7.8 9/115	3.9 9/230	Ciaravino *et al.*, 2001 All Groups Combined[3]
BioMedic transponder	2.2 2/90	6.6 6/90	4.2 8/190	First Frovatriptan study[3] Covance 7070-102
BioMedic transponder	7.7 1/13	16.7 2/12	12 3/25	Blanchard *et al.*, 1999[5] Control Groups Only
BioMedic transponder	11 8/73	21 10/48	14.8 18/121	Blanchard *et al.*, 1999[5,6] All Groups Combined[1]
BioMedic transponder Overall Incidences	3.8 19/503	9.0 43/478	6.3 62/981	All Studies Combined
— transponder	20 3/15	0 0/15	10 3/30	Present study[4,5] Control Groups Only
—| transponder	12.4 13/105	19.0 20/105	15.7 33/210	Present study[4,5] All Groups Combined[3]

[1] Percent of mice with sarcoma
[2] Incidence (number with sarcoma/total in the group)
[3] Combined group incidence as there was no effect of treatment
[4] Overall incidence all groups combined
[5] Only animals with a microscopic examination of the transponder site included.
[6] Table II of the Blanchard paper shows that 56 animals in the in their study were not examined microscopically. As there may have been additional tumors in the animals not examined microscopically, their data is re-presented above to show tumor incidence based only on the mice they examined microscopically.

Another issue discussed by the sponsor and reported in the literature of transponder site lesions is **"transponder migration"**. This sponsor stated that 32 of the 33 sarcomas in this study were *clearly* identified as transponder-related and only 1 out of 20 sarcomas in female mice could not be definitely identified as being intimately associated with the transponder. As a background information, transponders are usually implanted s.c. in the mid scapula area but can migrate to flanks and have been even found near the hind limbs. Such migration occurs as a result of physical handling of the animals for dosing purposes, physical exams etc. The sponsor continued to indicate that when migration occurs, it is impractical to identify and microscopically examine all s.c. sites that may have been in contact with the transponder. Therefore, examination of the final transponder site may fail to detect some microscopic sarcomas.

Genotoxic vs. Non-Genotoxic Mechanisms for Transponder-site Sarcomas:

The sponsor argued that the skin sarcomas in frovatriptan groups were induced via non-genotoxic mechanisms as supported by absence of higher incidence in the 2 genotoxic cpds, p-cresidine and MNU and induction of such lesions have been observed following administration of non-genotoxic cpds such as anti-oxidants or irritants or simply via ear tags (Youssef et al., 2000; Torti et al., 2001). The argument continued to state that molecular analysis of transponder-site sarcomas and loss of heterozygosity (LOH) to the p53 gene has provided inconsistent and unreliable data. The sponsor therefore concluded that using molecular analysis of transponder-site sarcomas from frovatriptan mice can not assist to determine if these lesions were directly caused by the drug, or are human relevant. LOH can not be done in the current study because the incidence of sarcoma was zero in control females and only 1 control male had a large enough sarcoma to be sampled at postmortem.

The sponsor continued to explain away the markedly high incidence of sarcomas in the present p53 study compared to concurrent as well as historical data by referencing the *overall* incidence observed following microscopic exam of transponder sites in males (11%) and females (21%) with 16.7% incidence in control females. These values compare to 12% and 19% incidences in males and females in the present study but, with 0 incidence in control females. The sponsor also contributed the differences in the sarcoma incidence between the 2 p53 studies to inter-lab variations, [redacted]

TK & Metabolism:

VML 251 concentrations in this study were measured on day 11 and end of dosing wk26. Plasma concentrations increased with increase in dose with females having slightly lower concentrations and exposure than males at the same doses. The increase was linear between the 2 lowest doses but non-linear and less than proportional to dose at higher doses. There seemed to be some drug accumulation with repeated dosing. Mean C_{max} and $AUC_{0\text{-}24hr}$ in males ranged between 1325-20,425ng/ml and 17,508-186,717ng.hr/ml and in females from 1117-10,594ng/ml and 11,764-146,190ng.hr/ml respectively. Based on "estimated" human exposure of 0.188ug.hr/ml ($AUC_{0\text{-}inf}$; 5mg/d), mouse to human exposure ratio at 400mg/kg/d VML 251 is >900 times and 90 times at 20mg/kg/d dose.

Metabolism of VML 251 was studied in excreta after single oral dose of radioactive frovatriptan to male and female mice on wk26 of study. Similar to CD-1 mice, most of radioactivity was eliminated in feces (52-94% of dose), and only relatively small amount excreted in 24hr urine (3-8% of dose). There was no difference in excretion profile with respect to dose or sex. The main urinary and fecal radioactivity was the unchanged drug in both sexes at 20 & 400mg/kg/d doses

and accounted for 85&100% of total radioactivity. Two metabolites, desmethyl VML 251 and the N-acetyl desmethyl VML 251 each accounted for 2-6% of urinary radioactivity and 0.1-1.5% of fecal radioactivity. There were 2 unidentified peaks, M1 & M2, in some urine and feces samples that accounted for 0.11-1.63% of total radioactivity. The metabolic profile in C57BL/6 mice in addition to be similar to that in conventional CD-1 mice, was also similar to the profile seen in humans.

CONCLUSION:

Although the sponsor's arguments and the data in the literature, support a transponder-related sarcomas, the incidence of sarcomas in frovatriptan groups particularly in the 2 highest doses (200&400mg/kg/d), was markedly higher than the concurrent control females and, several fold higher than historical data obtained from a number of independent studies. Such strong signal is not a trivial finding and it is this reviewer's opinion that through whatever mechanism, frovatriptan seem to have "***potentiated***" the induction of these lesions. This conclusion is also supported by absence of sarcomas in MNU group, a direct acting carcinogen and p-cresidine, an indirect acting carcinogen (minimal incidence was observed), as well as the low incidence in the 1st p53 mouse study with only 4.7% sarcoma incidence. Moreover, the concurrent control incidence of zero in females is within the historical range. This study was analyzed by our Statistical Division and they provided a final written report. A statistically significant **LINEAR TREND** was calculated in female mice for the skin sarcomas and the trend for the sarcomas among female mice when not all mid dose group of mice were examined, appeared to follow a quadratic function. Pair-wise comparison between the negative control and treated female mice reached statistical significance for the skin sarcomas. The argument by the sponsor that higher mesenchymal dysplasia in control females reflects the higher incidence of sarcoma in frovatriptan groups as being a precursor for neoplastic development, is not a valid one because: (1) the increase was small (5/15) compared to 2-3 out of 15 in drug groups and, (2) imposing an unsubstantiated time factor in female controls but not in female drug groups that these preneoplastic lesions are on there way to develop into sarcomas. In conclusion, this study is a valid study where both positive controls, MNU and p-cresidine, induced the appropriate types of tumors. The only tumor type observed in frovatriptan groups was the skin subcutis sarcomas particularly in female mice. Frovatriptan had no effect on mortality, food intake, B.wt, or organ wts. The skin sarcomas in general, were physically associated with transponder sites and considered by the sponsor not to be drug induced based on literature data. This reviewer concludes the following:

Frovatriptan in *this* p53 study may have *potentiated* the transponder-site sarcomas through an unidentified mechanism. The relevance of such tumors to humans is unknown. Note that the mouse to human AUC ratio at 200mg/kg/d dose of frovatriptan in this study is 1160 (2.5mg human dose).

cc:
/Div. File/Orig. NDA# 21-006
/B.Rosloff/A. Atrakchi/L. Chen

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Aisar Atrakchi
11/5/01 11:01:07 AM
PHARMACOLOGIST

Barry Rosloff
11/5/01 04:12:53 PM
PHARMACOLOGIST

APPEARS THIS WAY
ON ORIGINAL

Ph.D., **CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT**

AND

FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

NDA: 21-006 **DRUG CODE#:** VML 251; SB 29555 **DATE:** 10/31/00
DIVISION(s): HFD-120
DRUG NAME(s): [redacted] (frovatriptan succinate) tablets
THERAPEUTIC CATEGORY: Acute treatment of migraine
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Serotonin receptor 5-$HT_{1B/1D}$ partial agonist
SPONSOR: Vernalis Ltd. (formerly Vanguard prior to merger)
LABORATORY: [redacted]
SPONSOR CONTACT: [redacted]

MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay): Yes, clastogenic in cultured human lymphocyte tests
P/T REVIEWER(s): S. Stolzenberg
DATE SUBMITTED: 10/3/00
DATE OF CAC REVIEW: 10/31/00

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): Yes, 1/18/00, about a month after the study had been initiated. While the study was in progress, an unacceptable mortality rate was observed at 625 mg/kg/day. This dose was reduced to 400 mg/kg/day on Day 45 of treatment, as recommended by the executive-CAC after being informed of the deaths. The executive-CAC had no objection to any of the other frovatriptan doses or to the positive control.

BACKGROUND: The study was submitted in order to satisfy the final pre-clinical requirement for this NDA. The protocol for this study was reviewed and approved by the Agency. The minutes of the executive-CAC meeting of 3/14/00 indicated that if the results of the p53 study were clearly negative, the rat carcinogenicity study need not be repeated.

p53 MOUSE CARCINOGENICITY STUDY
STUDY DURATION (weeks): 26 weeks
STUDY STARTING DATE: 12/14/99 (Initiation of treatment)
STUDY ENDING DATE: 6/17/00 (Completion of necropsies)
MOUSE STRAIN: C57BL/6TacfBR-[KO]p53 N5 heterozygous (+/-).
ROUTE: Oral gavage
DOSING COMMENTS: Dosage is expressed as free base. Dosing volume was 10 mL/kg in carboxymethylcellulose suspension vehicle.

No. Rats in Control (C-1): 15 (Groups 1)
Low Dose (LD): 15 (Group 2)
High Dose (HD): 15 (Group 5)
Middle Dose 1 (MD-1): 15 (Group 3)
Middle Dose 2 (MD-2): 15 (Group 4)
Positive Control (MNU):15 (Group 6)

MOUSE DOSE LEVELS (mg/kg/day)

Mouse Low Dose: 20
Mouse High Dose: 625/400
Mouse Middle Dose 1: 62.5
Mouse Middle Dose 2: 200
Positive Control: N-methylnitrosourea (MNU) dosed at 90 mg/kg only on Day 1

There was an additional 18/sex/group in each of the frovatriptan treated groups designated for toxicokintics.

Basis for Doses Selected (M.T.D.; AUC ratio; saturation of absorption; maximum feasible dose): M.T.D. The M.T.D. was based on lethality and severe clinical signs observed in a 4 week dose-finding study after a single dose of 800 and 1600 mg/kg (⟵ Study No. 7070-101). Little or no toxicity was observed at 400 or 200 mg/kg/day.

p53 MOUSE CARCINOGENICITY (negative;positive;MF;M;F):
Negative for tumors in males
Negative for tumors in females
Negative for tumors in males and females of the positive controls

Because the results for tumor findings were negative in the positive control group, the validity of this study is in question.

The sponsor believes that an error in dosing of the positive control group had occurred and concluded that the study was acceptable. Nevertheless, a second p53 mouse study was initiated as soon as the present study was completed. The protocol is virtually the same as in the completed study, except the highest dose is 400 mg/kg/day from Day 1 of treatment and there are 2 positive control groups using 2 different known mutagenic carcinogens (MNU and *p*-cresidine).

NDA 21-006

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Sidney J. Stolzenberg
October 25, 2000

NDA AMENDMENT: NBP and **DATED**: 9/18/00 and 10/3/00
CENTER RECEIPT DATE: 9//19/00 and 10/4/00
REVIEWER RECEIPT DATE: 9/19/00 and 10/4/00

SPONSOR: Vernalis Ltd
Surrey
United Kingdom

The first dated submission is an unaudited report and the second submission is the audited report of the same data.

DRUG: Miguard™ (Frovatriptan succinate; SB 209509-AX; VML 251)

FORMULATION: 2.5 mg (as base) tablets for oral administration.

PHARMACOLOGICAL CLASS: Serotonin receptor subtype 5-$HT_{1B/1D}$ partial agonist

PROPOSED INDICATION: Acute treatment of migraine attacks

DOSAGE REGIMEN: One tablet

RELATED APPLICATION: IND [redacted]

26-Week Oral (Gavage) Oncogenicity Study with Frovatriptan in p53(+/-) C57BL/6 Mice

Study No: 7070-102 [redacted]

Quality Assurance: A signed statement of compliance with GLP is included.

Test Animals: C57BL/6TacfBR-[KO]p53 N5 heterozygous (+/-) mice, [redacted], 9-10 week old, (21.1-28.6 g males and 17.4-23.6 g females) at initiation of dosing, were used.

Test Substance: Batch number 60811-44; 99% purity.

Procedure: Animals in the main study were observed daily for clinical signs, morbidity and mortality, weekly for body weights and food consumption. Hematology parameters from Week 27 main study non-fasting animals in negative control and all 4 frovatriptan treated groups included erythrocyte count, hemoglobin, hematocrit, platelet count, leukocyte count, differential blood cell count and blood cell morphology. Postmortem evaluations of all animals surviving to term and decedents (including toxicokinetic mice) where possible, were subjected to routine gross pathology. At terminal sacrifice, weights of brain, heart, kidneys, liver with gallbladder, ovaries, testes with epididymides and thymus were obtained for main study animals. Left and right organs were weighed together, and bone marrow smears were made. For histopathology, samples of the following tissues were fixed in 10% neutral formalin: adrenals, aorta, brain, cecum, cervix, colon, duodenum, esophagus, eyes, femur with marrow at articular surface, liver with gall bladder, lesions, lungs, Harderian gland, heart, ileum, jejunum, kidneys, larynx, lungs, mammary glands, mandibular salivary gland, mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerves, seminal vesicles, skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach, testes with epididymides, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus and vagina. Microscopic evaluation included all indicated tissues from negative control, high dose, positive control and decedent animals, gross lesions from all animals. In addition, tissue from the low dose, 2 intermediate dose and high dose PK group were embedded in paraffin for possible histopathology evaluation. Tissue from kidney, liver, adrenal and thymus were collected from all main study dose group animals and embedded in paraffin for possible immunohistochemistry analysis. A sample of the tail from 5 mice/sex from control, high dose and NMU-treated was collected, frozen and sent to [redacted] for genotyping.

A satellite toxicokinetic study with 4 groups of 18 animals of each sex received the same doses. Procedure for toxicokinetics is given on page 15. Urine samples (0-6 hour and 6-24 hour) were collected and frozen from high dose toxicokinetic animals (dosed until Week 27) during week 26 for possible metabolic analysis, and blood samples for toxicokinetics were also obtained from these animals.

Results

Mortality: During the first 2 weeks of the study, 2 TK females at 20 mg/kg/day, 1 main study female and 1 TK male at 625 mg/kg/day were replaced with previously untreated mice. The following is sponsor's summary of mortality after replacement time.

Table 3
Summary of Mortality During In-Life Phase of the p53(+/-) Study[1]

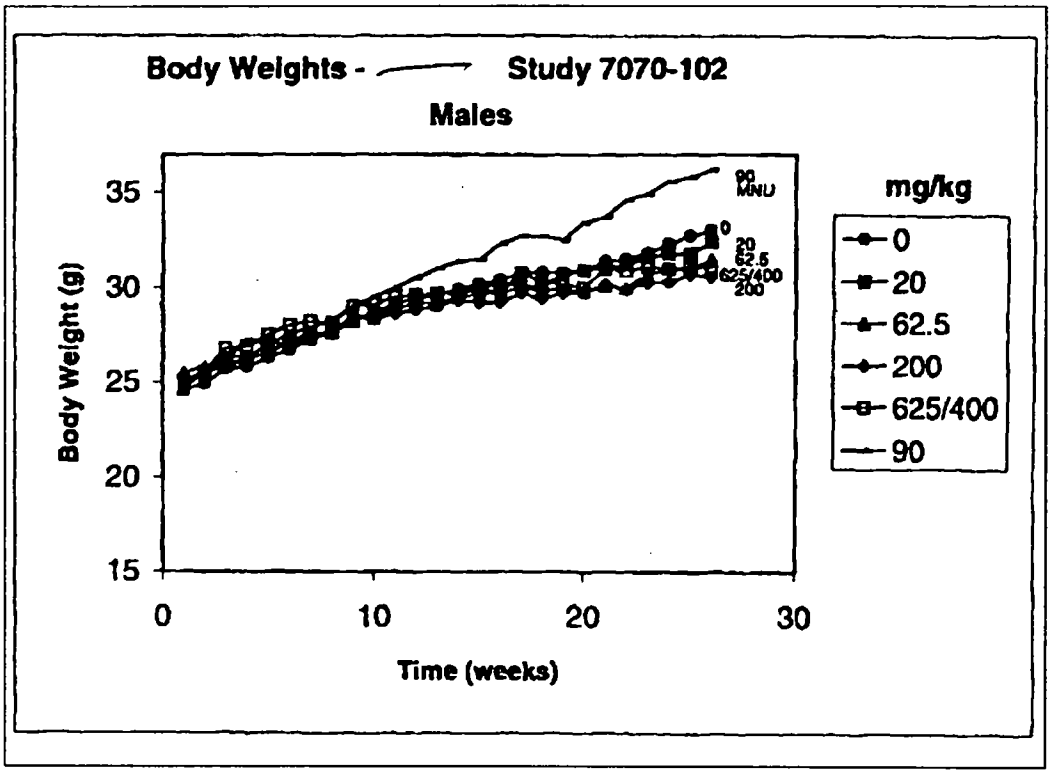
Animal No.	Dose level/sex	Week of death	Status	Cause of death
A86863	200mg/kg/day F (TK)	3	Found dead	Not established
A86895	625mg/kg/day F(TK)	3	Found dead	Not established
A86890	625mg/kg/day F (TK)	7	Found dead	Not established
A86896	400mg/kg/day F (TK)	7	Sacrificed moribund	Thymic mass
A86684	200mg/kg/day M	15	Sacrificed moribund	Not determined
A86715	400mg/kg/day M	16	Found dead	Urologic syndrome
A86908	MNU F	18	Found dead	Lymphoma
A86888	400mg/kg/day F (TK)	19	Sacrificed moribund	Not established
A86766	Neg. control F	23	Found dead	Not determined
A86769	Neg. control F	26+	Found dead	Lymphoma
A86884	400mg/kg/day F	26+	Found dead	Sarcoma

[1] Four other mice died during the first 2 weeks and were replaced

Six main study and 5 TK study mice in all 6 groups were sacrificed moribund or found dead. The sponsor claimed that subsequent to the reduction in high dose from 625 to 400 mg/kg/day, none of the unscheduled deaths was due to compound treatment. The pathologist concluded that the deaths occurred randomly with apparently no predilection for treatment group or sex, and lymphomas were determined to be the cause of death in 1 untreated control female and 1 positive control female of the main study animals.

In the main study, by Week 26, surviving males included 15 in groups 1, 2, 3 and 6, fourteen in groups 4 and 5; surviving females included 15 in groups 2, 3 and 4, fourteen groups 1 and 6. A footnote to Table 2 survival data indicates 1 additional female died in groups 1 and 5. The extra female in each of groups 1 and 5 apparently came from the toxicokinetic groups, as recommended by the executive-CAC.

Body Weight: Graphs and tables of mean body weights and tables of body weight gains are shown on the pages that follow. There was no statistically significant effect on a mean body weight, but there was a tendency for drug-related decrease in mean body weight gain among males.


Body Weights - Study 7070-102
Males
Body Weight (g)
35
30
25
20
15
0
10
20
30
Time (weeks)
90 MNU
0
20
62.5
625/400
200
mg/kg
0
20
62.5
200
625/400
90

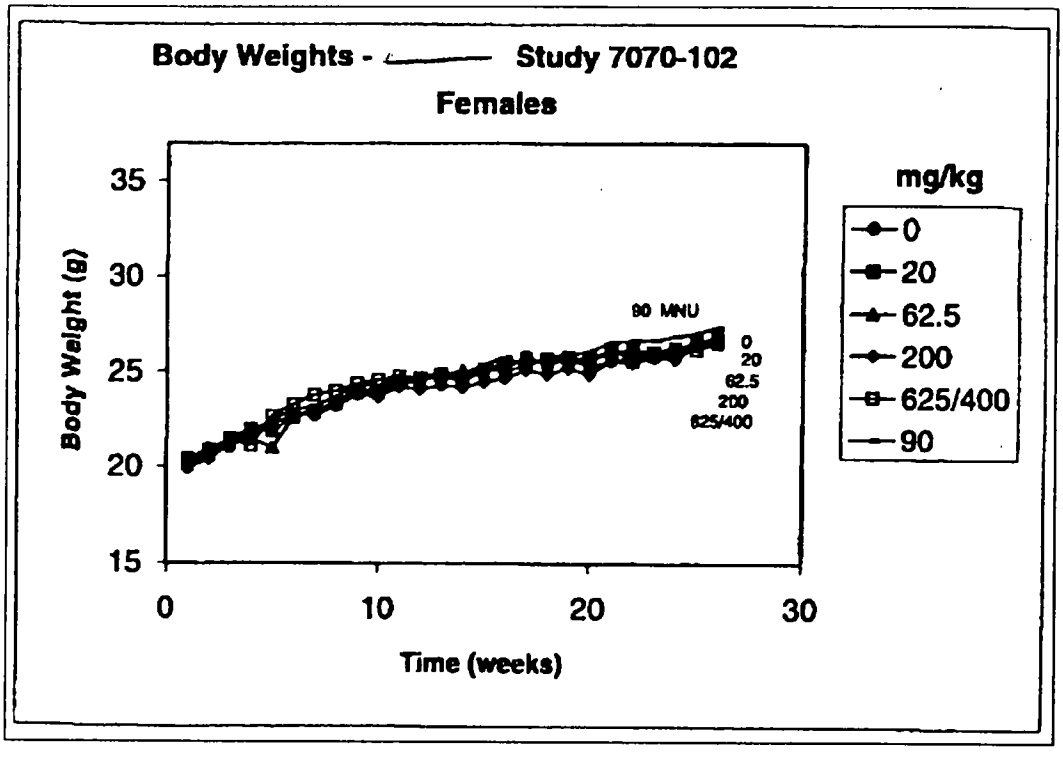

Body Weights - Study 7070-102
Females
Body Weight (g)
35
30
25
20
15
0
10
20
30
Time (weeks)
90 MNU
0
20
62.5
200
625/400
mg/kg
0
20
62.5
200
625/400
90

TABLE 6 (REVISED)
SUMMARY OF BODY WEIGHT DATA (G)
TWENTY-SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN
p53(+/-)C57BL/6 MICE

SEX: MALE

WEEK		GROUP: 1 DOSE: 0 MG/KG/DAY	2 20 MG/KG/DAY	3 62.5 MG/KG/DAY	4 200 MG/KG/DAY	5 400 MG/KG/DAY	6 90 MG/KG/DAY
1	N	15	15	15	15	15	15
	MEAN	24.6	25.1	25.5	25.0	24.6	25.0
	S.D.	1.46	1.56	1.48	1.50	0.82	1.31
	p-value		ns	ns	ns	ns	ns
7	N	15	15	15	15	15	15
	MEAN	27.3	27.5	27.3	27.5	28.2	27.9
	S.D.	1.76	1.91	1.35	1.31	0.97	1.11
	p-value		ns	ns	ns	ns	ns
13	N	15	15	15	15	15	15
	MEAN	29.6	29.6	29.1	29.0	29.7	31.0
	S.D.	2.04	2.10	1.46	1.82	1.19	1.75
	p-value		ns	ns	ns	ns	ns
16	N	15	15	15	14	15	15
	MEAN	30.4	30.3	29.8	29.2	29.7	32.3
	S.D.	2.44	2.46	1.36	1.66	1.90	2.40
	p-value		ns	ns	ns	ns	0.0403 + *
22	N	15	15	15	14	14	15
	MEAN	31.5	31.2	30.0	29.9	30.9	34.6
	S.D.	2.69	2.82	1.77	1.34	1.70	3.15
	p-value		ns	ns	ns	ns	0.0072 + **
26	N	15	15	15	14	14	15
	MEAN	33.0	32.4	31.5	30.6	31.1	36.2
	S.D.	3.10	3.31	1.94	1.79	2.08	3.76
	p-value		ns	ns	ns	ns	0.0168 + *

NOTE: CHANGE OF DOSE LEVEL FROM 625 MG/KG/DAY TO 400 MG/KG/DAY ON DAY 45 OF STUDY (WEEK 7)
NOTE: ns = STATISTICALLY NOT SIGNIFICANT VERSUS CONTROL AT P≥0.05.
NOTE: * = STATISTICALLY SIGNIFICANT VERSUS CONTROL AT P≤0.05.
NOTE: ** = STATISTICALLY SIGNIFICANT VERSUS CONTROL AT P≤0.01.
NOTE: + = INCREASED OVER CONTROL.

TABLE 6 (REVISED)
SUMMARY OF BODY WEIGHT DATA (G)
TWENTY-SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN
p53(+/-)C57BL/6 MICE

SEX: FEMALE

WEEK		GROUP: 1 DOSE: 0 MG/KG/DAY	2 20 MG/KG/DAY	3 62.5 MG/KG/DAY	4 200 MG/KG/DAY	5 400 MG/KG/DAY	6 90 MG/KG/DAY
1	N	15	15	15	15	15	15
	MEAN	20.3	20.2	20.1	19.9	20.4	20.3
	S.D.	1.20	1.12	1.35	1.22	1.76	1.32
	p-value		ns	ns	ns	ns	ns
7	N	15	15	15	15	15	15
	MEAN	23.0	22.9	22.9	22.7	23.8	23.2
	S.D.	1.39	1.17	1.80	1.15	2.16	0.80
	p-value		ns	ns	ns	ns	ns
13	N	15	15	15	15	15	15
	MEAN	24.8	24.8	24.8	24.3	24.9	25.0
	S.D.	1.59	1.27	2.70	1.51	2.24	0.97
	p-value		ns	ns	ns	ns	ns
16	N	15	15	15	15	15	15
	MEAN	25.2	25.5	25.5	24.7	25.0	25.8
	S.D.	1.74	1.62	3.40	1.10	2.01	1.27
	p-value		ns	ns	ns	ns	ns
22	N	15	15	15	15	15	14
	MEAN	25.8	25.6	26.1	25.5	26.2	26.7
	S.D.	1.92	1.57	3.94	1.64	2.46	1.38
	p-value		ns	ns	ns	ns	ns
26	N	14	15	15	15	15	14
	MEAN	27.0	26.8	26.8	26.6	26.6	27.4
	S.D.	2.72	1.96	4.98	1.97	2.74	1.78
	p-value		ns	ns	ns	ns	ns

TABLE 5 (REVISED)
SUMMARY OF BODY WEIGHT CHANGE DATA (G)
TWENTY-SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN
p53(+/-)C57BL/6 MICE

SEX:		MALE					
GROUP:		1	2	3	4	5	6
DOSE:		0	20	62.5	200	400	90
WEEK	UNITS:	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY
1-7	N	15	15	15	15	15	15
	MEAN	3.1	2.5	2.1	2.6	3.5	3.2
	S.D.	0.98	0.76	1.21	1.21	1.04	1.11
	p-value		ns	0.0258 * +	ns	ns	ns
7-13	N	15	15	15	15	15	15
	MEAN	2.6	2.3	2.2	1.8	1.3	3.5
	S.D.	1.06	1.02	0.77	0.73	1.07	1.37
	p-value		ns	ns	ns	0.0013 **+	ns
13-16	N	15	15	15	14	14	15
	MEAN	1.2	0.5	0.9	0.8	1.1	1.8
	S.D.	0.99	2.03	0.64	0.84	0.74	1.29
	p-value		ns	ns	ns	ns	ns
16-22	N	15	15	15	14	14	15
	MEAN	1.4	1.3	0.9	1.1	1.1	2.6
	S.D.	0.69	0.84	0.92	0.80	0.89	1.37
	p-value		ns	ns	ns	ns	0.0052 **+
22-26	N	15	15	15	14	14	15
	MEAN	1.6	1.2	1.6	1.3	0.8	2.1
	S.D.	1.17	1.12	0.80	0.85	0.48	1.27
	p-value		ns	ns	ns	ns	ns
1-26	N	15	15	15	14	14	15
	MEAN	8.5	7.3	6.1	6.3	7.0	11.8
	S.D.	2.56	2.53	2.20	1.71	1.75	3.50
	p-value		ns	0.0118 *+	0.0262 *+	ns	0.0064 **+

NOTE: CHANGE OF DOSE LEVEL FROM 625 MG/KG/DAY TO 400 MG/KG/DAY ON DAY 45 OF STUDY (WEEK 7)
NOTE: CHANGE OF DOSE LEVEL FROM 625 MG/KG/DAY TO 400 MG/KG/DAY ON DAY 45 OF STUDY (WEEK 7)
NOTE: ns = STATISTICALLY NOT SIGNIFICANT VERSUS CONTROL AT P≤0.05.
NOTE: * = STATISTICALLY SIGNIFICANT VERSUS CONTROL AT P≤0.05.
NOTE: ** = STATISTICALLY SIGNIFICANT VERSUS CONTROL AT P≤0.01.
NOTE: + = INCREASED OVER CONTROL.

TABLE 5 (REVISED)
SUMMARY OF BODY WEIGHT CHANGE DATA (G)
TWENTY-SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN
p53(+/-)C57BL/6 MICE

SEX:		FEMALE					
GROUP:		1	2	3	4	5	6
DOSE:		0	20	62.5	200	400	90
WEEK	UNITS:	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY	MG/KG/DAY
1-7	N	15	15	15	15	15	15
	MEAN	3.0	3.1	3.1	3.4	3.6	3.5
	S.D.	0.63	0.99	1.44	1.00	1.34	0.93
	p-value		ns	ns	ns	ns	ns
7-13	N	15	15	15	15	15	15
	MEAN	1.5	1.5	2.2	1.4	0.9	1.6
	S.D.	0.65	0.75	1.51	0.70	1.06	0.78
	p-value		ns	ns	ns	ns	ns
13-16	N	15	15	15	15	15	15
	MEAN	1.0	0.8	0.7	0.8	0.4	0.3
	S.D.	0.97	1.02	1.47	1.03	0.74	2.53
	p-value		ns	ns	ns	ns	ns
16-22	N	15	15	15	15	15	14
	MEAN	0.8	0.6	0.4	1.1	1.1	0.8
	S.D.	1.51	0.81	0.99	0.87	0.52	1.67
	p-value		ns	ns	ns	ns	ns
22-26	N	14	15	15	15	14	14
	MEAN	0.9	1.0	0.7	0.8	0.5	1.5
	S.D.	1.95	1.26	1.04	0.84	0.35	1.38
	p-value		ns	ns	ns	ns	ns
1-26	N	14	15	15	15	14	14
	MEAN	6.5	6.4	6.6	6.4	6.3	7.7
	S.D.	2.57	1.27	3.08	1.61	1.57	2.24
	p-value		ns	ns	ns	ns	ns

NOTE: CHANGE OF DOSE LEVEL FROM 625 MG/KG/DAY TO 400 MG/KG/DAY ON DAY 45 OF STUDY (WEEK 7)
NOTE: ns = STATISTICALLY NOT SIGNIFICANT VERSUS CONTROL AT P≤0.05.

Hematology: No compound related effect was noted.

Gross Pathology:

Apparently frovatriptan-related masses were seen in the subcutaneous tissue of 1 male in group 3, 1 male in group 4, in a total of 5 females in groups 3 and 5; one in the MNU treated group. These were not seen in males or females of group 1 negative controls. The pathologist indicated that the masses were seen "usually in the dorsolateral abdominal region" in close association with the implanted identification microchip. There were no other suggestions of a compound related gross pathology effect.

Text Table 3. Incidences of Macroscopic Findings for All Animals

Tissue and Macroscopic Finding		Males						Females					
	Group	1	2	3	4	5	6	1	2	3	4	5	6
	Number Examined	15	15	15	15	15	15	15	15	15	15	15	15
Brain	Soft	-[a]	-	-	-	-	-	1	-	-	-	-	-
Lung	Mottled	-	1	-	-	-	-	-	-	-	-	-	-
Liver	Mottled	-	-	-	-	-	-	1	-	-	-	-	-
Kidney	Mottled	-	-	-	-	-	-	1	-	-	-	-	-
Spleen	Enlarged	-	1	-	-	-	-	1	-	1	-	1	1
Pancreas	Cyst	-	-	-	-	-	-	-	-	-	1	-	-
Mesenteric Lymph Node	Enlarged	-	1	-	-	-	-	-	-	-	-	-	-
Thymus	Enlarged	-	-	-	-	-	-	3	-	-	-	-	-
	Mass	-	-	-	-	-	-	-	-	-	-	-	1
	Dark	-	-	-	-	-	-	1	-	-	-	-	-
Uterus	Distended	-	-	-	-	-	-	-	-	-	-	1	-
	Lumen, fluid	-	-	-	-	-	-	-	-	-	-	1	-
Skin, Other	Alopecia	1	-	1	1	-	-	2	3	1	-	1	3
	Mass	-	-	-	-	-	-	-	-	-	-	1[b]	-
Subcutaneous Tissue	Mass	-	-	1	1	-	-	-	-	2	-	2	1
Thoracic Cavity	Dark material	-	-	-	-	-	-	1	-	-	-	-	-

[a] "-" indicates zero incidence.
[b] This mass, described in the skin, was in the subcutaneous tissue.

Organ Weights: Summaries of absolute and relative (to body weights) organ weights for all 6 groups is shown in the pages that follow. The mean absolute and relative kidney weights were reduced for males in group 5, mean absolute heart and thymus weights were reduced in groups 3, 4 and 5 males, also mean relative heart weight in males at high dose was reduced, but there were no histopathology effects associated with these findings.

TABLE 8 (REVISED)

SUMMARY OF ORGAN WEIGHT DATA – MAIN STUDY ANIMALS – MALES - ABSOLUTE WEIGHT

TWENTY- SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN p53 (+ / -) C57BL/ 6 MICE

GROUP (mg/kg/day)		TERMINAL BODY WEIGHT (g)	BRAIN (g)	TESTES/ EPIDIDYMIS (g)	KIDNEY (g)	HEART (g)	LIVER/ GALLBLADDER (g)	THYMUS (g)
1	N	15	15	15	15	15	15	15
(0)	MEAN	32.7	0.45	0.37	0.50	0.18	1.70	0.05
	S. D.	3.1	0.03	0.03	0.05	0.02	0.22	0.01
2	N	15	15	15	15	15	15	15
(20)	MEAN	32.3	0.45	0.36	0.49	0.17	1.69	0.04
	S. D.	3.4	0.03	0.03	0.06	0.02	0.22	0.01
3	N	15	15	15	15	15	15	15
(62.5)	MEAN	31.4	0.45	0.37	0.46	0.17*	1.67	0.04*
	S. D.	2.4	0.01	0.02	0.03	0.02	0.16	0.01
4	N	14	14	14	14	14	14	14
(200)	MEAN	31.1	0.45	0.36	0.47	0.17*	1.56	0.04*
	S. D.	1.9	0.02	0.03	0.04	0.02	0.21	0.01
5	N	14	14	14	14	14	14	14
(625/400)	MEAN	31.8	0.45	0.36	0.45*	0.16*	1.68	0.04*
	S. D.	2.2	0.02	0.03	0.04	0.01	.24	0.01
6	N	15	15	15	15	15	15	15
(90)	MEAN	37.0	0.46	0.39	0.55	0.20	2.05	0.05
	S. D.	3.9	0.01	0.04	0.04	0.03	0.25	0.01

* - Significantly different from control group at $p \leq 0.05$

TABLE 8 (REVISED)

SUMMARY OF ORGAN WEIGHT DATA – MAIN STUDY ANIMALS – FEMALES – ABSOLUTE WEIGHT

TWENTY- SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN p53 (+ / -) C57BL/ 6 MICE

GROUP (mg/kg/day)		TERMINAL BODY WEIGHT (g)	BRAIN (g)	KIDNEY (g)	HEART (g)	LIVER/ GALLBLADDER (g)	THYMUS (g)	OVARIES (g)
1	N	13	13	13	13	13	13	13
(0)	MEAN	27.1	0.46	0.37	0.15	1.40	0.05	0.026
	S. D.	2.7	0.02	0.03	0.02	0.20	0.02	0.008
2	N	15	15	15	15	15	15	15
(20)	MEAN	26.8	0.47	0.36	0.14	1.40	0.05	0.027
	S. D.	1.6	0.02	0.02	0.02	0.11	0.01	0.012
3	N	15	15	15	15	15	15	15
(62.5)	MEAN	27.2	0.45	0.35	0.14	1.41	0.05	0.024
	S. D.	5.0	0.02	0.03	0.02	.27	0.01	0.007
4	N	15	15	15	15	15	15	15
(200)	MEAN	27.0	0.45	0.36	0.15	1.41	0.05	0.026
	S. D.	2.2	0.02	0.02	0.02	0.15	0.01	0.009
5	N	14	14	14	14	14	14	14
(625/400)	MEAN	27.1	0.46	0.36	0.15	1.42	0.05	0.024
	S. D.	2.5	0.02	0.05	0.02	0.24	0.01	0.006
6	N	14	14	14	14	14	14	14
(90)	MEAN	28.6	0.47	0.40	0.16	1.54	0.06	0.018
	S. D.	2.2	0.02	0.03	0.03	0.12	0.01	0.003

TABLE 8 (REVISED)

SUMMARY OF ORGAN WEIGHT DATA – MAIN STUDY ANIMALS – MALES – ORGAN TO BODY WEIGHT

TWENTY- SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN p53 (+ / -) C57BL/ 6 MICE

GROUP (mg/kg/day)		TERMINAL BODY WEIGHT (g)	BRAIN (%)	TESTES/ EPIDIDYMIS (%)	KIDNEY (%)	HEART (%)	LIVER/ GALLBLADDER (%)	THYMUS (%)
1	N	15	15	15	15	15	15	15
(0)	MEAN	32.7	1.383	1.144	1.528	0.559	5.208	0.145
	S. D.	3.1	0.173	0.106	0.095	0.038	0.482	0.027
2	N	15	15	15	15	15	15	15
(20)	MEAN	32.3	1.413	1.121	1.527	0.537	5.259	0.135
	S. D.	3.4	0.118	0.135	0.143	0.042	0.582	0.034
3	N	15	15	15	15	15	15	15
(62.5)	MEAN	31.4	1.429	1.165	1.469	0.526	5.330	1.125
	S. D.	2.4	0.106	0.081	0.106	0.037	0.331	0.031
4	N	14	14	14	14	14	14	14
(200)	MEAN	31.1	1.461	1.156	1.495	0.539	5.010	0.122
	S. D.	1.9	0.081	0.109	0.063	0.042	0.501	0.026
5	N	14	14	14	14	14	14	14
(625/400)	MEAN	31.8	1.415	1.121	1.425*	0.504*	5.259	0.116*
	S. D.	2.2	0.071	0.109	0.083	0.038	0.525	0.015
6	N	15	15	15	15	15	15	15
(90)	MEAN	37.0	1.256	1.069	1.495	0.542	5.543	1.129
	S. D.	3.9	0.118	1.173	0.134	0.075	0.224	0.027

* - Significantly different from control group at $p \leq 0.05$

TABLE 8 (REVISED)

SUMMARY OF ORGAN WEIGHT DATA – MAIN STUDY ANIMALS – FEMALES – ORGAN TO BODY WEIGHT

TWENTY- SIX WEEK GAVAGE ONCOGENICITY STUDY WITH VML 251 IN p53 (+ / -) C57BL/ 6 MICE

GROUP (mg/kg/day)		TERMINAL BODY WEIGHT (g)	BRAIN (%)	KIDNEY (%)	HEART (%)	LIVER/ GALLBLADDER (%)	THYMUS (%)	OVARIES (%)
1	N	13	13	13	13	13	13	13
(0)	MEAN	27.1	1.717	1.353	0.559	5.145	0.204	0.0980
	S. D.	2.7	0.140	0.103	0.066	0.376	0.063	0.0350
2	N	15	15	15	15	15	15	15
(20)	MEAN	26.8	1.744	1.350	1.531	5.240	0.192	0.1001
	S. D.	1.6	0.083	0.074	0.058	0.292	0.031	0.0439
3	N	15	15	15	15	15	15	15
(62.5)	MEAN	27.2	1.692	1.320	0.525	5.186	0.198	0.0881
	S. D.	5.0	0.236	0.128	0.074	0.694	0.028	0.0283
4	N	15	15	15	15	15	15	15
(200)	MEAN	27.0	1.661	1.321	0.561	5.215	0.187	0.0950
	S. D.	2.2	0.132	0.080	0.074	0.365	0.022	0.0350
5	N	14	14	14	14	14	14	14
(625/400)	MEAN	27.1	1.713	1.327	0.554	5.241	0.167	0.0889
	S. D.	2.5	0.102	0.078	0.062	0.609	0.036	0.0200
6	N	14	14	14	14	14	14	14
(90)	MEAN	28.6	1.654	1.416	0.550	5.376	0.200	0.0632
	S. D.	2.2	0.131	0.084	0.078	0.234	0.044	0.0118

Histopathology

Malignant sarcomas were seen in the subcutaneous tissue of 1 male in group 3, 1 male in group 4, in 3 females in group 5 and 1 female in group 6 (same animals with subcutaneous mass seen at gross pathology examination). The subcutaneous masses found in group 3 females were not microscopically examined, apparently because the masses found at gross pathology were considered to be associated with the implanted identification microchip, and not compound related, even though they occurred only in treated animals, not in controls. Two references are cited which support the investigator's claim that the subcutaneous tumors in p53 mice are caused by the implanted microchips (Blanchard et al, Toxicologic Pathol.27:519-527, 1999 and Johnson et al., 1996). Of fundamental importance is the low incidence of neoplasms in group 6, positive controls.

Text Table 4. Incidence of Neoplastic Findings

Tissue and Neoplastic Finding		Males					Females		
Group		1	3	4	5	6	1	5	6
Number Examined		15	1	1	15	15	15	15	15
Adrenal, Cortex	B-Adenoma	1	-[a]	-	-	-	-	-	-
Lung	B-Adenoma bronchiolar-alveolar	-	-	-	-	-	1	-	-
Harderian Gland	B-Adenoma	-	-	-	-	1	-	-	-
Epididymis	M-Fibrosarcoma	-	-	-	-	1	na[b]	na	na
Hematopoietic Neoplasia	M-Lymphoma	-	-	-	-	-	2	-	1
Subcutaneous Tissue	M-Sarcoma, NOS	-	1	1	-	-	-	3	1

M = malignant; B = benign; NOS = not otherwise specified

[a] "-" indicates zero incidence.

[b] na, non-applicable, gender specific tissue.

Non-neoplastic Proliferative Findings: None of these effects listed below were considered to be drug related.

Text Table 5. Incidence of Selected Nonneoplastic Proliferative Findings

Tissue and Microscopic Finding		Males			Females		
Group		1	5	6	1	5	6
Number Examined		15	15	15	15	15	15
Thymus	Atypical lymphoid hyperplasia	-[a]	-	-	-	-	1
Adrenal, Cortex	Subcapsular cell hyperplasia	4	2	6	15	15	15
Adrenal, Cortex	Focal hyperplasia	-	-	1	-	-	-
Pituitary	Hyperplasia	-	-	-	1	-	-
Lung	Hyperplasia, epithelial, alveolar/bronchiolar	-	-	-	-	-	2
Stomach, Glandular	Hypertrophy, mucosa	2	3	-	1	2	1

[a] "-" indicates zero incidence.

Compound-Related Non-neoplastic Effects

A compound related effect was observed only in kidneys, but tissues only from controls, high dose and MNU-treated mice were examined. There was an increase in incidence of minimal or slight renal tubular basophilia predominantly in males, as indicated in the table that follows. One male and 1 female at high dose had slight tubular degeneration/necosis. The group 5 male listed below with tubular degeneration/necrosis died of "uremic syndrome" during Week 16 (See Table 3 under Mortality on page 5). No other histopathology effects were considered to be compound related. The kidney appears to be a target organ for histopathology effects, previously seen in rats of the 3 and 6 month studies, but at higher doses; 750 and 1000 mg/kg/day.

Text Table 6. Incidence of Severity of Selected Microscopic Findings in the Kidney

Microscopic Finding and Severity Score		Males			Females		
	Group	1	5	6	1	5	6
	Number Examined	15	15	15	15	15	15
Tubule, basophilia	Minimal	1	8	3	1	3	1
	Slight	-*	2	1	-	-	-
Tubule, dilatation	Minimal	-	-	-	-	1	-
	Slight	-	1	-	1	-	-
Tubule, degeneration/necrosis	Slight	-	1	-	-	1	-
Infiltrate, lymphohistiocytic	Minimal	15	11	15	13	11	10
	Slight	-	2	-	1	-	1

* "-" indicates zero incidence.

The pathologist also indicated that testicular atrophy was noted in 2/15 males in group 5 and in 3/15 males in group 6 (positive control). Seminiferous tubule degeneration was noted in 1/15 males in group 5 and in 4/15 in group 6. The seminiferous tubular atrophy in the group 5 males was "minimal" and not considered as toxicologically significant.

The following is a summary of the background control incidence of tumors found in 4 different 26 week studies with the p53 mouse performed at the contract laboratory..

Table 2
Spontaneous Tumor Incidence in 26-Week Carcinogenicity Studies in p53(+/-) Mice ——— Control (vehicle animals)

Sex	Tissue	Diagnosis	Study Number 1	2	3	4
			Number of Animals per Sex 15	15	15	20
Male	Adrenal	Adenoma	-	1	-	-
Male	Hemato-Neoplasia	Lymphoma	-	1	-	-
Male	Subcutaneous Tissue	Sarcoma	-	-	1	-
Female	Thoracic Cavity	Osteosarcoma	1	-	-	-
Female	Hemato-Neoplasia	Lymphoma	-	-	1	-
Female	Lung	Adenoma	-	-	-	1
Female	Skin	Osteosarcoma	-	-	-	1
Female	Skin	Subcutis sarcoma	-	-	-	4
Female	Subcutaneous Tissue	Fibrosarcoma	1	-	-	-
Female	Subcutaneous Tissue	Sarcoma	-	1	1	-
Total tumor number across sexes (%)			2 (7%)	3 (10%)	3 (10%)	6 (15%)

Because the incidence of neoplasms was similar in the positive control group to the concurrent controls and within historical control incidence, the validity of the 6-month p53 mouse study is in question. A retrospective investigation pertaining to the MNU dosing was performed by the sponsor and investigator. It is claimed that a dosing error with MNU occurred. All statements found in the final report pertaining to MNU dosing is copied and shown in the Appendix. They are discussed in the Summary and Evaluation.

Toxicokinetics:

On Days 1 and 28, orbital sinus blood samples were collected from 3 animals per time point at 1, 2, 3, 4, 8 and 24 hours post-dosing (only 2 females from 200 and 625/400 mg/kg/day dose groups at the 3 hour time point on Day 28 because of mortalities). High dose animals were bled for toxicokinetics 7 full days following the change in dosing. At Week 27, additional blood samples were obtained from all remaining main study animals via cardiac puncture at the time of necropsy, for additional toxicokinetics. The table that follows indicates that dose-related high levels of systemic exposure to frovatriptan was evident at all 4 dose levels. For human blood levels at maximum recommended human dosage, we have previously conservatively estimated $AUC_{0-\infty}$ exposure levels of 0.141 ug.h/mL, which had been observed in a clinical study with elderly women.

Group number	Dose (mg/kg/day)	Dose Ratio	Day 1		Day 28		Week 8		Week 27	
			$AUC_{(0\text{-}24h)}$	AUC ratio	$AUC_{(0\text{-}24h)}$	AUC ratio	$AUC_{(0\text{-}24h)}$	AUC ratio	$AUC_{(0\text{-}24h)}$	AUC ratio
2	20	1	9.2	1	15.0	1				
3	62.5	3.1	23.4	2.5	31.7	2.1				
4	200	10	57.0	6.2	79.4	5.3				
5	625	31	166	18.0	354	23.6				
5*	400	20					228	24.8@		
5	400	20							245	16.3$
Main study #	20	1							16.0	1
	62.5	3.1							42.6	2.7
	200	10							129	8.1
	400	20							237	14.8

Note: The values above have been taken from Appendix 8 but have been converted from ng/mL to μg/mL and rounded to three significant figures.
* Measured 8 days after change of dose level from 625 to 400 mg/kg/day
# Blood samples taken from study mice at necropsy
@ AUC ratio calculated using AUC value from the low dose on Day 1
$ AUC ratio calculated using AUC value from the low dose on Day 28

SUMMARY AND EVALUATION

Frovatriptan was administered by daily oral gavage at doses of 0, 20, 62.5, 200 and 625/400 mg/kg/day. Initially, the highest dose was 625 mg/kg/day but the dosage had to be reduced on Day 45 because of unacceptable mortality. The 400 mg/kg/day dose was accepted by the Agency as the MTD. Positive controls received MNU (N-methyl-N-nitrosourea).

Malignant subcutaneous tissue sarcomas were seen in 1 male at 62.5 mg/kg/day, 1 male at 200 mg/kg/day, 3 females at 625/400 mg/kg/day and 1 in the MNU positive control group; none in solvent controls. The investigators cited 2 literature references where others have found that subcutaneous malignant sarcomas were associated with the

implanted identification microchips in p53 mice. The incidence of other neoplasms that occurred in this study was small in number and was randomly distributed across all control and treatment groups, including positive controls. There was no increase in incidence of other neoplasms in fropvatriptan or NMU positive control treated mice.

The predominant frovatriptan-related finding was kidney tubular basophilia in 13/30 mice at the highest dose, classified as minimal to slight, which occurred at greater incidence in males than in females. Of these, 1 male and a female had renal tubular degeneration/necrosis; the male died of "uremic syndrome" during Week 16 of the study. There was no compound related effect on survival after reduction of the high dose to 400 mg/kg/day and there were no other clear indications of drug related toxicity, including weight gain.

A toxicokinetic study conducted in satellite groups on Days 1 and 28 (also during Week 8 for the high dose treated groups) indicated dose-related high levels of systemic exposure to frovatriptan at all doses tested.

A report from [redacted], which included a signed statement of quality assurance authentication, confirmed the heterozygosity of the p53 mice used in this study. This test was based on DNA markers identified by [redacted] in the tail samples obtained at necropsy and submitted by the contract laboratory. It is claimed that in subsequent studies conducted by the contract laboratory, similar confirmations of heterozygosity were obtained. This is evidence that genetic drift did not occur in this or two other studies conducted at [redacted]. It is also claimed that genetic drift is not likely to occur in knockout mice, such as with p53(+/-) mice.

In a telcon on 8/9/00 between Drs. Joe DeGeorge (FDA), Chris Powell (Vernalis) and George Shopp (Elan), Chris explained that "the MNU in the dose preparation was very labile and very pH sensitive. An audit of the contract laboratory showed no overt errors, but the records are incomplete enough that there is a degree of doubt whether or not the mice were actually exposed to NMU. For example, the pH of the final MNU preparation was not determined and therefore could not be confirmed" (correspondence of 8/23/00).

The sponsor has concluded that the positive control group was not adequately dosed with MNU. It is claimed that the positive control was inadequate because evidence for a formulation error in preparation of the MNU was discovered. Thus, the mice did not receive a sufficiently high dose of MNU to induce tumors. NMU is administered at 90 mg/kg, only once on Day 1 of the study. The following is a statement by the contract laboratory, found in the report.

We believe that only one of these 3 criteria has been adequately satisfied. The genotyping of the tail tissue by [redacted] apparently confirmed the heterozygosity of the p53 mice used in this study. We also agree with sponsor's suggestion that it is unlikely that genetic drift to the wild type p53(+/+) occurred. In our opinion, the first and third criteria have not been satisfied. The study failed to generate data on reproducibility of the positive response and it failed to convincingly identify problems associated with the conduct of the study. The sponsor's conclusion that the lack of a positive response to MNU was due to a dosing error is based on a retrospective investigation and has not been adequately substantiated.

Further evidence for acceptability of the present study in spite of an inadequate positive control, as stated by the sponsor, is as follows:

1. ——— has shown through its experience, along with the experience of the ——— programs and other laboratories, that the p53(+/-) model is a reproducible, consistent model.

2. The evidence firmly supports the fact that the mice used in this study were indeed genotypically p53(+/-), and were from the same breeding stock as previous and subsequent studies performed by ——— and other testing laboratories in the U.S.

3. An audit of the study and investigations by ———, have concluded that formulation errors resulted in failure of the mice to receive MNU as indicated in the protocol. Importantly, the audit of the study did not reveal any other significant deviations from the procedures that ——— normally uses in the conduct of its p53(+/-) studies.

4. Toxicokinetics were performed on the test animals, which confirmed constant high levels of exposure to frovatriptan throughout the study. In addition, observations of renal histopathology confirmed exposure to high dose levels of frovatriptan.

We agree that the p53(+/-) model is a reproducible, consistent model and evidence supports the fact that the mice used in the present study were genotypically p53(+/-), based on the ——— test for genotype in tissues from mice used. Also, toxicokinetics and renal histopathology confirmed that adequate systemic exposure to frovatriptan had occurred. We also agree that formulation errors which may resulted in failure of the mice to receive MNU is a reasonable hypothesis, but it was not possible to substantiate this beyond reasonable doubt.

It was also claimed that in two previously performed tests with the p53 mouse, the contract laboratory conducted validation studies with MNU for both studies; Study Nos.2100-199 and 2100-225. Significant mortality due to malignant thyomas were observed and survival was reduced to 16 and 21 weeks, respectively. The mice used in the present study were similar in every respect to the mice in the previous two studies and the auditors verified that the mice were from the same breeding stock in all three studies. Age of the animals at the start of the present study, housing, food consumption and body weight gain were similar as in the previous studies with validation. Furthermore, positive responses to treatment with carcinogens have been subsequently obtained in two additional studies in p53 mice from the same animal suppliers.

The 2 previous studies conducted by ——— appear to have been adequately validated because positive responses occurred in the presence of MNU. However, the present study has not been adequately validated because a positive response was not obtained by treatment of the p53 mice with MNU.

Sponsor also claimed, "Positive control groups have been included in ——— studies, but have not necessarily been included in studies by ——— (Tenant et al. Environmental Health Perspectives 103:942-950, 1995; Dunnick et al. Toxicologic Pathol. 25:553-540, 1997; Eastin et al. Toxicologic Pathol. 26:461-473, 1998)."

This reviewer examined the publications cited above.. The primary objective in all 3 publications was to validate two transgenic mouse models {the p53 (+/-) and TG.AC} for possible use in 6-month bioassays with only 15/sex/group and to determine if they were reliable models for possible extrapolation to human risk. Compounds used in the cited 6-month bioassays included some that had previously shown cross-species carcinogenic potential in 2-year rodent assays, and included suspected human carcinogens based on epidemiology studies. Genotoxic and non-genotoxic compounds, such as melphalan, phenolphthalein, DES, cephalosporin A, TCDD, *p*-cresidine, etc., were tested with the 2 transgenic mouse strains. It also included a few known non-carcinogens (*p*-anisole, rotenone, etc.), as a test for specificity and reliability of the assays to distinguish between carcinogens and non-carcinogens. In the cited publications there were no concomitant positive controls. However, they were testing compounds that were known carcinogens or known non-carcinogens; they were not testing drugs or compounds that had never before been adequately tested for carcinogenicity, such as frovatriptan, which may also have genotoxic predisposition. These were not GLP studies for the purpose of evaluating the safety of new drugs.

CONCLUSIONS:

Because the p53(+/-) mouse is a relatively new short-term model for identifying genotoxic carcinogens and the paucity of validated historical data, an adequate positive control is required for this assay. Even if it were firmly established that the lack of response by the positive control group was caused by an error in dosing, in the reviewer's opinion, this assay would still not be valid in support of the NDA. However, we believe that the sponsor or the contract laboratory has failed to established beyond reasonable doubt that the lack of response to positive control was due to a dosing error.

Because the positive control was negative, it is not possible to conclude that frovatriptan lacks genotoxic or carcinogenic potential in the p53 mouse assay. This study does not fulfill the pre-clinical requirements for acceptability of the NDA.

RECOMMENDATION:

A final decision for the possible approval of this NDA should be postponed until acceptable completion of the 6-month test with the p53 mouse that is presently in progress.

Sidney J. Stolzenberg, PhD

cc:
HFD-120 Division File
HFD-120/JWare
HFD-120/GFitzgerald
HFD-120/AOliva
HFD-120/SStolzenberg
NDA21-006.ec5

APPENDIX

4 PAGE(S) REDACTED

/s/

Sidney Stolzenberg
12/15/00 02:44:13 PM
PHARMACOLOGIST

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NDA 21-006

REVIEW AND EVALUATION OF TOXICOLOGY DATA

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March 23, 2000

NDA AMENDMENT DATED: 1/21/00 & 3/9/00
CENTER RECEIPT DATE: 1/21/00 & 3/10/00
REVIEWER RECEIPT DATE: 1/21/00 & 3/14/00

SPONSOR: Vanguard Medica Ltd.
Chancellor Court, Surrey Research Park
Guildford, Surrey GU2 5SF
United Kingdom

DRUG: Miguard™ (Frovatriptan succinate; SB 209509-AX; VML 251)

FORMULATION: 2.5 mg (as base) tablets for oral administration.

PHARMACOLOGICAL CLASS: Serotonin receptor subtype 5-$HT_{1B/1D}$ partial agonist

PROPOSED INDICATION: Acute treatment of migraine attacks

DOSAGE REGIMEN: One tablet

RELATED APPLICATION: IND ——

RELATED COMPOUNDS: Imitrex® (sumatriptan succinate)

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I. BACKGROUND

At a meeting of the full CAC, sponsor was given various options for correcting the deficiencies in the present NDA. Sponsor has chosen to perform a 6-month p53 mouse carcinogenicity study and a second 3-month rat dose-finding study. If the dose-finding study demonstrated that the MTD is within 2-3 times the highest dose used in the previously performed 2-year rat carcinogenicity test (85 mg/kg/day), it was agreed that a second 2-year rat study would not be required (See minutes of meeting on 7/29/99, by A. Seifried). The occurrence of treatment-related renal lesions was considered to be a dose limiting effect because compound-related deaths attributed to nephropathy were observed at 1000 mg/kg/day in the 26-week rat study. Renal lesions of similar incidence and severity were also seen at1000 mg/kg/day in a 13-week study, but deaths had not yet occurred by 13 weeks. Because of the wide gap that existed between the NOEL (100 mg frovatriptan/kg/day) and the LOEL (1000 mg/kg/day) for renal lesions in both the 13- and 26-week studies, it was not possible to estimate an MTD. The purpose of the second 13-week study was to select doses between 100 and 1000 mg/kg/day in order to define the MTD, based on renal nephropathy.

In a teleconference on 8/31/99, followed by submissions of 8/26/99 and 8/30/99, sponsor informed the Division of their novel hypothesis that the genetic toxicity of frovatriptan was caused by high *in vitro* concentrations of frovatriptan binding to erythrocyte membranes, causing hemolysis and release of free heme. The free heme resulted in free radical formation which may then initiate peroxidative damage to other cells, including lymphocytes, and indirectly cause chromosomal damage. Based on this hypothesis, they reasoned that the positive findings in the previous *in vitro* human lymphocyte assays should be discounted because when administered to an intact animal or human, the resulting *in vivo* exposure of erythrocytes to the drug would be very low and insufficient to cause significant hemolysis. This hypothesis was based on a publication (R. Munday, J Appl Toxicol, 1985) which reported that diphenyl disulfide dicarboxylic acids incubated in whole blood was observed to bind to erythrocyte membranes, causing hemolysis and free radical formation by the resulting free heme.

Plans were made to conduct a study designed to investigate if the positive findings in the chromosome aberration studies were attributable to a drug-erythrocyte membrane interaction and release of free heme *in vitro*. In an amendment of 9/17/99, a protocol was provided to the Division in which frovatriptan would be tested in both whole blood and in separated lymphocyte cultures.

This amendment contains reports on 1) cultured human lymphocyte assays in whole blood and in separated lymphocytes and 2) a 13-week rat oral gavage study that was used to determine the MTD.

II. TOXICLOGY REVIEW

A. Induction of Chromosome Aberrations in Cultured Human Blood Lymphocytes Incubated With and Without the Presence of Erythrocytes

Study No. SV1012
Sponsor Reference: C09210

Performing Laboratory: [redacted]

Dates Study Performed: Initiated on 9/28/99 and completed 10/4/99

Quality Assurance: A statement of GLP compliance is included

Test Substance: Batch 60848-48

Procedure: Human peripheral whole blood and separated lymphocyte cultures were exposed to test solvent (control) and 3 concentrations of frovatriptan, as shown in sponsor's Table 2, which follows. Positive control consisted of mitocin C. Treatment of cultures with frovatriptan started 48 hours after initiation of incubation. All cultures were incubated without metabolic activation for a period of 20 hours.

Criteria for selection of doses for cytogenetic analysis: With both whole blood and separated lymphocytes, the highest doses selected were based on reductions in mitotic indices and cytotoxic effects observed in preliminary cell toxicity tests.

Results: In both whole blood and in separated lymphocytes, frovatriptan caused statistically significant increases in cells with chromosomal aberrations, which were even higher for separated lymphocytes.

Conclusion: The data do not support sponsor's hypothesis that the clastogenic effect of this drug is caused by membrane damage of the erythrocytes and a resulting release of free heme.

TABLE 2 - MEAN CHROMOSOMAL ABERRATIONS AND MITOTIC INDICES

Treatment		Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Whole blood			
Solvent Control	10 µl/ml.	0.50	9.4
Mitomycin C	0.2 µg/ml.	40.00**	4.7Δ
Frovatriptan			
	1000 µg/ml.	6.00**	6.8
	500 µg/ml.	1.00	6.5
	50 µg/ml.	0.50	8.2
Separated lymphocytes			
Solvent Control	10 µl/mL	4.00	5.1
Mitomycin C	0.2 µg/mL	48.00**	1.4Δ
Frovatriptan			
	500 µg/mL	12.00**	2.8
	250 µg/mL	6.00	4.8
	50 µg ml.	6.50	4.2

** Statistically significant increase in the percentage of aberrant cells at p< 0.01 using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

B. 13-Week Oral Gavage MTD-Finding Study in the Rat (Vol 3)

Report No: 1165/80-1050 ———

Performing Laboratory: [redacted]

Sponsor: Vanguard Medica Ltd.
Chancellor Court, Surrey Research Park
Guildford, Surrey GU2 5SF
United Kingdom

Dates Performed: Treatment initiated on 9/20/99, necropsies completed 12/22/99.

Quality Assurance: a signed statement, indicating that the study was conducted in accordance with GLP, is included.

Test Animals: Crl:CD(SD)IGBR rats, ———— were used. At initiation of dosing the rats were 6 week old and body weights were 152.4-192.2g for males and 123.6-159.9 g for females. In the previously performed carcinogenicity study and in the 3- and 6-month studies, the strain used was designated as Crl:CDBR. In reply to a request for an explanation of the strain difference, sponsor indicated that the new strain was derived from the same colony as the previously used strain but there was genetic drift due to the difference in time.

Test Substance: Batch number 60684-39; purity of 99.8%.

Procedure: The following table illustrates the design of the study.

Group Number	Description	Dose level (mg/kg/day)	Animals/group			
			Main Study		Satellite Study #	
			Male	Female	Male	Female
1	Control	0	10	10	9	9
2	Low	150	10	10	9	9
3	Intermediate-I	255	10	10	9	9
4	Intermediate-II	440	10	10	9	9
5	High	750	10	10	9	9

satellite animals used for toxicokinetic investigations - body weight, food consumption and clinical observations were recorded and a necropsy performed but no data has been reported

Dosage is expressed as free base. Animals were housed in groups of 5/cage and observed daily for clinical signs, morbidity and mortality, weekly for body weights and food consumption, at Weeks 6 and 12 for water consumption. Clinical pathology was

performed on blood samples obtained from the lateral caudal vein after an overnight fast in Week 13 from all main study animals. Hematology included hemoglobin concentration, red blood cell count, PCV, MCV, MCH, MCHC, platelet count total and differential white cell count, platelet count, PTT and APTT. A blood film for reticulocyte was obtained but not examined. Bone marrow smears were obtained at necropsy but not examined. Clinical chemistry included aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, sodium, potassium, calcium, inorganic phosphorus, chloride, total protein, albumin, globulin, A/G ratio, total cholesterol, glucose, urea, total bilirubin and creatinine. Urinalyses were performed from overnight samples collected in Weeks 1, 2, 6 and 12. Urinary parameters measured were volume, specific gravity, protein (UPPY), N-acetyl-B-D-glucosamidase (UNAG), alkaline phosphatase (UALK), creatinine (URCR) and total creatinine (T CREAT). At Week 13, rat alpha-glutathione S-transferase (RGST) was measured in the urine.

Blood samples for toxicokinetics were obtained on Day 1 andin Weeks 5 and 12 from 3 satellite rats/sex/group at 1, 2, 3, 4, 6, 8 and 24 hours after dosing.

Postmortem: Necropsies were performed on all main study and satellite study animals surviving to term and decedents, which included routine gross pathology of main study and satellite study animals. Organ weights were obtained from the following in the main study animals: adrenals, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, and testes with epididymides combined and thyroids with parathyroids. Tissues for histopathology was collected from around 50 organs from each animal of the main study. Microscopic examination included "target organ" tissues (kidney, adrenal and thyroid) from all 5 groups in the main study. Histopathology for other organs included all from high dose and most controls group animals in the main study, and gross lesions from all animals, including satellite goups.

Satellite Studies: The first 5 numbered main study animals/sex/group received bromodeoxyuridine (BDU) by s.c. injections (around 37 mg in males and 25 mg in females). The first 5 numbered satellite study animals/sex/group received BDU by means of —— minipumps implanted 1 week before scheduled necropsy. Tissue samples from the kidneys for measurement of cell proliferation by BDU labeling were obtained.

Rationale for Dose Selection: The study was designed to estimate the MTD and to determine how close this was to the highest dose (85 mg/kg/day) that had been selected for the rat carcinogenicity study. Based on results of the previous 3- and 6-month studies, a dose of 750 mg/kg/day was expected to result in renal lesions, which has previously been associated with mortality and a dose limiting effect. The 2 mid doses were 3- and 5.2- fold higher than the high dose in the carcinogenicity test.

Results: Only mortality and effects that may be compound related are listed.

Mortality: At 440 mg/kg/day, 1 male during Week 1 and 1 female during Week 4 in the main study, were found dead. In the satellite study, an additional female was found dead during the course of the study; time of death was not indicated. At 150 mg/kg/day, 1 male