characterized by cuboidal to columnar cells lining the thyroid follicles, with reduced amounts of colloid in the lumen"

TABLE 8

Group mean organ weights adjusted to overall mean necropsy body weight(g)
Terminal kill

Test article	Control		VML 251	
Group	1	2	3	4
Level (mg/kg/day)	0	10	100	1000

Group Sex	Body weight (g)		AD	KI	SP	LI	HT
1M	583.2	Adjusted	0.056	2.765	0.865	12.787	1.631
		Unadjusted	(0.056)	(2.764)	(0.865)	(12.783)	(1.631)
2M	582.7	Adjusted	0.056	2.774	0.878	12.998	1.629
		Unadjusted	(0.056)	(2.772)	(0.876)	(12.981)	(1.627)
3M	608.5	Adjusted	0.057	2.952	0.859	12.534	1.674
		Unadjusted	(0.059)	(3.031)	(0.898)	(13.125)	(1.722)
4M	552.8	Adjusted	0.069***	3.341***	1.016**	13.143	1.660
		Unadjusted	(0.067)	(3.246)	(0.968)	(12.423)	(1.602)
Statistics		A	C	C	C	C	C

* P<0.05
** P<0.01
*** P<0.001

A = ANOVA, regression and Dunnett's
C = ANCOVA and Dunnett's

TABLE 8

Group mean organ weights adjusted to overall mean necropsy body weight(g)
Terminal kill

Test article	Control		VML 251	
Group	1	2	3	4
Level (mg/kg/day)	0	10	100	1000

Group Sex	Body weight (g)		AD	KI	SP	LI	HT
1F	331.7	Adjusted	0.060	1.713	0.547	7.868	1.075
		Unadjusted	(0.059)	(1.710)	(0.546)	(7.841)	(1.074)
2F	329.5	Adjusted	0.069*	1.739	0.588	8.118	1.068
		Unadjusted	(0.069)	(1.731)	(0.584)	(8.048)	(1.065)
3F	336.6	Adjusted	0.069*	1.798	0.619*	8.356*	1.136
		Unadjusted	(0.069)	(1.806)	(0.623)	(8.426)	(1.140)
4F	334.3	Adjusted	0.074***	1.955***	0.666***	8.513**	1.122
		Unadjusted	(0.074)	(1.958)	(0.668)	(8.537)	(1.124)
Statistics		A	C	C	C	C	C

* P<0.05
** P<0.01
*** P<0.001

A = ANOVA, regression and Dunnett's
C = ANCOVA and Dunnett's

Incidence of selected histopathology findings in the kidney - terminal kill

Tissue and finding		Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
Kidney	Number examined	20	20	19	15	20	18	19	19
Tubular nephropathy	Grade -	20	20	19	3	20	18	19	11
	1	0	0	0	4	0	0	0	2
	2	0	0	0	1	0	0	0	2
	3	0	0	0	6	0	0	0	3
	4	0	0	0	1	0	0	0	1

Key: Grade - = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = marked.

Incidence of selected histopathology findings in the adrenal - terminal kill

Tissue and finding		Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
Adrenal	Number examined	20	20	19	15	20	18	19	19
Prominent zona glomerulosa	Grade -	20	20	1	1	20	17	7	2
	1	0	0	16	12	0	1	12	14
	2	0	0	2	2	0	0	0	3
Medullary atrophy	Grade -	20	20	19	2	20	18	19	2
	1	0	0	0	5	0	0	0	1
	2	0	0	0	4	0	0	0	3
	3	0	0	0	4	0	0	0	12
	4	0	0	0	0	0	0	0	1
Focal medullary hyperplasia	Grade -	20	20	18	11	20	18	19	10
	1	0	0	0	3	0	0	0	7
	2	0	0	1	1	0	0	0	2

Key: Grade - = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = marked.

Incidence of selected histopathology findings in the thyroid - terminal kill

Tissue and finding		Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
Thyroid	Number examined	20	20	19	15	20	18	19	19
Follicular cell hypertrophy	Grade -	13	12	9	1	17	13	16	4
	1	7	7	8	8	3	4	3	7
	2	0	1	2	6	0	1	0	8

Key: Grade - = finding not present, 1 = minimal, 2 = slight.

Toxicokinetics

Following is sponsor's summary tables of toxicokinetic parameters. Generally, C_{max} and AUC values for females were similar to males. Time to C_{max} varied between 2 and 7 hours post dosing, although at week 1 C_{max} was at 24 hours for high dose. The data demonstrate continuous exposure to parent compound at all dose levels in both sexes. A dose relationship that was sub-proportional was observed on day 1 for both C_{max} and AUC but by week 26 they were approximately dose proportional. C_{max} and AUC at week 26 were approximately twice as high as on day 1. The data for males and females were comparable.

Mean data for $AUC_{(0\ to\ 24\ h)}$ - Males

Dose (mg/kg/day)	Dose ratio	Day 1 (ng.h/mL)	AUC ratio	Week 26 (ng.h/mL)	AUC ratio
10	1	2334.2	1.0	4863.2	2.1
100	10	15090.1	6.5	41407.2	17.7
1000	100	124932.3	53.5	325498.3	139.4

Mean data for $AUC_{(0\ to\ 24\ h)}$ - Females

Dose (mg/kg/day)	Dose ratio	Day 1 (ng.h/mL)	AUC ratio	Week 26 (ng.h/mL)	AUC ratio
10	1	3355.9	1.0	3954.4#	1.2
100	10	23504.6	7.0	38795.6	11.6
1000	100	119751.3	35.7	355379.7	105.9

Mean data for C_{max} - Males

Dose (mg/kg/day)	Dose ratio	Day 1 (ng/mL)	C_{max} ratio	Week 26 (ng/mL)	C_{max} ratio
10	1	156.3	1.0	261.3	1.7
100	10	1341.9	8.6	2752.6	17.6
1000	100	9052.7	57.9	18052.1	115.5

Mean data for C_{max} - Females

Dose (mg/kg/day)	Dose ratio	Day 1 (ng/mL)	C_{max} ratio	Week 26 (ng/mL)	C_{max} ratio
10	1	200.5	1.0	328.2#	1.6
100	10	1367.2	6.8	3790.6	18.9
1000	100	6566.1	32.7	19420.9	96.9

Mean data for T_{max}

Dose (mg/kg/day)	males		females	
	Day 1 (h)	Week 26 (h)	Day 1 (h)	Week 26 (h)
10	2.0	3.0	7.0	4.0#
100	4.0	3.0	7.0	3.0
1000	24.0	3.0	24.0	7.0

omitting one result

104-Week Oral (Gavage) Oncogenicity Study in Rats (with Toxicokinetics) (Vol 1.26-1.30)

Report No: 1165/45-D6154 ———

Performing Laboratory: [ ]

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

Dates Performed: Treatment initiated on 4/12/96, necropsies completed 4/20/98.

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

Test Animals: Crl:CDBR rats, ———— At initiation of dosing the rats were 6 week old and body weights were 209-273 g for males and 156.3-196.6 g for females

Test Substance: Batch numbers 60467-05, 60475-06, 60532-12, 60514-10, 60531-11, 60587-14, 60597-15, 60684-39; purity of batches not indicated.

Procedure: 60 rats of each sex per group received frovatriptan at 8.5, 27 and 85 mg/kg/day (dosage based on free base), and there were 2 control groups of 60/sex. The dose volume was 5 mL/kg. Animals were housed 5/cage and observed daily for clinical signs, morbidity and mortality, and each animal was given a detailed physical examine weekly, which included palpation for tissue masses. Body weights and food consumption were obtained weekly during the first 16 weeks, every 4 weeks thereafter, until necropsy. Moribund animals were killed and necropsied.

Clinical pathology was limited only to hematology at terminal kill and decedents, where possible, and included only red and white blood cell counts.

Postmortem did not include any organ weights but did include gross pathology. For histopathology, samples of the following tissues were fixed in 10% neutral formalin, with the exception of eyes, which were fixed in Davidson's fluid: adrenals, aorta, brain, cecum, colon, duodenum, eyes, femur with marrow and articular surface, gross lesions, Harderian glands, head, heart, ileum, jejunum, kidneys, lacrimal glands, larynx, liver, lungs with mainstream bronchi, mammary glands, larynx, liver, mammary, mandibular lymph nodes, mesenteric lymph nodes, muscle (quadriceps), nasal turbinates, nasopharynx, esophagus, optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerves, seminal vesicles, skin, spinal cord (cervical, lumbar, thoracic), spleen, sternum with bone marrow, stomach, testes with epididymides, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus,

vagina and Zymbal glands, tissue masses. Microscopic evaluation included all indicated tissues from control, high dose and decedent animals and gross lesions from all animals. Subsequently, adrenals, kidneys and pituitary from low and mid dose males, and ovaries, uterus, mammary, adrenals, kidneys and adrenals from low and mid dose females, were microscopically examined.

Rationale for Dose Selection: Sponsor claimed that in the 13 week dose-range study, the high dose of 1000 mg/kg/day was associated with 2 deaths "possibly attributable to test article...and with treatment-related findings in the kidney with a possible slight effect at 100 mg/kg/day. The high dose was expected to give systemic exposure in animals that exceeded 25 times the AUC of that resulting in humans."

Compound Related Effects in the Main Study

Mortality

Survival to the start of terminal kill was as follows:

Group number	Group description	Dose level (mg/kg/day)	Survival Male	Female
1	control I	0	29 (48)	18 (30)
2	low	8.5	18 (30)*	12 (20)
3	intermediate	27	29 (48)	18 (30)
4	high	85	16 (27)**	24 (40)
5	control II	0	29 (48)	19 (32)
			DR**	

() - figures in brackets indicate percentage survival
DR dose response
* p<0.05
** p<0.01

Neoplastic findings were as follows:

Group incidence of selected histopathology findings: pituitary and adrenal

Tissue and finding	Group and sex: 1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
	Males					Females				
Pituitary Number examined	60	60	60	60	60	60	60	60	60	60
focal hyperplasia	10	16	16	13	21	12	9	14	14	10
B-adenoma - *M*	29	29	34	36	26	42	43	34	40	39
M-carcinoma - *M*	0	0	0	0	0	1	1	0	1	0
M - benign and malignant pituitary tumors merged for statistical analysis	29	29	34	36**/	26	43	44	34	41	39
Adrenal Number examined	60	60	60	60	60	60	60	60	60	59
focal medullary hyperplasia	13	14	18	25**/	12	11	13	16	30 ***/	11
B-benign pheochromocytoma - *M*	13	13	9	10	15	0	2	5	7	3
B-ganglioneuroma - *M*	0	1	0	0	0	0	0	0	0	0
M-malignant pheochromocytoma - *M*	2	0	3	1	0	0	0	0	0	0
M - adrenal medullary tumours merged for statistical analysis	15	14	12	11	15	0	2	5*/	7**/	3

* = P<0.05, ** = P<0.01
/ = Increasing dose response

The investigators claimed that the increased incidence of deaths in high dose males (P<0.01) was attributable to the increased incidence of pituitary tumors, but the 60% incidence of these tumors at high dose was within the high end of the background range (25-66%), whereas the incidence in the 2 control groups (43 & 48%), although within the incidence of background range, was lower than mean background incidence (51%). However, there was also an increased incidence of deaths in low dose males (P<0.05 compared to control; only 2 more deaths in high dose than in low dose males), and there was no concomitant increase in pituitary adenomas. On the other hand, there was an increase in incidence of pituitary adenomas in mid dose males (n.s. but only 2 less than at high dose) with no concomitant increase in mortality. In females, there was no effect of treatment on either incidence of pituitary adenomas or on mortality.

Body weight gain throughout the entire 104 weeks of the study was higher than control for mid (9%; n.s.) and high (17%; P<0.05) dose males, as well as for all 3 treated females groups (17, 13 and 5%, respectively; n.s. for any of the 3 female treated groups).

TABLE 4.2

Group mean body weight gains

Test Article	Control		VM, 251		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	8.5	27	85	0

Week of study		Mean body weight gains (g) for Group and Sex 1F	2F	3F	4F	5F	Statistics	
0 to 13	Mean	289.2	301.6	299.2	300.2	296.5	DR*	A
	SD	36.34	45.56	42.97	43.01	37.51		
0 to 104	Mean	517.3	501.7	572.4	613.4*	532.6		A
	SD	100.42	116.76	147.46	120.43	120.95		
13 to 24	Mean	74.9	73.7	79.8	76.8	74.5		A
	SD	17.49	21.97	22.25	22.54	19.08		
24 to 52	Mean	109.7	115.7	121.5	119.3	112.7	DR*	A
	SD	28.52	34.56	40.93	35.23	30.38		
52 to 76	Mean	48.1	45.0	48.3	48.6	52.0		A
	SD	43.54	37.30	44.76	37.79	44.12		
76 to 104	Mean	7.2	0.5	15.5	69.7	25.3		A
	SD	56.90	53.34	79.21	63.33	68.74		

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test

A = ANOVA, regression and Dunnett's

TABLE 4.2

Group mean body weight gains

Test Article	Control		VM, 251		Control
Group	6	7	8	9	10
Level (mg/kg/day)	0	8.5	27	85	0

Week of study		Mean body weight gains (g) for Group and Sex 6F	7F	8F	9F	10F	Statistics	
0 to 13	Mean	117.1	119.5	125.4*	130.2***	119.7		A
	SD	16.86	16.10	19.88	19.34	16.31		
0 to 104	Mean	312.0	335.4	362.9	385.2	329.4		A
	SD	84.17	69.37	138.19	90.15	104.79		
13 to 24	Mean	31.1	32.7	34.5	34.3	28.0	DR*	A
	SD	12.64	11.20	18.82	12.93	10.83		
24 to 52	Mean	80.8	87.0	88.7	88.9	75.1	DR**	A
	SD	35.08	42.13	70.85	39.92	39.78		
52 to 76	Mean	66.8	62.7*	68.5	75.2	66.3		A
	SD	34.28	34.36	29.46	25.84	30.69		
76 to 104	Mean	34.3	31.9	59.6	39.4	45.7		A
	SD	40.12	51.91	61.09	43.26	47.57		

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test

A = ANOVA, regression and Dunnett's

FIGURE 1

Group mean survival - males

Test Article	Control		VML 251		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	8.5	27	85	0

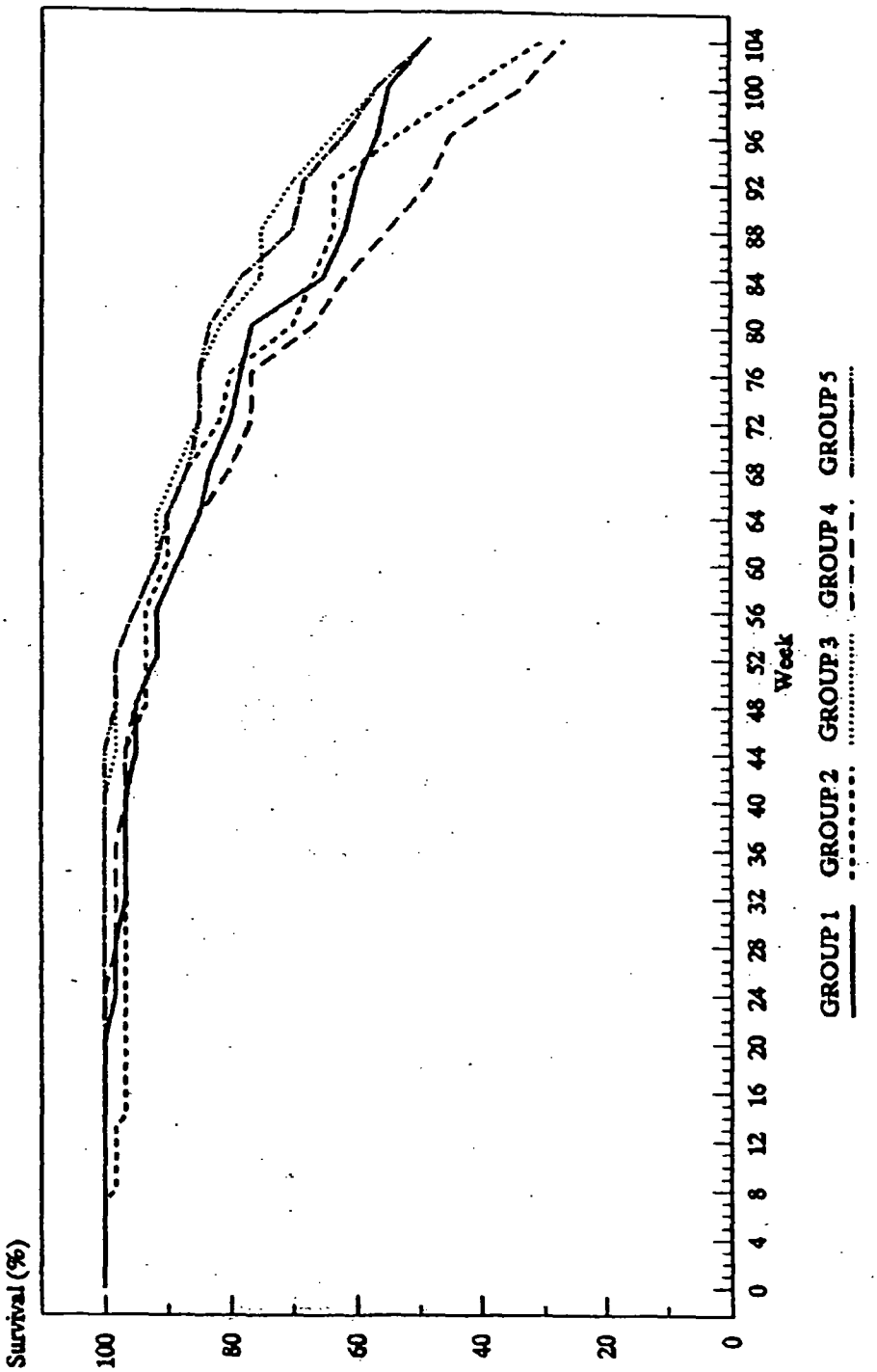


FIGURE 2

Group mean survival - females

Test Article	Control	VML 251			Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	8.5	27	85	0

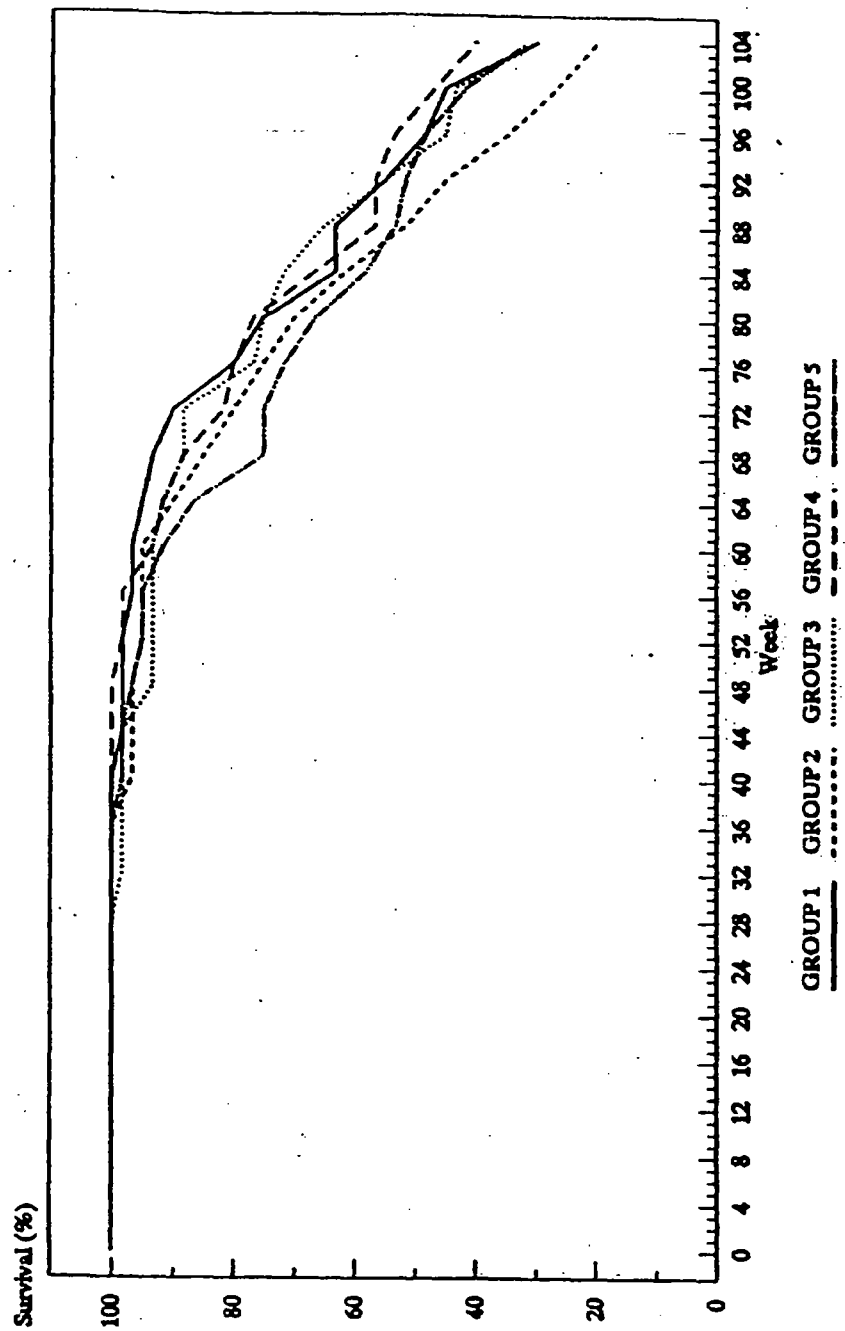


FIGURE 3

Group mean body weight - males

Test Article	Control	WL 251			Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	8.5	27	85	0

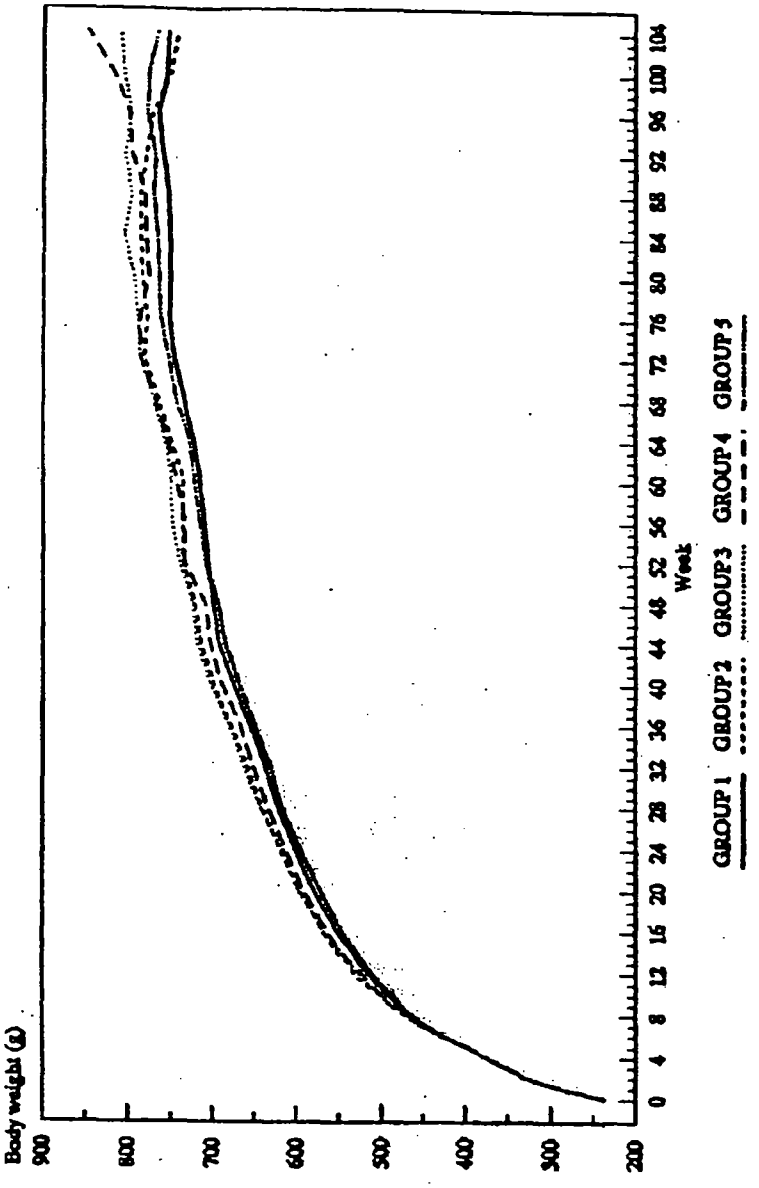


FIGURE 4

Group mean body weight - females

Test Article	Control	VML 251			Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	8.5	27	85	0

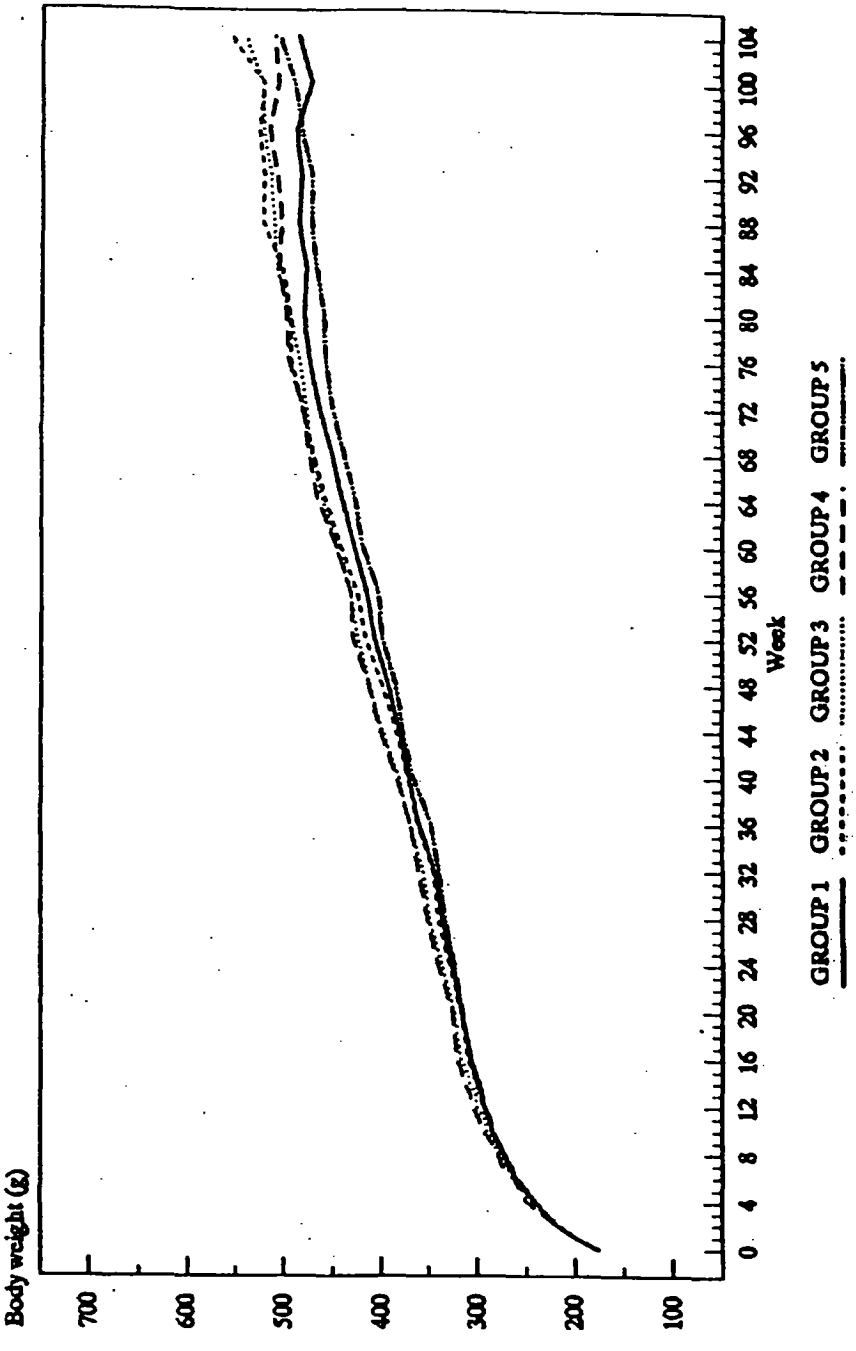


TABLE 4.2

Group mean body weight gains

Test Article		Control	VML 251			Control
Group		1	2	3	4	5
Level (mg/kg/day)		0	9.5	27	85	0

Week of study		Mean body weight gains (g) for Group and Sex 1M	2M	3M	4M	5M	Statistics	
0 to 13	Mean	289.0	301.8	299.2	300.2	285.5	DR*	A
	SD	36.34	40.56	42.97	43.01	37.51		
0 to 104	Mean	517.3	501.7	572.4	613.4*	532.6		A
	SD	100.82	116.70	147.46	120.43	120.95		
13 to 26	Mean	74.9	79.7	79.8	76.8	74.5		A
	SD	17.49	21.07	22.25	22.54	19.06		
26 to 52	Mean	103.7	115.7	121.5	119.3	112.7	DR*	A
	SD	28.52	34.56	48.93	35.27	30.38		
52 to 76	Mean	49.1	45.0	48.3	48.0	52.0		A
	SD	43.55	37.30	44.95	47.78	44.12		
76 to 104	Mean	7.2	0.5	15.5	60.7	25.3		A
	SD	56.99	54.34	79.21	63.38	66.74		

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test

A = ANOVA, regression and Dunnett's

TABLE 4.2

Group mean body weight gains

Test Article		Control	VML 251			Control
Group		1	2	3	4	5
Level (mg/kg/day)		0	9.5	27	85	0

Week of study		Mean body weight gains (g) for Group and Sex 1F	2F	3F	4F	5F	Statistics	
0 to 13	Mean	117.1	119.5	125.4*	130.2***	119.7		A
	SD	16.86	18.10	18.88	18.36	14.31		
0 to 104	Mean	310.0	375.4	362.9	335.2	329.8		A
	SD	84.17	89.37	108.18	50.15	104.79		
13 to 26	Mean	31.1	32.7	34.5	34.3	28.0	DR*	A
	SD	12.64	11.20	19.82	12.98	10.83		
26 to 52	Mean	80.8	87.0	88.7	88.8	75.1	DR*	A
	SD	35.01	42.13	39.88	39.62	39.38		
52 to 76	Mean	66.8	82.7*	68.5	75.2	66.3		A
	SD	34.21	34.35	29.46	25.84	38.99		
76 to 104	Mean	34.3	51.9	59.6	39.4	45.7		A
	SD	40.12	51.91	41.09	43.26	47.57		

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test

A = ANOVA, regression and Dunnett's

Gross Pathology: There was an increase in pituitary masses in high dose males, which correlated with an increase pituitary in adenomas, seen microscopically. A higher incidence of compression of the brain correlated with, and was considered to be, a direct consequence of pituitary adenomas.

Histopathology

Non-neoplastic findings: In adrenals, there was an increase in incidence of focal medullary hyperplasia in (See table that follows). This was characterized "by small aggregates of medullary cells with a more basophilic cytoplasm and smaller hyperchromatic nuclei than the surrounding medullary cells. The foci often arose near the corticomedullary junction, were less than 1.25 mm in diameter and exhibited minimal or no compression of surrounding tissue." In contrast, there was a reduction in incidence of various focal proliferative lesions characteristic in aging rats of this strain in all the male treatment groups ($P<0.001$), which was marginally reduced in high dose females (n.s.). Other effects in reproductive organs and mammary glands, observed in females, are shown in the table, which follows.

Table 5.3.2.2.G3

Incidence of Non-Neoplastic Findings Following 104-Weeks

Oral Dosing with Frovatriptan in Rats

	Males					Females				
Dose (mg/kg/day)	0	8.5	27	85	0	0	8.5	27	85	0
ADRENAL										
No. examined	60	60	60	60	60	60	60	60	60	59
Focal medullary hyperplasia	13	14	18	26**	12	11	12	14	29***	10
Cortical altered cell foci -	42	31*	26**	19***	37	37	34	37	31	38
OVARY										
No. examined	0	0	0	0	0	60	60	59	60	60
Acyclic follicular	0	0	0	0	0	34	27	20**	6***	33
UTERUS										
No. examined	0	0	0	0	0	60	60	59	60	60
Cystic glands	0	0	0	0	0	26	17*	17*	13**	27
Squamous metaplasia	0	0	0	0	0	13	7*	9	3**	15
Cervical/vaginal squamous hyperplasia	0	0	0	0	0	21	15	17	9***	25
Cervical/vaginal mucification	0	0	0	0	0	20	20	25	33**	18
MAMMARY GLAND										
No. examined	0	3	1	1	1	60	60	60	60	60
Acinar hyperplasia	0	0	0	0	0	15	17	19	34***	15
Cystic hyperplasia	0	1	0	0	0	41	38	38	24***	42

* $p<0.05$ ** $p<0.01$ ***$p<0.001$ compared with combined control group
- combined altered cell foci – incidence of animals with one or more subtypes of altered cell foci, eosinophilic, basophilic, vascuolated, normochromic or clear cell.

Neoplastic findings: In mid and high dose males there was an increase in incidence of pituitary adenomas (dose related but statistically significant at high dose), and in females there was a dose related increase in B-benign pheochromocytomas. Pheochromocytomas (including those in control animals) were characterized as "well delineated modules of medullary cells, commonly at the corticomedullary junction." See page 48 for table of neoplastic findings.

All of the treatment related effects occurred in endocrine or neuroendocrine related organs in which the background incidences are normally high for this species and strain. Sponsor points out that in the whole body autoradiographic distribution study — Study No. 1165/7), there was a considerable accumulation of frovatriptan in the adrenal medulla, and in aging rats, and this is an organ that is prone to develop proliferative lesions. It was also indicated that in the rat female fertility study — Study No. 1165/11), treatment with frovatriptan resulted in a slight prolongation of estrus and a consequent dose related reduction in estrous cycle frequency. To explain the microscopic findings in the ovaries of treated rats, sponsor suggested that as a result of decreases in number of estrous cycles during the lifetime of the treated female animals, there would be more follicles remaining in the ovaries. Consequently the ovaries would maintain normal estrogen levels and the rats would continue cycling longer than controls. However, this has not been proven. It seems reasonable to suggest that frovatriptan has an effect on the hypothalamo-pituitary-gonad hormonal axis.

TABLE A.6

[redacted]: Histopathology - Statistical Data - All Animals

Test article	Control		DAC 251		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	3.5	27	45	0

PRINTED: 26-OCT-90
PAGE: 1

STUDY NUMBER: 116545

		--- NUMBER OF ANIMALS AFFECTED ---									
TABLE INCLUDES:											
SEX=ALL;GROUP=ALL;WEEKS=ALL	SEX:	----------MALE----------					----------FEMALE----------				
DEATH=ALL;FIN=B,M;NOSET=T											
	TRUTH:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	50	50	50	50	50	50	50	50	50	50
** TOP OF LIST **											
SYS	NUMBER EXAMINED:	50	43	38	35	50	60	48	44	50	59
--B-BENIGN MESANOMA		0	0	0	0	0	1	0	0	0	0
SKIN + SUBCUTIS	NUMBER EXAMINED:	60	53	43	50	60	60	58	56	60	60
--B-DERMAL FIBROMA		8	7	5	7	5	0	0	0	0	0
--B-FIBROMA		2	6	6	6	3	2	4	4	3	1
--B-FIBROLIPOMA		1	1	0	0	0	0	0	0	0	0
--B-KERATOACANTHOMA		4	3	1	5	7	0	0	0	1	2
--B-LIPOMA		5	0	0	5	3	1	2	1	3	1
--B-SQUAMOUS CELL PAPILLOMA		2	2	0	3	2	0	0	0	0	0
--B-BASAL CELL ADENOMA		0	2	0	0	1	0	1	1	0	0
--B-SEBACEOUS CELL ADENOMA		0	1	0	0	0	0	0	0	0	0
--B-NEUROFIBROMA		0	1	0	0	0	0	0	0	0	0
--M-FIBROSARCOMA		1	0	0	0	1	0	0	1	1	0
--M-MALIGNANT SCHWANNOMA		2	2	1	0	1	1	0	0	1	1
--M-SARCOMA		1	3	0	0	2	0	0	2	0	3
--M-SQUAMOUS CELL CARCINOMA		1	0	1	1	0	1	0	0	0	0
--M-HISTIOCYTIC SARCOMA		0	1	1	1	2	0	0	1	0	1
--M-HAEMANGIOSARCOMA		0	0	0	0	1	0	0	0	0	0
MAMMARY GLAND	NUMBER EXAMINED:	0	3	1	1	1	60	60	60	60	60
--B-FIBROADENOMA		0	2	1	1	0	40	40	36	32	35
--B-ADENOMA		0	0	0	0	0	8	7	4	1	3
--B-ADENOLIPOMA		0	0	0	0	0	0	1	0	0	0
--M-CARCINOMA		0	0	0	0	1	14	6	7	9	5
--M-SARCOMA		0	0	0	0	0	0	1	0	0	0

APPEARS THIS WAY
ON ORIGINAL

TABLE A.6

Group Incidence Distribution - Neoplastic Data - All Animals

Test Article	Control		VNL 251		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	8.5	27	85	0

PRINTED: 26-OCT

PAGE: 2

STUDY NUMBER: 11454

TABLE INCLUDES:
SEX-ALL;FROM-ALL;WEEK-ALL
DEATH-ALL;FIND-ALL;SUBSET-T

		---NUMBER-OF-ANIMALS-AFFECTED---									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	60	60	60	60	60	60	60	60	60	60
LIVER	NUMBER EXAMINED:	60	60	61	59	60	60	59	58	59	60
--B-HEPATOCELLULAR ADENOMA		1	0	2	0	2	1	0	1	0	0
--M-HEPATOCELLULAR CARCINOMA		1	0	0	0	0	0	0	0	1	0
SPLEEN	NUMBER EXAMINED:	60	43	33	59	60	60	48	43	60	60
--M-SARCOMA		0	0	0	1	0	0	0	0	0	0
PANCREAS	NUMBER EXAMINED:	60	42	32	60	60	60	44	42	60	60
--B-ISLET CELL ADENOMA		4	0	1	2	2	0	0	2	1	0
--B-MIXED ACINAR-ISLET CELL ADENOMA		1	0	0	0	0	0	0	0	0	0
--B-ACINAR CELL ADENOMA		1	0	0	0	0	0	0	0	0	1
--M-ISLET CELL CARCINOMA		0	3	1	1	0	0	0	0	0	0
--M-ACINAR CELL CARCINOMA		0	0	0	1	0	0	0	0	0	0
MESENTERIC LN	NUMBER EXAMINED:	59	42	33	60	60	60	48	42	60	60
--B-LYMPHANGIOMA		0	0	1	2	1	0	0	1	0	0
--B-HAEMANGIOMA		1	1	0	0	1	0	0	0	1	0
--M-HAEMANGIOSARCOMA		0	1	0	0	0	0	0	0	0	0
STOMACH	NUMBER EXAMINED:	60	43	40	58	60	60	51	45	60	59
--B-SQUAMOUS CELL PAPILLOMA		0	0	0	0	0	0	0	0	1	0
--M-LEIOMYOSARCOMA		0	0	1	0	0	0	0	0	0	0
CAECUM	NUMBER EXAMINED:	52	39	27	50	56	60	46	41	57	56
--M-LEIOMYOSARCOMA		0	1	0	0	0	0	0	0	0	0

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

TABLE 9.[illegible]

[illegible]

Test article	Control	SML [illegible]			Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	0.5	17	85	0

[ ]

PRINTED: 26-OCT-98
PAGE: 3

STUDY NUMBER: 11n545

TABLE INCLUDES:
SEX-ALL;GROUP-ALL;WEEKS-ALL
DEATH-ALL;FINT-B,M;SUBSET-T

ORGAN AND FINDING DESCRIPTION		MALE -1-	MALE -2-	MALE -3-	MALE -4-	MALE -5-	FEMALE -1-	FEMALE -2-	FEMALE -3-	FEMALE -4-	FEMALE -5-
	NUMBER:	60	60	60	59	60	60	60	60	60	60
ADRENAL	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	59
--B-BENIGN PHAEOCHROMOCYTOMA		13	11	9	10	15	0	2	5	7	1
--B-GANGLIONEUROMA		0	1	0	0	0	0	0	0	0	0
--B-CORTICAL ADENOMA		0	1	0	0	0	1	0	0	0	0
--M-CORTICAL CARCINOMA		0	0	1	0	0	0	0	1	0	2
--M-MALIGNANT PHAEOCHROMOCYTOMA		2	0	3	1	0	0	0	0	0	0
KIDNEY	NUMBER EXAMINED:	60	60	60	59	60	60	60	60	60	60
--B-TUBULAR CELL ADENOMA		0	1	0	0	0	0	0	0	0	0
--B-LIPOMA		0	2	0	0	0	0	0	0	0	1
TESTIS	NUMBER EXAMINED:	60	44	33	60	60	0	0	0	0	0
--B-INTERSTITIAL CELL ADENOMA		1	2	0	0	2	0	0	0	0	0
--B-BENIGN MESOTHELIOMA		1	0	0	0	0	0	0	0	0	0
URINARY BLADDER	NUMBER EXAMINED:	60	41	32	59	60	60	48	41	60	60
--B-LEIOMYOMA		0	0	0	0	0	1	0	0	0	0
PROSTATE	NUMBER EXAMINED:	60	42	33	60	60	0	0	0	0	0
--M-CARCINOMA		0	0	0	0	1	0	0	0	0	0
OVARY	NUMBER EXAMINED:	0	0	0	0	0	60	60	59	60	60
--B-SEX CORD/STROMAL ADENOMA		0	0	0	0	0	1	0	0	0	1
--M-MALIGNANT THECOMA		0	0	0	0	0	0	1	0	0	0
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	60	60	59	60	60
--B-POLYP		0	0	0	0	0	2	7	2	4	5

** CONTINUED ON NEXT PAGE **

APPEARS THIS WAY
ON ORIGINAL

TABLE A.6

Group Incidence Distribution - Neoplastic Rate - All Animals

Test article	Control		DNL /51		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	5.5	27	45	0

[ ]

STUDY NUMBER: 119545

		--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---									
TABLE INCLUDES:											
SEX-ALL;GROUP-ALL;WEEKS-ALL	SEX:	----------MALE----------					----------FEMALE----------				
DEATH-ALL;FIND-B,M;NUMBER-C											
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	60	60	60	60	60	60	60	60	60	60
** FROM PREVIOUS PAGE **											
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	60	60	59	60	60
--B-FIBROMA		0	0	0	0	0	0	1	0	1	1
--B-BENIGN GRANULAR CELL TUMOUR		0	0	0	0	0	1	1	0	0	2
--M-SARCOMA		0	0	0	0	0	0	0	1	0	0
--M-MALIGNANT SCHWANNOMA		0	0	0	0	0	0	0	0	1	0
--M-STROMAL SARCOMA		0	0	0	0	0	0	1	0	0	0
--M-CARCINOMA		0	0	0	0	0	1	0	0	0	1
THYMUS	NUMBER EXAMINED:	59	59	50	60	59	59	47	41	56	60
--B-BENIGN THYMOMA		0	1	0	0	0	1	2	0	1	0
--M-MALIGNANT THYMOMA		0	1	0	0	0	0	0	0	0	0
LUNG	NUMBER EXAMINED:	60	48	36	60	60	60	49	44	60	60
--B-BRONCHIOLO-ALVEOLAR ADENOMA		0	1	0	1	0	0	0	0	0	0
--M-BRONCHIOLO-ALVEOLAR CARCINOMA		0	0	0	0	1	0	0	0	0	0
HEART	NUMBER EXAMINED:	60	43	32	60	60	60	48	42	60	60
--B-BENIGN SCHWANNOMA		0	1	0	0	0	0	0	0	0	0
THYROID	NUMBER EXAMINED:	55	40	32	56	59	60	47	43	58	60
--B-FOLLICULAR CELL ADENOMA		2	1	0	1	2	0	0	0	0	0
--B-C-CELL ADENOMA		9	2	6	6	8	9	3	1	8	5
--M-C-CELL CARCINOMA		0	1	1	0	1	0	1	1	0	0
PARATHYROID	NUMBER EXAMINED:	59	41	29	58	59	55	45	41	58	57
--B-ADENOMA		1	1	0	0	4	1	1	0	0	2

APPEARS THIS WAY
ON ORIGINAL

TABLE [illegible]

Group incidence: Histopath Body - neoplastic data - all animals

Test article	Control	VML 251			Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	9.5	27	65	0

[ ]

PRINTED: 29-OCT-98
PAGE: 9

STUDY NUMBER: 116545

TABLE INCLUDES:
SEX-ALL; GROUP-ALL; WEEKS-ALL
DEATH-ALL; FIND-B,M; SUBSET-T

ORGAN AND FINDING DESCRIPTION		MALE -1-	MALE -2-	MALE -3-	MALE -4-	MALE -5-	FEMALE -1-	FEMALE -2-	FEMALE -3-	FEMALE -4-	FEMALE -5-
	NUMBER:	60	60	60	60	60	60	60	60	60	60
PITUITARY	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60
--B-ADENOMA		29	29	34	36	26	42	43	34	40	39
--M-CARCINOMA		0	0	0	0	0	1	1	0	1	0
BRAIN	NUMBER EXAMINED:	60	42	32	60	60	60	45	42	60	60
--B-GRANULAR CELL MENINGIOMA		2	0	0	0	2	0	0	0	0	0
--M-MENINGEAL SARCOMA		1	0	0	0	0	0	0	0	0	0
--M-ASTROCYTIC GLIOMA		1	0	0	0	0	1	0	0	0	1
--M-OLIGODENDROCYTIC GLIOMA		0	1	0	0	0	0	0	0	0	0
SPINAL CORD	NUMBER EXAMINED:	60	42	32	60	60	60	45	42	60	60
--M-ASTROCYTIC GLIOMA		0	0	0	0	0	0	0	1	0	0
HAEM/LYMPH/RETIC	NUMBER EXAMINED:	60	42	32	60	60	60	49	43	60	60
--M-LYMPHOCYTIC LEUKAEMIA		1	1	1	1	2	0	1	1	0	0
--M-MIXED LYMPHOMA		0	0	0	0	0	0	0	0	0	2
--M-GRANULOCYTIC LEUKAEMIA		0	1	0	0	0	0	1	0	0	0
ABDOMINAL CAVITY	NUMBER EXAMINED:	5	3	1	1	5	2	2	2	1	0
--B-LIPOMA		0	0	0	0	1	0	0	1	0	0
--M-SARCOMA		0	1	0	0	0	0	0	0	1	0
--M-OSTEOSARCOMA		0	0	0	0	0	0	1	0	0	0
THORACIC CAVITY	NUMBER EXAMINED:	3	2	2	0	5	1	3	0	0	2
--M-HISTIOCYTIC SARCOMA		1	0	0	0	0	0	0	0	0	0

APPEARS THIS WAY
ON ORIGINAL

TABLE 9.6

Group incidence distribution by [illegible] data - all animals

Test article	Contr 1		5AC 253		Contr 2
Group	1	2	3	4	5
Level (mg/kg/day)	0	1.5	27	35	0

[ ]

PRINTED: 26-OCT-88
PAGE: 6

STUDY NUMBER: 116545

TABLE INDUCED:
SEX-ALL:GROUP-ALL:WEEKS-ALL
DEATH-ALL:FINT-B,M:SUBSET-T

---NUMBER-OF-ANIMALS-AFFECTED---

	SEX:	MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	60	60	60	60	60	60	60	60	60	60
NASAL CAVITY	NUMBER EXAMINED:	1	1	0	2	0	2	1	0	0	0
--M-SARCOMA		0	0	0	0	0	0	1	0	0	0
--M-SQUAMOUS CELL CARCINOMA		0	0	0	0	0	1	0	0	0	0
MUSCLE	NUMBER EXAMINED:	4	0	4	3	1	0	3	0	2	1
--M-HISTIOCYTIC SARCOMA		0	0	0	0	1	0	0	0	0	0
ORAL CAVITY	NUMBER EXAMINED:	4	1	0	4	10	9	7	5	4	9
--M-SARCOMA		0	0	0	0	0	1	0	0	0	0
PCOT/LEG	NUMBER EXAMINED:	10	15	10	12	4	3	6	4	3	6
--B-HISTIOCYTOMA		0	0	0	0	0	0	0	1	0	0
TAIL	NUMBER EXAMINED:	14	24	21	11	17	31	28	32	22	27
--B-KERATOACANTHOMA		1	1	0	0	0	0	0	0	0	0
--B-SQUAMOUS CELL PAPILLOMA		1	1	1	1	0	1	0	0	0	0
UAR	NUMBER EXAMINED:	5	0	4	1	2	2	0	1	0	3
--B-NEUROFIBROMA		1	0	0	0	0	0	0	0	0	0
CONNECTIVE TISS	NUMBER EXAMINED:	0	0	0	0	1	1	0	0	0	0
--M-SARCOMA		0	0	0	0	1	0	0	0	0	0

** END OF LIST **

APPEARS THIS WAY
ON ORIGINAL

Background incidence of Adrenal and Pituitary Tumors

			ADRENAL							
	Controls examined		Benign pheochromocytoma				Malignant pheochromocytoma			
Study	m	f	m	%	f	%	m	%	f	%
3	110	110	7	6.4	6	5.5	0	0.0	0	0.0
4	100	100	18	18.0	2	2.0	2	2.0	0	0.0
5	100	99	7	7.0	2	2.0	2	2.0	0	0.0
6	60	60	17	28.3	1	1.7	1	1.7	0	0.0
7	50	50	11	22.0	5	10.0	2	4.0	2	4.0
8	100	100	18	18.0	3	3.0	1	1.0	0	0.0
9	120	120	12	10.0	6	5.0	3	2.5	0	0.0
10	100	99	16	16.0	3	3.0	1	1.0	0	0.0
11	100	99	15	15.0	5	5.1	1	1.0	0	0.0
13	60	60	12	20.0	2	3.3	0	0.0	0	0.0
61	130	130	19	14.6	7	5.4	0	0.0	0	0.0
62	120	120	11	9.2	3	2.5	0	0.0	0	0.0
64	60	60	4	6.7	3	5.0	0	0.0	0	0.0
65	96	100	5	5.2	2	2.0	0	0.0	0	0.0
66	100	100	15	15.0	2	2.0	0	0.0	0	0.0
107	120	119	13	10.8	2	1.7	0	0.0	0	0.0
Total	1526	1526	200	13.1	54	3.54	13	0.85	2	0.131

			PITUITARY							
	Controls examined		Adenoma				Carcinoma			
Study	m	f	m	%	f	%	m	%	f	%
3	110	110	57	51.8	78	70.9	0	0.0	2	1.8
4	100	100	62	62.0	69	69.0	0	0.0	1	1.0
5	100	100	50	50.0	73	73.0	1	1.0	0	0.0
6	60	60	23	38.3	45	75.0	1	1.7	1	1.7
7	50	50	27	54.0	40	80.0	0	0.0	2	4.0
8	99	100	56	56.6	71	71.0	0	0.0	1	1.0
9	120	119	55	45.8	91	76.5	0	0.0	2	1.7
10	100	100	55	55.0	65	65.0	0	0.0	3	3.0
11	100	100	66	66.0	83	83.0	0	0.0	0	0.0
13	60	60	27	45.0	45	75.0	0	0.0	0	0.0
61	128	130	75	58.6	99	76.2	0	0.0	4	3.1
62	120	120	58	48.3	82	68.3	0	0.0	1	0.8
64	60	60	34	56.7	45	75.0	0	0.0	1	1.7
65	96	100	40	41.7	70	70.0	1	1.0	1	1.0
66	100	100	53	53.0	80	80.0	0	0.0	1	1.0
107	120	120	30	25.0	63	52.5	0	0.0	2	1.7
Total	1523	1529	768	50.4	1098	71.9	3	0.2	22	1.439

End of study dates			
Study code	Study end	Study code	Study end
3	28/10/92	11	13/08/91
4	20/11/92	13	03/08/92
5	20/08/93	61	14/11/95
6	03/05/94	62	24/01/96
7	16/03/90	64	30/04/97
8	04/02/90	65	01/10/97
9	22/09/94	66	27/11/93
10	31/07/94	107	09/10/97

APPEARS THIS WAY
ON ORIGINAL

Toxicokinetics

Blood samples for toxicokinetic evaluation were obtained from the lateral caudal vein from 3 rats/sex/group at 1, 2, 3, 4, 6, 8 and 24 hours after dosing at Weeks 1, 2, 13, 52 and 78. The analyses were done at ——— The table which follows is sponsor's summary of toxicokinetic parameters derived from the concentrations found in blood and a comparison of doses administered and exposures in rat and man.

Unfortunately, Week 1 blood samples were not analyzed because of technical difficulties in blood collection at that time interval. It should be noted from the AUC and C_{max} toxicokinetic data of the 13- and 26-week rat studies (pages 35 and 44) that there were very large increases of frovatriptan concentrations in blood at Weeks 13 or 26, compared to Day 1. Similarly, very large increases in blood concentrations were also observed in the 4-week rat study following 4 weeks of treatment, compared to blood concentrations on Day 1. The data in the tables below generally indicates that in this 2-year study, blood concentrations in both males and females after treatment for 2, 13, 52 and even 78 weeks were continuously increasing with time, indicating bioaccumulation, and that steady state does not appear to occur even after treatment for 52 or 78 weeks of treatment.

In general, there was apparent dose proportionality for AUC and C_{max} values for both sexes at the
Week 2 and Week 13 sampling periods. For the Week 52 and 78, the AUC values were generally greater than proportional, particularly between the intermediate and high dose levels.

The mean T_{max} varied from 1.0 to 8.0 h with no apparent differences in terms of sampling
period, dose or sex.

The last table on the page that follows is sponsor's comparison of doses in rat and man, based on mg/kg, mg/m^2 and ratio of systemic exposures based on AUC found in blood of rats at start of the carcinogenicity study (presumably Week 2) and end of the study.

Sponsor's summary of toxicokinetic parameters is as follows:

Table 5.3.2.2.G5

Mean C_{max} (µg/mL) from 104-Week Rat Oncogenicity Study

Dose	Week 2		Week 13		Week 52		Week 78	
(mg/kg/day)	Male	Female	Male	Female	Male	Female	Male	Female
8.5	0.20	0.25	0.21	0.44	0.26	0.25	0.63	0.54
27	0.78	0.87	1.14	0.99	1.64	1.44	1.53	1.18
85	2.33	2.40	2.65	1.70	5.95	3.99	4.70	4.19

Table 5.3.2.2.G6

Mean AUC (µg.h/mL) from 104-Week Rat Oncogenicity Study

Dose	Week 2		Week 13		Week 52		Week 78	
(mg/kg/day)	Male	Female	Male	Female	Male	Female	Male	Female
8.5	3.09	3.40	2.90	3.60	3.87	3.39	9.25	8.19
27	9.81	10.88	14.88	11.11	15.87	14.47	18.42	18.96
85	31.86	37.56	44.29	28.33	75.38	58.56	60.43	87.67 (56.94)

Table 5.3.2.2.G7

Mean t_{max} (h) from 104-Week Rat Oncogenicity Study

Dose	Week 2		Week 13		Week 52		Week 78	
(mg/kg/day)	Male	Female	Male	Female	Male	Female	Male	Female
8.5	2.0	6.0	1.0	2.0	2.0	2.0	8.0	3.0
27	4.0	3.0	6.0	6.0	4.0	3.0	3.0	3.0
85	3.0	4.0	6.0	2.0	4.0	4.0	8.0	8.0

Table 5.2.2.1C

Comparison of Doses Administered and Exposures

to Frovatriptan in Rat and Man

Species	Dose Administered mg/kg/day	Dose Administered mg/m²	Exposure to Frovatriptan in Blood AUC µg.h/mL	
			Start of study	End of study
Rat	8.5	55	3.1	7.5
	27	176	10.9	17.0
	85	553	33.4	72.3
Man	0.04	1.6	0.094	-
Multiple Rat : Man				
8.5 mg/kg/day	213	34	33	80
27 mg/kg/day	675	110	106	180
85 mg/kg/day	2125	346	355	750

3. Dog

52-Week Oral (Capsule) Dog Study with a 26 Week Interim Kill (Vol 35-36)

Report No: 1165/32 ———

Performing Laboratory [ ]

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

Dates Performed: Treatment initiated on 4/18/96, necropsies completed 4/20/97.

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

Test Animals: Pure beagle dogs (from ———————————— . At initiation of dosing the dogs were 8-9 months old and body weights were 5.90-9.05 kg for males and 6.00-7.70 kg for females.

Test Substance: Batch number 60467-05, 60475-06, 60532-12, 60514, 60531-11 and 60587-14; purity of 98.2, 103.1, 98.6, 99.7, 100.8 and 97.7%, respectively, for the 6 different batches.

Procedure: Frovatriptan was administered in capsules to 6 dog/sex/group at doses of 0, 1, 5 and 12.5 mg/kg/day. Two/sex/group were killed following 26 weeks of treatment and the remaining 4/sex/group were killed following 52 weeks of treatment. Owing to the absence of clinical signs, the high dose level was increased to 15 mg/kg/day in week 8, then 17.5 mg/kg/day in week 12 and finally 20 mg/kg/day in week 29. Dosage is based on free base. Animals were observed daily for clinical signs, morbidity and mortality, weekly for body weights and food consumption. Ophthalmology was performed on all animals at pretreatment, in Week 25 and 51. Electrocardiography was performed on all animals at pre-treatment, in weeks 6, 12, 25, 33 and 51. Clinical pathology and urinalyses were performed from jugular vein blood and urine samples collected from all dogs at pre-treatment, in weeks 13, 26 and 52. Hematology included hemoglobin concentration, red blood cell count, PCV, MCV, MCH, MCHC, total and differential white cell count, platelet count, prothrombin time and activated partial thromboplastin time. Bone marrow smears were obtained at necropsy for reticulocyte count but not examined. Clinical chemistry included aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, GGT, sodium, potassium, calcium, inorganic phosphorus, chloride, total protein, albumin, globulin, A/G ratio, total cholesterol, triglycerides, glucose, urea, total bilirubin and creatinine. For toxicokinetics, blood samples were drawn from

the jugular vein of all animals at time points at pre-treatment and 1, 2, 3, 4, 8 and 24 hours after dosing on Day 1, Weeks 26 and 52.

Postmortem evaluations of all animals included routine gross pathology and weighing of adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, and testes with epididymides combined thyroids with parathyroids and uterus. Left and right organs were weighed together. For histopathology, samples of the following tissues were fixed in 10% neutral formalin, with the exception of eyes and optic nerves, which were fixed in Davidson's fluid: adrenals, aorta, brain, cecum, colon, duodenum, eyes and optic nerves, femur with marrow and articular surface, gall bladder, gross lesions, heart, ileum, jejunum, kidneys, lacrimal glands, liver, lungs with mainstream bronchi, mammary glands, liver, lungs, mammary glands of females only, mandibular lymph nodes, mesenteric lymph nodes, muscle (quadriceps), nasal turbinates, nasopharynx, esophagus, optic nerves, ovaries, pancreas, pituitary, prostate, rectum with anus, salivary glands, sciatic nerves, seminal vesicles, skin, spinal cord (cervical, lumbar, thoracic), spleen, sternum with bone marrow, stomach, testes with epididymides, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus and vagina. Microscopic evaluation included all indicated tissues from all animals.

Rationale for Dose Selection: The initial high dose of 12.5 mg/kg/day was based on data from a previous 14 day dog study — study 1165/36) in which doses of 15 and 20 mg/kg/day initially caused 50-130% increases in heart rate and in heart strength compared to pre-treatment for up to 6 to 8 hours post dosing which remained 25-40% higher at 24 hours post dosing. This effect was not observed at 10 mg/kg/day in the same study and in 28-day dog study (1165/6), there were no indications of toxicity at 10 mg/kg/day. Severely adverse reactions were seen in 2 dogs following a single 50 mg/kg dose which persisted to 48 hours when the dogs had to be killed and adverse reactions were seen in a dose escalating study up to 20 mg (Study No. T93602).

Results

Main Study (Compound related effects)

Body Weight: All animals gained less body weight than expected during the first week of treatment, based on the 4 week pretreatment period, but the effect was most marked in drug treated dogs. Generally, this difference in mean body weight between treated and controls remained throughout the study, although body weight gains after the first week were generally similar. There were no statistically significant differences in body weight or body weight gain due to treatment. See Figures 1 and 2.

Electrocardiography: There were no clear treatment related effects on ECG parameters at any time.

Other Effects: There were no effects on mortality or morbidity, no consistent effects on hematology, clinical chemistry, urinalyses, organ weights, gross or microscopic pathology.

FIGURE 1
Males
Group mean body weight (kg)

Test article Control VML 251
Group 1 2 3 4
Level (mg/kg/day) 0 1 5 12.5/15/17.5/20+
+ dose increased to 15 in Week 8, to 17.5 in Week 12 and to 20 in Week 29

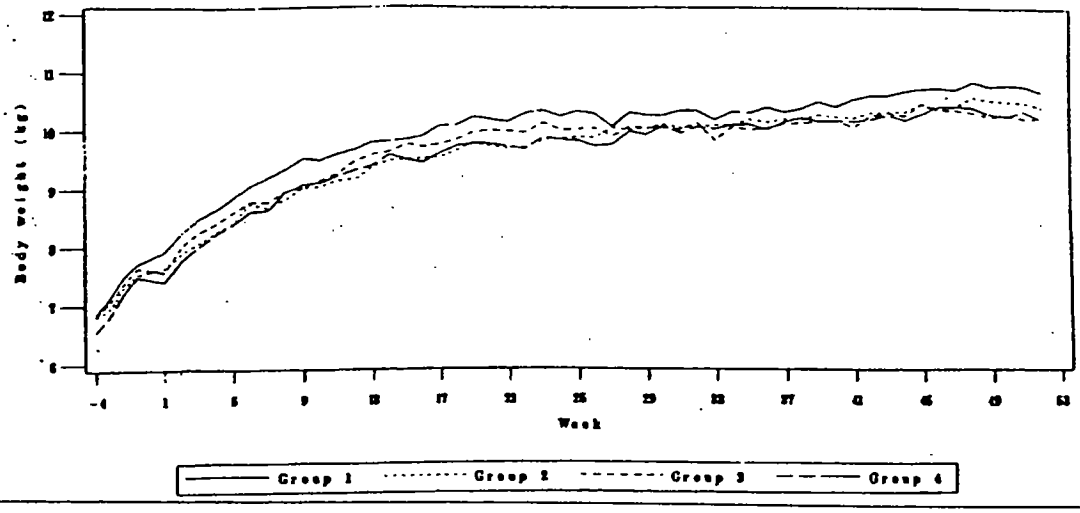
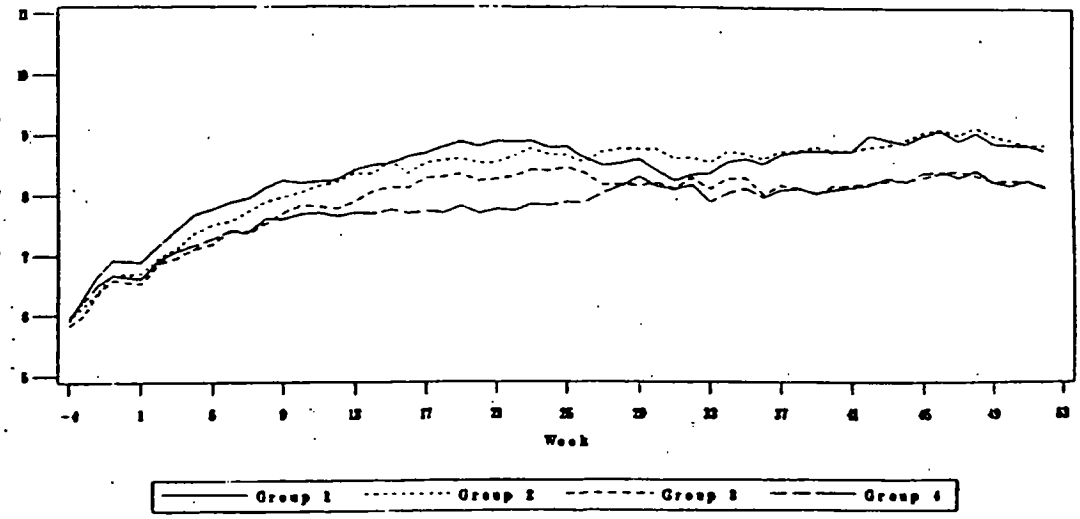


FIGURE 2
Females
Group mean body weight (kg)

Test article Control VML 251
Group 1 2 3 4
Level (mg/kg/day) 0 1 5 12.5/15/17.5/20+
+ dose increased to 15 in week 8 and to 17.5 in Week 12 and to 20 in Week 29



Toxicokinetics:

Following is sponsor's summary of toxicokinetic data.

Mean data for Males

Dose	AUC (ng.h/mL)			C_{max} (ng/mL)			T_{max} (h)		
(mg/kg/day)	Day 1	Week 26	Week 52	Day 1	Week 26	Week 52	Day 1	Week 26	Week 52
1	-	812.4	4939.8	31.3	89.4	255.5	4.2	2.2	16.0
5	1999.3	4522.8	6776.5	254.9	363.1	809.5	2.8	3.3	1.3
12.5/15/17.5/20	5231.7	12436.4	11632.1	565.9	1100.0	1392.1	3.8	2.8	3.3

- unsuitable data to calculate

Mean data for Females

Dose	AUC (ng.h/mL)			C_{max} (ng/mL)			T_{max} (h)		
(mg/kg/day)	Day 1	Week 26	Week 52	Day 1	Week 26	Week 52	Day 1	Week 26	Week 52
1	-	768.3	824.5	44.6	57.5	63.3	3.5	4.3	8.3
5	1775.2	4380.2	4833.9	169.8	405.6	430.2	3.8	3.2	2.5
12.5/15/17.5/20	4855.6	13474.9	12540.4	423.6	1220.5	1102.0	4.0	3.7	4.5

- unsuitable data to calculate

Blood levels of frovatriptan were generally dose proportional with no apparent differences between males and females. AUC and Cmax values in weeks 26 and 52 were approximately twice the day 1 values, indicating bioaccumulation during the first 6 months.

The following is a comparison of doses administered and exposures to frovatriptan in dogs and man.

Species	Dose mg/kg/day	Dose mg/m^2/day	Exposure to Frovatriptan in Blood[1] ug.hr/mL	
			Day 1	Week 52
Dog	1	20	-	824.5
	5	100	1999.3	6776.5
	12.5 and 20	250 & 400	5231.7	12540.4
Man	0.04	1.5	0.094	
Ratio Dog:Man	25	13.3	-	
	125	66.7	21,266	
	312.5 & 500	166.7	55,656	

[1] Based on AUC found in male, except Day 1 high dose where value found for females was used because of an apparently unrealistically high value found in the males.

[2] High dose was 12.5 mg/kg/day on Day 1 but it had been increased to 20 mg/kg/day by Week 29

C. Mutagenicity Tests (Vol 44 and 45)

a. Bacterial Reverse Mutation Studies

Performing Laboratory: [ ]

Quality Assurance: Statements of GLP compliance are included.

Tester strains: *S typhimurium* TA 98, TA 100, TA 1535 and TA 1537, *E coli* strains WP2 pKM101 and WP2 pKM 101

In the first study, a clearly positive response for mutagenicity for *E coli* strain WP2 uvra pKM 101 was obtained only in the absence of Aroclor 1254-induced rat liver S9. Therefore, three additional tests (total of four studies) with four different preparations of frovatriptan were performed. All four studies were identical in procedure. There were 2 experiments in each of the 4 studies. In Experiment 1, concentrations tested were 8, 40, 200, 1000 and 5000 ug/plate, both in the presence and absence of S9. In Experiment 2, concentrations of SB209509 tested were 1000, 2000, 3000, 4000 and 5000 ug/plate and there was a modified metabolic activation condition consisting of a pre-incubation step with S9. For pre-incubation, the test compound at each of the 4 concentrations and controls were mixed together with S9 and bacteria, then incubated for 1 hour. The molten agar was added to the pre-incubation mix before addition to the plates as an overlay. In this way, it was hoped that there would be an increase in metabolically derived and possibly mutagenic compounds. Triplicate plates were used at each concentration. Negative controls were done with quintuplet plates using solvent, both with and without S9. Positive controls were with triplicate plates, both with and without S9, using appropriate mutagenic substances for each bacterial strain.

Following is sponsor's summary of all four studies, which is taken from sponsor's briefing document, dated July 7, 1999.

APPEARS THIS WAY
ON ORIGINAL

Table 1: Summary of the results of the bacterial mutation studies with frovatriptan

Study No. (year)	Strain	Experiment 1[1]		Experiment 2[1]		Conclusions by study
		+S9	-S9	+S9	-S9	
Study No. 1165/16.1 (1995)	TA98	-	-	1.7x at 4000 1.5x at 5000	-	Except for *E. coli (uvrA)* all responses were less than 2 times control, and none were reproduced between experiments. Judged negative. For *E. coli (uvrA)*, -S9 responses were greater than 2 times control, but the extent of dose response was not reproduced between experiments. Judged positive.
	TA100	-	1.3x at 5000	-	-	
	TA1535	-	-	-	1.6x at 4000	
	TA 1537	-	-	-	-	
	WP2 pKM101	-	-	1.5x at 2000 1.7x at 4000	-	
	WP2 *uvrA* pKM101	-	*2.6x at 5000*	-	*1.7x at 1000* *2.4x at 2000* *2.6x at 3000* *3.3x at 4000* *3.4x at 5000*	
Study No. 1165/16.2 (1995)	TA98	-	-	-	-	The single response was less than 2 times control and not reproduced between experiments. Judged negative.
	TA100	-	-	-	-	
	TA1535	-	-	-	-	
	TA 1537	-	-	-	-	
	WP2 pKM101	-	-	-	-	
	WP2 *uvrA* pKM101	-	1.6x at 5000	-	-	
Study No. 1165/107 (1997)	TA98	-	-	-	-	All responses were less than 2 times control, and none were reproduced between experiments, except for -S9 high dose *E. coli (uvrA)*. Judged negative.
	TA100	-	-	-	-	
	TA1535	-	-	-	-	
	TA 1537	-	-	-	-	
	WP2 pKM101	-	-	1.35x at 3000 1.42x at 4000 1.63x at 5000	1.29x at 4000 -	
	WP2 *uvrA* pKM101	-	1.37x at 5000	1.25x at 4000 1.22x at 5000	1.31x at 5000	
Study No. 1165/176 (1998)	TA98	-	-	-	-	Judged negative.
	TA100	-	-	-	-	
	TA1535	-	-	-	-	
	TA 1537	-	-	-	-	
	WP2 pKM101	-	-	-	-	
	WP2 *uvrA* pKM101	-	-	-	-	

With Batch HP 1 (Study No. 1165/16.1), a positive mutagenic response in an Ames test for *E coli* strain WP2 uvra pKM 101 was obtained only in the absence of Aroclor 1254-induced rat liver S9, at concentrations of 1000 to 5000 ug/plate (dose related, $P<0.001$, with a 1.7-to 3.4-fold increases in revertant colonies per plate at $\geq$1000 ug/plate). The Ames test was negative or equivocal for 5 different strains of *S typhymurium* and for *E coli* WP2 pKM101 both in the absence and presence of rat liver S9. This test was repeated three times using different batches of frovatriptan, each test with the same six bacterial strains, and sometimes comparing it to batch HP 1. In two of these tests, the responses for *E coli* strain WP2 uvra pKM 101 obtained only in the absence of S9 were equivocal; i.e., statistically significant but less than a 2-fold increase in revertant colonies. In the fourth test with "degraded and undegraded" frovatriptan, the results were negative.

——— which is the ——— of frovatriptan and a possible contaminant in frovatriptan preparations, and the desmethyl metabolite of frovatriptan, were tested in the exact same test systems but were found to be non-mutagenic.

b. Induction of Chromosome Aberrations in Cultured Human Blood Lymphocytes

HE Study No. 1165/17-1052

Performing Laboratory: [redacted]

Dates Study Performed: 10/12/94-11/17/94

Quality Assurance: A statement of GLP compliance is included

Test Substance: SB209509, Batch HP1 (94.2% purity, synthesized by SmithKline Beecham).

Procedure: Human lymphocytes were obtained from a healthy, non-smoking male (used in Experiment 1) and female (used in experiment 2) for two independent but similar studies. In experiment 1, concentrations tested in duplicate cultures for cell toxicity were 47.51, 67.87, 96.96, 138.5, 197.9, 282.7, 403.8, 576.9, 824.2, 1177, 1682 and 2408 ug/mL (concentrations expressed as free base). In Experiment 2, concentrations tested in duplicate cultures were 57.09, 76.12, 101.5, 135.3, 180.4, 240.6, 320.8, 427.7, 570.2, 760.3, 1014, 1352, 1802 and 2403 ug/mL. In both experiments, the incubation periods in cultures with the absence of S9 were for 20 and 44 hours (4 cultures total, 2 for each time period). In the presence of S9, the incubation period was for 3 hours (pulse treatment) plus 17 and 41 hours of recovery period, to the time of cell harvest. Sponsor estimates that the highest concentration of 2403 ug/mL [which caused 50-80% reduction in mitotic index (MI)] is equivalent to 10 mM of SB209509. Negative and positive controls were incubated in quadruplicate and harvested at 20 or 3 + 17 hours. Positive control consisted of 4-nitroquinoline 1-oxide (NQO) without S9 and cyclophosphamide (CPA) with S9.

At least 25 cells from each negative or positive control culture and 50 from each drug-treated culture was analyzed and scored in a blinded fashion. Aberrant cells were categorized for cells with 1) gaps, 2) structural aberrations excluding gaps and 3) polyploidy, endoreduplicated or hyperdiploidy. Probability values of ≤ 0.05 or better, compared to control, were accepted as significant using Fisher's exact test. Criteria for a positive response were; 1) a statistically significant increase of cells with structural aberrations (excluding gaps) occurred at 1 or more concentrations, 2) proportion of cells with structural aberrations at such doses exceeded the normal range, and 3) the results were confirmed in Experiment 2.

Criteria for selection of doses for cytogenetic analysis: In both experiments the highest dose selected for the 20 hour or 3 + 17 hour treatments was to be the one which caused a 50-80% reduction in mitotic index or the highest dose incubated. Slides from this and the next 2 lower doses were taken for microscopic analysis. If negative or equivocal results were obtained in Experiment 1, a single dose from the delayed harvest (44 or 3 + 41 hour) was scored in Experiment 2 corresponding to the top dose in Experiment 1.

Compound Related Effects: Batch HP 1 in the absence of S9 induced increased frequencies of cells with aberrations (excluding gaps) in both replicates at 403.8 ug/mL (highest concentration tested) in Experiment 1 (n.s., but exceeded historical control). Concentration of 576.9 and 403.8 ug/mL induced a reduction in MI of 79.2 and 64%, respectively. In Experiment 2, increased frequencies of cells with aberrations (excluding gaps) were seen at 570.2 ($P \leq 0.05$), 760.3 (n.s.) and 1014 ($P \leq 0.01$) ug/mL (exceeded historical control at all 3 concentrations), which sponsor claimed occurred in the presence of cytotoxicity. Reduction in MI at 1014 ug/mL in this study was 63%. In the presence of S9, no increases in cells with aberrations were found at concentrations up to 2403 ug/mL (which induced no reduction in MI).

APPENDIX 1

SB209509: cells with structural aberrations

TABLE 1
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 1
Donor sex: Male

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Signifi-cance §	Mitotic index (mean)
Solvent	A	100	4	3		4.0
	B	100	5	3		5.6
	Totals	200	9	6		(4.8)
197.9	A	100	5	3		2.5
	B	100	0	0		3.2
	Totals	200	5	3	NS	(2.9)
282.7	A	100	1	1		2.5
	B	100	3	1		2.1
	Totals	200	4	2	NS	(2.3)
403.8	A	100	[illegible]	[illegible]		2.0
	B	100	4	[illegible]		1.5
	Totals	200	12	9	NS	(1.8)
NQO, 2.5	A	25	[illegible]	[illegible]		
	B	25	[illegible]	[illegible]		
	Totals	50	26	24	p ≤0.001	

§ Statistical significance

APPEARS THIS WAY
ON ORIGINAL

NS = not significant

Numbers highlighted exceed historical negative control range (Appendix 7)

APPENDIX 1 (continued)

SB209509: cells with structural aberrations

TABLE 3
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2
Donor sex: Female

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	2	1		3.0
	B	100	2	1		3.5
	Totals	200	4	2		(3.3)
570.2	A	100	[illegible]	3		3.0
	B	100	[illegible]	[illegible]		2.1
	Totals	200	18	8	p ≤0.05	(2.6)
760.3	A	100	[illegible]	3		2.0
	B	100	[illegible]	2		1.8
	Totals	200	18	5	NS	(1.9)
1014	A	94	[illegible]	[illegible]		1.3
	B	100	[illegible]	[illegible]		1.1
	Totals	194	40	17	p ≤0.001	(1.2)
NQO, 2.5	A	50	[illegible]	[illegible]		
	B	25	[illegible]	[illegible]		
	Totals	75	18	9	p ≤0.001	

§ Statistical significance

APPEARS THIS WAY
ON ORIGINAL

NS = not significant

Numbers highlighted exceed historical negative control range (Appendix 7)

c. Induction of Chromosome Aberrations in Cultured Human Blood Lymphocytes
—— Batch —/5310/03)

HE Study No. 1165/40-1052

Performing Laboratory: [redacted]

Dates Study Performed: 7/13/95-8/23/95

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, Batch — 5310/03 (99.58% purity). The reviewing pharmacologist for IND —— indicated that this batch, according to sponsor's statement, was intended for human use (Review #1 by KC Wadhwani, dated 12/11/95).

Procedure: Procedure was exactly the same as for the previous study, again using human lymphocytes obtained from a healthy, non-smoking male (used in Experiment 1) and female (used in experiment 2) donor for two independent but similar studies. There was one exception; Experiment 2 did not include a 44-hour treatment in the absence of S9. In experiment 1, concentrations tested in duplicate cultures for cell toxicity were 46.96, 67.09, 95.84, 136.9, 195.6, 279.4, 399.2, 570.2, 814.6, 1164, 1663 and 2375 ug/mL (concentrations expressed as free base). In Experiment 2, concentrations tested in duplicate cultures were 163.2, 204.0, 318.8, 398.5, 498.1, 622.6, 778.3, 979.9, 1216, 1520, 190 and 2373 ug/mL.

Compound Related Effects: — 5310/03 in the absence of S9 induced increased frequencies of cells with aberrations (including and excluding gaps) in both replicates at 1164 ug/mL (highest concentration tested in Experiment 1 (exceeded historical control; $P \leq 0.001$ for excluding gaps). In Experiment 2, increased frequencies of cells with aberrations (excluding gaps) were seen at 773.8 ($P \leq 0.001$ and exceeded historical control concentrations), which sponsor claimed occurred in the presence of cytotoxicity in both experiments. Reduction in MI at highest doses in both experiments was around 63%. In the presence of S9, no increases in cells with aberrations were found at concentrations up to 2375 ug/mL (which induced no reduction in MI).

APPENDIX 1

SB209509 ——— Batch —/5310/03): cells with structural aberrations

TABLE 1
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 1
Donor sex: Male

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Signifi-cance §	Mitotic index (mean)
Solvent	A	100	1	1		2.9
	B	100	2	0		3.4
	Totals	200	3	1		(3.2)
S70.2	A	100	2	2		1.4
	B	100	4	2		2.4
	Totals	200	6	4	NS	(1.9)
814.6	A	100	5	1		1.1
	B	100	5	4		1.7
	Totals	200	10	5	NS	(1.4)
1164	A	100	19	13		1.0
	B	100	11	10		1.1
	Totals	200	30	23	p ≤0.001	(1.1)
NQO, 2.5	A	25	6	6		
	B	25	4	4		
	Totals	50	10	10	p ≤0.001	

§ Statistical significance (Appendix 5a)

NS = not significant

APPEARS THIS WAY ON ORIGINAL

Numbers highlighted exceed historical negative control range (Appendix 6)

APPENDIX 1

SB209509 ——— Batch –/5310/03): cells with structural aberrations

TABLE 3
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2
Donor sex: Female

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Signifi-cance §	Mitotic index (mean)
Solvent	A	100	2	1		3.5
	B	100	2	1		3.6
	Totals	200	4	2		(3.6)
662.6	A	100	6	5		1.3
	B	100	3	2		1.4
	Totals	200	9	7	NS	(1.4)
778.3	A	100	10	5		0.6
	B	100	13	9		1.5
	Totals	200	23	14	p ≤0.001	(1.1)
972.9	A	100	4	2		0.8
	B	100	7	3		1.3
	Totals	200	11	5	NS	(1.1)
NQO, 2.5	A	25	11	11		
	B	24	6	6		
	Totals	49	17	17	p ≤0.001	

§ Statistical significance (Appendix 5b)

NS = not significant

APPEARS THIS WAY
ON ORIGINAL

Numbers highlighted exceed historical negative control range (Appendix 6)

d. (Batch 60532-12) Induction of Chromosome Aberrations in Cultured Human Blood Lymphocytes

HE Study No. 1165/108-1052

Performing Laboratory [redacted]

Dates Study Performed: 3/3/97-5/7/97

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, Batch No. 60532-12 (98.6% purity).

Procedure: Procedure was basically the same as for the previous study, again using human lymphocytes obtained from a healthy, non-smoking male (used in Experiment 1) and female (used in experiment 2) donor for two independent but similar studies. In experiment 1, concentrations tested in duplicate cultures for cell toxicity were 101.3, 135.0, 180.0, 240.0, 320.1, 426.7, 569.0, 758.6, 1012, 1349, 1798 and 2398 ug/mL (concentrations expressed as free base). In Experiment 2, concentrations tested in duplicate cultures were 105.5, 131.8, 164.8, 206.0, 257.5, 321.9, 402.3, 502.9, 602.6, 758.7, 982.2, 1228, 1535, 1918 and 2398 ug/mL.

Compound Related Effects: In Experiment 1, Batch No. 60532-12 in the absence of S9 did not induced increased frequencies of cells with aberrations at thee 3 concentrations tested (240, 320.1 and 426.7 ug/mL). In Experiment 2, slightly increased frequencies of cells with aberrations (excluding gaps) were seen at 982.2 ug/mL ($P \leq 0.05$ and exceeded historical control), which occurred in the presence of cytotoxicity. In Experiment 2, following the prolonged incubation period of 44 hour, the number of cells with aberration exceeded historical control, but the increase compared to experimental control was not statistically significant. In the presence of S9, no increases in cells with aberrations were found at concentrations up to 2398 ug/mL (which induced no reduction in MI).

As indicated on page 74, one of the 3 criteria for a positive response in this assay is that the results have to be confirmed in 2 experiments. Although the results of this study are equivocal because a positive response was obtained in only 1 of the 2 experiments, they support the positive results obtained for frovatriptan in the two preceding studies.

APPENDIX 1

VML251 (batch number 60532-12): cells with structural aberrations

TABLE 1
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 1
Donor sex: male

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	3	1		4.4
	B	100	1	1		4.1
	Totals	200	4	2		(4.3)
240	A	100	0	0		2.3
	B	100	4	2		2.7
	Totals	200	4	2	NS	(2.5)
320.1	A	100	3	2		2.4
	B	100	3	2		2.3
	Totals	200	6	4	NS	(2.4)
426.7	A	100	2	2		1.0
	B	100	4	4		1.6
	Totals	200	6	6	NS	(1.3)
NQO, 5	A	100	[redacted]	[redacted]		
	B	100				
	Totals	200	77	74	p ≤0.001	

§ statistical significance
NS not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

APPENDIX 1

VML251 (batch number 60532-12): cells with structural aberrations

TABLE 3
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2
Donor sex: female

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	4	2		6.5
	B	100	4	2		4.0
	Totals	200	8	4		(5.3)
628.6	A	100	4	4		2.2
	B	100	7	[illegible]		3.3
	Totals	200	11	9	NS	(2.8)
785.8	A	100	[illegible]	4		2.8
	B	100	7	4		2.8
	Totals	200	15	8	NS	(2.8)
982.2	A	100	[illegible]	[illegible]		2.3
	B	100	[illegible]	[illegible]		1.8
	Totals	200	23	12	p ≤0.05	(2.1)
NQO, 2.5	A	100	[illegible]	[illegible]		
	B	100	[illegible]	[illegible]		
	Totals	200	51	44	p ≤0.001	

§ statistical significance
NS not significant
Numbers highlighted exceeded historical negative control ranges (Appendix 6)

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

e. Mutation at the Thymidine Kinase (tk) Locus in Mouse Lymphoma L5178Y Cells Using the ———————

HE Study No. 1165/26-1052

Performing Laboratory [ ]

Dates Study Performed: 11/24/94-1/23/95

Quality Assurance: A statement of GLP compliance is included

Test Substance: SB209509, Batch No. —/5310/01 of — (purity not specified).

Culture Used: L5178Y $TK^{+/-}$ mouse lymphoma cells, obtained from ———————

Procedure: Following a cytotoxicity range-finding test, concentrations selected in experiment 1 for mutation assessment were 0, 500, 1000, 1500, 2000, and 2455 ug/mL. In experiment 2, concentrations selected were 1000, 1500, 2000 and 2455 ug/mL. Positive control was 4-nitroquinoline 1-oxide (-S9) or benzo(a)pyrene (+S9). After incubation of the cultures for 2 days (expression period for TK^{-} mutations) in the presence of test compound or control, all doses were plated for viability and 5-trifluorothymidine resistance. The highest concentrations yielded 64.1 102.1% cell survival in the absence and presence of S9, respectively.

Results: No increase in mutant frequency was observed both in the absence of presence of S9.

f. Induction of Micronuclei in Bone Marrow of Mice

Study No. 1165/27-1052

Performing Laboratory: [redacted]

Dates Study Performed: 11/29/94-6/21/95

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, Batch — 5310/01

Procedure: CD-1 mice, from [redacted] were used. A range-finder study was conducted to determine highest dose that could be administered (based on clinical signs and body weight gain). For the main study the animals were 39-46 days old; males were weighed 24-32 g, females weighed 19-25 g. Control or test substance was administered in a 1% methyl cellulose suspension for 2 consecutive days by oral gavage to 10/sex/group at 371, 742 and 1484 mg/kg/day; dose volume of 20 mL/kg. Bone marrow for cytogenetic analysis was obtained from 5/sex/group at 24 and 48 hours after dosing. Total numbers of polychromatic (PCE) and normochromatic (NCE) erythrocytes and micronuclei in each animal were counted. The individual and group frequencies of micronuclei in 1000 (PCE), based on approximately 2000 pce for the 24 hour samples and 4000 pce for the 48 hour samples, were determined and compared to control and background incidence. The ratio of PCE/NCE was obtained to determine if there was a decrease in groups of treated animals, which could be taken as evidence of bone marrow toxicity.

Compound Related Effects: There was no compound related increase in micronuclei.

Samples of blood taken from a satellite group of 10 animals of each sex on the highest dose level at 0, 1, 2, 3 and 24 hours post-dosing following the initial dose revealed significant levels of drug in the blood up to 24 hours after dosing.

k. Induction of Micronuclei in Bone Marrow of Mice —— Batch —/5310/03)

Study No. 1165/41-1052

Performing Laboratory: [redacted]

Dates Study Performed: 6/1/95-8/18/95

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, —— Batch — /5310/03 (Intended for human use)

Procedure: The procedure for this study was similar the previous one (1165/40-1052). Doses tested for the main study were 1000, 1250 and 1500 mg/kg/day.

Compound Related Effects: There was no compound related increase in micronuclei due to treatment with —— Batch — /5310/03.

f. Unscheduled DNA Synthesis in Rat Liver Using *an Vivo/In Vitro* Procedure (——— Batch ~ '5310/03)

Study No. 1165/35-052

Performing Laboratory [ ]

Dates Study Performed: 5/24/95 to 9/20/95

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, ——— Batch – '5310/03 (Intended for human use)

Procedure: Male Wistar rats, from ——————— were housed in groups of 3. A series of range-finder studies were conducted to determine highest dose that could be administered (based on clinical signs and body weight gain). For the main studies, the animals were 43-53 days old and weighed 186-266g. Control or test substance was administered in a 1% methylcellulose suspension at 10 mL/kg to 5 animals per group (Experiments 1 and 2) or 4 animals per group (Experiment 3). Doses tested were 402 and 1272 mg/kg in Experiments 1 and 2, and 2000 mg/kg in Experiment 3. Positive control was 2-acetamidofluorene (2-AAF) in Experiment 1 or dimethylnitrosamine (DMN) in Experiments 2 and 3. Hepatocytes were prepared from 3 of the 4 or 5 animals per group that were successfully dosed. Animals were killed 12-14 hours after dosing for Experiment 1 and 2-4 hours after dosing for Experiment 2. For Experiment 3, animals were killed at both the 12-14 and 2-4 hour time intervals (4/group at each of the 2 time intervals). The liver of each rat was perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from 3 animals in each group which were then treated with [^{3}H]-thymidine. Six slides were prepared from each animal and autoradiographs were prepared from at least 3/group. After development of the emulsion and staining, the net grain count (NNG) was determined (number of grains present in the nucleus minus mean number of grains in 3 equivalent areas of the cytoplasm) for 2 of the 3 slides for each animal.

Results: Oral treatment of male rats with SB 209509 at doses of 402, 1272 or 2000 mg/kg did not result in increased UDS in hepatocytes obtained from rats that were killed approximately 12-14 hours or 2-4 hours after dosing.

D. Reproductive Toxicology

1. Fertility

a. Oral Gavage Male Fertility Study in the Rat

Study No. 1165/79-1050

Performing Laboratory [redacted]

Dates Study Performed: 12/2/96-3/7/97

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, Batch numbers 60532-12, 60514-10 and 60531-11; purities of 98.6, 99.7 and 100.8%, respectively.

Test Animals: Crl:CDBR rats, [redacted] At initiation of dosing, male rats were 11 weeks old and body weights were 335.3-440.1 g; females were 12-13 weeks old.

Doses Administered: 0, 100, 500 and 1000 mg/kg/day

Procedure: Frovatriptan was administered daily to 24 males/group starting 4 weeks prior to pairing with untreated females. Controls received 1% aqueous methylcellulose vehicle. Dosing was continued during and after the dosing period, for a total of 14 weeks. During the treatment period, males were examined daily for mortality and clinical signs, body weights and food consumption were measured weekly. Mating was confirmed by the presence of a vaginal plug or vaginal sperm.

On the day following the final dose, the males were examined by gross necropsy. A small sample of seminal fluid was extruded from the left epididymis for assessment of sperm viability and sperm count under a light microscope. Sperm morphology was evaluated from eosin Y stained slide preparations in control and high dose groups. Volume of seminal fluid in each male was measured. Histopathology was performed on testes, epidiymides, seminal vesicles, prostate, coagulating gland and lesions from 6 males in control and high dose groups.

On gestation day (GD) 20, females were C-sectioned for determination of pregnancy status, corpora lutea count, number and intrauterine positions of implantations, live fetuses, early and late intrauterine deaths. If pregnancy was not detected, the uteri were stained with 10% ammonium sulfide. Live fetuses were weighed, sexed and examined externally for anomalies.

Results: Only clinical signs in the majority of males (red extremities, salivation, paddling) following each dose were seen; no effects on male fertility parameters were observed.

b. Oral Gavage Female Fertility and Early Embryonic Development in the Rat

Study No. 1165/11-1050

Performing Laboratory: [redacted]

Dates Study Performed: Treatment initiated 2/27/95, necropsies completed 4/15/95

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, Batch number HP-1; purity of 94.2%.

Test Animals: Crl:CD(SD)BR rats, [redacted]. At initiation of estrous cycle monitoring, the rats were 12-13 weeks old and body weights were 238.6-285.7 g in females and 345.7-447.0 g in males.

Doses Administered: 0, 96, 479 and 958 mg/kg/day (dosage based on free base)

Rationale for Dose Selection: Based on results of the 28 day rat study [redacted] Study No. 1165/5). In the 28-day study, the investigators concluded, "The administration of SB 209509-AX .... at dose levels up to and including 500 mg/kg/day was well tolerated".

Procedure: Frovatriptan was administered daily to 24 females/group starting 14 days prior to pairing for up to 15 days with untreated males. Dosing of the females was continued during the pairing period to GD 6. Controls received 1% aqueous methylcellulose vehicle and the dosing volume for all groups was 10 mL/kg.

The females were examined daily for mortality and clinical signs. Body weights and food consumption were measured weekly during the pairing period, then at 7 time intervals between GD 0 and GD 20. Water intake was recorded daily and reported weekly. Estrous cycles were monitored starting 14 days before initiation of treatment until mating was confirmed. The females were killed on GD 20 for assessment of uterine content. Data recorded included pregnancy status, corpora lutea count, number and intrauterine positions of implantations, live fetuses, early and late intrauterine deaths. If pregnancy was not detected, the uteri were stained with 10% ammonium sulfide. Live fetuses were weighed, sexed and examined externally for anomalies. All females were subjected to macroscopic pathology examination. Calculations included mating, fertility, fecundity indices, percentage of pre-implantation and post-implantation loss, and percent male fetuses.

Results:

Dams: Clinical signs consisting of red extremities for a few hours after dosing, salivation after dosing in all 3 treated groups, and a marginal decrease in body weight (1-2% before mating, 3%

by GD 6) and body weight gain compared to controls at high dose was associated with a slight decrease in food intake. Water intake during the treatment period was lower in the intermediate and high dose groups than control and predosing period.

Mating Performance and Pregnancy Rate: The tables that follow indicate that there were no treatment-related effects on fertility and fecundity indices. However, more females in the 3 treated groups mated on the first day of pairing than controls but there was an increase in number of females in the treated groups that did not mate during the estrous stage (no dose relationship). Females of the treated groups remained at the estrous stage of the cycle (vaginal cornification) longer than controls. The mating index (number of females that copulated/number of estrous cycles required for copulation X 100) showed a significantly increasing dose relationship ($P<0.05$, Cochran-Armitage test).

Caesarian Data: The mean numbers of corpora lutea, implantations and live fetuses were lower than controls ($P<0.05$, dose response). While mean corpora lutea count in the treated groups were within background range (17.4-19.5), pre-implantation losses in mid and high dose groups were 5.4 to 12.7% higher than expected from background control data. Consequently, the mean number of implantations in the mid and high dose groups were also below the background control range. Postimplantation losses in control and high dose groups were higher than expected. Since the percentage of live fetuses/implantation count was comparable to control in all treated groups, and since dosing was concluded on GD 6 (around the time of implantation), sponsor concluded that the dose-related reduction in mean litter size was due to a lower corpora lutea count (which is most likely due to a reduction in ovulation rate).

Skin masses were found at necropsy in 2 mid dose animals. Histopathology revealed that they were mammary carcinomas, which is unusual for this strain at such an early age (approximately 20 weeks). One carcinoma showed evidence of rapid growth with areas of necrosis and numerous mitotic figures. Sponsor nevertheless suggests that this was a fortuitous finding.

Fetal Data: No compound related effects.

In the discussion, sponsor points out that anesthetics, such as barbituates, and noradrenergic blocking agents (such as chlorinated pesticides) are known to extend the estrous cycle. They also known to impair ovulation by inhibiting the neural trigger which causes a mid-cycle LH surge that results in ovulation. In addition, delayed ovulation may be induced by substances that have a direct effect on the ovaries due to alteration of estrogen activity (endocrine disrupters). It has also been shown that constant estrus can occur when 5-HT_1 synthesis is blocked by *p*-chlorophenylalanine, an inhibitor of tryptophan hydroxylase.

TABLE 6

Group mating data

Test article	Control	SR 209509 - AX		
Group	1	2	3	4
Level (mg/kg/day)	0	96	479	958

6.2 Mating performance

		Group 1	Group 2	Group 3	Group 4
Group size at pairing:					
Male		24	24	24	24
Female		24	24	24	24
Number mating on Day of pairing:	1	4	15	18	10
	2	9	1	3	3
	3	6	1	2	3
	4	4	2		6
	5		2		2
	6				
	7				
	8		1	1	
	9				
	10				
	11				
	12				
	13				1
	14				
	15				
	Total:	24	22	24	24
Median pre-coital time (days)		2	1	1	2.5

TABLE 7

Group mean caesarian data

Test article	Control	SB 209503 - AX	
Group	1	3	4
Level (mg/kg/day)	0	479	958

7.1 Uterine/implantation data

	Group 1	Group 3	Group 4
Number of pregnant females with live foetuses at Day 20 gestation	23	20	22
Number of corpora lutea Mean number per female	436 19.0	378 18.9	395 18.0	383 17.6*DRI
Number of implantations Mean number per female	391 17.0	321 16.1	342 15.5	329 15.0*DRI
% pre-implantation loss	10.3	15.1	13.4	15.2
Number of early intrauterine deaths Mean number per female	30 1.3	17 0.9	16 0.7	29 1.3
Number of late intrauterine deaths Mean number per female	1 0.0	1 0.0	2 0.1	0 0.0
Number of dead foetuses Mean number per female	0 0.0	0 0.0	0 0.0	0 0.0
% post-implantation loss	7.9	5.6	5.3	8.8
Number of foetuses Mean number per female	360 15.7	303 15.2	325 14.8	300 13.6*DR
% of implantations	92.1	94.4	95.0	91.2

‡ excludes two animals with undetermined day of mating
*DRI p<0.05, decreasing dose response, Terpstra-Jonckheere test

c. Oral Gavage Developmental Toxicity Study in the Rat

Study No. 1165/13-1050

Performing Laboratory: [redacted]

Dates Study Performed: Treatment initiated 1/9/95, necropsies completed 1/27/95

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, Batch number —5310/02; purity not indicated.

Test Animals: Time mated Crl:CD(SD)BR rats, ———————— , approximately 9 weeks old, weighing 2.63 to 4.00 kg, were used..

Doses Administered: 0, 100, 500 and 1000 mg/kg/day (dosage based on free base)

Rationale for Dose Selection: Based on results of the 28 day rat study (— Study No. 1165/5). In the 28-day study, the investigators concluded, "The administration of SB 209509-AX .... at dose levels up to and including 500 mg/kg/day was well tolerated".

Procedure: Frovatriptan was administered daily to 30 females/group between GD 7 and GD 17. Controls received 1% aqueous methylcellulose vehicle and the dosing volume for all 4 groups was 10 mL/kg. The females were examined twice daily for morbidity and mortality, daily for clinical signs. Body weights and food consumption and water intake were measured 9 times between GD 3 and GD 20. C-sections were performed on GD 20 for assessment of pregnancy status, gravid uterine weight, corpora lutea count, number and intrauterine positions of implantations, live fetuses, early and late intrauterine deaths and dead fetuses. If pregnancy was not detected, the uteri were stained with 10% ammonium sulfide. Live fetuses and placentas were weighed, the fetuses were sexed and examined externally for anomalies. Approximately half the fetuses in each litter were dissected for visceral examination. They were then eviscerated and the carcasses processed to stain the ossified skeleton (Alizarin technique). The remaining fetuses were fixed and partially decalcified to obtain serial coronal sections through the nasal, orbital and cranial regions of the head.

Result; Dams:

Mortality: A control female was killed due to poor condition on GD 16. No macroscopic abnormality was detected at necropsy.

Clinical Signs: Red extremities in majority of females in all treated groups beginning 0.5 hours after dosing persisting for more than 4 hours but no longer present by 24 hours after dosing.

Body Weight, Food and Water Intake: At high dose there was an initial decrease in body weight gain from GD 6 to GD 7 ($P<0.001$) but the difference in mean body weight was only around 2%. Mean food intake for all 3 treated groups was significantly lower than control at various time periods during treatment.

Cesarian Data: The numbers of early and late uterine deaths, and the number of litters showing at least one intrauterine death was greater in the mid and high dose groups than in control (See sponsor's Table 7.1 which follows). Sponsor considered this relationship to treatment as unlikely because the percentage of post-implantation loss was within background control range (2.7 to 7.5%) and was marginally higher than concurrent controls.

Fetal Effects: The increase in mean placental weight ($P<0.05$) of low dose group (sponsor's Table 7.2) is considered a spurious effect. Litter and mean fetal weights in all treated groups were lower than control for all treated groups but sponsor concluded that they were not related to treatment because the differences were marginal and not dose related Table 7.2).

Sponsor's Table 7.3 is a summary of all external, visceral and skeletal fetal defects combined. Increased incidences of external and visceral variations were seen at mid and high doses. Dose related increased incidences (including low dose) of dilated ureters (a variation), unilateral and bilateral pelvic cavitation (a variation), hydronephrosis (a malformation) were observed, and one at high dose had hydroureter (a malformation). This indicates that the drug caused a syndrome of inter-related effects on the kidney. Incomplete ossification of sternebrae and subcutaneous jaw/mouth hemorrhage (table 8) and a tendency for treatment related increased incidences of incomplete ossification of skull and nasal bones, all of which are indicative of slight delay in fetal maturation. The total incidence of fetal malformations was higher at high dose than in other groups, which included hydronephrosis and anophthalmia, both of which may be treatment related.

NOAEL for maternal effects: 500 mg/kg/day based on marginal decrease in mean body weights.
NOAEL for fetal effects: <100 mg/kg/day, based on dose related increases in fetuses with visceral and skeletal variations and slightly delayed maturation.

TABLE 7

Group mean caesarian data

Test article	Control	SB 209509 - AX		
Group	1	2	3	4
Level (mg/kg/day)	0	100	500	1000

7.1 Uterine/implantation data

	Group 1	Group 2	Group 3	Group 4
Number of females with live foetuses at Day 20 gestation	28	29	30	29
Number of corpora lutea	434	433	468	440
Mean number per female	15.5	14.9	15.6	15.2
Number of implantations	394	393	422	403
Mean number per female	14.1	13.6	14.1	13.9
% pre-implantation loss	9.2 (13)	9.2 (17)	9.8 (20)	8.4 (15)
Number of early intrauterine deaths	20	19	24	26
Mean number per female	0.7	0.7	0.8	0.9
Number of late intrauterine deaths	1	2	2	6
Mean number per female	0.0	0.1	0.1	0.2
Number of dead foetuses	0	0	0	0
Mean number per female	0.0	0.0	0.0	0.0
% post-implantation loss	5.3 (14)	5.3 (13)	6.2 (19)	7.9 (18)
Number of foetuses	374	372	396	371
Mean number per female	13.3	12.8	13.2	12.8
% of implantations	94.9	94.7	93.8	92.1

( ) number of litters affected

TABLE 7

Group mean caesarian data

Test article	Control	SB 209509 - AX		
Group	1	2	3	4
Level (mg/kg/day)	0	100	500	1000

7.2 Foetal data

		Group 1	Group 2	Group 3	Group 4
Number of male foetuses		193	184	207	193
Number of female foetuses		181	188	189	178
% male foetuses		51.6	49.5	52.3	52.0
Mean litter weight (g)		52.14	49.07	49.95	48.87
Mean placental weight (g)	A	0.52	0.58*	0.55	0.54
Mean foetal weight (g)		3.94	3.84	3.80	3.83
Mean foetal weight (g) - males only		4.06	3.94	3.88	3.96
Mean foetal weight (g) - females only		3.83	3.74	3.71	3.68

A = ANOVA, regression and Dunnett's tests

* p<0.05
** p<0.01
*** p<0.001

TABLE 7

Group mean caesarian data

Test article	Control	SB 209509 - AX		
Group	1	2	3	4
Level (mg/kg/day)	0	100	500	1000

7.3 Foetal defect data

		Group 1	Group 2	Group 3	Group 4
EXTERNAL AND VISCERAL DEFECTS					
Number of foetuses examined		374	372	396	371
Number of litters examined		28	29	30	29
Number showing malformations		3	0	1	5
% of foetuses examined		0.8	0.0	0.3	1.3
Number of litters affected		2	0	1	5
Number showing variations		56	76	97	124
% of foetuses examined	C	15.0	20.4	24.5*	33.4***
Number of litters affected		23	25	26	28
SKELETAL DEFECTS					
Number of foetuses examined		192	193	205	192
Number of litters examined		28	29	30	29
Number showing malformations		0	0	0	2
% of foetuses examined		0.0	0.0	0.0	1.0
Number of litters affected	D	0	0	0	2 DR*
Number showing variations		149	166	187	177
% of foetuses examined		77.6	86.0	91.2	92.2
Number of litters affected	D	28	29	30	29 DR**
Total number of foetuses showing malformations		3	0	1	6
% of foetuses examined		0.8	0.0	0.3	1.6
Number of litters affected	D	2	0	1	6 DR*

C = Kruskal Wallis, Terpstra-Jonckheere and Wilcoxon Rank-Sum tests
D = Cochran-Armitage and Fisher-Irwin tests

* $p<0.05$
** $p<0.01$
*** $p<0.001$
DR = significant dose response