in the satellite study was found dead during the course of the study. Causes of deaths could not be determined, but sponsor believes they are compound-related at the higher dose. None of the deaths were associated with nephropathy. There were no deaths at 250 or 750 mg/kg/day.

Clinical Signs: The usual effects (red extremities, salivation, paddling) in all treated groups starting around 30 minutes post-dosing were seen. In addition, arched gait appeared dose-related with incidences of around 20% at 150 mg/kg/day and 100% at 750 mg/kg/day.

Body Weight Gain: During the first 8 weeks, there was an increase in body weight gain in all treated groups compared to control, which we estimated as 9.8, 18.1, 16.1 and 13.8% for males, 9.0, 12.4, 16.4 and 18.4% for females, for low to high doses, respectively (see sponsor's Table 2.2 and Figure 1 which follow). Between Weeks 8 and 13, the males dosed with 440 and 750 mg/kg/day and the females dosed with 750 mg/kg/day gained less than controls; 36.2 and 61.6% for the 2 highest doses in males, respectively, and 46.8% for highest dose females. However, these effects on body weight gain did not produce any significantly different mean body weight values. By week 13, mean body weights of all male and female treated groups remained higher than controls, except for high dose males, which was about the same as controls. See sponsor's Table 2.2 and Figure 1, which follows.

Table 2.2

Group mean body weight gains

Test article	Control	VML 251			
Group	1	2	3	4	5
Level (mg/kg/day)	0	150	255	440	750

Week of study		Mean body weight gains (g) for Group: 1M	2M	3M	4M	5M	Statistics
Start to 4	Mean	168.1	176.8	192.5*	191.8*	188.0	A
	SD	11.33	27.14	17.93	17.57	14.40	
Start to 13	Mean	296.6	312.2	340.6**	315.5	297.0	A
	SD	21.69	42.47	32.86	27.83	23.88	
4 to 8	Mean	74.6	89.7	94.3*	89.3	88.3	A
	SD	10.92	16.66	13.48	14.31	13.76	
8 to 13	Mean	53.9	45.7	53.8	34.4**	20.7***	A
	SD	11.08	18.87	11.80	12.70	8.88	
		1F	2F	3F	4F	5F	Statistics
Start to 4	Mean	82.3	88.4	86.8	93.9	92.5	A
	SD	10.40	11.59	15.22	10.00	17.41	
Start to 13	Mean	130.1	148.8	146.3	149.6	146.1	A
	SD	18.67	21.40	20.21	11.38	21.18	
4 to 8	Mean	30.5	35.5	39.8*	37.4	41.5**	A
	SD	6.92	8.58	4.19	8.86	7.58	
8 to 13	Mean	17.3	24.9	19.8	17.3	12.1	A
	SD	5.86	13.39	6.57	4.98	13.50	

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

A = ANOVA, regression and Dunnett's

Figure 1

Group mean body weights - males

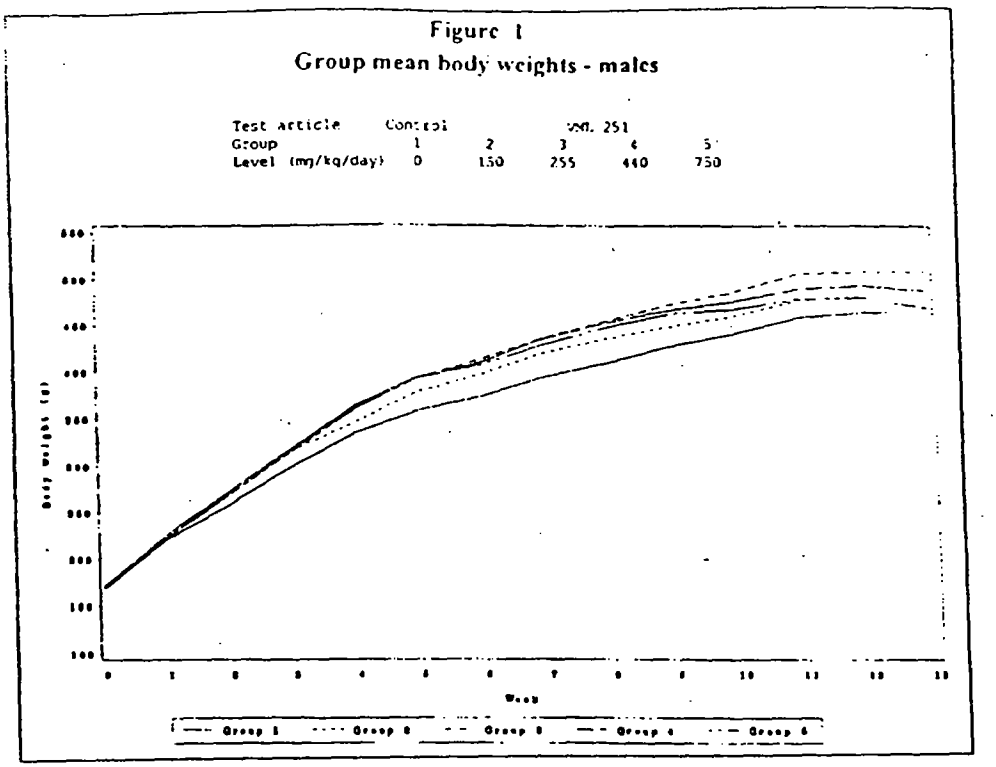
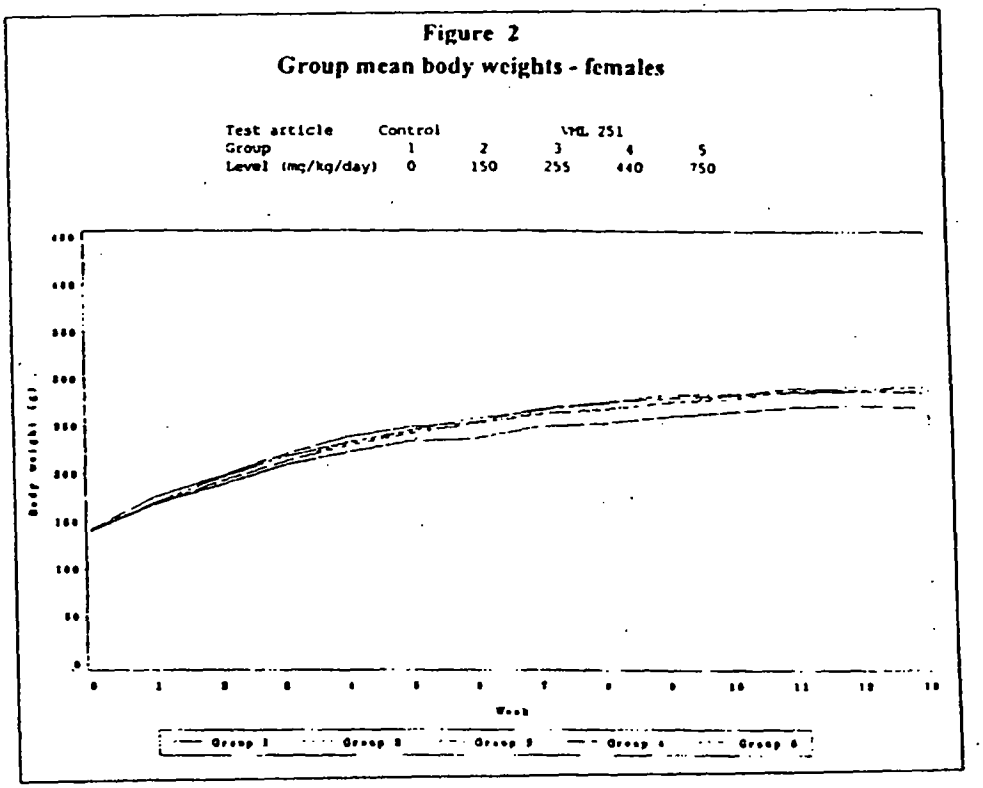


Figure 2

Group mean body weights - females



Water Consumption: At Week 12, there was an increase in water consumption (about 45% higher than controls) in both males and females of the 2 highest dose treated groups.

Clinical Pathology: Statistically significant changes were seen in several parameters, such as increases in PT of all treated males groups (dose related), decrease in APPT in high dose males, minor increases in AST and ALT in high dose males, but no consistent pattern of toxicological importance for any other parameters.

Urinalysis: Urinary parameters measured at weeks 2, 6 and 12 were volume, specific gravity, protein (UPPY), N-acetyl-B-D-glucosamidase (UNAG), alkaline phosphatase (UALK), creatinine (URCR) and total creatinine (T CREAT) (See sponsor's Table 3 on page 11). At Week 13, rat alpha-glutathione S-transferase (RGST) was measured in the urine. UNAG and UALK are "enzyme markers for kidney tubule damage and may be associated with histopathological changes in the kidney". Transient, but statistically significant increase in urine volume were seen at Week 2 in females with decreases in specific gravity at the three highest doses. These effects, with the exception of an increase in urine volume in 440 mg/kg/day treated females at Week 12, were not seen again during weeks 6 and 12. Decreases in UNAG and UALK activity were noted in both sexes during treatment. However, sponsor points out that on examination of the individual animal data, generally a higher volume of urinary output was associated with a decrease in UNAG. In conclusion, there are no clear urinary effects that could be associated with renal toxicity in animals of any treated group.

Organ Weights: Presented in terms of "adjusted to overall mean necropsy body weight and unadjusted weight". No effect was noted.

Table 3: Summary of Group Mean Urine Analysis Data (selected parameters)

VML 251			Males				Females			
Gp	Dose (mg/kg/day)	Week	Vol mL	SP GR	UNAG IU/L	UALK IU/L	Vol mL	SP GR	UNAG IU/L	UALK IU/L
1	0	-1	4.2	1.030	20.2	176	3.1	1.033	20.7	225
		2	5.9	1.029	24.7	688	3.2	1.047	26.9	592
		6	7.6	1.032	20.8	498	5.5	1.031	21.3	326
		12	6.0	1.034	25.6	438	3.5	1.036	27.0	338
2	150	-1	3.8	1.032	22.5	217	2.4	1.037	24.7	284
		2	5.0	1.036	27.7	486	4.3	1.038	20.9	655
		6	5.9	**1.044$_a$**	**27.2$_a$**	598	5.2	**1.043$_a$**	26.4	448
		12	7.3	1.033	20.1	381	4.5	1.035	25.4	306
3	255	-1	3.5	1.030	22.2	539	2.7	1.032	18.5	193
		2	6.4	1.028	20.0	391	4.7	**1.033$_a$**	**18.3$_a$**	596
		6	8.4	1.031	17.2	386	5.7	1.030	17.7	359
		12	6.8	1.035	21.4	328	4.6	1.032	**21.2$_b$**	213
4	440	-1	3.6	1.033	20.9	215	2.1	1.037	19.5	375
		2	6.1	1.032	18.1	327	4.3	**1.032$_a$**	**18.6$_a$**	646
		6	5.9	1.039	18.6	521	5.2	1.033	20.8	464
		12	8.0	1.032	**13.6$_b$**	238	**6.2$_b$**	1.030	**17.9$_b$**	**153$_b$**
5	750	-1	4.3	1.028	19.6	262	2.3	1.033	20.9	419
		2	7.3	1.026	**15.0$_a$**	336	**6.7$_c$**	**1.027$_c$**	**12.5$_c$**	**383$_c$**
		6	7.9	1.034	**11.0$_b$**	309	5.6	1.037	15.8	299
		12	6.4	1.043	**12.4$_c$**	298	4.5	1.039	**18.7$_a$**	261

a - $P < 0.05$
b - $P < 0.01$
c - $P < 0.00$

The tables that follow are sponsor's summaries of incidences and severity of histopathology findings in the in the kidneys, adrenals and thyroids.

At high dose (750 mg/kg/day), an increase in incidence and severity of focal tubular basophilia/regeneration and tubular dilation in males (8/10) was clearly evident, but only a small increase in number of females (2/10) with either a moderate or severe lesion. At 440 mg/kg/day, only 1 of 9 males had a renal lesion that was minimally or slightly more severe than in controls, whereas no effect on renal histopathology was evident in females. A dose of 440 mg/kg/day (the NOEL for renal lesions in females and probably in males) is 5.2-fold higher than 85 mg/kg/day (highest dose in the rat carcinogenicity study). If this is considered to be the MTD, it does not meet CAC's criteria (of within 2 to 3 fold) for acceptability of the 2-year study.

The renal finding "was characterized by an increased basophilia of cytoplasm of tubular cells, with thickening of the basement membrane and occasional mitotic figures. Minor cases were seen as small foci of affected tubules in the cortex, whilst in the more severe cases, there were multiple fine wedges of affected tubules extending from the cortex to the outer medulla, often with associated tubular dilation and intraluminal granular casts. There was also a slightly reduced incidence of hyaline droplets in the kidneys of high dose males, possibly associated with altered metabolic pattern due to tubular pathology."

In the adrenals, a prominent zona glomerulosa (minimal or slight) was seen in all treated male groups, but in females only at high dose. In affected animals, the z. glomerulosa was more distinct from the z. fasciculata because of increased fine cytoplasmic vacuolation and/or increased width of the zona.

Adrenal medullary atrophy was also seen, affecting all high dose males and females, and 5/9 males or females at 440 mg/kg/day. "This finding was characterized by smaller medullary cells with more densely granular appearance, separated by increased interstitial or fibular tissue."

In the thyroid, there was an increased incidence of minor follicular cell hypertrophy (minimal or slight) in high dose males and females. "This was characterized by slightly basophilic cuboidal to columnar cells lining the thyroid follicles, with generally reduced amount of colloid."

Incidence of selected microscopic findings in the kidney – terminal kill

Tissue and finding		Males 1M	2M	3M	4M	5M	Females 1F	2F	3F	4F	5F
Kidney	No. examined:	10	10	10	9	10	10	10	10	9	10
focal tubular basophilia/regeneration	Grade -	6	6	4	2	0	8	6	5	4	7
	1	3	3	6	6	2	2	4	5	5	1
	2	1	0	0	1	4	0	0	0	0	0
	3	0	0	0	0	3	0	0	0	0	1
	4	0	1	0	0	1	0	0	0	0	1
tubular dilatation	Grade -	10	10	10	8	5	10	10	10	9	8
	1	0	0	0	1	3	0	0	0	0	2
	2	0	0	0	0	2	0	0	0	0	0
granular casts	Grade -	10	10	10	9	7	10	10	10	9	9
	1	0	0	0	0	2	0	0	0	0	0
	2	0	0	0	0	1	0	0	0	0	1
hyaline droplets	Grade -	1	1	2	3	5	10	10	10	9	10
	1	7	9	8	4	3	0	0	0	0	0
	2	2	0	0	1	2	0	0	0	0	0
	3	0	0	0	1	0	0	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe

Incidence of selected microscopic findings in the adrenal – terminal kill

Tissue and finding		Males 1M	2M	3M	4M	5M	Females 1F	2F	3F	4F	5F
Adrenal	No. examined:	10	10	10	9	10	10	10	10	9	10
Prominent zona glomerulosa	Grade -	10	3	3	0	0	10	10	10	9	5
	1	0	7	7	9	9	0	0	0	0	4
	2	0	0	0	0	1	0	0	0	0	1
Medullary atrophy	Grade -	10	10	10	4	0	10	10	10	4	0
	1	0	0	0	5	8	0	0	0	5	2
	2	0	0	0	0	2	0	0	0	0	8

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

Incidence of selected microscopic findings in the thyroid – terminal kill											
		Males					Females				
Tissue and finding		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Thyroid	No. examined:	10	10	10	9	10	10	10	10	9	10
follicular cell hypertrophy	Grade -	9	10	8	7	4	9	9	8	8	5
	1	1	0	2	2	4	1	1	2	1	5
	2	0	0	0	0	2	0	0	0	0	0
Key: "-" = finding not present, 1 = minimal, 2 = slight											

Toxicokinetics: Sponsor's summary of results is shown in the tables and Figure 1 on pages 15 to 17 that follow. Comparisons of rat to human doses are based on a 50 kg human receiving a maximum dose of 7.5 mg (3 tablets within 24 hours).

At all three time, including Day 1, Week 5 and Week 12 of treatment, blood AUC levels and C_{max} values of frovatriptan were dose proportional (See table on page 15 and Figure 1 on page 16). At most time periods, blood AUC and C_{max} values tended to be somewhat higher in females than in males (Table on page 15), but T_{max} was generally lower in females (Table on page 16). Sponsor indicated that AUCs increased 2.1 to 2.7 times between Day 1 and Week 5, and a further AUC 1.2- to 1.5-fold increase was found between Week 5 and Week 12. These findings indicate blood levels were increasing with time after daily administration of frovatriptan but they may also suggest that a plateau was being reached or may even have been achieved by week 12 of dosing.

Very high ratios of rat:human dosage, based on mg/kg or mg/m^2, were evident, and high ratios of systemic exposures, based on rat:human AUCs, were obtained at all dose levels (See Table on 17).

$AUC_{(0-24)}$ values

Group number	Dose (mg/kg/day)	Male		
		Day 1 (ng.h/mL)	Week 5 (ng.h/mL)	Week 12 (ng.h/mL)
2	150	24616	46877	57685
3	255	43544	115576@	137960
4	440	62136	167498	236279
5	750	108518	299228	420331

@ the mean female concentration was used as the 0h and 24h concentrations for the calculation of AUC

Group number	Dose (mg/kg/day)	Female		
		Day 1 (ng.h/mL)	Week 5 (ng.h/mL)	Week 12 (ng.h/mL)
2	150	30116	69425	83394
3	255	51574	112656	137277
4	440	69188	182713	268090
5	750	128111	327290	426408

C_{max} values

Group number	Dose (mg/kg/day)	Male		
		Day 1 (ng/mL)	Week 5 (ng/mL)	Week 12 (ng/mL)
2	150	2278	3898	3879
3	255	2842	7197	9836
4	440	3267	10391	17076
5	750	5853	20842	26219

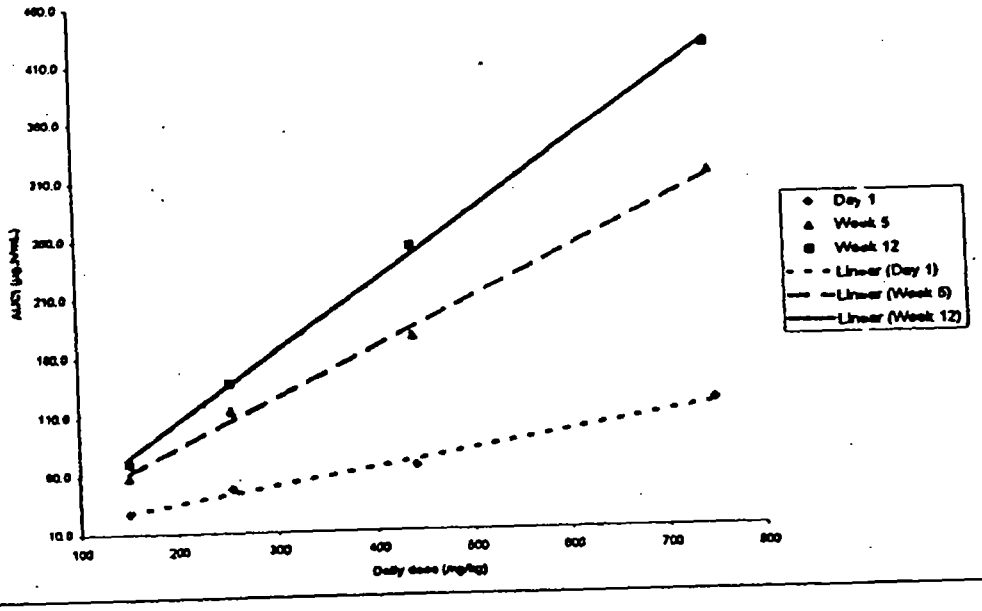
Group number	Dose (mg/kg/day)	Female		
		Day 1 (ng/mL)	Week 5 (ng/mL)	Week 12 (ng/mL)
2	150	1743	5075	5097
3	255	2956	6504	11361
4	440	4100	12145	16772
5	750	8678	19204	28639

T_{max} values

Group number	Dose (mg/kg/day)	Male Day 1 (h)	Male Week 5 (h)	Male Week 12 (h)
2	150	4	6	3
3	255	6	4	3
4	440	8	3	3
5	750	24	8	4

Group number	Dose (mg/kg/day)	Female Day 1 (h)	Female Week 5 (h)	Female Week 12 (h)
2	150	3	1	3
3	255	4	3	2
4	440	6	4	1
5	750	6	3	2

Figure 1 Oral administration of frovatriptan in the rat - Dose-AUC relationship (linear)



Study No.	Sex	Daily dose (mg/kg)	Dose ratio (mg/kg) rat/human	Daily dose (mg/m^2)	Dose ratio (mg/m^2) rat/human	AUCτ (μg.h/mL) Day 1	AUCτ (μg.h/mL) Week 5	AUCτ (μg.h/mL) Week 12	AUC rat/AUC human, at absolute maximal clinical exposure level# Day 1	AUC rat/AUC human, at absolute maximal clinical exposure level# Week 5	AUC rat/AUC human, at absolute maximal clinical exposure level# Week 12	AUC rat/AUC human, at realistic clinical exposure level* Day 1	AUC rat/AUC human, at realistic clinical exposure level* Week 5	AUC rat/AUC human, at realistic clinical exposure level* Week 12
13 week rat Tox														
1165/221	m	150	1000	975.0	163	24.6	46.9	57.7	74	140	173	1106	2106	2591
		255	1700	1657.5	276	43.5	115.6	138.0	130	346	413	1956	5192	6198
		440	2933	2860.0	477	62.1	167.5	236.3	186	502	708	2791	7525	10614
		750	5000	4875.0	813	108.5	299.2	420.3	325	896	1259	4875	13442	18883
	f	150	1000	975.0	163	30.1	69.4	83.4	90	208	250	1353	3119	3746
		255	1700	1657.5	276	51.6	112.7	137.3	154	337	411	2317	5061	6167
		440	2933	2860.0	477	69.2	182.7	268.1	207	547	803	3108	8208	12044
		750	5000	4875.0	813	128.1	327.3	426.4	384	980	1277	5755	14703	19156
						single dose			single dose			single dose		
clinical study VML 251/96/12	human (f) (50 kg)	0.15	1	6.0	1	0.3339			1			1		

mg/m^2 = mg/kg x 6.5 for rat and 40 for human (ICH S 1 C (R))

AUChuman = AUC(0-inf) adjusted for a dose of 7.5 mg (3 x 2.5 mg) and body weight of 50 kg (ie 0.15 mg/kg) = 333.9 ng.h/mL (=31.8 x 7.5 x (70/50))

31.8 ng.h/mL = AUC(0-inf) normalised for dose (1 mg) and body weight (70 kg) in healthy, young females (Study No VML 251/96/12)

Based upon absolute maximum clinical dose of 3 x 2.5 mg tablets frovatriptan/day, continuously, for a 50 kg female

* Based upon average clinical dose of 1.8 x 2.5 mg frovatriptan tablets/ migraine attack and an incidence of 3.3 migraine attacks/month, = a dose of 0.5 mg/day, over a 30 day period

III. SUMMARY AND EVALUATION

The data for the human lymphocyte study do not support sponsor's hypothesis that the clastogenic effect of frovatriptan is caused by membrane damage of the erythrocytes and a resulting release of free heme. In both whole blood and in separated lymphocytes, frovatriptan caused statistically significant increases in cells with chromosomal aberrations. The genetic toxicity of frovatriptan in cultured human lymphocytes has been confirmed. Additional studies, as proposed by the full CAC on 7/29/99 and agreed upon by the sponsor, are required.

A composite of three tables with kidney histopathology findings in 3 different studies with frovatriptan is shown on the page 20. Compound-related deaths (incidence of 10%) attributed to tubular nephropathy had been observed at 1000 mg/kg/day in the 26-week rat study. Although a similar increase in incidence and severity of nephropathy at 1000 mg/kg/day had been observed in the first 13-week study, deaths had not yet occurred within the 13 weeks of the study (See 2nd and 3rd table on page 20). It was reasonable to suggest that with increasing time of high systemic exposure to frovatriptan and with the persistence of renal lesions, there would be increasingly more deaths with time of dosing, which eventually would result in unacceptable mortality during the course of a 2 year study. During the meeting of 7/29/99, the full CAC agreed that the occurrence of treatment-related renal lesions may be considered a dose limiting effect for possible use in estimating the MTD.

In the 13-week MTD-finding study, the incidences and severity of renal lesions in males at 750 mg/kg/day appeared to be generally similar to those observed at 1000 mg/kg/day in the previous 13- and 26-week studies. The slight increase in incidence (1/9) with a minimal or slight increase in severity in males at 440 mg/kg/day may be considered questionable or minimal for estimating a dose limiting effect. In females, there was only a small increase in incidence (2/9) of moderate or severe kidney lesions at 750 mg/kg/day but no renal effect at 440 mg/kg/day or lower. Thus, a NOEL for renal lesions is suggested to be 440 mg/kg/day in males and between 440 and 750 mg/kg/day in females. A dose of 440 mg/kg/day is 5.2-fold higher than 85 mg/kg/day (highest dose in the rat carcinogenicity study). If this is considered to be the MTD, it does not meet CAC's criteria (of within 2 to 3 fold) for acceptability of the 2-year study.

Toxicokinetic parameters in the MTD-finding study, particularly AUC and C_{max} findings, were generally higher in females than in males on Day 1, Weeks 5 and 12, whereas T_{max} values were higher in males at all 3 time periods. Therefore, the higher incidence of renal lesions in males cannot be attributed to higher systemic exposures than in females. In the previous 4-, 13-, 26 and 104-Week studies, there were generally no consistent differences in AUC or C_{max} values between males and females.

In all repeat dose studies with frovatriptan (listed in Table 5 on page 21), AUC and C_{max} values were found to increase with time, indicating bioaccumulation of frovatriptan. In the 13-week MTD-finding study, AUC values increased 2.1 to 2.7 times between Day 1

and Week 5, and a further AUC 1.2- to 1.5-fold increase was found between Week 5 and Week 12. It was reasoned by the sponsor that nephrotoxicity would become progressively more severe with time due to increasing levels of systemic exposure. According to sponsor's extrapolations, blood levels of frovatriptan would be expected to range between 131.8 ug.hr/mL for a dose of 150 mg/kg/day to 790.8 ug.hr/mL for a dose of 750 mg/kg/day (See Table 5).

Sponsor's predicted values for Week 52 assumes an approximate 1.9-fold increase in AUC levels between Week 12 and Week 52. Based on this assumption (which has not been experimentally established), AUC values by Week 52 for the 255 and 440 mg/kg/day treated groups would be as high or even higher than those found for the 440 and 750 mg/kg/day treated groups, respectively, at Week 12. As a result, the MTD by 52 weeks would occur at lower dose levels than that observed after only 13 weeks.

Although it is possible that blood levels may still be increasing with time after 12 or 13 weeks of treatment, we see no clear and consistent indications for an increase at a rate predicted by the sponsor beyond Week 12 or 13 of dosing. In the 2-year rat study, systemic exposure at the highest dose of 85 mg/kg/day increased from 35.3 ug.hr/mL at Week 13 to 66 ug.hr/mL by Week 52 (mean value for males and females combined); a 1.9-fold increase, which supports sponsor's hypothesis. On the other hand, with doses of 8.5 and 27 mg/kg/day, systemic exposures were 3.3 and 13.0 ug.hr/mL at Week 13 and 3.6 and 14.2 ug.hr/mL by Week 52; 1.1-fold increases for both doses. In the first 13-week and in the 26-week studies, doses given in both studies were 0, 10, 100 ad 1000 mg/kg/day. Blood AUC levels at 26 weeks were generally even lower than at 13 weeks (See Table 5 on page 21).

3-Month MTD-Finding Study (Report No. 1165/80-1050). Doses administered were 0, 150, 255, 440 & 750 mg/kg/day in groups 1-5, respectively.

Incidence of selected microscopic findings in the kidney – terminal kill

Tissue and finding		Males 1M	2M	3M	4M	5M	Females 1F	2F	3F	4F	5F
Kidney	No. examined:	10	10	10	9	10	10	10	10	9	10
focal tubular basophilia/regeneration	Grade -	6	6	4	2	0	8	6	5	4	7
	1	3	3	6	6	2	2	4	5	5	1
	2	1	0	0	1	4	0	0	0	0	0
	3	0	0	0	0	3	0	0	0	0	1
	4	0	1	0	0	1	0	0	0	0	1
tubular dilatation	Grade -	10	10	10	8	5	10	10	10	9	8
	1	0	0	0	1	3	0	0	0	0	2
	2	0	0	0	0	2	0	0	0	0	0

3-Month Dose-Finding Study (Report No 1165/80-1050). Doses administered were 0, 10, 100 & 1000 mg/kg/day in groups 1-4, respectively.

Incidence and severity of tubular basophilia/regeneration in the kidney

Tissue and finding		Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
Kidney	Number examined	10	10	10	10	10	10	10	10
Number with tubular basophilia/regeneration		1	2	3	8	2	2	3	10
	Grade								
	1	1	2	3	3	2	2	3	3
	2	0	0	0	2	0	0	0	5
	3	0	0	0	3	0	0	0	2

Key : Grade 1 = minimal, 2 = slight, 3 = moderate

6-Month Rat Study (Report No 1165/33-1050). Doses administered were 0, 10, 100 & 1000 mg/kg/day in groups 1-4, respectively.

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.

Table 5 : Frovatriptan AUC comparisons in rat carcinogenicity and toxicity studies (based on combined male and female data)

Study No. (duration)	Daily dose (mg/kg)	AUCτ (µg.h/mL)						
		Day 1	Week 2	Week 4/5	Week 12/13	Week 26	Week 52	Week 78
1165/5 (4 weeks)	5	1.0		1.8				
	50	10.9		18.7				
	500	61.7		227.0				
1165/80 (13 weeks)	10	2.6			5.4			
	100	27.1			36.2			
	1000	105.1			609.8			
1165/33 (26 weeks)	10	2.8				4.4		
	100	19.3				40.1		
	1000	122.3				340.4		
1165/45 (2 years)	8.5		3.2		3.2		3.6	7.7
	27		10.3		13.0		15.2	17.7
	85		34.7		35.3		66.0	58.7
1165/221 (13 weeks)	150	27.4		58.2	70.55		**131.8***	
	255	47.6		114.1	137.61		**257.0***	
	440	65.7		175.1	252.20		**471.0***	
	750	118.3		313.3	423.40		**790.8***	

*** Predicted week 52 AUC = Week 52 AUC x 66.0/35.3**
(Week 52 AUC / Week 13 AUC at 85 mg/kg/day)

V. CONCLUSION:

Based on renal histopathology alone, the MTD is found to be 440 mg/kg/day in male and between 440 and 750 mg/kg/day in female rats. However, this reviewer suggests that the MTD is 255 mg/kg/day in males and 440 mg/kg/day in females based on the following observations and evidence:

1) at the 750 mg/kg dose, the incidence and severity of renal lesions were considerably greater in males than in females,
2) blood AUC and C_{max} levels of frovatriptan would be expected to increase with time of dosing due to bioaccumulation and may reach levels that cause renal lesions,
3) by week 52 of dosing, renal lesions may occur at even lower doses than those found in the present 13-week study as a result of bioaccumulation,
4) with increasing time of systemic exposure to frovatriptan, renal lesions would become more and more severe, and
5) mortality due to renal lesions, which had not been observed even at the highest doses in the two 13-week studies, were observed in the 26-week study and would be expected to increase with time of dosing at sufficiently high levels systemic exposures.

If the MTD is accepted as 255 mg/kg/day, it falls within 3 times the highest dose used in the previously performed 2-year rat carcinogenicity test (85 mg/kg/day). Therefore, a second 2-year rat study may not be required. Conversely, if the MTD is accepted as 440 mg/kg/day, a second 2-year rat carcinogenicity test may be required.

There is presently an ongoing 6-month p53 mouse carcinogenicity bioassay in progress. Is believed that there is little to be gained by an additional 2-year rat carcinogenicty test for evaluation of the safety of this drug if the results in the p53 study are negative.

V. RECOMMENDATIONS

[ ]. The executive-CAC concluded that the MTD for males "may be around 255 mg/kg/day, but for females it exceeded 440 mg/kg/day (more than 2 to 3-fold the high dose used in the carcinogenicity study"). They recommended that a rat carcinogenicity study need not be repeated provided there is a clearly negative study in the p53 mouse.

The Division is not in agreement with the exec-CAC and considers the MTD to be greater than 440 but less than 750 mg/kg/day. Therefore, a rat carcinogenicity study should have to be repeated in order to satisfy the Division requirements for this NDA.

/S/

Sidney J. Stolzenberg, Ph.D.

3/23/00

cc:
HFD-120 Division File
HFD-120/LChen See Team Leader memo
HFD-120/GFitzgerald /S/ 4/5/00
HFD=120/AOliva
HFD-120/SStolzenberg
IND ———

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Chen

JAN 18 2000

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

NDA: 21-006 **DRUG CODE#:** VML 251; SB 29555 **DATE:** January 18, 2000
DIVISION(s): HFD-120
DRUG NAME(s): MIGARD™ (frovatriptan succinate monohydrate; SB 209509-AX; VML 251
THERAPEUTIC CATEGORY: Acute treatment of migraine
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Serotonin receptor 5-$HT_{1B/1D}$ partial agonist
SPONSOR: Vangard Medica Ltd. (England)
LABORATORY: [redacted]
SPONSOR CONTACT: [redacted]

P/T REVIEWER(s): S. Stolzenberg
DATE SUBMITTED: 1/11/2000
DATE OF CAC REVIEW: 1/18/2000
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): No.

Background

During a meeting of 7/29/99, the full CAC concluded that although the studies normally required for an NDA had been completed, this NDA could not be approved. Doses selected for the 104-week rat and the 84-week mouse carcinogenicity studies were based on the >25-fold AUC option, but this option was not considered appropriate for frovatriptan because the drug was found to be genotoxic in the human lymphocyte test.

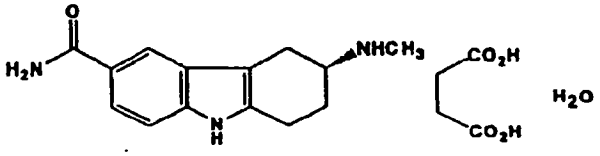
[redacted]

At a teleconference on November 12, 1999, sponsor had agreed to perform a 26-week p52 mouse bioassay as part of the requirements to satisfy the NDA. A summary of the results of a 1-month dose-finding study in p53+/- mice, rationale for the dose levels chosen for the 26-week study and the proposed protocol for the definitive study is presently reviewed for evaluation and for consideration by the exec-CAC. A 3-month rat dose-finding study is also being performed in order to determine a MTD and the possible acceptability of the previously performed rat carcinogenicity study.

REVIEW AND EVALUATION OF TOXICOLOGY DATA

NDA AMENDMENT DATED: 12/10/99
CENTER RECEIPT DATE: 12/10/99
REVIEWER RECEIPT DATE: 12/17/99

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



MW: 379.41

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

4-Week Oral (Gavage) Dose-Finding Study in the P53 Mouse (with Toxicokinetics)

Dates Performed: 10/13/99 to 11/13/99. Final data became available on 12/7/99.

Quality Assurance: The study was performed in accordance with GLP.

Test Animals: P53 heterozygous C57 B1/6 mice; 6/sex/group in the main study and 18/sex/group in the satellite study for toxicokinetic analysis. Age of animals was 7-9 weeks body weights and body weights averaged around 23.5 for males and 18.2 for females at initiation of dosing.

Test Substance: Lot number AO 60811

Doses: 0, 200, 400, 800 and 1600 mg frovatriptan/kg/day.

Rationale for Dose Selections: Based on toxicity results observed in the 13-week, 84-week and acute toxicology studies.

Gross pathology was performed on all animals on test. Forty different tissues from each animal were collected in formalin but only 10 were histologically examined, including adrenals, heart, kidney, liver, gall bladder, lung, ovary, pituitary, spleen, thyroid and macroscopic abnormalities.

RESULTS:

Mortality: A dose level of 1600 mg/kg/day was acutely lethal with the majority of male and female mice dying within the first 24 hours after the first dose. The surviving animals at this dose were killed on Day 3 of dosing. Three males and 1 female died

within 2 days following a single dose of 800 mg/kg. Therefore, this dose was reduced to 600 mg/kg/day on the third day of dosing.

Table 1: Mortality

Dose (mg/kg/day)	Number of animals that died or were killed *in extremis*			
	Males		Females	
	Main study	TK group	Main study	TK group
0	0/6	0/3	0/6	0/3
200	0/6	0/18	0/6	0/18
400	0/6	0/18	0/6	0/18
800/600	2/6	2/18	0/6	3/18
1600	5/6	14/18	3/6	9/18

TK = toxicokinetic

Clinical chemistry parameters measured were not indicated but 5 of them, indicated in sponsor's Table 2, were decrease (not generally dose-related). It is claimed that there was a high level of variance for some parameters in control animals and that the effects noted are not likely to be of toxicological significance.

Table 2: Clinical chemistry data: Male animals

Dose (mg/kg/day)	AST # U/L	ALT # U/L	Chol. mg/dL	Triglyc. mg/dl.	Urea mg/dL
0	190 26.1	118 31.2	101 2.6	166 21.8	34 2.7
200	244 128.1	71 33.5	98 4.5	148 2.1	31 2.3
400	78* 13.9	30* 2.5	86* 2.9	132 10.7	25* 2.2
600/800	84* 12.0	34* 1.9	82* 2.8	129 10.2	24* 1.1

Data quoted are the mean and SEM of 4-6 animals/group

* - Significantly different from control value, $p \leq 0.05$ (Student's t test)

- A similar treatment related decrease, noted in female animals

Histopathology: It is claimed that in H/E stained sections of 10 different tissues from animals in the main study that survived to termination, there was no indication of any clear compound-related target organ toxicity. In male animals that died following a single dose of 800 mg/kg frovatriptan, "renal medullary tubules containing proteinaceous droplets and scattered subcapsular cortical tubules from some animals containing exfoliated cells and epithelial debris" were seen. These animals also had lymphocytic necrosis in white pulp of the spleen (which sponsor indicates is probably related to stress) and vacuolation of the sinusoidal face of midzonal to periportal hepatocytes (indicative of increased level of lipid). Sponsor claims that the renal lesions in decedent animals were treatment-related.

Toxicokinetics: On Day 28 of treatment, blood samples from satellite animals were drawn at 6 time points, up to 24 days post-dosing, from 3/sex/group. Sponsor's summary of toxicokinetic parameters in mice of this study is shown in Table 3 that follows. The ratios of mouse:human systemic exposures, based on AUC during Week 4 in mice and Day 1 in humans, is shown in their Table 4. Sponsor's Table 4 is not shown in this review because bioaccumulation is know to occur in all species where tested, including humans, following administration of multiple doses of frovatriptan. Therefore, we believe it is more appropriate to compare to compare Day 1 of treatment in the rodent to Day 1 of treatment in humans. In the protocol for the 6-month study, blood samples for toxicokinetics are scheduled for both Days 1 and 28 of treatment so that comparisons on both days of treatment in the mouse can be made.

Table 3: Whole blood toxicokinetic parameters at Week 4 following daily oral administration of frovatriptan in C57 Bl/6 mice

Sex	Daily dose (mg/kg)	Dose ratio	t_{max} (h)	C_{max} (µg/mL)	C_{max} ratio[1]	C_{24h} (µg/mL)	C_{24h} ratio[1]	AUCτ (µg.h/mL)	AUCτ ratio[1]	AUC norm (µg.h/mL)
m	200	1	2	7.98	1	2.04	1	87.3	1	0.44
m	400	2	3	22.2	2.8	3.7	1.8	173.1	2.0	0.43
m	600/800	3	3	31.9	4.0	4.6	2.2	281.2	3.2	0.47
f	200	1	2	8.23	1	1.91	1	84.9	1	0.42
f	400	2	2	12.3	1.5	4.4	2.3	161.6	1.9	0.40
f	600/800	3	2	24.62	3.0	NR	NR	NR	NR	NR

[1] Ratios are relative to respective low dose values
AUCτ = AUC over a 24-h dose interval
AUCnorm = AUCτ /daily dose
NR = no result

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Protocol for the 26-Week Oncogenicity Study in p53(+/-)C57BL/6 Mice

Quality Assurance: The study will be performed in accordance with GLP

Test Animals: C57BL/6TacfBR-[KO]p53 N5 heterozygous (+/-). Age of animals will be 8-10 weeks at initiation of dosing. Body weights will be obtained before dosing and at initiation of dosing. Source of animals will be [redacted].

Test Substance: Batch 44 (99.4% purity)

Group Designations and Dose Levels

Group	Main No. of Animals		Toxicokinetic No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Male	Female	Male	Female		
1 (Control)	15	15	--	--	0	0
2 (Low)	15	15	18	18	20	2
3 (Mid 1)	15	15	18	18	62.5	6.25
4 (Mid 2)	15	15	18	18	200	20
5 (High)	15	15	18	18	625	62.5
6 (Positive Control)[a]	15	15	--	--	90	9.0

[a] Methylnitrosourea (MNU, 90 mg/kg in citrate buffered saline, pH=4.5). The positive control animals will be dosed once on Day 1.

Rationale for Dose Selections: Based on lethality and clinical signs observed in the dose-finding study.

Procedure: The animals will be housed in individual cages and dosed daily by oral gavage. Clinical observations will be conducted twice daily, body weights and food consumption will be obtained weekly. Hematology on main study animals (excluding positive controls) will be conducted on orbital sinus blood samples collected after an overnight fast at termination (Week 26). It will include red cell count, HB, HCT, platelet count, leukocyte count and differential leukocyte count with blood cell morphology. Additional blood samples for toxicokinetic analysis will be obtained by heart puncture in main study animals.

Postmortem: Gross pathology will be performed on all main study animals. Organ weights will include brain, heart, kidneys, liver with gallbladder, ovaries, testes with epididymides and thymus. Tissues from 39 organs listed below and from observable masses will be preserved in buffered formalin. Histopathology will be performed only on high dose and control animals and on all animals that die or are sacrificed prior to termination. The thymus and gross lesions from each animal in positive control group will be examined microscopically.

adrenal (2)	mammary gland (female)
aorta	ovary (2)
brain	pancreas
cecum	pituitary
cervix	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
esophagus	sciatic nerve
eye (2)	seminal vesicles
femur with bone marrow (articular surface of the distal end)	skin
	spleen
Harderian gland	spinal cord (cervical, thoracic, lumbar)
heart	stomach
ileum	testis with epididymis (2)
jejunum	thymus
kidney (2)	thyroid (2) with parathyroid
lesions	trachea
liver with gallbladder	urinary bladder
lung	uterus
lymph node (mesenteric)	vagina

Toxicokinetics: To be performed on orbital sinus blood from satellite animals (3/sex/group at each time point), collected on Day 1 (after the first dose) and on Day 28, at 1, 2, 3, 4, 8 and 24 hours after dosing.

All toxicokinetic samples, including those collected from main study animals, will be shipped to [redacted] at -10 to -30 degrees Centigrade under dry ice and will be analyzed for blood concentrations of frovatriptan in accordance with GLP.

CONCLUSION

In the absence of any dose limiting histological or clinical pathology effects that could be attributed to compound administration, the MTD selected, 625 mg frovatriptan/kg/day, was based on lethality observed in the 1-month dose-finding study. A slightly higher dose level, 800 mg/kg/day, caused lethality in 3/24 males and 1/24 females within the first 2 days with a resulting reduction of the dose to 600 mg/kg/day on the third scheduled day. An additional male and female died within a day or 2 after reduction of the dosage. A dose of 1600 mg/kg/day was acutely lethal, with the majority of males and females dying within the first 24 hours.

Doses selected for the 6-month study in the p53 mouse model are 0, 20, 62.5, 200 and 625 mg/kg/day. Sponsor claims that a higher dose, 700 mg/kg/day, was considered as a possible MTD or highest dose, but this was rejected because of the modest group size with genetically engineered mice and the probability that mortality would compromise the outcome of the study.

NDA 21-006

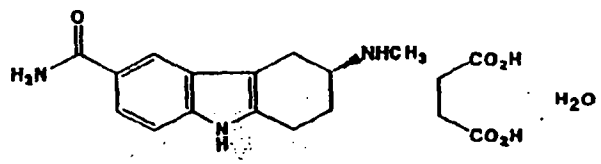
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Sidney J. Stolzenberg
November 5, 1999

ORIGINAL NDA DATED: 1/29/99
CENTER RECEIPT DATE: 1/29/99
REVIEWER RECEIPT DATE: 3/1/99

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

DRUG: Miguard™ (Frovatriptan succinate; SB 209509-AX; VML 251)
Empirical Formula: $C_{14}H_{17}N_3O.C_4H_6O_4.H_2O$
(+)-3-methylamino-6-carboxamido-1,2,3,4-tetrahydrocarbazole succinate, hydrate



MW: 379.41

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

Excipients per tablet include lactose, microcrystalline cellulose, colloidal silicon dioxide, sodium starch glycollate, magnesium stearate, hydroxypropyl methyl cellulose, titanium dioxide, polyethylene glycol 3000, triacetin

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

Miguard tablets, containing 3.91 mg frovatriptan succinate, equivalent to 2.5 mg frovatriptan, is being developed for use in acute treatment of migraine headaches. Like other triptans of its class, frovatriptan is considered to be a selective agonist at the 5-$HT_{1B/1D}$ receptor. It is believed to act selectively on extracerebral, intracranial arteries to inhibit excessive dilation of these vessels in migraine.

A protocol for a 2-year rat carcinogenicity study in which the doses proposed were 8.5, 27 and 85 mg/kg/day, was submitted to the executive-CAC on 6/11/96. The high dose was based on the "AUC ratio option", even though the drug was found to be clastogenic in a human lymphocyte test. The exec-CAC concluded that the data provided at that time were not sufficient for the committee to provide a recommendation for a 2-year rat study. However, by 6/19/96, sponsor had been informed by telephone and subsequently by mail that the high dose for a 2-year rat study had to be based on toxicity and that a 3-month dose-finding study at sufficiently high doses was required. Upon reviewing the data for NDA 21-006, we found that the doses for the rat carcinogenicity study were the same as those originally proposed by the sponsor. The exec-CAC met again on 4/27/99 to decide on the adequacy of the 2-year rat and an 82-week mouse carcinogencity studies. The committee agreed that the drug was positive in the genotoxicity study and that dose selection should not be based on the AUC ratio option. At a meeting of the full CAC on 7/29/99, sponsor was given various options for correcting the deficiencies in the present NDA. Although the NDA is not approvable, this review is being completed because of the considerable effort involved in reviewing the pre-clinical studies to this date and sponsor has initiated studies designed to overcome the deficiencies, to be submitted to FDA at the earliest possible time.

II. PHARMACOLOGY

A. Pharmcodynnamics; Sponsor's Summary

Frovatriptan is a potent vasoconstrictor in isolated cerebral arteries from several species, including human. The activity of frovatriptan as a vasoconstrictor was investigated in isolated cerebral blood vessels and compared with sumatriptan. In human middle cerebral arteries, frovatriptan was a potent partial agonist (relative to 5-HT) and was at least 5-fold more potent than sumatriptan. Frovatriptan was a full agonist in human basilar arteries, being 8.3-fold more potent than sumatriptan. Frovatriptan was 23- and 3-fold more potent than sumatriptan in the rabbit basilar artery and cat middle cerebral artery, respectively In the rabbit, frovatriptan was a partial agonist whilst sumatriptan was a full agonist with respect to 5-HT. In the cat, frovatriptan and sumatriptan were both partial agonists relative to 5-HT.

Frovatriptan is a potent, but low efficacy, vasoconstrictor in isolated coronary arteries. Frovatriptan was 6.6- to 9.8-fold more potent than sumatriptan in dog isolated coronary arteries. In human isolated coronary arteries, from recipient and donor hearts, frovatriptan was up to 7.2- and up to 2.4-fold more potent than sumatriptan, respectively. With respect to 5-HT, frovatriptan was a partial agonist in all coronary arteries studied whilst sumatriptan was a full agonist. On the basis of their respective EC50 values, the relative activities of frovatriptan and sumatriptan as vasoconstrictors in human cerebral and coronary arteries were similar.
By virtue of its apparent partial agonist activity in human isolated coronary arteries, frovatriptan demonstrates a functional selectivity for cerebral arteries over coronary arteries. Frovatriptan produced a "bell-shaped" concentration-effect curve in coronary arteries; contraction followed by relaxation at higher concentrations. The relaxant phase may be a contributory factor in its low efficacy in coronary arteries and so conferring a functional selectivity for the cerebral vasculature. The mechanism which mediates the relaxant activity of frovatriptan in coronary arteries is unclear, but it is possibly related to 5-HT7 receptor activation, since the compound has been shown to have moderate agonist activity at 5-HT7 receptors and SB-209509/—— 100 —— 71).

Frovatriptan exhibited high affinity for human 5-HT1Da and 5-HT1Db (subsequently renamed as 5-HT1D and 5-HT1B) binding sites (pKi 8.4 and 8.6, respectively). Radioligand binding studies were conducted to establish the extent of interaction of frovatriptan with a range of receptors. A summary of the binding results (apparent affinities of frovatriptan, pKi) is shown in the table that follows.

Receptor Binding of Frovatriptan

Receptor	Species	pK_i
5-HT_{1A}	Rat	7.3
5-$HT_{1D\alpha}$	Human	8.4
5-$HT_{1D\beta}$	Human	8.6
5-HT_3	Rat	< 6
Dopamine D_1, D_2, D_3	Human	< 5
Adrenergic α_1	Rat	< 5
Histamine H_1	Guinea-pig	< 5

With respect to its affinity at 5-HT1Da/5-HT1Db binding sites, frovatriptan displayed > 10-fold selectivity over 5-HT1A receptors and > 1000-fold selectivity over other 5-HT, dopamine, adrenergic and histamine receptors. In other radioligand binding studies, frovatriptan displayed only weak inhibition of [3 H]ketanserin binding to the cloned human 5-HT2A receptor with a pKi of < 5.3. Frovatriptan was also a weak inhibitor of 3 H-mesulergine binding to the cloned human 5-HT2C (pKi < 5.3) receptors and so would only be expected to interact with these receptors at greater than micromolar concentrations. Frovatriptan bound to cloned human 5-HT1E and 5-HT1F receptors with pK's of 5.6 and 7.0, respectively. It showed > 600-fold selectivity for 5-HT1D over 5-HT1E receptors and markedly greater selectivity than sumatriptan and GR85548 (naratriptan) for 5-HT1D over 5-HT1F receptors (> 25-fold). The ability of frovatriptan (3 nM to 100 mM) to modulate the GABAA receptor chloride channel complex in rat cerebral cortex, using [35 S] – TBPS binding was examined. Frovatriptan did not interact with the TBPS binding site on the GABAA chloride channel complex and did not, therefore, alter the functional state of the channel.

In cell-based assays in vitro, frovatriptan is a potent, full agonist at human recombinant 5-HT1B and 5-HT1D receptors and a moderately potent, partial agonist at 5-ht1F receptors. Unlike sumatriptan, frovatriptan is a moderately potent, full agonist at 5-HT7 receptors. Functional activities of frovatriptan and sumatriptan at recombinant human 5-HT receptor subtypes (5-HT1A, 5-HT1B, 5-HT1D, 5-ht1E, 5-ht1F, and 5-HT7 receptors) were examined. The concentrations of compounds causing half maximal stimulation of activity corresponded to pEC50 (-log10 EC50) values at 5-HT1B and 5-HT1D receptors, respectively, as follows: frovatriptan: 7.8 ± 0.1 and 8.3 ± 0.1; sumatriptan: 7.1 ± 0.1 and 8.0 ± 0.1. Both compounds were weak (pEC50 5.8 ± 0.2 and 5.2 ± 0.1) full agonists at 5-HT1A receptors. Frovatriptan was a moderately potent partial agonist at 5-ht1F receptors (pEC50: 6.5 ± 0.1) and, like sumatriptan only a weak (pEC50: 5.5) partial agonist at 5-ht1E receptors. At 5-HT7 receptors, frovatriptan was a full agonist of moderate potency (pEC50: 6.2 ± 0.1), unlike sumatriptan which was a very weak (pEC50: 5.2 ± 0.1) partial, agonist.

Further confirmation that frovatriptan has its principal affinity at the human recombinant 5-HT1B and 5-HT1D receptors was provided. Selectivity for 5-HT1B/1D binding sites with respect to human recombinant 5-HT1A, 5-HT1F, and 5-HT7 receptors was between 10- and 25-

fold. Frovatriptan was inactive at other neurotransmitter receptors tested. This study, which was conducted with the succinate salt, also confirmed previous work with different salt forms of frovatriptan showing that the salt form has little influence on the affinity of frovatriptan for neurotransmitter receptors.

The desmethyl metabolite of frovatriptan (SB 205555-A) exhibits affinity for 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT1F and 5-HT7 binding sites with pKi values approximately 0.5 units lower than those for frovatriptan, whereas the N-acetyl desmethyl metabolite (SB 210199) exhibits no significant affinity at these sites. The frovatriptan metabolites, SB 205555-A (hydrochloride salt of desmethyl frovatriptan) and SB 210199 (free base of N-acetyl desmethyl frovatriptan) were evaluated in radioligand binding studies for their affinities at a range of neurotransmitter recombinant receptor binding sites. The pKi values of SB 205555-A at each site were 7.9 (5-HT1B), 8.0 (5-HT1D), 6.7 (5-HT1A), 6.7 (5-HT7), and 5.8 (5-HT1F). The compound exhibited very low affinity at other binding sites evaluated (5-HT2A, 5-HT2B, 5-HT2C, 5-HT4, 5-HT6, D2(longform), D3 and SB-204269 [novel anticonvulsant]). In contrast, SB 210199 had pKi values of < 5.3 in these assays.

b. Safety (General Pharmacology) Studies; Sponsor's Summary

Frovatriptan is a potent constrictor of the carotid vascular bed in the cat and dog in vivo. The effects of frovatriptan (alone and compared to sumatriptan) on the cardiovascular system have been investigated in the cat and dog. Of relevance to its proposed therapeutic use in the treatment of migraine, frovatriptan produced dose related constriction of the carotid artery vascular bed. Successive, increasing IV (intravenous) doses of frovatriptan (0.2 μg/kg to 2 mg/kg) or sumatriptan (0.1 μg/kg to 1.0 mg/kg) were administered to anesthetized cats at 5-minute intervals. In a separate group of cats, successive increasing ID (intraduodenal) doses of frovatriptan (0.6 μg/kg to 2 mg/kg) or sumatriptan (0.3 μg/kg to 3 mg/kg) were administered at 30-minute intervals. Both drugs, administered IV, evoked dose-dependent and rapid increases in carotid vascular resistance with concomitant reductions in carotid blood flow, the maximum effect occurring after 20 μg/kg of frovatriptan and 100 μg/kg of sumatriptan. Higher doses (60 μg/kg to 2 mg/kg) of frovatriptan tended to reduce mean arterial blood pressure and raise heart rate slightly, although these effects were somewhat transient in nature. Intraduodenal administration of both drugs produced dose dependent increases in carotid vascular resistance which were of longer duration than after IV administration. Blood pressure was unaffected, apart from a small reduction following administration of the highest dose. Heart rate was slightly reduced by both drugs after the completion of the dose regimen. Sumatriptan was less potent than frovatriptan; when drug doses are expressed in molar terms, the doses of frovatriptan producing a 50% increase in carotid vascular resistance were 7 and 5 times lower than that of sumatriptan for the IV and ID routes, respectively.

Similar results were observed in anesthetized dogs receiving successive, increasing IV doses of frovatriptan (0.1 μg/kg to 1.0 mg/kg) or sumatriptan (0.1 μg/kg to 0.3 mg/kg). Different animals received successive, increasing ID doses of frovatriptan (0.1 μg/kg to 1.0 mg/kg) or sumatriptan (0.3 μg/kg to 0.3 mg/kg). Following IV dosing, both drugs resulted in rapid and dose-related increases in carotid vascular resistance, with the maximum effect occurring at 0.03 mg/kg of frovatriptan and 0.3 mg/kg of sumatriptan. There was, however, little change in blood pressure or

heart rate. Dose-related increases in carotid vascular resistance were also observed after ID administration, with the maximum effect occurring following administration of the highest dose of each drug. These responses developed over the 30-minute period following dosing. Sumatriptan was less potent than frovatriptan; when drug doses are expressed in molar terms, the doses of frovatriptan producing a 50% increase in carotid vascular resistance were 12- and 1.9-fold lower than those for sumatriptan for the IV and ID routes, respectively.

When pentobarbitone-anesthetized dogs received IV bolus injections of 0.02, 0.2, or 2 mg/kg of frovatriptan, a dose of 0.02 mg/kg caused a 2 to 3 mm Hg increase in systolic pressure which was not considered to be of biological significance. Similarly, no biologically significant effects on diastolic or mean blood pressure, heart rate or femoral blood flow were noted. Although no statistically significant differences were noted in respiratory rate, transient hyperpnea was observed during and immediately after dosing in all animals. Administration of the higher doses (0.2 and 2.0 mg/kg) also resulted in an increase in systolic blood pressure.

The effects of IV bolus doses of frovatriptan (0.1 μg/kg to 1 mg/kg) and sumatriptan (0.1 μg/kg to 1 mg/kg) on coronary hemodynamics were further examined in anesthetized, open-chest dogs. Both frovatriptan and sumatriptan induced dose-related decreases in carotid artery blood flow, with concomitant increases in carotid artery vascular resistance. Frovatriptan was approximately 10-fold more potent than sumatriptan. Both compounds had little effect on coronary artery blood flow or vascular resistance.

Studies were designed to compare the hemodynamic effects of frovatriptan and sumatriptan administered IV and into the coronary artery (IC) of open chest, anesthetized dogs. Results confirmed that in contrast to sumatriptan, frovatriptan did not elicit any significant hemodynamic effects over the dose range of 0.0001 to 1.0 mg/kg, when administered IV, and only a negligible change in TPR (total peripheral resistance) when administered IC. These data indicate a favorable profile for frovatriptan relative to sumatriptan with respect to potential hemodynamic liabilities.

In a standard model of myocardial infarction in open-chest, anesthetized dogs subject to cardiac ischemia and reperfusion was used. The effects of frovatriptan (0.1 mg/kg) and sumatriptan (1 mg/kg), administered IV 15 minutes before occlusion of the left circumflex coronary artery, on the coronary hemodynamics and myocardial infarction were compared. In vehicle treated animals, infarct size was 12.97% ± 3.78% of the left ventricle. In the frovatriptan treated animals, infarct size was not statistically significantly different from the vehicle group (12.06% ± 2.58%). Sumatriptan treatment resulted in a trend toward increased infarct size (17.17% ± 2.74%) but this did not reach statistical significance. The CBF (coronary blood flow) did not differ among the three experimental groups prior to occlusion of the coronary artery. However, during the reperfusion period, sumatriptan treatment resulted in a significant decrease ($p < 0.05$) of CBF compared to baseline levels. The CBF tended to be higher in the frovatriptan treated group compared to the sumatriptan group during the reperfusion period, although the difference did not reach statistical significance. The CVR was elevated in the sumatriptan treated group as compared to baseline and higher than the CVR of the vehicle treated animals, although these differences did not reach statistical significance. In sumatriptan treated animals blood flow was significantly decreased but was well maintained in frovatriptan-treated animals.

Frovatriptan is devoid of pronounced or dose-related gross behavioral effects in the mouse. Gross behavioral effects were assessed in mice administered frovatriptan IP (intraperitoneally) at doses of 1 to 30 mg/kg and using a simplified version of the Irwin Profile to determine outcome. At 15 minutes after dosing, no major or dose-related behavioral, neurological, or autonomic effects were noted, although piloerection was observed in all animals (including controls). The profile was similar at 60 minutes after dosing; only slight increases in spontaneous activity, visual placing, tail elevation, and skin color were observed, accompanied by slight decreases in locomotor activity and body temperature. Writhing was observed at low doses between 30 and 60 minutes after dosing. None of these changes was pronounced or dose-related. Piloerection was exhibited by some animals at all doses (including vehicle) and was still evident 21 to 24 hours after dosing.

Frovatriptan attenuates carrageenan-induced thermal hyperalgesia but does not possess anti-nociceptive activity in the mouse. These properties of frovatriptan and sumatriptan were examined in a mouse abdominal constriction study based on the ability of phenyl-p-quinone dosed IP to induce a writhing syndrome. Analgesic compounds such as morphine and acetylsalicylic acid inhibit this syndrome. In this study, mice were dosed SC (subcutaneously) with frovatriptan (1, 10, or 30 μg/kg), sumatriptan (10, 100, or 300 mg/kg), or vehicle. Neither compound was associated with a significantly different latency from the respective control group and, thus, did not possess anti-nociceptive activity in this mouse model. The effects of frovatriptan were determined in a mouse model of acute (thermal) nociception and thermal hyperalgesia (post-carrageenan or olvanil treatment) and in carrageenan-induced inflammation. Frovatriptan (3 to 100 mg/kg, IP, 60 minutes prior to testing or 0.1 to 3 mg/kg, IP, 90 minutes prior to testing) had no significant effect on acute thermal nociception, but a dose of 30 mg/kg, IP, significantly attenuated post-carrageenan thermal hyperalgesia when administered 15 minutes prior to testing. These latter effects were blocked by the 5-HT1B/1D and 5-HT1D antagonists SB-213646G (3 mg/kg, IP) and ← -15572 (10 mg/kg, IP), but not by the 5-HT1B antagonist SB-224289 (10 mg/kg, IP). In contrast, frovatriptan (3 to 100 mg/kg, PO) had no effect on olvanil-induced thermal hyperalgesia, nor did it modify the change in paw volume induced by carrageenan when 30 mg/kg, IP, was administered 30 minutes before and 150 and 240 minutes after the carrageenan dose.

C. Pharmcokinetics

Absorption: Absorption studies have been done in mouse, rat, rabbit, dog and humans in single dose and repeat dose experiments, using the hemisuccinate and hydrochloride salt forms of frovatriptan. The ^{3}H-labelled form, with the label situated on the N-methyl group, was used in initial studies, but it was found that this methyl group could be metabolically removed; thus measurement of total radioactivity may not have reflected metabolic pool but may have given a closer approximation of parent compound. Subsequently, a ^{14}C-label, located in a metabolically stable aromatic ring, was used.

Frovatriptan is soluble in aqueous solutions over a wide range of pH, with a pKa of about 10. Therefore, it should be predominantly in ionized form in acid or neutral environment of the GI tract, which may be a limiting factor in absorption.

As shown in the table which follows, taken from sponsor's summary, absorption of frovatriptan is generally low; estimated as 7 to 19% in rat, 35 to 65% in dog (estimated by comparison of radioactivity after oral and IV dosing in these two species), 9% in mouse and 4% in rabbit (based on recovery in urine after oral administration). In humans (not shown in the table), absorption was 22% and 30% in healthy males and females, respectively, at a dose of 2.5 mg. In the rabbit, this estimate is probably low because it is based on urinary recovery up to 24 hours when the blood levels were still elevated.

Table 5.2.3.1

Estimates of Oral Absorption of Frovatriptan

Species	Dose (mg /kg)	Sex	No. Kinetics	Route	F % Blood	F % Plasma	No. Excretion	Urine % Dose	Urine Adjusted
Mouse 1165/172	40	m	n/a[1]	Oral	n/a	n/a	5	9.0	n/a
Rat DR93074	2.5	m	5	Oral	18.7	17.1	n/a	n/a	n/a
Rat 1165/7	5	m	3	IV	100	100	4	79.7	100
Rat 1165/7	5	m	3	Oral	19	7	4	10.8	13.6
Rat 1165/63	5	m	3	Oral	8	9	6	8.6	10.8
Rabbit[2] 1165/134	20	f	n/a	Oral	n/a	n/a	1	3.8	n/a
Rabbit[3] 1165/180	20	f	n/a	Oral	n/a	n/a	2	7.2	n/a
Dog 1165/8	0.3	m f	2 1	IV	100	100	3	66.6	100
Dog 1165/8	0.5	m f	2 1	Oral	63	65	3	42.6	64.0

[1] n/a = not applicable
[2] urine collected for 24 h after single radioactive dose
[3] urine collected for 24 hours after single radioactive dose preceded by 13 daily doses of 20 mg/kg non radioactive frovatriptan

The extent of absorption in humans (and rabbits) seemed more variable than other species. The comparatively high absorption in the dog is suggested by the investigators to be due to the relatively high gastric pH, whereas the rabbit has a particularly low gastric pH.

Pre-systemic metabolism in the gut is considered to be unlikely or very small because of limited metabolism after oral administration in the mouse, rat and dog and because of the similar degree of metabolism after IV and oral administration in the rat and dog. Sponsor suggests that there is slightly greater metabolism in rabbit and man, and suggests that pre-systemic metabolism may have slightly influenced oral bioavailability in these two species.

In view of the low level of metabolism in the rat and dog, sponsor claims that concentrations of total radioactivity provides a close reflection of parent compound for use in estimating bioavailability.

Blood levels of parent compound rose proportionately with dose over a large range (based on C_{max} and AUC), both after single and repeated daily administration to either sex in the mouse (doses of 4-500 mg/kg/day), rat (doses of 5-1000 mg/kg/day) and dog (doses of 0.5-20 mg/kg/day). In pregnant rabbits (doses of 5-200 mg/kg/day), the limited data available

suggested that dose proportionality was achieved following a single dose, but disproportionately higher levels were seen after 13 daily doses of 20 mg/kg than after 5 mg/kg/day.

Following either oral or IV administration in all species, the blood:plasma ratio of frovatriptan and total radioactivity concentrations increased to attain an equilibrium typically in the range 1:1 to 2:1. A slightly longer time was taken to reach equilibrium following oral administration (usually by between 1 and 2 hours post-dose). Sponsor suggested that this was probably due to the more prolonged input of drug into the systemic circulation after oral administration than after IV infusion. This was interpreted as parent drug and possibly metabolites being preferentially distributed into the cellular fraction. In later metabolism studies it was observed that the blood:plasma ratios of desmethyl frovatriptan were similar to those of the parent drug whereas those of N-acetyl desmethyl frovatriptan were lower indicating less blood cell binding for the latter metabolite.

Sponsor indicates that gender differences in pharmacokinetics were observed in humans, but there was no pattern of gender differences for pharmacokinetic parameters observed in animal studies. After repeated daily doses, bioaccumulation ratios, ranging from ——— were seen in the toxicokinetic studies with mice, rats or dogs. Sponsor indicates that accumulation ratios were consistent with daily dosing and half-lives. There were no apparent time-dependent changes in pharmacokinetic parameters in mice, rats or dogs.

Protein binding: Protein binding is serum, estimated by dialysis equilibrium at concentrations ranging from 10 to 5000 nM, was found to be 17.1±3.1%, 14.9±2.6%, and 15.3±2.6% in rat, dog and human, respectively. Sponsor concluded that there was no difference in proportion of protein binding between the 3 species and there was no evidence of significant concentration dependence in any of the 3 species. Furthermore, it was claimed that the low binding and lack of concentration dependence is unlikely to influence the PK and PD of frovatriptan in vivo.

Blood binding: In two equilibrium dialysis studies, binding of frovatriptan to the cellular fraction of blood was found to 52%, 60%, 70% and 70% in rat, cat, dog and human, respectively, and the extent of binding to the cellular fraction was linear in all 4 species between concentrations of around 21 to 2066 ng/mL. Using purified preparations of erythrocytes and platelets, binding in human blood was predominantly to erythrocytes (18.5%-25.4%) and only weakly to platelets (0-3%).

Tissue distribution: In non-pregnant, pregnant and lactating female rats and in male rats, tissue concentrations following a single dose of frovatriptan that were greater than blood were seen in salivary glands, adrenal medulla, spleen, bone marrow, uveal tract, skin and milk; it is especially high in the adrenal medulla where it is slowly released, with a half-life for release of about 450-600 hours. Thus, considerable accumulation in the adrenal medulla would be expected after repeated daily doses, which sponsor suggests was responsible for the toxicity in the adrenal medulla observed in the rat carcinogenicity study. Sponsor suggests (but has not established) that such extensive bioaccumulation by the adrenal medulla is not likely to occur in humans because it did not occur in rabbits and dogs. Concentrations in gut, liver and kidney (organs associated with excretion) also had higher than blood concentrations of frovatriptan. Low levels were observed in rat and dog brain (around 10% of those in blood) and in placenta of rat and rabbit;

below quantifiable limits in the fetus. Sponsor suggests that high blood concentrations of frovatriptan observed in the mouse clastogenicity study was indicative of high exposure in the bone marrow.

Clearance: The following is sponsor's review of clearance. Both renal and metabolic clearance mechanisms are involved in the elimination of frovatriptan from the circulation. The relative importance of renal and metabolic clearance varies among species with renal elimination playing a major role in the elimination of unchanged parent drug in the mouse, rat, and dog. Elimination by metabolism is more important in the rabbit and in man.

The rates of both renal and metabolic clearance are relatively slow in all species examined leading to a slow overall clearance and long half-life. This implies that blood binding rather than the elimination capacity itself may control the clearance of frovatriptan.

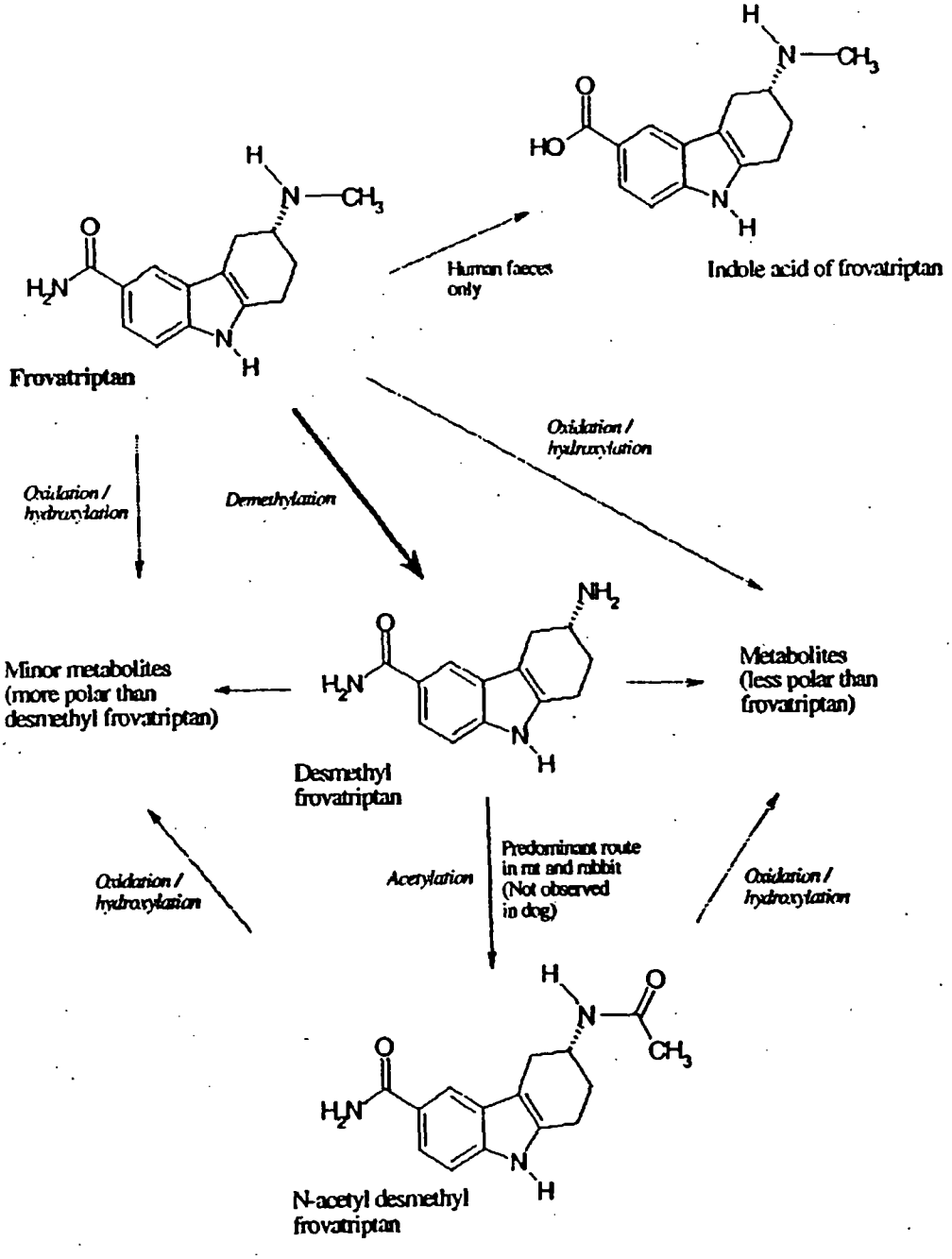
The kidney is capable of excreting both parent frovatriptan and its metabolites. In each species examined the metabolite profiles found in urine are qualitatively similar to those of blood and plasma. Quantitative similarities were generally observed between the profiles of circulating and urinary metabolites. The minor differences in the relative quantities of circulating and urinary metabolites may have resulted not only from differences in the renal clearance, CL_R of the individual metabolites but also differences in Vd, in renal metabolism or in recovery prior to analysis.

Following IV administration of [^{14}C] frovatriptan in the rat and dog about 18% to 19% of the administered radioactivity was recovered in feces, demonstrating that fecal excretion is also a route of elimination. The metabolic profile in the feces after IV administration resembled the blood and urinary metabolite profiles with the presence of the N-acetyl desmethyl and desmethyl metabolites in the rat and dog, respectively. The proportion of radioactivity attributed to parent frovatriptan in feces was greater following oral administration indicative of incomplete absorption by this route. The high levels of radioactivity in the bile and gall bladder of the dog following oral administration suggest biliary excretion of parent drug and/or metabolite may be the route by which they arrive in the feces. In man only low levels of circulating metabolites were found in feces after oral administration and about 40% to 50 of % of the administered dose was recovered in urine after IV administration. Taken together these findings indicate that, at most, biliary excretion only plays a minor role in elimination in man.

Frovatriptan and its metabolites were also excreted in the milk of lactating rats. Maximum concentrations in milk were about 4-fold greater than maximum blood concentrations. Whilst this is of little significance in terms of bulk administration for the mother, it is a source of exposure to the drug for the neonate. However, there were no indications in a pre/postnatal development study that the neonate was affected by this exposure.

A schematic of the proposed metabolic pathways, based on metabolites isolated and identified in blood, urine and feces of man, mouse, rat, rabbit and dog, is illustrated by the sponsor and shown on the page that follows.

Proposed Metabolic Pathways for Frovatriptan



Sponsor's Table 5.2.3.E1, which follows, is a summary of metabolites isolated in blood and urine following a single oral dose, which allows for a comparison in both blood and urine of 5 species. The doses selected for this table represent the human dose and the highest dose orally used in each animal species for the carcinogenicity tests (mouse and rat) or the 1-year dog study. Sponsor's summary table of metabolites found in plasma is not shown but is covered in the text that follows.

Table 5.2.3.E1

Frovatriptan: Metabolite Profiles in Blood and Urine

Blood 4 h post-dose			HUMAN mean of 4 (2m+2f)		MOUSE pool of 5 (m)		RAT pool of 3 (m)		RABBIT mean of 2 (f)		DOG mean of 2 (m)	
Dose			2.5 mg		40(mg/kg)		100 (mg/kg)		20 (mg/kg)		12.5 (mg/kg)	
	Fraction HB	RT (min)	% of sample	Conc (ng eq/mL)	% of sample	Conc (ng eq/mL)	% of sample	Conc (ng eq/mL)	% of sample	Conc (ng eq/mL)	% of sample	Conc (ng eq/mL)
DMF	3	31.4	12.05	0.83	22.43	25.6	x		16.89	94.1	7.65	111.5
F	4	34	46.13	2.50	62.05	70.7	83.97	1416.0	58.74	301.4	68.09	1031.5
	5	45.8	17.01	1.01	0.39	0.4	1.53	25.8	x		0.90	10.0
					0.99	1.1			3.52	18.0	1.71	19.5
(NADMF)					1.57	1.8	3.35	56.5	7.40	40.3	x	
					0.76	0.9	x		1.58	9.1	1.16	22.0
	6	50.6	5.29	0.28	1.00	1.1	2.82	47.6	1.13	6.5	x	
	others		19.52	1.13	10.81	12.3	8.33	140.2	8.75	44.2	20.50	323.5
Total				5.56		114.0		1686.0		513.4		1518.0

Urine	Sampling period			0-48 h		0-24 h		0-4 h		4-24 h		0-24 h	
	Fraction HU	RT (min)	% of sample	% of dose	% of sample	% of dose	% of sample	% of dose	% of sample	% of dose	% of sample	% of dose
DMF	7	32.8	8.17	2.38	4.58	0.45	x		2.97	0.11	8.93	2.49
F	8	35.5	33.08	9.62	84.36	7.63	88.28	0.56	22.46	0.86	74.20	26.67
	11	42.3	0.75	0.22	0.29	0.03	x		x		3.51	1.26
	12	44.3	4.09	1.19	0.42	0.04	x		9.38	0.36	7	
	13	46.1	16.47	4.79	1.06	0.09	0.99	0.01	4.16	0.16	3.58	1.29
(NADMF)	14	46.9	12.51	3.64	3.30	0.30	7.01	0.04	30.39	1.54	1.28	0.46
	15	48.0	8.59	2.50	x		x		3.62	0.14	x	
	16	50.3	2.13	0.62	0.50	0.05	x		x		x	
others			14.24	4.14	5.12	0.46	3.72	0.02	17.06	0.65	10.49	3.77
Total				29.09		9.04		0.63		3.81		35.94

x = no corresponding radioactivity peak discernible in this species

* = no corresponding radioactivity peak discernible in the 4-h sample, but observed at other time points

* = Identification by ———— only, no corresponding radioactivity peak discernible

Retention times (RT; ... more accurate identification of frovatriptan (F), desmethyl frovatriptan (DMF) or N-acetyl desmethyl frovatriptan (NADMF) and of unknown metabolites by ——

Desmethyl frovatriptan was observed in the blood of human, mouse, rabbit and dog, parent compound was observed in blood of all 5 species, and N-acetyl desmethyl frovatriptan was observed in the blood of human, mouse, rat, and rabbit. Sponsor suggests that when present in a species, the concentrations of these metabolites in blood of animals given doses corresponding to those used in the toxicology studies greatly exceeded the concentrations estimated after administration of the intended clinical dose of 2.5 mg. In addition, accumulation of the metabolites on repeated daily administration of frovatriptan in toxicity studies, should at least equal that seen with the parent frovatriptan and will probably exceed it in the case of desmethyl frovatriptan due to its longer t1/2. (This can be inferred from the increase in ratio of desmethyl frovatriptan to frovatriptan with time.)

Generally, there was a close resemblance between plasma or blood and urinary metabolite profiles. In human urine, radioactivity recovered between 0 to 48 hours post-dose corresponded to about 29% of the administered dose and was separated into at least 17 discernible fractions by chromatography. Fractions identified as parent frovatriptan (HU8), desmethyl frovatriptan (HU7)

and N-acetyl desmethyl frovatriptan (HU14), hydroxylated frovatriptan (HU13) and hydroxylated N-acetyl desmethyl frovatriptan (HU15) accounted for about 33.1%, 8.2%, 12.5%, 16.5% and 8.6%, respectively, of the recovered urinary radioactivity. Other fractions accounted individually for less than 5% of the sample radioactivity.

As shown in the table above, desmethyl frovatriptan was observed in the urine of the human, mouse, rabbit and dog. Parent compound was observed in urine of all 5 species, and N-acetyl desmethyl frovatriptan was observed in the urine of all 5 species.

Due to very low levels of radioactivity in blood and plasma and proximity of retention times, a group of less polar metabolite fractions including those with the same retention times as N-acetyl desmethyl frovatriptan, hydroxylated frovatriptan and hydroxylated N-acetyl desmethyl frovatriptan could not be adequately separated for quantification. Sponsor suggests there is a high probability that the group of less polar metabolites seen in blood and plasma contained N-acetyl desmethyl frovatriptan and the hydroxylated metabolites in an approximately similar ratio to those found in urine.

A metabolite fraction designated as HF3 was found only in fecal samples of man (20%) and was also found in feces of dogs (not present in blood or urine). Sponsor suggests that the intestinal bacterial flora was responsible for this metabolite.

Sponsor summarized IV administration animal studies as follows. In general, the proportion of parent drug recovered in urine or in feces closely reflected the relative circulating levels. The proportion of parent drug metabolized and in circulation after oral administration appeared to be unaffected by dose in the species where different dose levels have been studied. There were no apparent gender differences in the extent or in the nature of metabolism in mouse, rat and dog (only females used in rabbit studies) in keeping with the similarity in male and female blood levels of parent frovatriptan observed in toxicokinetic studies. The following general species ranking in terms of extent of metabolism can be made: rabbit > human > dog > rat » mouse.

Enzymes Involved in Metabolism (Sponsor's summary)

The involvement of cytochrome P450 (CYP) enzymes in the metabolism of frovatriptan was studied using cDNA-expressed human cytochromes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4) and cryopreserved human hepatocytes. Frovatriptan was weakly metabolized in the 2 test systems. CYP1A2 appears to be involved in frovatriptan metabolism, based on the observations that a) frovatriptan was metabolized in the presence of human CYP1A2, and b) a total inhibition of the metabolism was observed when frovatriptan was co-incubated with furafylline, a specific CYP1A2 inhibitor. It was concluded that CYP1A2 plays a major role in frovatriptan biotransformation. Therefore in the clinical situation, drugs metabolized by CYP1A2 or inhibitors of CYP1A2 could decrease frovatriptan metabolism and inducers of CYP1A2 may increase the metabolism of frovatriptan.

N-acetyl desmethyl frovatriptan formation from desmethyl frovatriptan was greater in high acetylators in human studies, indicating the role of N-acetyl transferase in this transformation. However, the concentrations of parent frovatriptan appear to be unaffected by acetylator status.

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. Microscopic evaluation included all indicated tissues from control, high dose and decedent animals and gross lesions from all animals.

Rationale for Dose Selections: The maximum dose of 40 mg/kg was expected to result in systemic exposure that would exceed 25 times the AUC of that resulting in humans at maximum therapeutic dose. The low dose was expected to result in systemic exposure that would be a small multiple of the human dose and the intermediate dose was the geometric mean of the low and high doses.

Compound Related Effects in the Main Study

Mortality

In the males, mortality in control group 5 was significantly higher than control group 1 ($P<0.01$). The mortality in each treated group was not significantly different from the combined control groups, but when compared to each control separately, mortality of the 13 mg/kg/day males was higher than control group 1 ($P<0.05$ after Bonferroni adjustment).

Survival to the Start of Terminal Kill in the 84-Week Mouse Oncogenicity Study

Dose	Survival	
(mg/kg/day)	Male	Female
0	37 (73)	32 (63)
4	32 (63)	30 (59)
13	26 (51)	36 (71)
40	30 (59)	33 (65)
0	24 (47)	28 (55)

( ) figures in brackets indicate percentage survival

Clinical Signs:

Red extremities were observed after dosing at high dose throughout the study and at mid dose from 52 weeks on.

There were no effects on other parameters measured in this study, including gross and histopathology.

Figure 1

Group mean survival - males

Test article	Control I		VML 251		Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	4	13	40	0

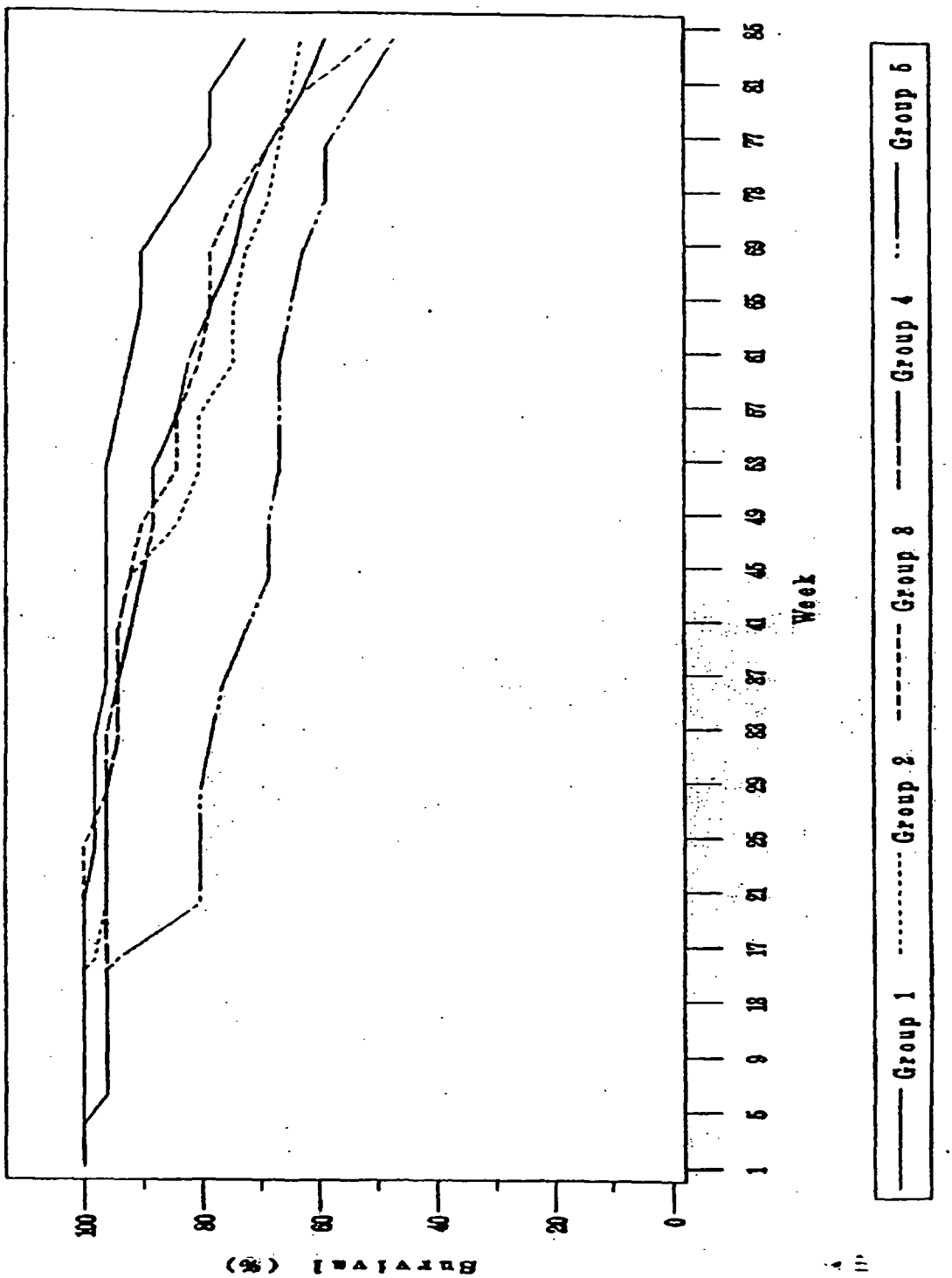


Figure 2

Group mean survival - females

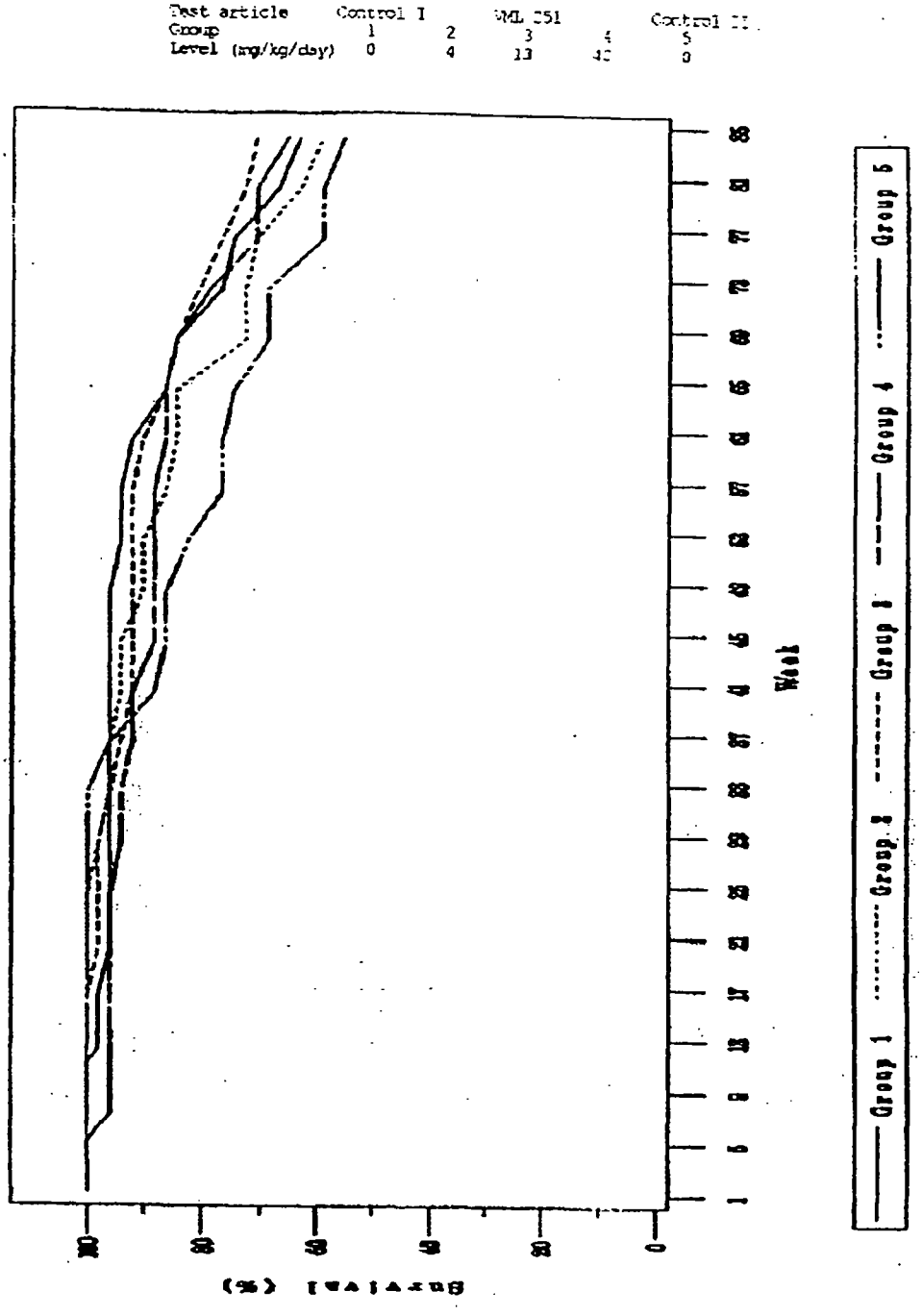


Figure 3

Group mean body weight - males

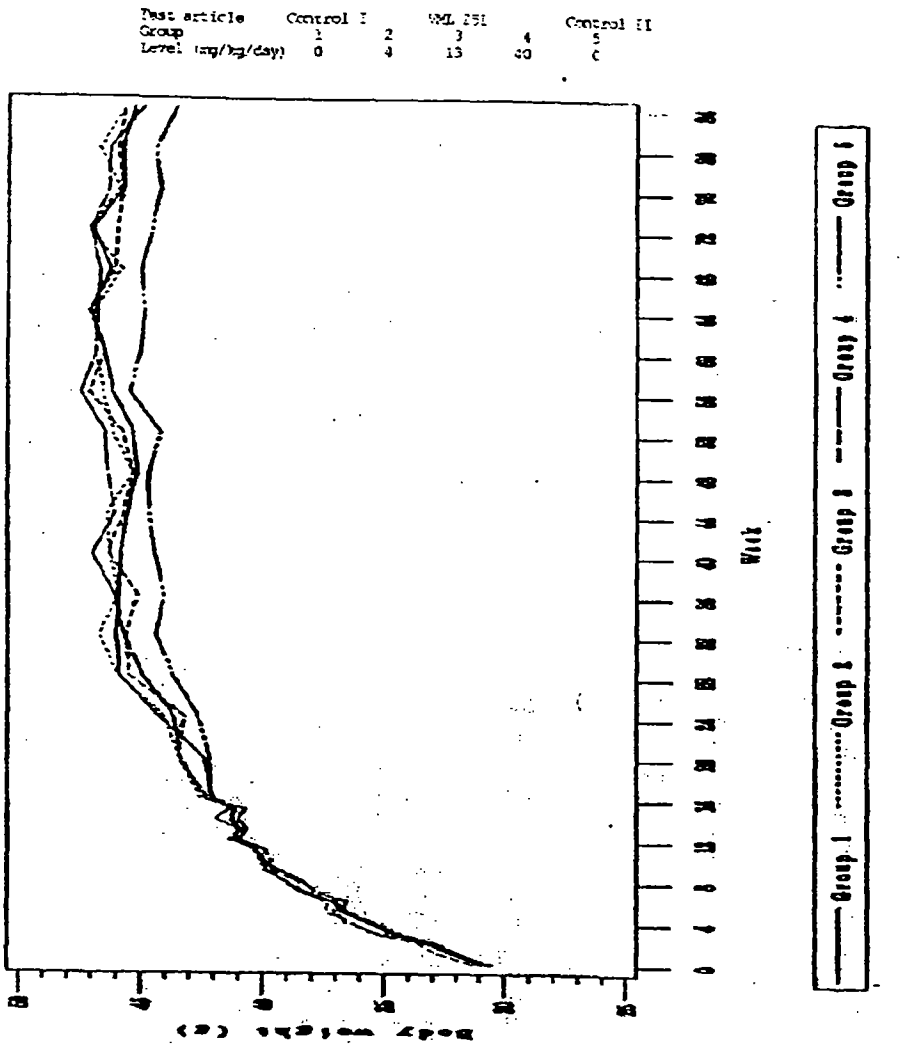


Figure 4

Group mean body weight - females

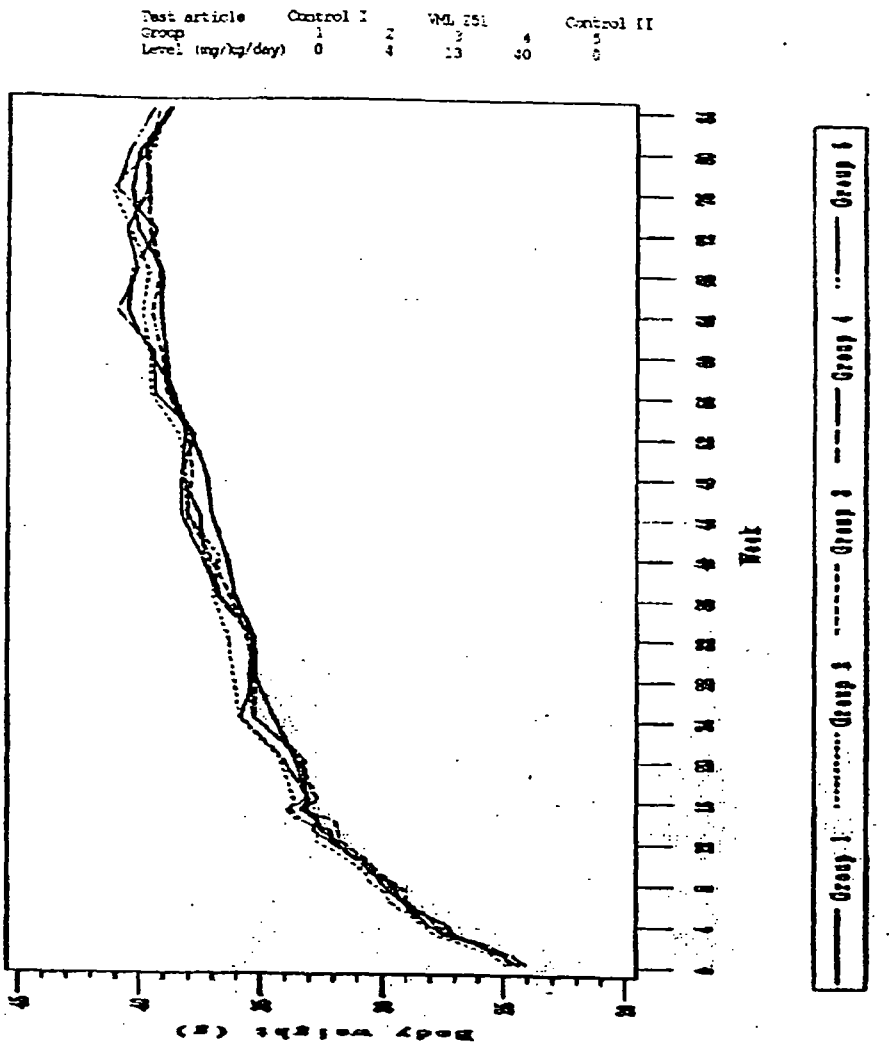


TABLE 3.6

Group incidence: histopathology - neoplastic data - combined

Test article	Control		AML 251		
Group	1	2	3	4	5
Level (mg/kg/day)	0	4	13	40	0

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STUDY NUMBER: 11

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

		--- N U M B E R - O F - A N I M A L S - A F F E C T									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	51	51	51	51	51	51	51	51	51	51
** TOP OF LIST **											
SKIN + SUBCUTIS	NUMBER EXAMINED:	51	22	29	51	51	51	26	26	51	51
--B-SQUAMOUS CELL PAPILLOMA		0	0	0	0	0	0	0	0	0	1
--M-OSTEOSARCOMA		0	0	0	0	0	0	1	0	0	0
--M-SARCOMA		1	1	1	0	1	1	1	0	0	0
MAMMARY GLAND	NUMBER EXAMINED:	0	0	0	0	0	51	20	15	48	51
--M-CARCINOMA		0	0	0	0	0	1	1	0	0	2
LIVER	NUMBER EXAMINED:	51	36	33	51	51	51	26	21	51	51
--B-HEPATOCELLULAR ADENOMA		9	7	9	11	4	0	0	0	0	0
--M-HEPATOCELLULAR CARCINOMA		1	1	1	1	0	0	0	0	0	0
PANCREAS	NUMBER EXAMINED:	51	27	25	49	50	50	20	16	51	51
--B-ISLET CELL ADENOMA		1	0	0	0	0	0	0	0	0	0
DUODENUM	NUMBER EXAMINED:	48	16	15	44	41	48	17	11	48	50
--B-ADENOMA		0	0	0	0	0	0	0	1	0	0
COLON	NUMBER EXAMINED:	49	17	23	47	45	47	17	14	49	51
--M-CARCINOMA		0	0	0	0	1	0	0	0	0	0
GALL BLADDER	NUMBER EXAMINED:	48	17	19	44	36	43	20	21	48	47
--B-PAPILLOMA		0	1	0	0	0	0	0	0	0	0
ADRENAL	NUMBER EXAMINED:	51	19	25	51	51	51	22	15	51	51
--B-CORTICAL ADENOMA		1	0	0	0	0	1	0	0	0	0

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TABLE 8.6

Group incidence: histopathology - neoplastic data - combined

Test article	Control		VHL 251		
Group	1	2	3	4	5
Level (mg/kg/day)	0	4	13	40	0

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PAGE: 2

STUDY NUMBER: 11

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

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECT									
SEX:		----------MALE----------					----------FEMALE----------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		51	51	51	51	51	51	51	51	51	51
KIDNEY	NUMBER EXAMINED:	51	26	36	51	51	51	22	16	51	51
--B-TUBULAR CELL ADENOMA		0	0	0	0	1	0	0	0	0	1
TESTIS	NUMBER EXAMINED:	51	21	26	51	51	0	0	0	0	0
--B-RETE TUBULAR ADENOMA		0	0	0	1	0	0	0	0	0	0
--B-INTERSTITIAL CELL ADENOMA		1	1	1	1	1	0	0	0	0	0
OVARY	NUMBER EXAMINED:	0	0	0	0	0	50	50	49	51	50
--B-CYSTADENOMA		0	0	0	0	0	1	0	0	1	0
--B-BENIGN LUTEOMA		0	0	0	0	0	1	0	0	0	0
--B-BENIGN GRANULOSA CELL TUMOUR		0	0	0	0	0	0	1	0	0	0
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	51	41	46	51	51
--B-ADENOMA		0	0	0	0	0	0	0	0	1	1
--B-POLYP		0	0	0	0	0	3	3	2	2	4
--B-LEIOMYOMA		0	0	0	0	0	1	1	1	1	1
--M-LEIOMYOSARCOMA		0	0	0	0	0	1	0	0	1	0
THYMUS	NUMBER EXAMINED:	49	20	22	49	49	49	27	18	51	48
--B-BENIGN THYMOMA		0	0	0	0	0	1	0	0	0	0
LUNG	NUMBER EXAMINED:	51	31	32	51	51	51	22	17	51	51
--B-BRONCHIOLO-ALVEOLAR ADENOMA		8	11	7	10	4	5	1	1	4	6
--M-BRONCHIOLO-ALVEOLAR CARCINOMA		3	1	3	2	1	0	0	0	2	3
PITUITARY	NUMBER EXAMINED:	51	18	23	50	48	51	22	16	51	49
--B-ADENOMA		0	0	0	0	0	0	2	0	0	2

TABLE 8.6

Group incidence: histopathology - neoplastic data - combined

Test article	Control			VML 251	
Group	1	2	3	4	5
Level (mg/kg/day)	0	4	13	40	0

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STUDY NUMBER:

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

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:		----------MALE----------					----------FEMALE----------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		51	51	51	51	51	51	51	51	51	51
BRAIN	NUMBER EXAMINED:	51	19	25	51	51	51	21	15	51	51
--B-MENINGIOMA		0	0	0	0	0	0	0	0	0	1
HAEM/LYMPH/RETIC	NUMBER EXAMINED:	51	21	25	51	51	51	22	17	51	51
--M-LYMPHOCYTIC LYMPHOMA		0	1	0	2	0	0	0	1	2	1
--M-MIXED LYMPHOMA		0	2	1	0	1	1	2	1	2	1
--M-LYMPHOMA		0	0	0	0	0	1	0	1	1	0
--M-LYMPHOCYTIC LEUKAEMIA		0	1	0	0	0	1	1	2	1	1
--M-LEUKAEMIA		1	1	0	0	0	0	0	0	0	0
--M-GRANULOCYTIC LEUKAEMIA		0	0	0	0	0	0	0	0	1	0
--M-MAST CELL SARCOMA		0	0	0	0	0	0	0	0	1	0
--M-HISTIOCYTIC SARCOMA-LIVER		1	0	0	0	0	1	1	1	0	2
--M-HISTIOCYTIC SARCOMA-UTERUS		0	0	0	0	0	1	1	0	0	1
BLOOD VESSEL	NUMBER EXAMINED:	51	20	25	51	51	51	22	17	51	51
--B-HAEMANGIOMA - UTERUS		0	0	0	0	0	2	0	0	0	2
--B-HAEMANGIOMA - LIVER		0	1	0	0	1	0	1	0	0	1
--B-HAEMANGIOMA - SPLEEN		1	0	0	0	1	1	0	1	2	2
--B-HAEMANGIOMA - MESENTERIC LYMPH NODE		0	0	0	0	1	0	0	0	0	0
--M-HAEMANGIOSARCOMA - BONE		0	1	0	0	0	0	0	0	0	0
--M-HAEMANGIOSARCOMA - TESTIS		0	0	0	1	0	0	0	0	0	0
PREPUT/CLIT GL	NUMBER EXAMINED:	16	11	10	8	15	2	0	1	2	2
--M-CARCINOMA		0	0	0	0	0	0	0	0	0	1
BONE	NUMBER EXAMINED:	0	1	0	1	2	0	2	0	1	1
--B-CHONDROMA		0	0	0	0	1	0	0	0	0	0

** CONTINUED ON NEXT PAGE **

TABLE 8.6

Group incidence: histopathology - neoplastic data - combined

Test article	Control			VHL 251	
Group	1	2	3	4	5
Level (mg/kg/day)	0	4	13	40	0

[ ]

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PAGE: 4

STUDY NUMBER: 1

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

		--- NUMBER-OF-ANIMALS-AFFECT									
	SEX:	----------MALE----------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	51	51	51	51	51	51	51	51	51	51
** FROM PREVIOUS PAGE **											
BONE	NUMBER EXAMINED:	0	1	0	1	2	0	2	0	1	1
--B-OSTEOMA		0	0	0	0	0	0	0	0	0	1
--M-OSTEOSARCOMA		0	0	0	0	1	0	1	0	0	0
HARDERIAN GLAND	NUMBER EXAMINED:	0	0	0	0	0	0	1	0	1	0
--B-ADENOMA		0	0	0	0	0	0	1	0	1	0
** END OF LIST **											

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TABLE 9

Statistical analysis of tumours

Tumour incidence in males and females: groups 1, 4 and 5 analysed

Test article	Control			VML 251	
Group	1	2	3	4	5
Level (mg/kg/day)	0	4	13	40	0

Results of tests for increasing and decreasing dose response

Tumour type		Number of tumour bearing animals in Group 1	4	5	Included in analysis	Groups 1,5	Groups 1+5v4	Method of analysis
Males								
Testis interstitial cell adenoma	NF	1	1	1	NF	NS	NS	P
HLR lymphoid tumours	NF	0	0	1				
	F	1	2	0				
	ALL	1	2	1	ALL	NS	NS	P
Liver hepatocellular tumours	NF	7	11	4				
	F	1	1	0				
	ALL	8	12	4	ALL	NS	NS	L
Lung alveolar epithelial tumours	NF	10	11	5				
	F	1	1	0				
	ALL	11	12	5	ALL	NS	NS	L
Blood vessel tumours	NF	1	0	3				
	F	0	1	0				
	ALL	1	1	3	ALL	NS	NS	P
Females								
Mammary gland carcinoma	NF	0	0	2				
	F	1	0	0				
	ALL	1	0	2	ALL	NS	NS	P
Uterus polyp	NF	3	2	4	NF	NS	NS	P
HLR lymphoid tumours	NF	1	3	2				
	F	2	3	1				
	ALL	3	6	3	ALL	NS	NS	L
Lung alveolar epithelial tumours	NF	5	5	6				
	F	0	1	3				
	ALL	5	6	9	ALL	NS	NS	L
Uterus smooth muscle tumours	NF	1	2	1				
	F	1	0	0				
	ALL	2	2	1	ALL	NS	NS	P
Blood vessel tumours	NF	2	2	5				
	F	1	0	0				
	ALL	3	2	5	ALL	NS	NS	L
HLR histiocytic sarcoma	NF	0	0	2				
	F	2	0	1				
	ALL	2	0	3	ALL	NS	NS	P

F = fatal
NF = non-fatal
L = large sample tests
P = permutational tests

NS = not significant for increasing or decreasing dose response (P>0.05)

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Toxicokinetics

Blood samples from 2 animals/sex on the satellite study for toxicokinetic analysis were taken from 2 animals/sex/group at 1, 2, 3, 4, 8 and 24 hours after dosing, on Day 1, Week 13, 25, 52 and 80. The analyses were done at ——— The tables that follows (which does not include data for Week 13) is sponsor's summary of toxicokinetic parameters derived from the concentrations found in blood.

The data generally indicates that blood concentrations (based on C_{max} and AUC) in both males and females after treatment for 25 and 52 weeks were continuously increasing with time, indicating bioaccumulation. There was apparently no further increase between 52 and 78 weeks of treatment.

Excluding AUCs for low dose, both C_{max} and AUC were approximately dose proportional at all time intervals, and T_{max} (with the exception of mid dose males at Week 13, which had an outlier) ranged from 1 to 4 hours.

The last table on page 29 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 Week 2) and end of the study.

Mean data for C_{max} – Males

Dose (mg/kg/day)	Dose Ratio	Day 1 (ng/mL)	C_{max} ratio	Week 25 (ng/mL)	C_{max} ratio	Week 52 (ng/mL)	C_{max} ratio	Week 80 (ng/mL)	C_{max} Ratio
4	1.0	257.4	1.0	259.1	1.0	241.7	0.9	303.7	1.2
13	3.3	527.0	2.0	626.7	2.4	1076.6	4.2	1278.4#	5.0
40	10.0	2165.9	8.4	2010.5	7.8	3161.3#	12.3	2677.1	10.4

value based upon single result only

Mean data for C_{max} – Females

Dose (mg/kg/day)	Dose ratio	Day 1 (ng/mL)	C_{max} ratio	Week 25 (ng/mL)	C_{max} ratio	Week 52 (ng/mL)	C_{max} ratio	Week 80 (ng/mL)	C_{max} ratio
4	1.0	238.4	1.0	270.4	1.1	397.9	1.7	337.5	1.4
13	3.3	869.8	3.6	797.1	3.3	1041.8	4.4	897.4	3.8
40	10.0	1493.5	6.3	2346.0	9.8	3564.3#	15.0	2699.7	11.3

value based upon single result only

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

Dose (mg/kg/day)	Dose ratio	Day 1 (ng.h/mL)	AUC ratio	Week 25 (ng.h/mL)	AUC ratio	Week 52 (ng.h/mL)	AUC ratio	Week 80 (ng.h/mL)	AUC Ratio
4	1.0	716.3	NA	3224.0	1.0/1.0	4085.1	1.3/1.0	3072.6	1.0/1.0
13	3.3	6626.7	1.0	11295.2	1.7/3.5	10324.6	1.6/2.5	12058.3	1.8/3.9
40	10.0	14817.8	1.0	31412.5	2.1/9.7	38584.4	2.6/9.4	40200.7	2.7/13.1

NA = Proportionality not calculable, due to non-quantifiable levels of VML-251 at 24 hours in Day 1, low dose group

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

Dose (mg/kg/day)	Dose ratio	Day 1 (ng.h/mL)	AUC ratio	Week 25 (ng.h/mL)	AUC ratio	Week 52 (ng.h/mL)	AUC ratio	Week 80 (ng.h/mL)	AUC Ratio
4	1.0	988.9	NA	3174.1	1.0/1.0	5407.3	1.7/1.0	3866.3	1.2/1.0
13	3.3	6370.8	1.0	8717.6	1.4/2.7	10423.3	1.6/1.9	10591.0	1.7/2.7
40	10.0	13057.6	1.0	32222.3	2.5/10.2	38445.3	2.9/7.1	39604.1	3.0/10.2

NA = Proportionality not calculable, due to non-quantifiable levels of VML-251 at 24 hours in Day 1, low dose group

Mean data for T_{max} – Males

Dose (mg/kg/day)	Day 1 (h)	Week 25 (h)	Week 52 (h)	Week 80 (h)
4	3.0	4.0	2.0	2.0
13	2.0	24.0#	1.0	1.0
40	1.0	4.0	1.0	8.0#

value due to one sample with high concentration at this timepoint

Mean data for T_{max} – Females

Dose (mg/kg/day)	Day 1 (h)	Week 25 (h)	Week 52 (h)	Week 80 (h)
4	1.0	1.0	4.0	1.0
13	1.0	2.0	3.0	2.0
40	1.0	1.0	2.0	2.0

Sponsor's Table 5.2.2.1B
Comparison of Doses Administered and Exposures to Frovatriptan in Mouse 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
Mouse	40	172	19	38.6
Man	0.04	1.6	0.094	-
Multiple Mouse : man	1000	106	138	410

2. Rat

13-Week Oral (Gavage) Range-Finding Study in the Rat (Vol 23)

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 8/15/96, necropsies completed 11/18/96.

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 187.1-221.1g for males and 146.8-182.3 g for females.

Test Substance: Batch number 60467-05, 60475-06 and 60532-12; purity of 98.2, 103.1 and 98.6%, respectively, for the 3 different batches.

Procedure: In the main study, 15 rats/sex/group received frovatriptan at 0, 10, 100 and 1000 mg/kg/day. 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 animal at pretreatment, but only on control and high dose animals in Week 12. Clinical pathology was performed from blood samples obtained in Week 13. 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. 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 Week 13. A satellite toxicokinetic study, consisting of 3 groups of 10 animals of each sex, received 10, 100 or 1000 mg/kg/day, and 5/sex in the control group, was included.

Postmortem evaluations of all animals surviving to term and decedents where possible, included routine gross pathology and weighing of adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, and testes with epididymides combined and thyroids with parathyroids. 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, 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, mammary glands, larynx, 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, 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. Microscopic evaluation included all indicated tissues from control, high dose and all decedent animals, gross lesions from all animals, and, in addition, kidneys from low and intermediate dose animals.

Results (Except for mortality, only compound related effects are listed)

Main Study

Mortality: One control male (due to dosing error during week 13), 1 high dose male and 1 high dose female (both during week 10); causes of deaths in the latter 2 animals could not be determined but there was no evidence of specific target organ toxicity.

Clinical Signs: Red extremities in all animals after dosing in mid and high dose groups; a few on low dose only on the first 2 days of dosing. Salivation in all animals almost every day in high dose animals, occasionally in mid dose animals. Paddling (exaggerated movement of forelimbs) occasionally seen at high dose.

Clinical Pathology: There were slight increases in a few red blood cell parameters (HB, PCV, MCH and MCHC) mainly in high dose treated animals. Females at high dose showed slightly longer prothrombin times ($P<0.01$) relative to controls. High dose males had slightly increased total bilirubin ($P<0.01$), high dose females had slightly elevated ALP ($P<0.001$) and slightly decreased total cholesterol ($P<0.01$) relative to controls.

Organ Weights: Weights of a few organs, adjusted for terminal body weight, were higher than control only at high dose, including adrenal (20 and 23% in males and females, respectively; $P<0.05$), kidneys (12%; $P<0.05$ and 22%; $P<0.001$ for males and females, respectively), heart (10 and 18% for males and females, respectively; $P<0.01$ only for females) liver (12% in females; $P<0.001$), ovaries (31%, $P< 0.05$), pituitary (31% only in females; $P<0.05$) but prostate was slightly lighter (16%, $p<0.05$).

Histopathology: The only compound related effect appeared to be an increase in incidence and severity of tubular basophilia/regeneration in the kidney of the high dose group animals compared with control. The incidence and severity of this finding in intermediate and low dose groups were similar to that in controls. Except for the kidneys, there were no histopathology changes associated with increased organ weights (see table below).

The NOAEL is considered to be 100 mg/kg/day, based on effects on the kidneys.

TABLE 6

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

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

Group Sex	Body weight (g)		AD	KI	SP	LI	HT	BR	PI	TY
1M	491.3	Adjusted	0.054	2.480	0.835	14.157	1.442	2.106	0.012	0.025
		Unadjusted	(0.053)	(2.444)	(0.823)	(13.717)	(1.415)	(2.095)	(0.012)	(0.024)
2M	512.9	Adjusted	0.057	2.640	0.771	13.054	1.398	2.068	0.013	0.022
		Unadjusted	(0.059)	(2.694)	(0.789)	(13.717)	(1.438)	(2.085)	(0.013)	(0.023)
3M	503.4	Adjusted	0.059	2.468	0.800	14.323	1.518	2.091	0.014	0.022
		Unadjusted	(0.060)	(2.495)	(0.809)	(14.652)	(1.538)	(2.099)	(0.014)	(0.022)
4M	475.0	Adjusted	0.065*	2.772*	0.820	14.133	1.586	2.065	0.013	0.025
		Unadjusted	(0.064)	(2.718)	(0.802)	(13.471)	(1.545)	(2.048)	(0.013)	(0.024)

Statistics	A	C	C	C	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 6

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

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

Group Sex	Body weight (g)		AD	KI	SP	LI	HT	BR	PI	TY
1F	299.3	Adjusted	0.060	1.584	0.576	7.034	0.964	1.979	0.013	0.015
		Unadjusted	(0.061)	(1.602)	(0.580)	(7.122)	(0.979)	(1.994)	(0.013)	(0.015)
2F	283.1	Adjusted	0.065	1.606	0.554	7.142	1.091*	1.998	0.015	0.017
		Unadjusted	(0.064)	(1.558)	(0.539)	(6.902)	(1.051)	(1.983)	(0.015)	(0.017)
3F	296.9	Adjusted	0.062	1.661	0.629	7.431	1.027	1.971	0.016	0.016
		Unadjusted	(0.062)	(1.673)	(0.633)	(7.487)	(1.037)	(1.975)	(0.016)	(0.017)
4F	299.2	Adjusted	0.074*	1.940***	0.651	7.987***	1.139**	2.026	0.017*	0.016
		Unadjusted	(0.074)	(1.962)	(0.658)	(7.995)	(1.156)	(2.033)	(0.017)	(0.017)

Statistics	A	C	C	C	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

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26-Week Oral (Gavage) Study in the Rat (Vol 24 and 25)

Report No: 1165/33-1050

Performing Laboratory: [redacted]

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

Dates Performed: Treatment initiated on 9/17/96, necropsies completed 3/24/97.

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

Test Animals: Crl:CDBR rats, [redacted] At initiation of dosing the rats were 6 week old and body weights were 183.0-227.5g for males and 116.5-187.8 g 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: In the main study, 20 rats/sex/group received frovatriptan at 0, 10, 100 and 1000 mg/kg/day, the dose volume was 5 mL/kg/day. Dosage is based on free base. Animals were observed daily for clinical signs, morbidity and mortality, weekly for body weights and food consumption. Detailed physical exams were given weekly. Ophthalmology was performed on all animal at pretreatment, but only on control and high dose animals in Week 25. Clinical pathology was performed from blood samples obtained in Week 13 and 25. 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 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 12 and 25. A satellite toxicokinetic study, consisting of 3 groups of 10 animals of each sex, received 10, 100 or 1000 mg/kg/day, and 5/sex were in the control group. Blood samples were obtained at pre-treatment, 2, 3, 4, 7 and 24 hours after dosing on Day 1 and Week 26.

Postmortem evaluations of all animals surviving to term and decedents where possible, included routine gross pathology and weighing of adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, and testes with epididymides combined and thyroids with parathyroids. 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, 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, mammary glands, larynx, 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, 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. Microscopic evaluation included all indicated tissues from control, high dose and all decedent animals, gross lesions from all animals, and, in addition, liver, spleen and kidneys from low and mid dose animals. Subsequently, tissues from adrenals and thyroid from low and mid dose animals were also included because of effects noted with these 2 tissues and with kidneys at high dose.

Results

Main Study (Compound related effects)

Mortality: Compound related deaths occurred in 4 animals at high dose (3M and 1F) in Weeks 3, 5, 19 and 23; cause of death for all of them was considered to be renal or urogenital tract lesions. The male that died in Week 19 and the female that died in Week 23 also had adrenal medullary atrophy. Both of these histopathology findings were observed as treatment related in terminal kill animals. Other main study findings in these 4 animals included atrophy of the thymus, lymph nodes and spleen, hypertrophy of the adrenal cortex. Two additional high dose male deaths were considered not compound related (1 in Week 5 of undetermined cause but "possibly cardiovascular lesion" and 1 in Week 25 due to eye damage during blood sampling). Other deaths included 2 females at low dose (1 in Week 13 of undetermined, possibly cardiac lesion and 1 in Week 19 with a thymic tumor), and 1 of each sex at mid dose (the male at week 25 with eye damage due to blood sampling and the female in Week 26 with a hemolymphoreticular tumor.

Clinical Signs: Red extremities throughout the course of the study in all animals within a half hour after dosing each day in mid and high dose groups, still noted at 4 hours after dosing, but generally resolved by the end of the day. Salivation in all animals almost every day in high dose animals immediately after dosing but resolved by 30 minutes, occasionally in mid dose animals. Paddling (exaggerated movement of forelimbs) was seen immediately after dosing at high dose, but resolved by 30 minutes.

Clinical Pathology: Slight increases in a few red blood cell parameters (HB, PCV, MCH and MCHC) in high dose males at both time periods compared to control; also high dose females had slightly increased red blood cell parameters by Week 25. Lower platelet count in high dose males at both time periods. Higher neutrophil count was seen in high dose females at Week 13 and in both high dose males and females by Week 25. Decreased urea in high dose females in Week 13, higher inorganic P in high dose females, lower Cl in high dose males females in Week 25, slightly lower albumin and protein concentration in high dose females in Week 25, ≤ cholesterol levels were lower in high dose females in Week 13 and in high dose of both sexes in

Week 25. AAT in high dose males were higher in Week 25, and total bilirubin in high dose males in Week 13.

Urinalyses: Increased incidence of protein in urine in Weeks 13 and 25 of high dose males and in high dose females in Week 25. Decreased phosphate crystals and increased incidence of amorphous debris in urine of high dose males in Week 25.

Organ Weights: (adjusted for terminal body weight) were higher than control for adrenal (23% in high dose males; P<0.001, 15, 15 and 23% in low, mid and high dose females, respectively; P<0.001), high dose kidneys (21% and 14 % for males and females, respectively; P<0.001), spleen (17% in high dose males; P<0.01, 13 and 22% in mid and high dose females P<0.05 and 0.001, respectively), liver (6 and 8% in mid and high dose females; P<0.05 and 0.01), pituitary (24% only in high dose females; P<0.05, not shown in table) and brain (3% only in high dose females; P<0.05, not shown in table).

Histopathology: Compound related effects were found in the kidneys and adrenals and thyroid. Except for kidneys and adrenals, there was no histopathology associated with increases in weight of other organs.

In the kidneys, tubular nephropathy characterized by multiple foci of degenerating or regenerating basophilic tubules, appearing as fine wedges extending from the capsule to the outer medulla. In some cases, the tubules were dilated and/or sclerotic, with mineralization, but intraluminal casts were not a common feature. The severity of the lesion was very variable, with some high dose animals unaffected, and the lesion was more common in males than in females (see table below). The investigators point out, "Higher kidney weights in high dose animals compared to control values may be related to tubular nephropathy in the kidney of these animals. The changes is plasma chemistry (increased phosphorus and lower plasma chloride, total protein and albumin concentrations) and parameters in urine (decreased phosphate and increased urinary protein and amorphous debris) are also indicative of kidney damage." Four compound related deaths that occurred were associated with renal and urogenital lesions.

"In the adrenal cortex, prominent zona glomerulosa was seen in most mid and high dose animals, particularly males. In affected animals the zona glomerulosa was more distinct because of increased cytoplasmic fine vacuolation and/or increased width of the zona."

"Adrenal medullary atrophy was found in many high dose animals. The overall cross-sectional area of the medulla was reduced, the medullary cells tended to be smaller with a dense granular appearance and were separated by increased interstitial or fibrillar elements. Against this background of general medullary atrophy, focal medullary hyperplasia was seen in some high dose animals. The finding was characterized by sporadic nests of small basophilic medullary cells with occasional mitotic figures." In tissue distribution studies, it was shown that following repeated injections frovatriptan accumulates in the adrenal medulla where it is slowly released, with a half-life for release of about 450-600 hours.

"In the thyroid, there was an increased incidence of follicular cell hypertrophy in high dose animals, and possibly in intermediate dose males, compared with controls. The hypertrophy was