TABLE 8

Group incidence of defects

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

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
	EXTERNAL AND VISCERAL DEFECTS					
	Head/skull					
18#	Internal hydrocephaly	malformation	1 (0.5)			
26	Haemorrhage - subcutaneous: head	variation	3 (0.8)	2 (0.5)	7 (1.8)	3 (0.8)
	Eyes					
49	Eye(s) medially displaced	malformation	1 (0.3)			
51	Anophthalmia	malformation				2 (0.5)
52	Microphthalmia	malformation	1 (0.3)			1 (0.3)
57	Iridial haemorrhage	variation			1 (0.3)	
60	Retinal dysplasia	malformation	1 (0.3)			
70	Lenticular lesion(s) (marked)	malformation	1 (0.3)			
74#	Lens(es) misshapen (marked)	malformation	1 (0.5)			
	Jaws/tongue/teeth					
90	Haemorrhage - subcutaneous: jaw/mouth	variation	5 (1.3)	7 (1.9)	13 (3.3)	15 (4.0)
	Ears					
104	Haemorrhage - subcutaneous: ear(s)	variation	2 (0.5)	4 (1.1)	1 (0.3)	

Figures in brackets denote the percentage of affected foetuses/foetuses examined
A foetus with multiple defects will appear more than once in this tabulation
observed in animals processed in Bouin's solution; number of foetuses examined for Groups 1 to 4 was 182, 179, 191 and 179 respectively

TABLE 8

Group incidence of defects

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

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
	Trunk					
131	Haemorrhage - subcutaneous: dorsal fat pad region	variation			1 (0.3)	
132	Haemorrhage - subcutaneous: trunk	variation	4 (1.1)	1 (0.3)	5 (1.3)	5 (1.3)
140	Oedema - subcutaneous: trunk	variation	1 (0.3)			
	Duplications					
408	Partial conjoined twin	malformation				1 (0.3)
409	Dipygus tetrapus	malformation				1 (0.3)
	Limbs					
163	Haemorrhage - subcutaneous: digit(s)	variation	1 (0.3)	1 (0.3)	2 (0.5)	2 (0.5)
164	Haemorrhage - subcutaneous: limb(s)	variation	13 (3.5)	10 (2.7)	8 (2.0)	22 (5.9)
178	Forelimb ankylosis	malformation	2 (0.5)			
	Tail					
186	Haemorrhage - subcutaneous: tail	variation	3 (0.8)	4 (1.1)		2 (0.5)
190	Filamentous tissue at tip of tail	variation			1 (0.3)	
	Heart and great vessels					
202	Atrium/atria enlarged	variation	1 (0.3)			
260	Origins of ascending aorta and pulmonary trunk transposed	malformation				1 (0.3)

Figures in brackets denote the percentage of affected foetuses/foetuses examined
A foetus with multiple defects will appear more than once in this tabulation

TABLE 8

Group incidence of defects

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

Key	Type of defect	Classification		Group 1	Group 2	Group 3	Group 4
	Thorax						
268	Atelectasis	variation					1 (0.3)
	Abdomen						
304	Incomplete closure of abdominal muscular layer	variation					1 (0.3)
346	Hydroureter	malformation					1 (0.3)
369	Dilated ureter(s)	variation	C	21 (5.6)	51 (13.7)*	58 (14.6)	83 (22.3)**
372	Hydronephrosis	malformation				1 (0.3)	2 (0.5)
373	Increased renal pelvic cavitation (bilateral)	variation		2 (0.5)	5 (1.3)	5 (1.3)	11 (3.0)
374	Increased renal pelvic cavitation (unilateral)	variation		17 (4.5)	17 (4.6)	14 (3.5)	31 (8.3)
379	Gall bladder contents pale	variation			1 (0.3)	4 (1.0)	1 (0.3)
423	Adrenal dark	variation		1 (0.3)			2 (0.5)
424	Spleen - pale	variation				1 (0.3)	1 (0.3)
425	Red foci - urinary bladder wall	variation			1 (0.3)		

Figures in brackets denote the percentage of affected foetuses/foetuses examined
A foetus with multiple defects will appear more than once in this tabulation
C = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank-Sum tests

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

d. Oral Gavage Developmental Toxicity Study in the Rabbits (Vol 42)

Study No. 1165/12-1050

Performing Laboratory: [redacted]

Dates Study Performed: Treatment initiated 1/14/95, necropsies completed 2/10/95

Quality Assurance: A statement of GLP compliance is included.

Test Substance: SB209509, Batch number —'5310/01; purity not indicated.

Test Animals: Time naturally mated Crl:NZW/Kbl BR New Zealand White rabbits, ——— ——— approximately 9 weeks old, were used..

Doses Administered: 0, 5, 20 and 80 mg/kg/day (dosage based on free base)

Rationale for Dose Selection: Based on results of the dose finding study with pregnant rabbits, 7/sex/group with doses of 0, 50, 100 and 200 mg/kg/day (— Study No. 1165/21). In the dose finding study, blood samples for toxicokinetic evaluation were obtained. Administration of frovatriptan at 200 mg/kg/day resulted in unacceptable toxicity [marked weight loss, diarrhea, mucoid discharge from anus, severely decreased food (75%) and water (80%) intake, dark urine, spontaneous abortions, gaseous distension of the cecum]. Animals at high dose were killed on GD 20, "owing to severity of effects". The investigators concluded that minimal maternal and embryo toxicity were evident at 100 mg/kg/day with a NOAEL for both effects at 50 mg/kg/day.

Procedure: Frovatriptan was administered daily to 24 females/group between GD 6 and GD 19. Controls received 1% aqueous methylcellulose vehicle and the dosing volume for all 4 groups was 5 mL/kg. The females were examined twice daily for morbidity and mortality, daily for clinical signs. Body weights were measured 10 times between GD 3 and GD 29, food and water intake were recorded daily between GD 3 and GD 29. C-sections were performed on GD 29 for assessment of pregnancy status, gravid uterine weight, corpora lutea count, number and intrauterine positions of implantations, live fetuses, early and late intrauterine deaths and dead fetuses. If pregnancy was not detected, the uteri were stained with 10% ammonium sulfide. Live fetuses and placentas were weighed, sexed and examined externally for anomalies. After internal examination for visceral anomalies, all fetuses were eviscerated, then half of the fetal heads in each litter were fixed and partially decalcified to obtain serial sections for evaluation of the heads by the Wilson technique. The brains from remaining intact fetuses were removed from the skull and sliced for examination by the Wilson technique. Bodies from intact and decapitated fetuses were processed for staining (Alizarin technique) for skeletal evaluation.

Result; Maternal:

Mortality: Nine high dose females were killed 7 to10 days after initiation of treatment due to marked anorexia (associated with adipsia) and one had red discharge from the vulva. Necropsy revealed all to be pregnant but 6 of the 9 showed a high proportion of intauterine deaths and 3 of the 9 had "dark contents" in the urinary bladder. At mid dose, 1 female was killed on GD 12 due to prolonged inappetence and anorexia. At low dose, 1 was killed on GD 13 due to inappetence and anorexia, 1 was killed on GD 18 because of spontaneous abortion, and 1 was killed due to gavage problems (necropsy revealed esophageal reddening and thickening). Two controls were killed, 1 because of poor clinical condition and 1 due to abortion problem.

Clinical Signs: No treatment related effect.

Body Weight, Food and Water Intake: At initiation of treatment, transient decreases in body weight loss (dose related) was observed in all treated groups but there was "general evidence of recovery" from GD 8 or 9. In high dose animals that were killed before scheduled necropsy, weight loss was 8-16% of body weight. In all treated groups, including high dose rabbits that were allowed to survive to scheduled necropsy, there was an initial decrease in body weight gain, restricted between GD 6 and 8, but after GD 8 there was a general pattern of recovery, but mean body weights at high dose remained substantially below control to scheduled necropsy (see Figure 1). Mean food and water intake for all 3 treated groups was significantly lower than control (dose related), particularly during the initial stage of treatment (GD 6- GD 12), but exceeded control intake after the treatment period.

The mean gravid uterine weight of high dose animals that survived to termination was lower than control, which sponsor attributed to smaller litter size.

Cesarian Data: Post-implantation loss (early and late intrauterine deaths combined) was higher in mid (16.1%) and high (20.5%) doses than in controls (11.0%), exceeding background control incidence (10-17%) at high dose. Mean number of live fetuses/litter was decreased at mid and high doses (dose related). These differences were not statistically significant.

Fetal Effects: Although there was a dose related decrease in percent of male fetuses at mid and high doses, which sponsor considers a spurious effect, there were no effects on incidence of external, visceral or skeletal anomalies or variations.

NOAEL for maternal effects: 5 mg/kg/day based on decrease in mean body weights.
NOAEL for fetal effects: 5 mg/kg/day, based on increased postimplantation loss and decreased number of live fetuses/litter.

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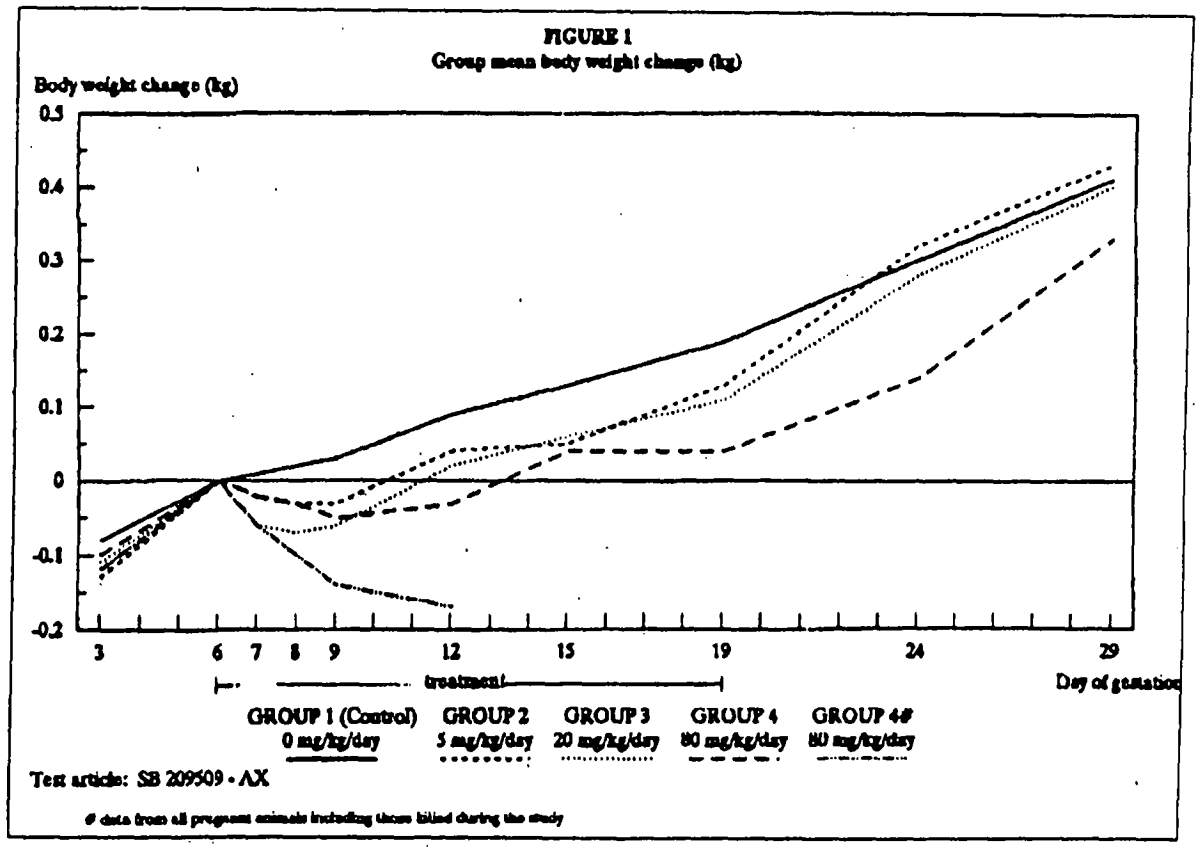

TABLE 7

Group mean caesarian data

Test article	Control	SB 209509 - AX		
Group	1	2	3	4
Level (mg/kg/day)	0	5	20	80

7.1 Uterine/implantation data - females with live foetuses

	Group 1	Group 2	Group 3	Group 4
Number of pregnant females with live foetuses at Day 29 gestation	21	15	19	13
Number of corpora lutea	211	162	213	142
Mean number per female	10.0	10.8	11.2	10.9
Number of implantations	191	151	180	112
Mean number per female	9.1	10.1	9.5	8.6
% pre-implantation loss	9.5 (10)	6.8 (6)	15.5 (13)	21.1 (8)
Number of early intrauterine deaths	11 (6)	8 (6)	24 (9)	20 (7)
Mean number per female	0.5	0.5	1.3	1.5
Number of late intrauterine deaths	10 (6)	5 (4)	5 (4)	3 (1)
Mean number per female	0.5	0.3	0.3	0.2
Number of dead foetuses	0	1	0	0
Mean number per female	0.0	0.1	0.0	0.0
% post-implantation loss	11.0 (10)	9.3 (7)	16.1 (9)	20.5 (7)
Number of foetuses	170	137	151	89
Mean number per female	8.1	9.1	7.9	6.8
% of implantations	89.0	90.7	83.9	79.5

( ) number of litters affected

TABLE 7

Group mean caesarian data

Test article	Control	SB 209509 - AX		
Group	1	2	3	4
Level (mg/kg/day)	0	5	20	80

7.2 Foetal data - females with live foetuses

		Group 1	Group 2	Group 3	Group 4	
Number of male foetuses		93	77	67	40	
Number of female foetuses		77	60	84	49	
% male foetuses	C	54.7	56.2	44.4	44.9	DR*
Mean litter weight (g)		314.0	343.0	300.7	272.5	
Mean placental weight (g)		5.37	5.09	5.45	5.53	
Mean foetal weight (g)		40.1	38.2	38.3	42.4	
Mean foetal weight (g) - males only		40.1	38.2	39.0	43.2	
Mean foetal weight (g) - females only		39.6	37.9	38.1	41.2	

C = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon Rank-Sum tests

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

TABLE 7

Group mean caesarian data

Test article	Control	SB 209509 - AX		
Group	1	2	3	4
Level (mg/kg/day)	0	5	20	80

7.3 Foetal defect data

	Group 1	Group 2	Group 3	Group 4
Number of foetuses examined	170	137	151	89
Number of litters examined	21	15	19	13
EXTERNAL AND VISCERAL DEFECTS				
Number showing malformations	3	1	9	2
% of foetuses examined	1.8	0.7	6.0	2.2
Number of litters affected	2	1	6	2
Number showing variations	59	40	50	28
% of foetuses examined	34.7	29.2	33.1	31.5
Number of litters affected	17	12	14	13
SKELETAL DEFECTS				
Number showing malformations	4	8	7	2
% of foetuses examined	2.4	5.8	4.6	2.2
Number of litters affected	3	5	5	2
Number showing variations	167	134	146	87
% of foetuses examined	98.2	97.8	96.7	97.8
Number of litters affected	21	15	19	13
Total number of foetuses showing malformations	7	9	12	2
% of foetuses examined	4.1	6.6	7.9	2.2
Number of litters affected	5	5	7	2

Toxicokinetics: Blood samples were collected from surviving rabbits (6 or 7 animals/group) on GD 13 in the dose finding study (Report No. 1165/21-1050) at 1, 2, 4, 8 and 24 hours after dosing. The table that follows is a summary of mean blood levels found at each of the collection periods, obtained from Appendix 9 in the report. Obviously, because blood levels were still as high at 24 hours post dosing, the sponsor did not calculate pharmacokinetic parameters.

Mean Blood Concentration (ng/mL) of Frovatriptan on Day 13 of Gestation

Dosage mg/kg	Timepoint (Hours After Dosing)				
	1	2	4	8	24
0	0	0	0	0	0
50	3327.13	3773.55	3561.02	2884.42	2879.02
100	17759.91	19293.97	19497.14	18837.18	14813.89
200	36186.23	44986.65	45865.29	33002.55	33897.31

Blood AUC level found in humans following a clinical dose of 0.04 mg/kg was found to be 94 ng.hr/mL.

e. Oral Gavage Pre- and Post-natal Development Study in the Rat (Vol 43)

Study No. 1165/78-1050

Performing Laboratory [redacted]

Dates Study Performed: Treatment initiated 1/13/97, necropsies completed 3/2/97

Quality Assurance: A statement of GLP compliance is included.

Test Substance: Batch nos. 60514-10 and 60531-11; 99.7 & 100.8% pure, respectively.

Test Animals: Time mated Crl:CDBR rats, [redacted], approximately 9 weeks old, weighing 180-289 g at the time of mating, delivered to the test facilities on GD 3, were used..

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

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

Procedure: Frovatriptan was administered daily to 24 F_0 females/group between GD 7 and postpartum day (PPD) 21. Controls received 1% aqueous methylcellulose vehicle and the dosing volume for all 4 groups was 10 mL/kg. For the F_1 generation, 20 males and 20 females were randomly selected from available litters. All animals on test were examined twice daily for morbidity and mortality, daily for clinical signs. Body weights of F_0 dams were measured 9 times between GD 3 and GD 20 of gestation, on PPD 1, 4, 7, 14 and 21; food consumption was measured at 8 time periods during gestation and twice weekly between PPD 1 and 21. After allowing the F_0 dams to deliver, the following were recorded; length of gestation, number of pups born (dead and alive), daily live litter size and sexes (reported on PPDs 1, 4, 7, 14 and 21), daily clinical observations, individual pup weights (PPDS 1, 4, 7, 14 and 21), and necropsy findings on dead and culled pups. On PPD 4, the litters were culled to a maximum of 8 pups (4 of each sex, if possible). Pups considered unlikely to survive were pre-selected for cull and a random selection procedure was used. Developmental parameters measured for each F_1 litter included pinna unfolding, incisor eruption, eye opening, surface righting reflex on PPD 1, air righting reflex on PPD 17, grip strength, pupillary reflex, auditory response and visual placing response on PPD 21,vaginal opening and balano-preputial separation were assessed around PPD 30 or 40, respectively. In control and high dose F_1 rats, learning ability in a swimming maze during week 4 and 5 and motor activity in an open field arena during week 4 were measured. Mating (1 male and 1 female of the same treatment group but not siblings) during week 12 was assessed. F_1 mated females were C-sectioned on GD 13 for determination of pregnancy status, corpora lutea count and examination of uterine contents. Finally, all F_1 animals and F_0 females were necropsied for macroscopic examination. The uteri of all F_0 females were stained with

ammonium sulfide and implantation sites were counted. Tissue retention from all adult animals included ovaries, uterus, cervix, vagina, pituitary, testes, epididymides, seminal vesicles, prostate, coagulating gland and lesions.

Result; F_0Dams:

Mortality: A total of 4 high dose females were found dead or killed in extremis; 1 found dead on GD 21 and 3 killed in extremis on GD 21 and PPD 1. One mid dose female was killed in extremis on GD 21. No explanation is given as to why all the animals died at about the time of expected parturition. All were pregnant, body weight and food intake had been similar to controls throughout gestation. Clinical signs prior to death included prostration, cold, shallow respiration, hunched, squinting and lethargy. No macroscopic abnormalities were detected at necropsy.

Clinical Signs: Red extremities in majority of females in all treated groups beginning 0.5 hours after dosing, persisting for more than 4 hours but no longer present by 24 hours after dosing. Also, salivation and paddling behavior after dosing were noted, particularly in mid and high dose groups. The latter 2 clinical signs are considered to be reactions to gavage by the sponsor.

Body Weight: Mid and high dose dams gained significantly less weight than controls during the first day of treatment but subsequently gained more weight than controls and there were no longer any differences in body weight by GD 20 (see figure 10. On PPD 1, control lactating dams were lighter than treated dams. Subsequently, there was a slight, dose related reduction in body weight gain among treated groups compared to control. Generally, there was a dose related decrease in food intake to GD 18, but not during lactation.

Pup Numbers and Survival: One dam at mid dose and 3 at high dose were not pregnant. This was considered to be a chance occurrence because treatment didn't start until GD 6, which is after implantation. Total litter loss occurred in 1 control, 1 low dose, 2 mid dose and 1 high dose. The gestation index (proportion of pregnant females with live pups) was lower than control at mid and high doses; attributed to female deaths in these groups. The mean number of implantations in high dose group was higher than control. While the mean number of live born pups were higher at high dose, there was a marginally higher incidence of post-implantation loss in this group. Sponsor claims that mean pup weight at birth from high dose dams were marginally lower than control and remained lighter to PPD 14, after which a significantly increasing, dose related pup body weight increases occurred. However, these effects were not statistically significant and the difference in mean pup weight at birth was only 0.2 g (2%). Therefore, we conclude that the only litter effect noted was a marginal increase in postimplantation loss at high dose (see tables which follows).

Developmental and Maturational Effects: Pinna unfolding and eye opening was generally noted earlier in the treated groups than in control; at high dose, 75% of the pups had eye opening significantly earlier than control ($P<0.01$).

TABLE 5

Group mean litter data - P generation

Test article	Control		VML 251	
Group	1	2	3	4
Level (mg/kg/day)	0	100	500	1000

5.1 Pup numbers - females rearing young to weaning

	Group 1	Group 2	Group 3	Group 4	Statistics
Number of females with live pups at Day 21 *post-partum*	23	23	20	16	X
Mean duration of gestation (days)	22.5	22.6	22.8	22.7	X
Mean number implantation sites	12.7	13.3	13.1	13.8	J
Mean number pups born	11.8	12.1	12.1	12.3	J
Mean number pups alive Day 1	11.4	11.7	11.5	11.9	X
Mean % male pups Day 1	50.6	53.6	52.1	47.3	J
Mean number pups alive Day 4 before culling	10.6	10.5	11.1	11.2	X
Mean number pups culled	3.1	2.9	3.1	3.5	X
Mean number pups alive Day 4 after culling	7.4	7.7	8.0	7.7	X
Mean number pups alive Day 7	7.4	7.7	8.0	7.7	X
Mean number pups alive Day 14	7.3	7.6	8.0	7.5	X
Mean number pups alive Day 21	7.3	7.6	7.9	7.5	X

J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon rank sum tests
X = Not analysed

TABLE 5

Group mean litter data - F generation

Test article	Control	VML 251		
Group	1	2	3	4
Level (mg/kg/day)	0	100	500	1000

5.2 Gestation and neonatal survival indices - females rearing young to weaning

	Group 1	Group 2	Group 3	Group 4	Statistics
Gestation index	100.0	100.0	95.7	85.7	X
Post-implantation survival index %	93.1	91.7	92.4	89.8	F-
Live birth index %	97.1	96.1	96.0	97.4	F-
Viability index 1 %	93.0	91.1	96.9	93.2	F-
Viability index 2 %	100.0	100.0	100.0	100.0	X
Viability index 3 %	98.9	98.8	100.0	97.2	F-
Viability index 4 %	100.0	100.0	98.1	100.0	F-

F- = Cochran-Armitage and Fisher's Exact tests, one-sided for decreasing trend, on proportion with index = 100%
X = Not analysed

TABLE 5

Group mean litter data - P generation

Test article	Control		VML 251	
Group	1	2	3	4
Level (mg/kg/day)	0	100	500	1000

5.3 Mean pup weights (g) - females rearing young to weaning

		Group 1	Group 2	Group 3	Group 4	Statistics
Mean weight (g) Day 1:	male	6.5	6.4	6.6	6.3	J
	female	6.2	6.0	6.1	5.9	J
	combined	6.3	6.2	6.3	6.1	J
Mean weight (g) Day 4:	male	8.9	8.7	8.8	8.6	J
	female	8.6	8.6	8.4	7.9	J
	combined	8.7	8.5	8.6	8.3	J
Mean weight (g) Day 7:	male	14.3	14.2	14.4	14.2	J
	female	14.0	14.2	13.8	13.1	J
	combined	14.1	13.9	14.1	13.7	J
Mean weight (g) Day 14:	male	30.2	30.2	30.5	30.4	J
	female	29.8	30.2	29.2	29.7	J
	combined	29.9	29.8	29.9	29.9	J
Mean weight (g) Day 21:	male	48.3	48.4	50.0	50.6	J
	female	47.5	48.1	47.7	48.8	J
	combined	47.9	47.7	48.9	49.6	J
% weight change Days 1 - 21:	combined	662.1	669.7	675.9	717.4 DR*	J

J = Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon rank sum tests

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

DOSAGE THRESHOLDS FOR ADVERSE EFFECTS IN REPRODUCTION STUDIES

Study Type	Fertility & Early Embryonic Development[1]	Prenatal-Postnatal Development	Embryo-Fetal Development	Embryo-Fetal Development
Animal	S-D Rat	S-D Rat	S-D Rat	N-Z Rabbit
Dose (mg/kg/day)	0, 96, 479, 958	0, 100, 500, 1000	0, 100, 500, 1000	0, 5, 20, 80
Days Administered	14 days pre-mat to GD 7	GD 6 to PPD 20	GD 7 to GD 17	GD 6 to GD 19
Day of C-Section	20	N/A	GD 20	GD 29
Number/Group	24	24	30	24
Mortality, Dams	>958	>100≤500	>1000	>20≤80
↓Maternal Weight Gain	>958	>100≤500	>500≤1000[2]	≤5
↑Postimplantation Loss	[4]	>500≤1000[3]	>100≤500[3]	>5≤20
↓Corpora Lutea Count	≤96	N/A	>1000	>80
↓Viable Fetuses/Dam	≤96	N/A	>1000	>5≤20
↓Fetal Weight	N/A	N/A	>1000	>80
↓Fetal Ossification	N/A	N/A	≤100[5]	>80
↑Fetal Visceral Anomalies	N/A	N/A	≤100[6]	>80
↑Fetal Bone Anomalies	N/A	N/A	>1000	>80
↓Pup Viability	N/A	>1000[7]	N/A	N/A
↓Pup Weight at Birth	N/A	>1000	N/A	N/A

[1]A slightly extended length of the estrous cycle was observed (not dose related) at all 3 dose levels.
[2]Small, transient effect; included ↓ food and water intake.
[3]Marginal effect
[4]↑Pre-implantation loss, dose related in all treated groups
[5]Incomplete ossification (dose related) of sternebrae 5-6, hindlimb phalanges, jaw/mouth, skull and nasal bones
[6]Dilated ureters, unilateral and bilateral pelvic cavitation, hydronephrosis; dose related
[7]Proportion of dams with live pups (gestation index) was lower than control at mid and high doses, but these effects were attributed to maternal deaths in these groups. No effects of live pups/litter at birth or pup survival to PPD 21

IV. OVERALL SUMMARY AND EVALUATION

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. The recommended dose is a single tablet.

Pharmacology

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 series of *in vitro* and *in vivo* studies relating to the proposed indication have been conducted with frovatriptan. This drug was found to be a potent vasoconstrictor in isolated cerebral arteries from several species, including human. Frovatriptan was found to be considerably more potent than sumatriptan as a partial agonist relative to 5-HT in human middle cerebral arteries, and as a full agonist in human and rabbit basilar arteries. Relative to 5-HT, frovatriptan was a partial agonist whereas sumatriptan was a full agonist in the rabbit, but both drugs were partial agonists in the cat. Frovatriptan was more potent than sumatriptan as a vasoconstrictor in dog and human isolated coronary arteries. **However, on the basis of EC_{50} values, both drugs were similar as vasoconstrictors in human cerebral and coronary arteries.** Sponsor suggests that by virtue of its apparent partial agonist activity in human isolated coronary arteries, frovatriptan demonstrates a functional selectivity for cerebral arteries over coronary arteries.

In cat and dog models, successive increases in i.v. doses of frovatriptan or sumatriptan produced dose-related constriction of carotid artery vascular bed, increases in carotid vascular resistance and concomitant reductions in carotid blood flow. Similar effects but of longer duration, were obtained in both species when the drugs were administered by the intraduodenal (i.d.) route . On a mg/kg or molar basis, sumatriptan was less potent than frovatriptan. Generally, little or no effects of frovatriptan were noted on blood pressure, heart rate or femoral blood flow in cats or dogs. In a dog model subject to myocardial ischemia, neither frovatriptan nor sumatriptan caused a significant increase in size of the infarct. Prior to the occlusion in these dogs, neither drug caused an increase in coronary blood flow.

ADME, Distribution, Metabolism and Pharmacokinetics

After an oral dose, absorption of frovatriptan has generally been found to be low; 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 (based on recovery in urine after an oral dose), 22% and 30% in healthy males and females, respectively, at a dose of 2.5 mg. 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 rat tissue distribution studies, tissue concentrations following a single oral 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.

The schematic for metabolic pathway suggested for all 5 species where tested is shown on page 11 and the metabolic profiles in blood and urine is shown on page 12. Both unchanged frovatriptan and desmethyl frovatriptan were observed in the blood of human, mouse, rabbit and dog but not in rat, parent compound was observed in blood of all 5 species. N-acetyl desmethyl frovatriptan was observed in the blood of mouse, rat, and rabbit, not in humans or dog. Generally, there was a resemblance between plasma or blood and urinary metabolite profiles. Fractions identified as parent frovatriptan, desmethyl frovatriptan and N-acetyl desmethyl frovatriptan, hydroxylated frovatriptan and hydroxylated N-acetyl desmethyl frovatriptan accounted for about 33.1%, 8.2%, 12.5%, 16.5% and 8.6%, respectively, of the recovered urinary radioactivity in humans; in rats, these urinary recoveries were 88.3, 0, 7.0, 1.0 and 0, respectively, whereas in mouse they were 84.4, 5.0, 3.3, 1.1 and 0%, respectively. Other fractions generally accounted individually for less than 5% of the sample radioactivity in these 3 species. The following general species ranking in terms of extent of metabolism can be made: rabbit > human > dog > rat > mouse.

In view of the low level of metabolism in the rat and mouse, we agree with sponsor's suggestion that concentrations of total radioactivity provides a close reflection of parent compound for use in estimating bioavailability in these two species. However, metabolism in man is considerably higher than in dogs, rats and mice. Therefore, the possibility that one or more of the metabolites in human blood may have binding affinity to the 5-$HT_{1B/1D}$ receptor sites and may possess pharmacological activity similar to frovatriptan has to be considered.

In order to compare differences in systemic exposures based on blood AUC between rats or mice and humans in the carcinogenicity studies, the differences in blood levels, and possible differences in binding affinities and pharmacological activities of the metabolites should also be taken into consideration. Desmethyl frovatriptan has around half the binding capacity to 5-$HT_{1B/1D}$ receptor sites as frovatriptan whereas N-acetyl desmethyl frovatriptan has little binding capacity to these sites. The remaining metabolites have not been tested for their affinities to the 5-$HT_{1B/1D}$ receptor sites. We are not aware of any studies in which the pharmacological activities of any of the metabolites have been tested. It is reasonable to believe that desmethyl frovatriptan may have significant pharmacological activity because of its affinity for the 5-$HT_{1B/1D}$ receptor sites. As noted in the table, there are differences in blood and urinary concentrations of desmethyl frovatriptan among these 3 species with measurable amounts in blood and urine of humans and mice (considerably higher levels in humans) and virtually none in rats. The half-life of desmethyl frovatriptan is considerably higher than frovatriptan. Considerably higher levels of N-acetyl desmethyl frovatriptan was found in human urine than in urine of rats or mice. However, this metabolite was reported to have low binding capacity to 5-$HT_{1B/1D}$ receptor sites, which suggests insignificant pharmacological activity. Fractions identified as hydroxylated frovatriptan and hydroxylated N-acetyl desmethyl frovatriptan were present in human urine at levels of 16.5% and 8.6%, respectively, and very little of these 2 metabolites were found in urine of rats and mice. When an intravenous dose of 5 mg was given to rats, around 80% was recovered in urine which may suggest biliary excretion of up to 20%, but there appears to be no indication that fecal recoveries were measured following an i.v. dose

CYP1A2 appears to be involved in frovatriptan metabolism, based on the observations that frovatriptan was metabolized in the presence of human CYP1A2, and a total inhibition of the metabolism was observed when frovatriptan was co-incubated with furafylline, a specific CYP1A2 inhibitor. 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. There was no evidence of liver enzyme induction; based on studies with rats and with human microsomal enzymes.

Following i.v. administration of frovatriptan, the proportion of parent drug recovered in urine closely reflected the relative circulating levels. The proportion of parent drug metabolized and found 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.

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.

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.

Protein binding:

Protein binding is serum, estimated by dialysis equilibrium, was found to be 17.1, 14.9 and 15.3% in rat, dog and human, respectively, thus, no difference in proportion of protein binding between the 3 species. Because of low binding and lack of concentration dependence is unlikely to influence the PK and PD of frovatriptan in vivo.

Blood binding:

Binding of frovatriptan to the cellular fraction of blood was found to 52, 60, 70 and 70% in rat, cat, dog and human, respectively. 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%).

TOXICOLOGY

The oral LD_{50} in mice and was >2000 mg/kg. No clinical signs were evident in mice but in rats, reddening of the extremities (indicative of vasodilation) was noted within 1 hour after dosing, which persisted to the following day in males and for 5 hours after dosing in females. By the i.v. route, deaths were observed at 50 mg/kg in both mice and rats. It should be noted that frovatriptan succinate is known to have low oral bioavailability.

A protocol for a 104-week rat oncogenicity study for which the doses selected (0, 8.5, 27 and 85 mg/kg/day) were based on the >25-fold AUC option, was submitted to the executive-CAC on 6/11/96. On 6/19/96, sponsor was informed by telephone that it had been concluded the drug was genotoxic, based on the human lymphocyte test, and the 104-week rat study had to be based on toxicity. They were also informed that a 3-month dose finding study at sufficiently high doses was required for selection of a MTD. Nevertheless, in the study submitted with this NDA, the doses selected were the same as those indicated in the protocol, which they had been advised by the Agency would not be acceptable. At the executive-CAC meeting of 4/27/99, it was concluded that the 104-week rat oncogenicty study was unacceptable in support of the NDA.

A protocol for the mouse carcinogenicity study was not submitted to FDA. However, doses selected for the 84-week mouse oncogenicity study (0, 4, 13 and 40 mg/kg/day) were also based on the >25-fold AUC option, but this option is similarly not appropriate because the drug is genotoxic in the human lymphocyte test. Furthermore, since survival in all male and female treated groups was $\geq$48%, the 84-week study was not in accord with current standards for study duration which calls for a 2-year mouse study. At the meeting of 4/27/99, the executive-CAC agreed that the 84-week mouse oncogenicty study was unacceptable in support of the NDA for the reasons cited above.

Doses selected for both the 13- and 26-week rat studies were 10, 100 and 1000 mg/kg/day. In both studies, increased incidence and severity of renal tubular basophilia degeneration and other effects on the kidney were observed at the 1000 mg/kg/day dose, which resulted in 10% mortality in the 6-month study. At 10 and 100 mg/kg/day, the incidence and severity of this dose-limiting renal effect was the same as in controls. Because of the wide spread in NOAEL and LOAEL for these effects, it is difficult to select an appropriate MTD for the rat 2-year study, and this study may have to be repeated.

Other histopathology effects worthy of mention were noted in the 26-week rat study. In the adrenal cortex, prominent zona glomerulosa was seen in most mid and high dose animals, particularly males. In the adrenal medulla, atrophy was found in many high dose animals. It should be noted that 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 characterized by cuboidal to columnar cells lining the thyroid follicles, with reduced amounts of colloid in the lumen, which suggests TSH stimulation.

With doses of 5, 50 and 500 mg/kg/day in the 13-week mouse dose finding study, there was no evidence toxicity or a dose limiting effect. Therefore, this study would have to be repeated for selection of a MTD in a mouse carcinogenicity study.

In a 52-week dog study with a 26-week interim kill, the initial doses were 0, 1, 5 and 12.5 mg/kg/day but owing to the absence of clinical signs, the high dose level was increased to 15 mg/kg/day in week 8, then 17.5 mg/kg/day in week 12 and finally 20 mg/kg/day in week 29. There were no indications of toxicity even with the highest doe of 20 mg/kg/day. However, the rationale for dose selection was based on a 14 day range finding study in which doses of 15 and 20 mg/kg/day initially caused 50-130% increases in heart rate and in heart strength compared to pre-treatment for up to 6 to 8 hours post dosing which remained 25-40% higher at 24 hours post dosing. Severely adverse reactions had also seen in 2 dogs following a single 50 mg/kg dose, which persisted, to 48 hours when the dogs had to be. This suggests that the drug that an initial or single dose of frovatriptan may cause severe effects on heart rate and possibly on ECG.

A battery of genotoxicity test for frovatriptan has been performed which included 5 reverse mutation assays each with 6 strains of bacteria, 3 chromosome aberration tests in cultured human blood lymphocytes, a mouse lymphoma test using L5178Y cells, two micronucleus test in mice and an unscheduled DNA synthesis test in rat liver using an *in vivo/in vitro* procedure. These tests are considered satisfactory in support of the NDA. Positive responses were obtained in all of 3 human lymphocyte tests only without the presence of Aroclor induced rat liver S9 but not in its presence. In the reverse mutation test, a positive response was obtained for *E coli* WP2 uvra pKM 101 in one of these tests, also in the absence of rat liver S9 but not in its presence. Equivocal results (statistically significantly higher than control but not a doubling or greater of bacterial cell colony count per plate) were obtained in 2 of these tests. Chiefly on the basis of the positive results in the human lymphocyte test, this drug was considered genotoxic by the reviewing toxicologists, and by the CAC. Positive and equivocal results in the reverse mutation assays serve as supporting evidence that the drug is genotoxic. However, genotoxicity was not observed in the UDS, mouse lymphoma and mouse micronucleus tests.

Doses selected for the reproductive toxicology studies in rats were 0, 100, 500 and 1000 mg/kg/day in rats and 0, 5, 20 and 80 mg/kg/day in rabbits. The only effects in males treated at these doses (paired to untreated females) were clinical signs (red extremities, salivation and paddling) that persisted for a few hours following each dose.

In the fertility-early embryonic development study, considerably more females in the 3 treated groups mated (to untreated males) on the first day of pairing than controls. There appeared to be a disruption of the estrous cycles. Females of the 3 treated groups remained at the estrous stage of the cycle (vaginal cornification) longer than controls, but there was an increase in number of females in the treated groups that did not mate during the estrous stage. The mating index (number of females that copulated/number of estrous cycles required for copulation X 100) showed a significantly increasing dose relationship ($P<0.05$, Cochran-Armitage test). The mean numbers of corpora lutea, implantations and live fetuses were lower than controls ($P<0.05$, dose response). It is reasonable to agree with sponsor's conclusion that the dose-related reduction in mean litter size was due to a lower corpora lutea count, caused by a decrease in ovulation rate. Many structurally unrelated compounds, such as barbituates, tranquilizing agents and

noradrenergic blocking agents (such as chlorinated pesticides) are known to extend the estrous cycle. They also known to impair or cause a delay in ovulation by inhibiting the neural trigger which causes a mid-cycle LH surge that results in ovulation.

In the rat developmental toxicity study, there was a marginal (2%) decrease in body weight gain of the dams during the initial period of treatment and food intake was significantly lower than control at a few time points. The number of early and late uterine deaths and the number of litters with at least one intrauterine death (post-implatation loss) was marginally higher in mid and high dose groups compared to control but the effect was not dose related. Increased incidences of external and visceral variations were seen at mid and high doses. Dose related increased incidences of dilated ureters, unilateral and bilateral pelvic cavitation and hydronephrosis were noted. There was also a tendency for treatment related increased incidences of incomplete ossification of the sternebrae, skull and nasal bones, all of which are indicative of slight delay in fetal maturation. The total incidence of fetal malformations was higher at high dose than in other groups, which included hydronephrosis and anophthalmia, one or both of which may be treatment related. The NOAEL for maternal effects is considered to be 1000 mg/kg/day because the only maternal treatment related effect noted was marginal decrease in mean body weight. The NOAEL for fetal effects was $\leq$100 mg/kg/day, based on increases in fetuses with visceral anomalies and with visceral and skeletal variations.

In the rabbit developmental toxicity study, there was a high incidence of maternal deaths (killed *in extremis*) at high dose (9 of 24 rabbits; 37.5%) within 7 to 10 days after initiation of treatment due to marked anorexia and adipia, resulting in a 8-16% loss in body weight. This group showed a high proportion of intrauterine deaths and the mean gravid uterine weight was lower than control, attributed to smaller litter size. In all treated groups, including high dose, in does that survived to scheduled necropsy, there was an initial decrease in body weight gain, but after GD 8 (3rd day of treatment), there was a general pattern of recovery. Food and water intake during treatment were reduced in all treated groups. Postimplantation loss and decrease in number of live fetuses per litter at C-section exceeded controls were seen in mid and high doses, but the differences were not statistically significant and there was no increased incidence of fetal external, visceral or skeletal abnormalities or variations. The NOAEL for maternal effects is considered to be 5 mg/kg, based on decrease in body weight gain, whereas the NOAEL for fetal effects is considered to be 20 mg/kg/day, based on intrauterine deaths in moribund females.

In the prenatal-postnatal development study, 4 on high dose and 1 on mid dose were found dead or killed *in extremis* on GD 21 or PPD 1. No explanation is given as to why all the animals died at about the time of expected parturition, but all were pregnant, body weight and food intake had been similar to controls throughout gestation and severe clinical signs prior to death. In high dose dams, an initial, transient, small decrease in mean body weight compared to control (around 2%) and food intake was seen during the initial days of dosing, but throughout the lactation period mean body weight of treated groups were higher than control. There were no significant effects on pup litter or viability indices.

IV. RECOMMENDATIONS:

1. Although all the pharmacology and toxicology studies normally required for a NDA have been completed, this application cannot be approved. Doses selected for the 104-week rat and 84-week mouse carcinogenicity studies were based on the >25-fold AUC option, but this option is not appropriate for frovatriptan because the drug was found to be genotoxic in the human lymphocyte test.

[

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2. Sponsor should be asked to provide data for binding affinities to 5-$HT_{1B/1D}$ receptor sites for two metabolites: hydroxylated frovatriptan and hydroxylated N-acetyl desmethyl frovatriptan. In addition, they should determine if there is pharmacological activities similar to that of frovatriptan for desmethyl frovatriptan, N-acetyl desmethyl frovatriptan, hydroxylated frovatriptan and hydroxylated N-acetyl desmethyl frovatriptan.

Reason: The requested data could not be found in the NDA submission. Substantially more of the drug is metabolized in humans than in rats or mice and they are the chief metabolites in humans. The requested data will provide us with a better understanding of the differences in systemic blood AUC exposure ratios, particularly if one or more of the metabolites have pharmacological activities similar to frovatriptan. They may be particularly important for comparisons of systemic exposures between rats or mice to humans in the carcinogenicity studies.

V. LABELING

Sponsor has indicated that they will complete any studies required for approval of this NDA. Based on animal data that are presently available, the following changes and additions in draft labeling are recommended.

[

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WITHHOLD 2 PAGE (S)

Draft Labeling

Reasons:

1. [redacted] For pregnancy category labeling, *Pregnancy Category C* more appropriately satisfies the guidelines of 21 CFR 201.57(6).

2. We believe that the remaining recommended changes more accurately reflect the results of the developmental toxicity studies performed for the sponsor.

/S/

Sidney J. Stolzenberg, PhD

cc:
HFD-120 Division File
HFD-120/LChen
HFD-120/GFitzgerald /S/ 4/3/00
HFD-120/SStolzenberg
NDA21-006.rv1

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

NDA: 21-006 **DRUG CODE#:** VML 251; SB 29555 **DATE:** 4/21/99
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: ———
SPONSOR CONTACT: ———
P/T REVIEWER(s): S. Stolzenberg
DATE SUBMITTED: 1/29/99
DATE OF CAC REVIEW: 4/27/99

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): No. Sponsor ignored the recommendations of exec-CAC for the rat carcinogenicity study, as proposed at a meeting of 6/11/96. The exec-CAC was not asked to review the protocol for the mouse carcinogenicity study.

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

Chromosome Aberrations in Cultured Human Blood Lymphocytes

In two replicate tests for induction chromosome aberrations in cultured, freshly obtained lymphocytes, frovatriptan (batch HP 1) induced increased frequencies of cells with aberrations (excluding gaps) only without the presence of S9. In the first experiment, frovatriptan induced increased frequencies of cells with aberrations (excluding gaps) in both replicates at 403.8 ug/mL, the highest concentration tested (n.s., but exceeded historical control), and it induced a reduction in mitotic index (MI) by 64%. In the second experiment, increased frequencies of cells with aberrations (excluding gaps) were seen at 570.2 ($P \leq 0.05$), 760.3 (n.s.) and 1014 ($P \leq 0.01$) ug/mL (exceeded historical control at all 3 concentrations), which sponsor claimed occurred in the presence of cytotoxicity. Reduction in MI at 1014 ug/mL was 63%. In the presence of S9, no increases in cells with aberrations were found at concentrations up to 2403 ug/mL (which induced no reduction in MI).

Batch — 5310/03 (the — product, apparently intended for human use) induced increased frequencies of cells with aberrations (excluding gaps) at the highest doses tested in both replicates, which exceeded historical control ($P<0.001$ in two duplicate experiments). Highest doses tested were 1164 and 773.8 ug/mL in the first and second experiments, respectively. Reduction in MI at highest doses tested in both experiments was around 63%. In the presence of S9, no increases in cells with aberrations were found at concentrations up to 2375 ug/mL (which induced no reduction in MI).

Batch 60532-12 did not induce increased frequencies of cells with aberrations at the highest dose tested in the first experiment (426.7 ug/mL; with a reduction in MI of 69%). In the second experiment, the drug caused a slight increase in frequency of cells with aberrations ($P<0.05$) at a concentration of 982.2 ug/mL, that "marginally" exceeded historical controls, and induced a reduction in MI of 61%.

Reverse Mutation (Ames) Tests

With Batch HP 1 (synthesized by SmithKline Beecham), a positive mutagenic response in an Ames test for *E coli* strain WP2 uvra pKM 101 was obtained only in the absence of Aroclor 1254-induced rat liver S9, at concentrations of 1000 to 5000 ug/plate (dose related, $P<0.001$, with a 1.7-to 3.4-fold increases in revertant colonies per plate at $\geq$1000 ug/plate). The Ames test was negative for 4 different strains of *S typhymurium* and for *E coli* WP2 pKM101 both in the absence and presence of rat liver S9. This test was repeated three times using different batches of frovatriptan, in each test with the same six bacterial strains, and sometimes comparing it to batch HP 1. In two of these tests, the positive response for *E coli* strain WP2 uvra pKM 101 obtained only in the absence of S9 was equivocal; i.e., statistically significant but less than a 2-fold increase in revertant colonies. In the third test with "degraded and undegraded" frovatriptan, the results were negative. [redacted] which is the [redacted] of frovatriptan and a possible contaminant in frovatriptan preparations, and the desmethyl metabolite of frovatriptan, were tested in the exact same test systems but were found to be non-mutagenic in all of them.

Other studies conducted with [redacted] preparation for mutagenic or genotoxic activity included mutation at the tk locus in mouse lymphoma L5158Y cells, two separate *in vivo* tests for induction of micronuclei in bone marrow of mice, and UDS in rat liver using an *in vivo/in vitro* procedure. Frovatriptan tested negative in all of these studies.

RAT CARCINOGENICITY STUDY

RAT STUDY DURATION (weeks): 104 weeks
STUDY STARTING DATE: 4/12/96 (Initiation of treatment)
STUDY ENDING DATE: 4/20/98 (Completion of necropsies)
RAT STRAIN: Crl:CDBR ————————
ROUTE: Oral gavage
DOSING COMMENTS: Dosage is expressed as free base. Dosing volume was 5 mL/kg (vehicle used not specified).

It should be noted that treatments for this 2-year rat study was initiated 2 months before the Exec-CAC meeting of 6/11/96 when a decision was made by the committee on the adequacy of the doses proposed by the sponsor. It should also be noted that treatments for the rat 13-week dose-range and the 26-week studies, which should have served as a basis for dose selection in the 2-year rat study, were initiated on 8/15/96 and 9/17/96, respectively; 4 and 5 months after treatments for the carcinogenicity test had begun. The Exec-CAC had recommended a 3-month dose range study as the basis for selection of doses in the carcinogenicty test if the AUC ratio option could not be used as a basis for dose selection.

No. Rats in Control#1 (C1): 60
Control#2 (C2): 60
Low Dose (LD): 60
Middle Dose (MD): 60
High Dose (HD): 60
High Dose (HD #2): NA

RAT DOSE LEVELS (mg/kg/day)

Rat Low Dose: 8.5
Rat Middle Dose: 27
Rat High Dose #1: 85
Rat High Dose #2: NA

Basis for Doses Selected (M.T.D.; AUC ratio; saturation of absorption; maximum feasible dose): Sponsor claimed that in the 13-week dose-range study, the high dose of 1000 mg/kg/day was associated with 2 deaths "possibly attributable to test article" and with treatment-related findings in the kidney. It was suggested that there was "a possible slight effect (in the kidneys) at 100 mg/kg/day", but this claim was not substantiated. Sponsor also stated, "The high dose of 85 mg/kg/day was expected to give systemic exposure in animals that exceeded 25 times the AUC of that resulting in humans", which was obviously the basis for dose selection in the 2-year rat study.

RAT CARCINOGENICITY (negative;positive;MF;M;F):
Positive for pituitary tumors in males
Positive for B-benign pheochromocytomas in females

RAT TUMOR FINDINGS:

The tables that follows are sponsor's summaries of (1) neoplastic findings which includes hyperplasia in organs with neoplastic findings and (2) survival data.

Group incidence of selected histopathology findings: pituitary and adrenal

Tissue and finding	Group and sex: Males 1M	2M	3M	4M	5M	Females 1F	2F	3F	4F	5F
Pituitary Number examined	60	60	60	60	60	60	60	60	60	60
focal hyperplasia	10	16	16	13	21	12	9	14	14	10
B-adenoma - *M*	29	29	34	36	26	42	43	34	40	39
M-carcinoma - *M*	0	0	0	0	0	1	1	0	1	0
M - benign and malignant pituitary tumours merged for statistical analysis	29	29	34	36**I	26	43	44	34	41	39
Adrenal Number examined	60	60	60	60	60	60	60	60	60	59
focal medullary hyperplasia	13	14	18	26**I	12	11	13	16	30 **I	11
B-benign phaeochromocytoma - *M*	13	13	9	10	15	8	2	5	7	3
B-ganglioneuroma - *M*	0	1	0	0	0	0	0	0	0	0
M-malignant phaeochromocytoma - *M*	2	0	3	1	0	0	0	0	0	0
M - adrenal medullary tumours merged for statistical analysis	15	14	12	11	15	8	2	5*I	7**I	3

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

Survival to the start of the terminal kill was as follows:

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

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

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

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

TABLE 4.2

Group mean body weight gains

Test Article	Control		VBL 251		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	4.5	27	65	0

Week of study		Mean body weight gains (g) for Group and Sex					Statistics
		1M	2M	3M	4M	5M	
0 to 13	Mean	269.9	301.0	299.2	330.2	265.5	1M* A
	SD	36.34	30.56	42.97	43.01	37.51	
0 to 104	Mean	517.5	561.7	572.4	613.4*	532.6	A
	SD	100.82	110.74	147.46	120.43	120.45	
13 to 24	Mean	78.9	79.7	74.6	76.9	78.5	A
	SD	17.40	21.07	22.25	22.54	19.06	
24 to 52	Mean	103.7	115.7	121.5	119.3	112.7	DR* A
	SD	28.52	34.56	44.93	35.27	30.39	
52 to 76	Mean	49.1	45.8	44.3	48.0	42.0	A
	SD	43.55	37.30	44.96	47.78	46.12	
76 to 104	Mean	7.2	0.5	15.5	20.7	25.1	A
	SD	56.98	54.24	79.21	63.18	64.74	

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test
A = ANOVA, regression and Dunnett's

TABLE 4.2

Group mean body weight gains

Test Article	Control		VBL 251		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	4.5	27	65	0

Week of study		Mean body weight gains (g) for Group and Sex					Statistics
		1F	2F	3F	4F	5F	
0 to 13	Mean	117.1	119.5	125.4*	130.2***	119.7	A
	SD	16.06	16.10	18.59	19.36	16.51	
0 to 104	Mean	310.0	335.6	362.3	375.2	324.8	A
	SD	44.17	56.33	106.10	58.15	104.79	
13 to 24	Mean	51.1	61.7	64.5	64.3	49.0	1M* A
	SD	12.64	11.30	19.02	13.48	10.63	
24 to 52	Mean	80.8	97.6	96.7	94.9	75.1	1M* A
	SD	15.01	42.13	15.95	15.42	13.36	
52 to 76	Mean	44.8	45.7*	48.5	35.2	46.3	A
	SD	34.21	44.55	23.84	25.64	30.99	
76 to 104	Mean	24.3	51.9	56.4	45.4	45.7	A
	SD	40.12	51.91	41.09	43.26	33.57	

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test
A = ANOVA, regression and Dunnett's

FIGURE 1

Group mean survival - males

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

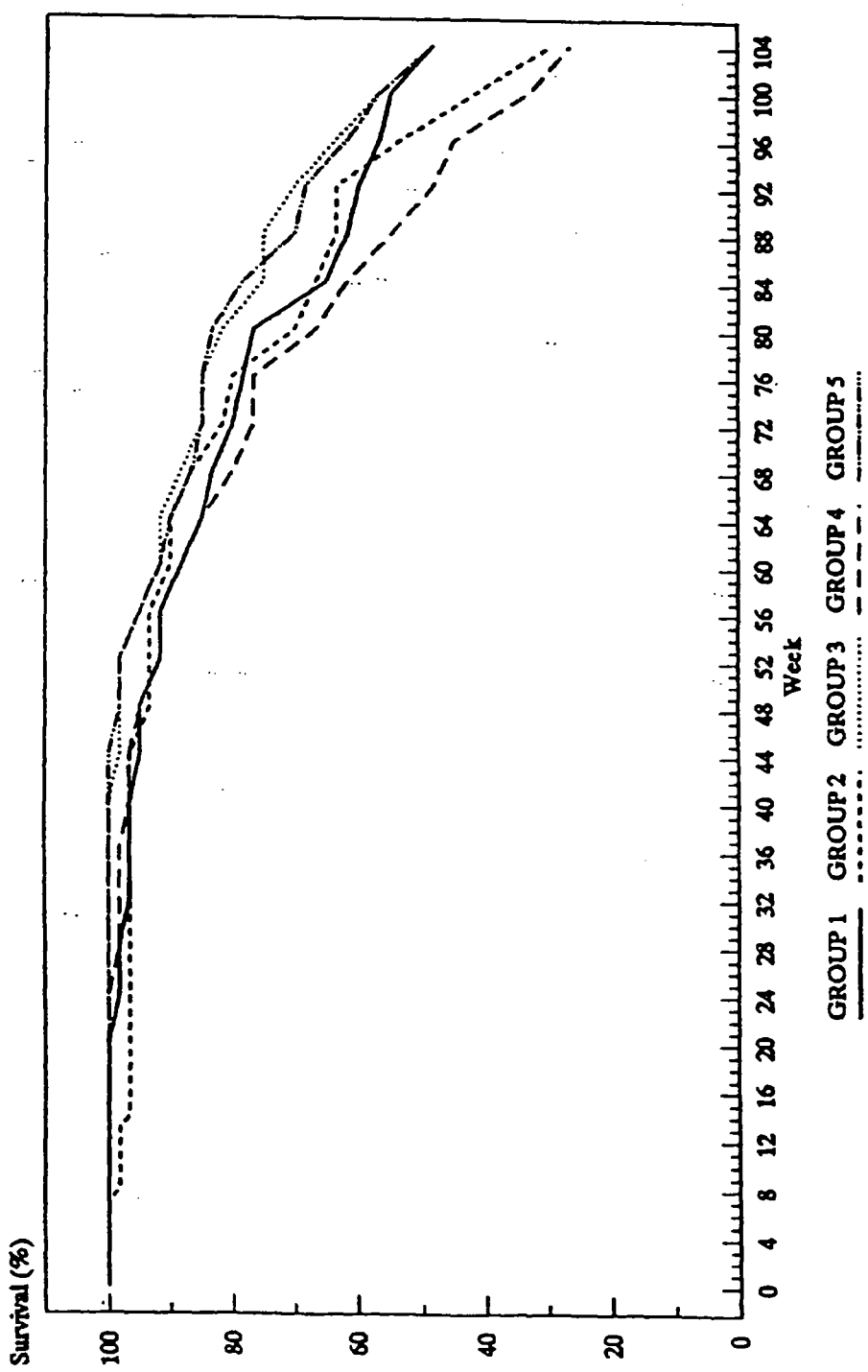


FIGURE 2

Group mean survival - females

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

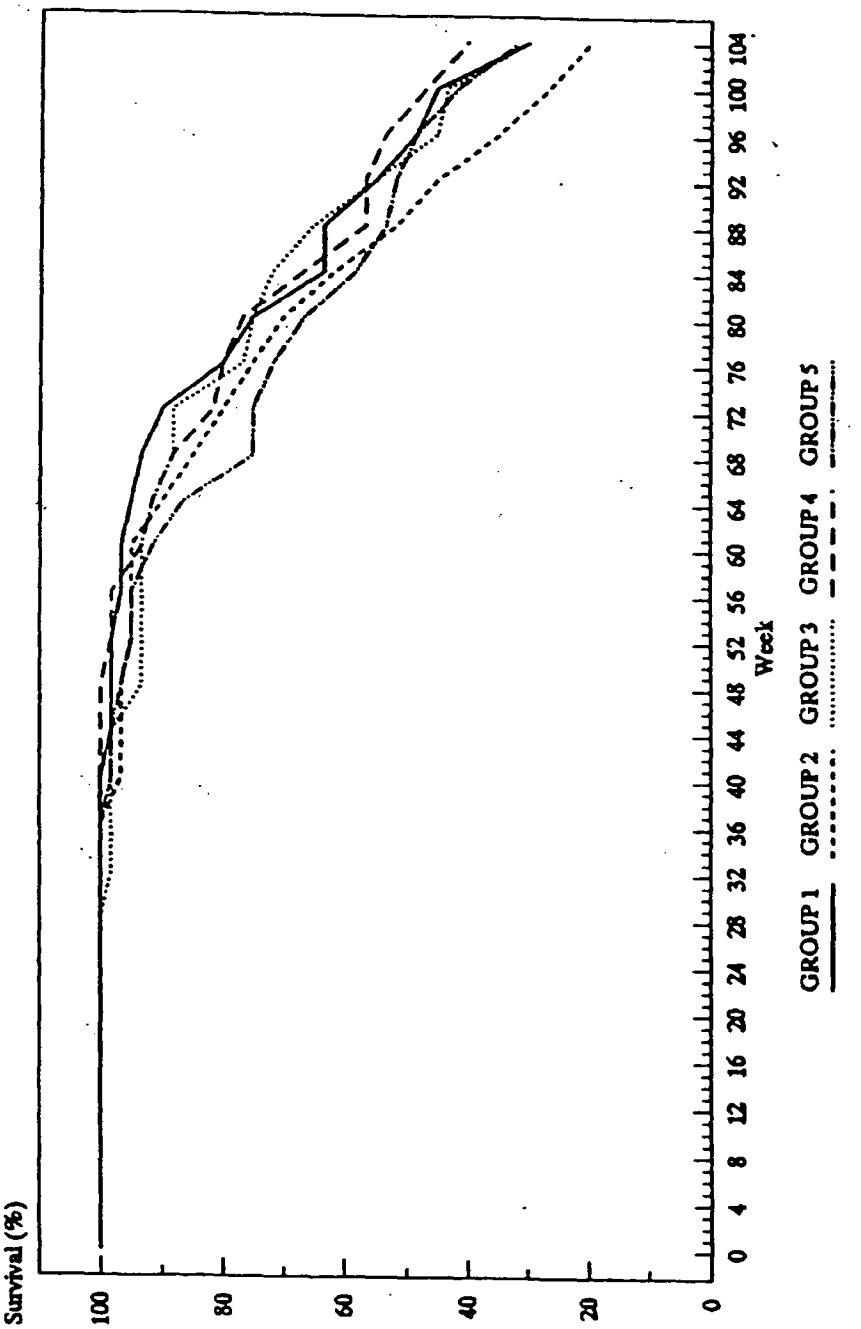


FIGURE 3

Group mean body weight - males

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

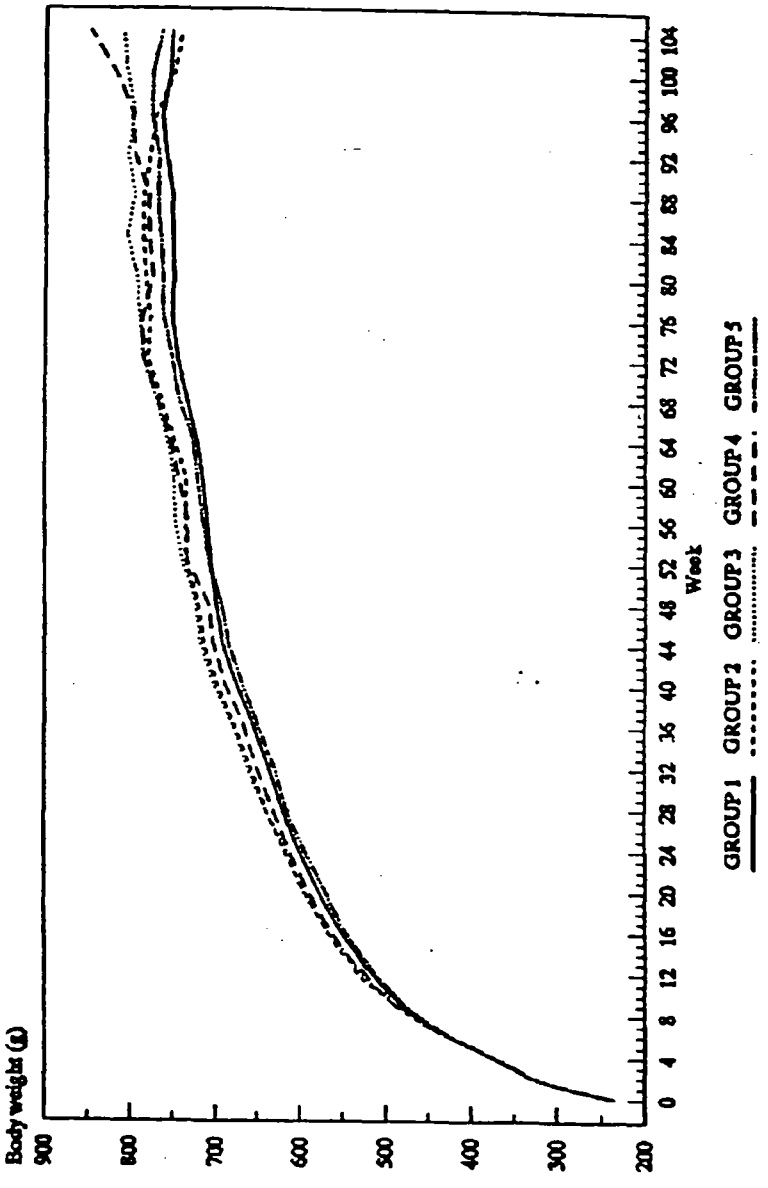
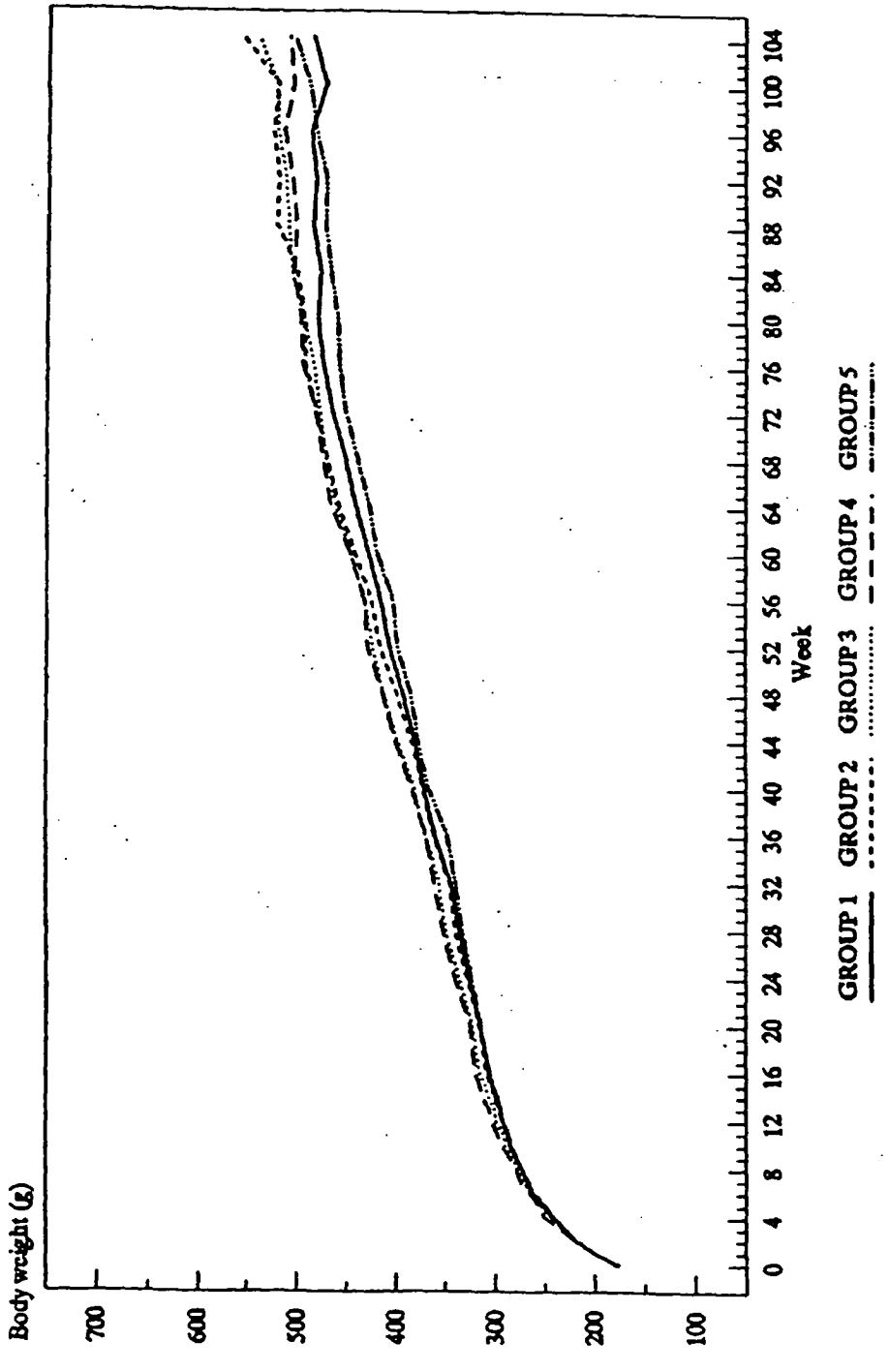


FIGURE 4

Group mean body weight - females

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



The following 2 tables summarize AUC findings in the carcinogenicity study. The third table provides a comparison of rat to human doses, based on mg/kg, mg/m^2 and AUC.

Mean data for AUC - Males

Dose (mg/kg/day)	Dose ratio	Week 2 (ng.h/mL)	AUC ratio	Week 13 (ng.h/mL)	AUC ratio	Week 52 (ng.h/mL)	AUC ratio	Week 78 (ng.h/mL)	AUC ratio
8.5	1.0	3087.5	1.0	2901.8	0.9	3866.1	1.3	9250.8	3.0
27	3.2	9814.6	3.2	14878.2	4.8	15871.8	5.1	18418.6	6.0
85	10.0	31857.0	10.3	44285.7	14.3	75359.0	24.4	60428.8	19.6

Figures in parentheses are calculated omitting anomalous results

Mean data for AUC - Females

Dose (mg/kg/day)	Dose ratio	Week 2 (ng.h/mL)	AUC ratio	Week 13 (ng.h/mL)	AUC ratio	Week 52 (ng.h/mL)	AUC ratio	Week 78 (ng.h/mL)	AUC ratio
8.5	1.0	3401.5	1.0	3597.2	1.1	3332.0	1.0	6194.3	1.8
27	3.2	10380.7	3.2	11113.8	3.3	14470.3	4.3	16979.5	5.0
85	10.0	37558.4	11.0	26334.2	7.7	56545.6	16.6	87672.0	25.8
								(56937.0)	(16.7)

Figures in parentheses are calculated omitting anomalous results

Table 5.2.2.1C

Comparison of Doses Administered and Exposures

to Frovatriptan in Rat and Man

Species	Dose Administered mg/kg/day	Dose Administered mg/m^2	Exposure to Frovatriptan in Blood AUC µg.h/mL	
			Start of study	End of study
Rat	8.5	55	3.1	7.5
	27	176	10.0	17.0
	85	553	33.4	72.3
Man	0.04	1.6	0.094	-
Multiple Rat : Man				
8.5 mg/kg/day	213	34	33	80
27 mg/kg/day	675	110	106	190
85 mg/kg/day	2125	348	355	750

Note: "Start of study" was Week 2 because of difficulties encountered during Day 1 bleedings. These ratios on Day 1 were consistently lower than during later time periods in the 4-week, 3- and 6-month studies in rats because of bioaccumulation, which is known for this drug.

RECOMMENDATIONS/COMMENTS:

The rat carcinogenicity study should be repeated.

Sponsor failed to select doses in accordance with the recommendations by FDA. The high dose of 85 mg/kg/day was based on the "AUC ratio option" rather than on toxicity observed in a 3-month rat study. The AUC option for dose selection is not valid because this drug was found to induce chromosome aberrations in cultured human lymphocytes.

The increase in incidence of lesions in the kidney and urogenital tract, observed at 1000 mg/kg/day in the 3- and 6-month rat studies, and was considered to be the cause of death in the 6-month study. Renal lesions were not seen even at the highest dose (85 mg/kg/day) in the 2-year study.

Pituitary adenomas, adrenal medullary hyperplasia and pheochromocytomas are common finding in aging rats, including this strain. We suggest that the increased incidence of pituitary adenomas and deaths associated with it (which occurred in high dose males), was fortuitous. There was an increased incidence of deaths in low dose males which was not associated with increased incidence of pituitary adenomas. The increased incidence of pheochromocytomas in females was not associated with an effect on mortality. Therefore, we believe that the increased incidence of pituitary adenomas in males, or pheochromocytomas in females, should not be considered a valid indicator of an MTD.

MOUSE CARCINOGENICITY STUDY

MOUSE STUDY DURATION (weeks): 84 weeks
STUDY STARTING DATE: 6/27/96 (Initiation of treatment)
STUDY ENDING DATE: 2/21/98 (Completion of necropsies)
MOUSE STRAIN: Crl: CD-1 (ICR) BR
ROUTE: Oral gavage
DOSING COMMENTS: Dosage is expressed as free base. Dosing volume was 5 mL/kg (vehicle used not specified).

No. mice in Control#1 (C1): 51
Control#2 (C2): 51
Low Dose (LD): 51
Middle Dose (MD): 51
High Dose (HD): 51
High Dose (HD #2): NA

MOUSE DOSE LEVELS (mg/kg/day)

Mouse Low Dose: 4
Mouse Middle Dose: 13
Mouse High Dose #1: 40
Mouse High Dose #2: NA

Basis for Doses Selected (M.T.D.; AUC ratio; saturation of absorption; maximum feasible dose): Based on toxicokinetic findings in the 3-month dose-finding study in mice, the high dose was expected to result in systemic exposure that would exceed 25-times the AUC of that resulting in humans receiving a therapeutic dose.

MOUSE CARCINOGENICITY (negative;positive;MF;M;F):
Negative in males
Negative in females

MOUSE TUMOR FINDINGS:

No treatment related increased incidence in tumors of any kind in males or females. Except for red extremities at mid and high doses for no more than a few hours after dosing each day, there were no compound related effects on any parameters measured, including morbidity, survival to term, hematology, body weight, food consumption, gross and microscopic pathology.

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

Figure 1
Group mean survival - males

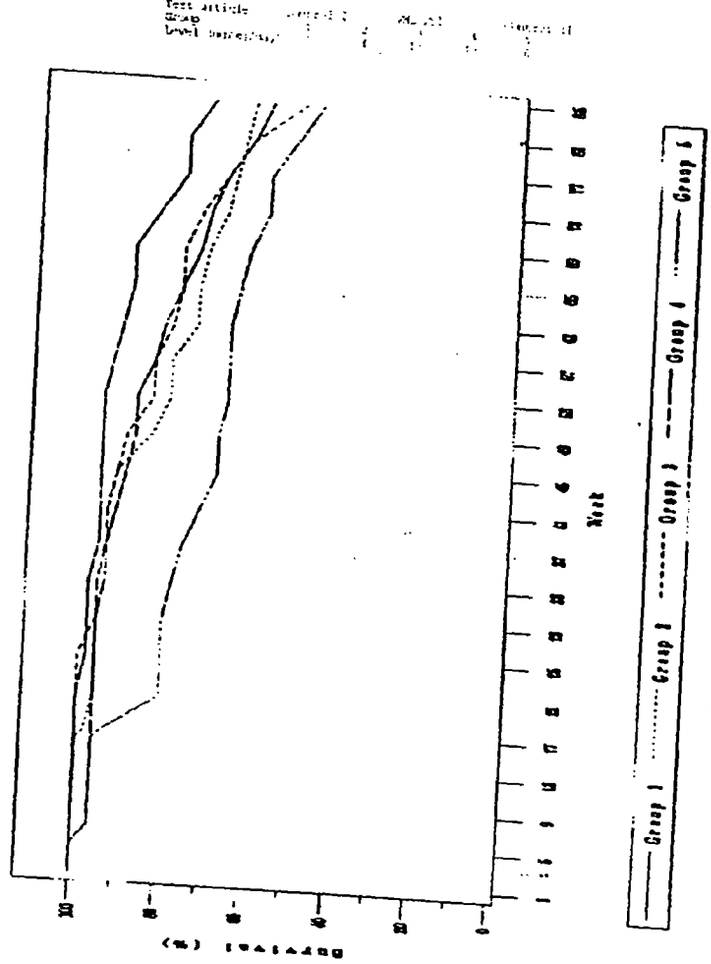


Figure 2
Group mean survival - females

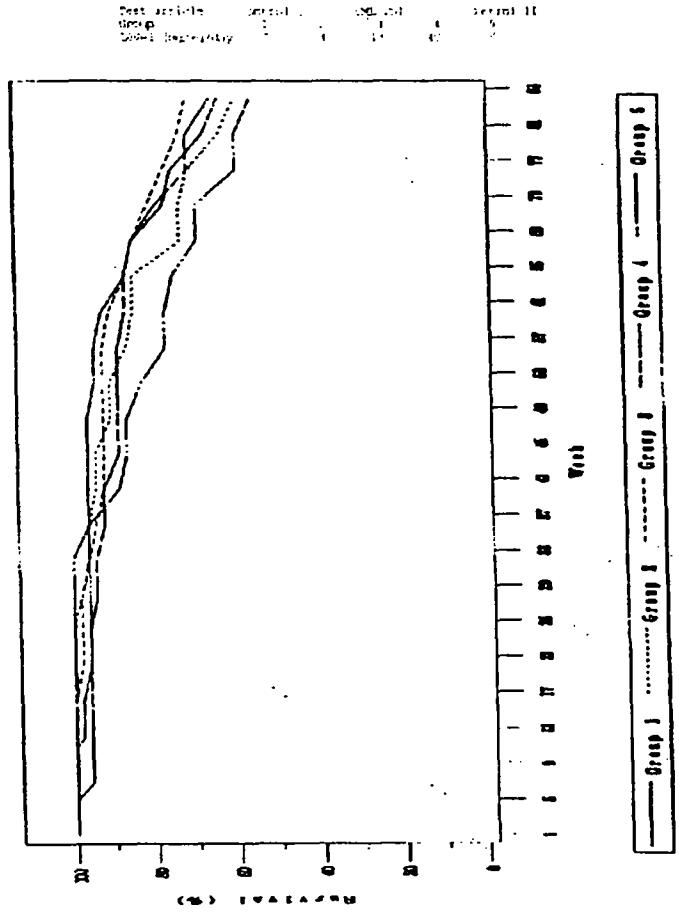


Figure 3

Group mean body weight - males

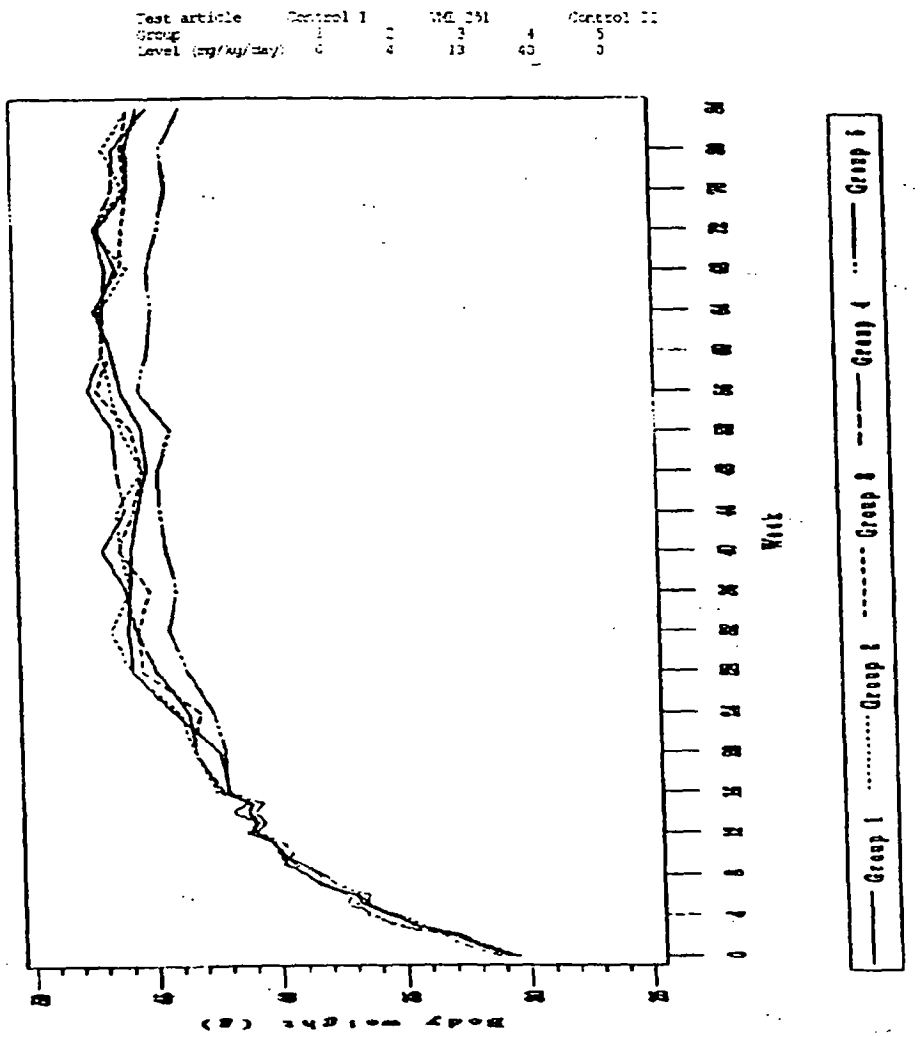


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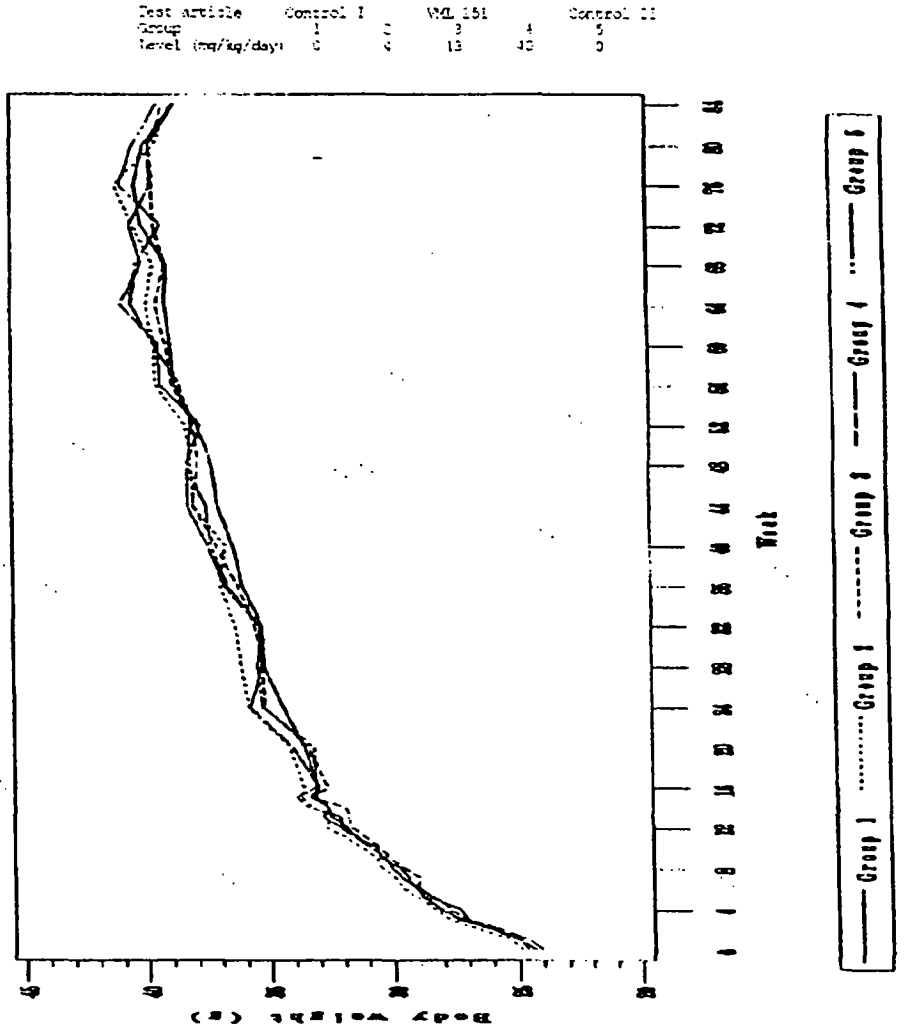
Figure 4

Group mean body weight - females



The following 2 tables summarize AUC findings in the carcinogenicity study. The third table provides a comparison of rat to human doses, based on mg/kg, mg/m^2 and AUC

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

Species	Dose Administered mg/kg/day	Dose Administered mg/m^2	Exposure to Frovatriptan in Blood AUC µg.h/mL	
			Start of study	End of study
Mouse	40	172	13	38.5
Man	0.04	1.6	0.094	-
Multiple Mouse : man	1000	108	138	410

RECOMMENDATIONS/COMMENTS:

The mouse carcinogenicity study should be repeated and the duration of this study should be for 2 years. The dosage in a second carcinogenicity test should be based on MTD observed in a 3- or 6-month dose-finding study,

The high dose of 40 mg/kg/day was based on the "AUC ratio option" rather than on toxicity observed in a 3-month rat study. The AUC option for dose selection is not valid because this drug was found to induce chromosomal aberrations in cultured human lymphocytes. With a dose of 40-mg frovatriptan/kg/day it is obvious that a MTD had not been attained in this 84-week study because there was no treatment-related effect of any kind.

SUMMARY OF MOST SIGNIFICANT FINDINGS IN THE 13-WEEK RAT STUDY

15 rats/sex/group received 0, 10, 100 and 1000 mg frovatriptan/kg/day by oral gavage.

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) 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 kidneys of the high dose animals compared to control. The incidence and severity of this finding in mid and low dose groups were similar to control. Except for the kidneys, there were no histopathology changes associated with increased organ weights.

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

The NOAEL was claimed to be 100 mg/kg/day.

Note: Graphs for mean body weights are not shown for the 13-week rat study. They are shown for the 26-week study, which follows.

SUMMARY OF MOST SIGNIFICANT FINDINGS IN THE 26-WEEK RAT STUDY

20 rats/sex/group received 0, 10, 100 and 1000 mg frovatriptan/kg/day by oral gavage.

Mortality: There were 4 compound related deaths, 3M (Weeks 3, 5 and 19) and 1F (Week 23). Cause of death for all of them was considered to be renal or urogenital tract lesions.

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; $P<0.05$) and brain (3% only in high dose females; $P<0.05$). Except for kidneys and adrenals, there was no histopathology associated with increases in weight of other organs.

Histopathology: Compound related effects were found in the kidneys (tubular nephropathy characterized by multiple foci of degenerating or regenerating basophilic tubules) only in high dose treated males and females. Some changes is plasma chemistry (increased phosphorus and lower plasma chloride, decreased total protein and albumin concentrations) and parameters in urine (decreased phosphate and increased urinary protein and amorphous debris) were considered to be indicative of kidney damage. Prominent adrenal z. glomerulosa (with sporadic nests of small basophilic medullary cells and occasional mitotic figures) was seen in majority of males and females at mid and high doses, adrenal medullary atrophy and thyroid follicular cell hypertrophy was found in most male and female animals at high dose.

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 from the adrenals of about 450-600 hours.

FIGURE 1
Males
Group mean body weight (g)

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

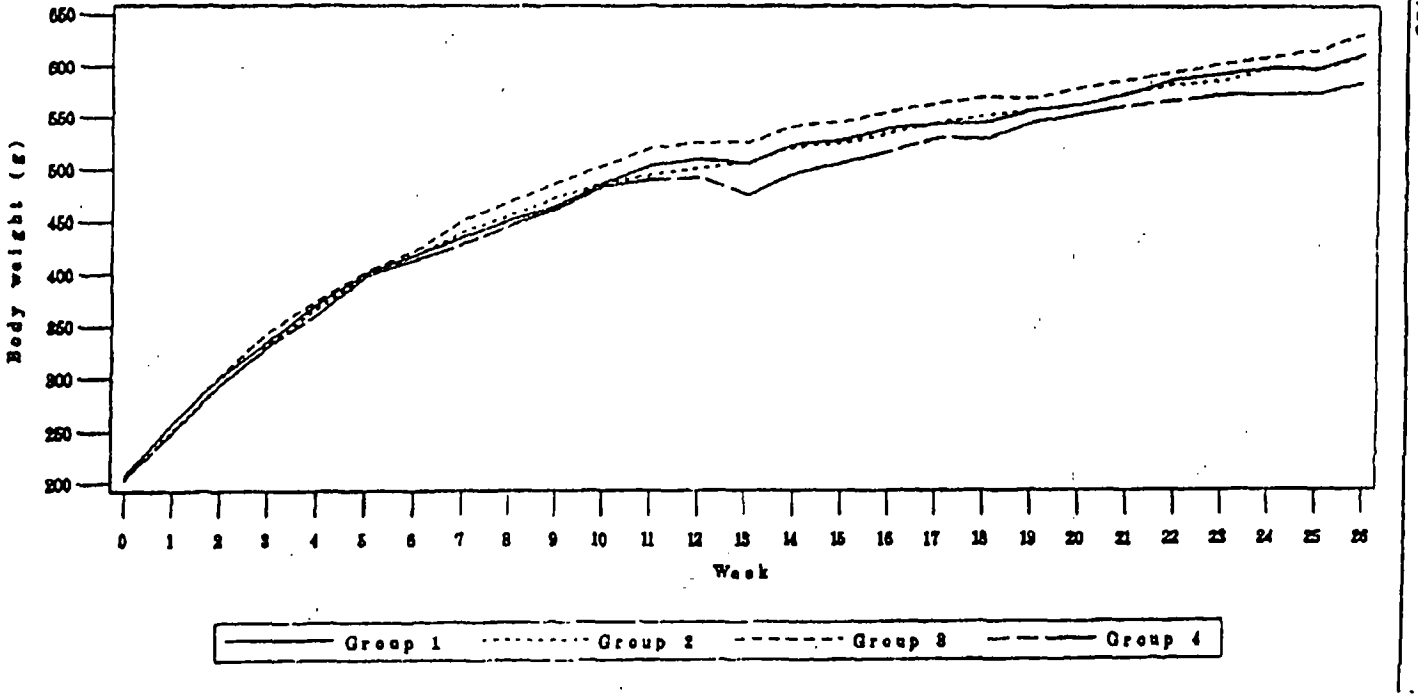
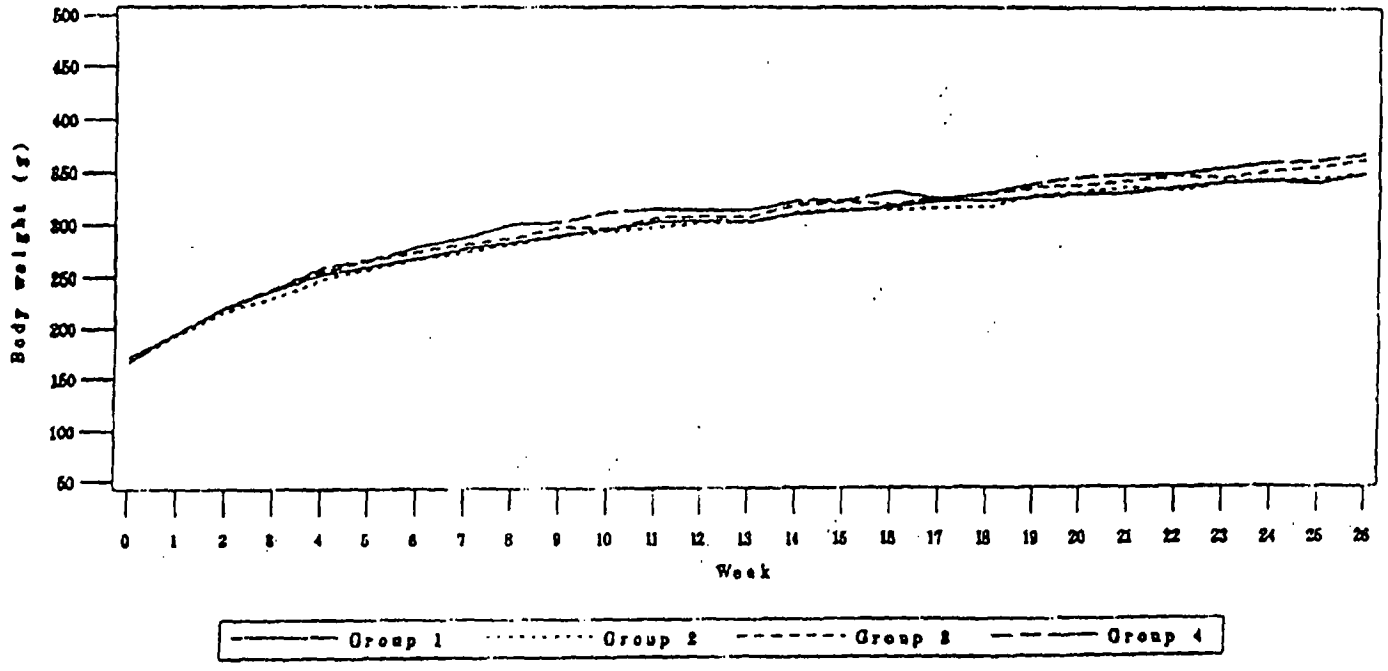


FIGURE 2
Females
Group mean body weight (g)

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



CONCLUSION

If the exec-CAC recommends that the rat 2-year rat study should be repeated, doses suggested by the present reviewer are 100, 300 and 500 mg frovatriptan/kg/day.

Unless a 3- or 6-month dose-finding study is repeated, a dose of 500 mg/kg/day seems like a reasonable estimate of the MTD. At a dose of 1000 mg/kg/day in a 2-year study, compound related deaths would probably be unacceptably high since this dose resulted in a mortality rate of 10% (4/40 rats) within 6 months of the study. The no-effect level for kidney tubular nephropathy in both rat studies and associated deaths in the 6-month study was 100 mg/kg/day, since no intermediate doses between 100 and 1000 mg/kg/day were tested.

We believe that the increased incidence of pituitary adenomas in males or pheochromocytomas in females, observed in treated rats of the 2-year study, should not be considered a valid indicator of a MTD. We question if the increased incidence of pituitary adenomas and in mortality due to enlargement of the pituitary gland are even of compound related effects, for reasons given above. There was no increase in mortality or indication of morbidity associated with pheochromocytomas.

SUMMARY OF MOST SIGNIFICANT FINDINGS IN THE 13-WEEK MOUSE STUDY

15 rats/sex/group received 0, 5, 50 and 500 mg frovatriptan/kg/day.

Deaths unrelated to dosing errors: At mid dose, 1M, 3F (one F had a renal lesion that was not described but was considered to be the cause of death). At high dose, 4M and 1F. Causes of all other deaths could not be determined.

There were no treatment related adverse effects on clinical signs, body weight, food consumption, morbidity, gross observations at necropsy or histopathology. See graphs which follow.

RECOMMENDATION

This dose range study in mice should be repeated with higher doses than those presently used.

There was no clear indication of a MTD or compound related effects in mice, even at the highest dose tested in the 13-week study.

FIGURE 1
Males
Group mean body weight (g)

Test article	Control	SB 209569-AX		
Group	1	2	3	4
Level (mg/kg/day)	0	5	50	500

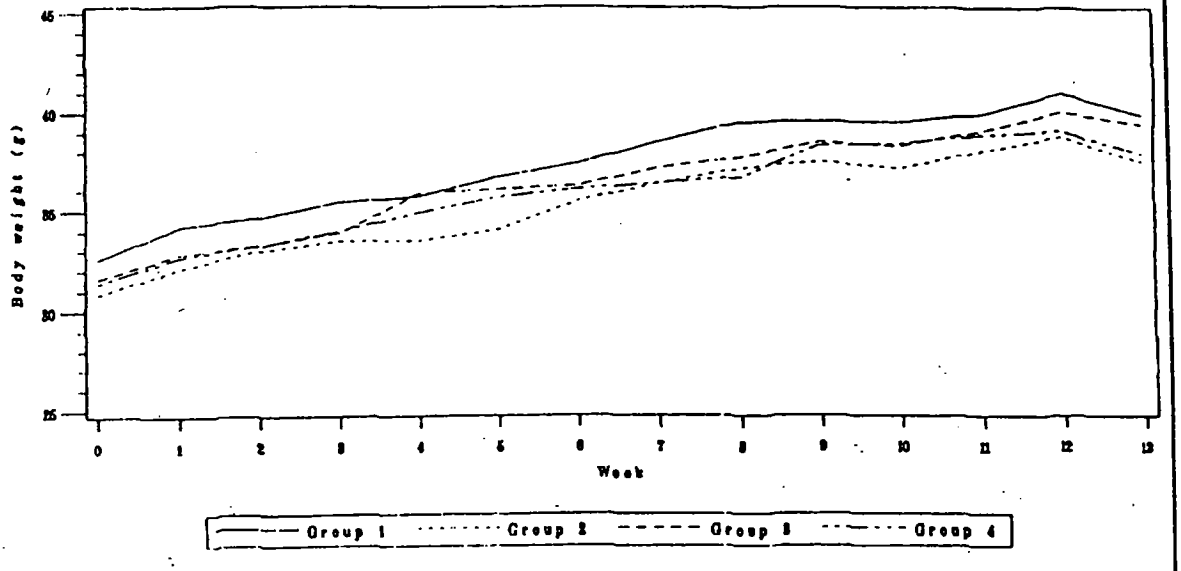
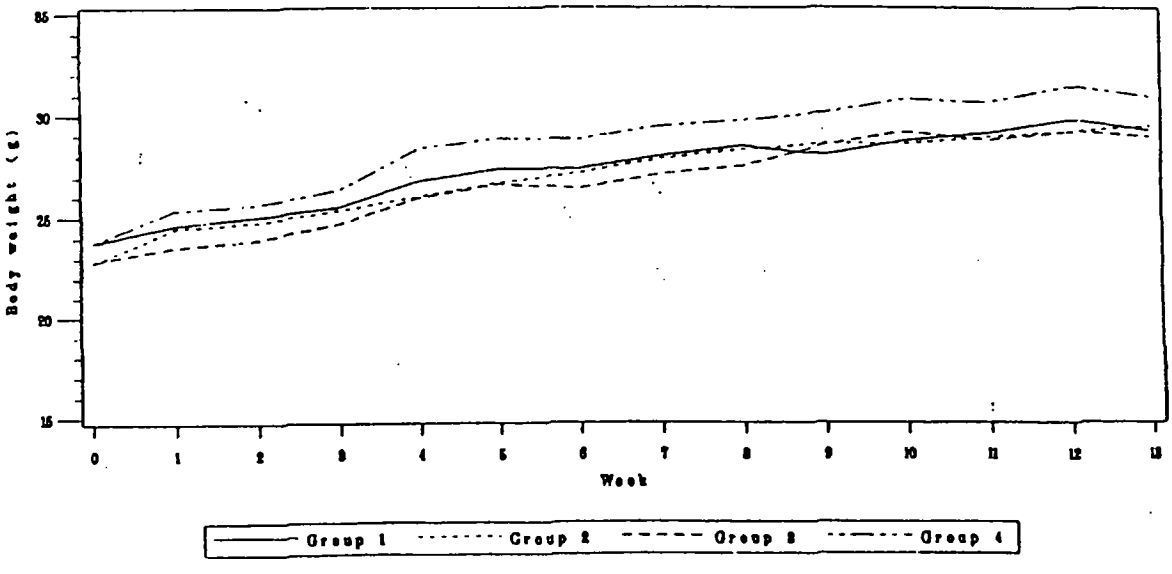


FIGURE 2
Females
Group mean body weight (g)

Test article	Control	SB 209569-AX		
Group	1	2	3	4
Level (mg/kg/day)	0	5	50	500



METABOLISM AND PHARMACOKINETICS

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	0.63	22.43	25.6	x		18.89	94.1	7.65	111.5
	F	4	34	46.13	2.50	62.05	70.7	83.97	1418.0	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		1688.0		513.4		1518.0

	Sampling period			0-48 h		0-24 h		0-4 h		4-24 h		0-24 h	
Urine		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	4.96	0.45	x		2.97	0.11	6.93	2.49
	F	8	35.5	33.06	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	1.05	0.09	0.99	0.01	4.10	0.16	3.58	1.29
	(NADMF)	14	46.9	12.51	3.64	3.30	0.30	7.01	0.04	40.39	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	17.08	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, [illegible] identification of frovatriptan (F), desmethyl frovatriptan (DMF) or N-acetyl desmethyl frovatriptan (NADMF) and of unknown metabolites by ——

Sponsor's Table 5.2.3.E1, which is shown above, 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 oral 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.

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.

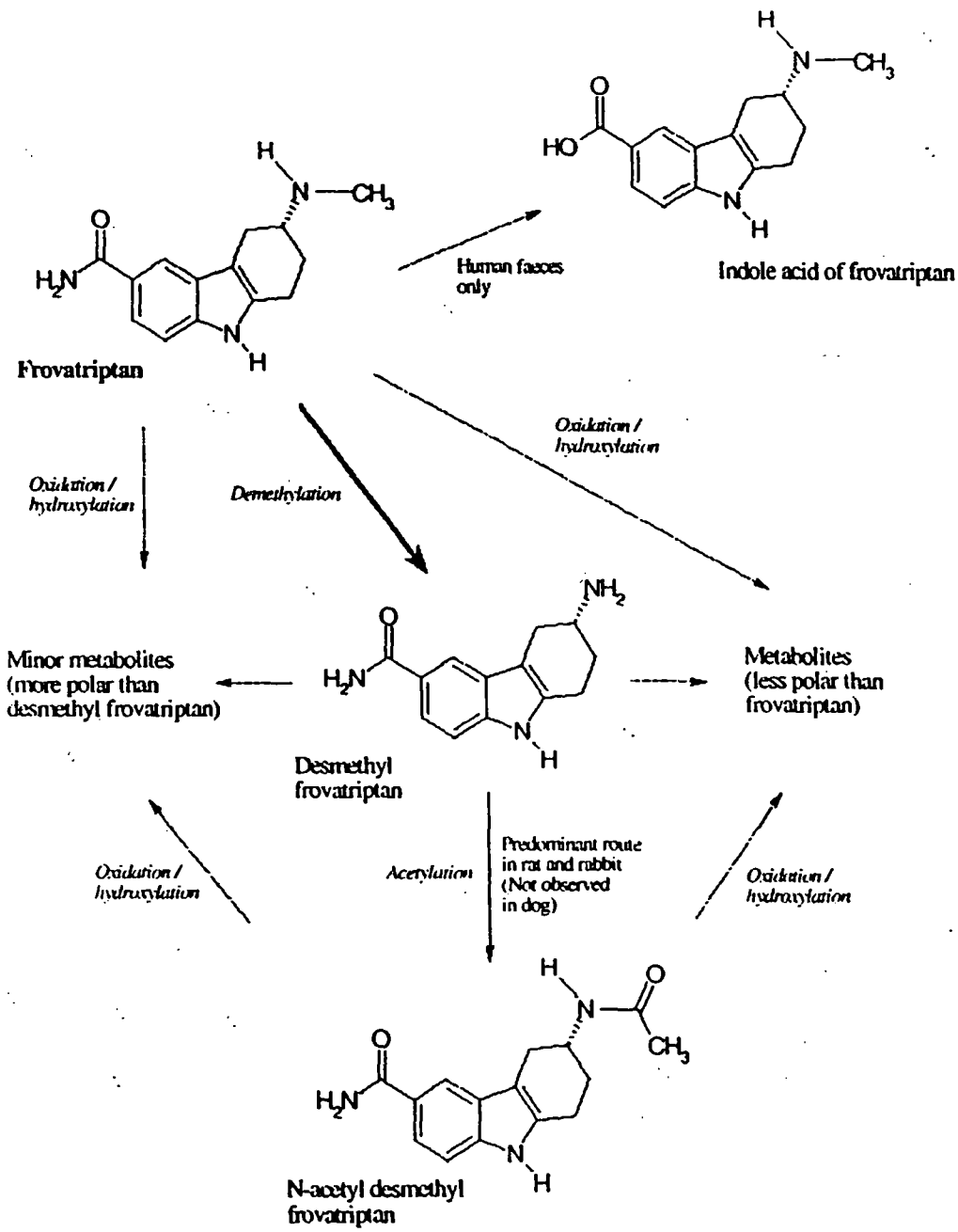
As shown in the table above, desmethyl frovatriptan was observed in the urine of the human, mouse, rabbit and dog, parent compound was observed in urine of all 5 species, and N-acetyl desmethyl frovatriptan was observed in the urine of all 5 species.

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.

Following IV administration of [14 C] frovatriptan in the rat 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. 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.

Proposed Metabolic Pathways for Frovatriptan



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% and 15.3±2.6% in rat and human, respectively. Sponsor concluded that there was no difference in proportion of protein binding between the 2 species and there was no evidence of significant concentration dependence in both 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% and 70% in rat and human, respectively, and the extent of binding to the cellular fraction was linear in both 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%).

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.

APPENDIX

Executive CAC
June 11, 1996

Committee:
James Farrelly, PhD, HFD-530, Acting Chair
Alex Jordan, PhD, HFD-580
Charles Resnick, PhD, HFD-110
Glenna Fitzgerald, PhD, HFD-120
Sharon Olmstead, HFD-006, Exec Sec

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VML 251
Vanguard Medica Ltd

The sponsor proposed a 2-year oral gavage carcinogenicity study in rats using 8.5, 27, and 85 mg/kg/day based on a 28-day toxicity study (HD 500 mg/kg). AUC multiples were provided for weeks 1 and 4. The sponsor believes the increased plasma levels reported over time in the 28-day study support the HD selection. Data from a 10-day toxicity study (100, 600, 1000, and 2000 mg/kg/day) reported 1 death at 1000 mg/kg but no deaths at 2000 mg/kg, although the dose was lowered to 600 mg/kg. The sponsor claims that the metabolism of the product is similar in humans and rats without providing supporting data. The sponsor also claimed that the increase in chromosomal aberrations reported with human lymphocytes was at cytotoxic doses. This claim could not be substantiated since data were not provided.

The committee expressed concern with the lack of data to support both the dose selection for the 2-year carcinogenicity and the other claims made by the sponsor. The committee agreed that a 90-day dose-ranging study with PK data would clarify the proposed dose selection. However, the committee felt the sponsor might be able to justify the dose selection with additional data.

Recommendations:
The data provided (28-day toxicity study) to support the proposed 2-year rodent carcinogenicity study were not sufficient for the committee to provide a recommendation on the dose selection. The committee suggested the following:

1. Information should be provided to justify the 25-fold AUC difference at 85 mg/kg. Data to support the sponsor's claim that the metabolism of the drug is similar in rats and humans (e.g., metabolite profiles) should be provided. The sponsor should also provide data to support their claim that an increase in chromosomal aberration with human lymphocytes was observed at cytotoxic doses.

2. Or the sponsor may conduct a 90-day dose-finding study with pharmacokinetic data to support the proposed dose range for the carcinogenicity study.

/S/
James Farrelly, Ph.D., HFD-530
Acting Chair, CAC

cc: IND file
Division file
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