BM 21.0955.NA: EMBRYO-FETAL TOXICITY STUDY IN THE RAT (INTRAVENOUS INJECTION) SEGMENT II– CAESAREAN SECTION

Report No. K12
Study period 1992
Batch Nr. 780-462-59A (formulation for injection)
Segment II- Caesarean Section

RAT STUDY (Segment II, IV dosing)

Species, strain	RAT, Crl: CD BR Sprague Dawley				
Route	IV injection (2x1ml/day)				
N	25 females/grp				
Dosing	GD6-GD15 (females)				
Dose selection	Based on MTD determined in study with 1, 2, 5, 10 mkd. Dystocia occurred at 1mkd, lethality at 2 mkd (K11).				
Procedure	F0 females C-sectioned and sacrificed on GD21				
Group		Control	LD	MD	HD
Dosing	Doses (mg/kg/day)*	0	0.1	0 4	1.5

*Doses are free-acid equivalents

Definitions

Postimplantation loss		# living pups at 1st check/#implantation sites

RESULTS

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.1	0 4	1.5
F0 dams	Pregnant females	24	25	21	24
	Premature decedents		1(#115, GD21)	1(#202, GD9)	
	F0 females evaluated	24	24	20	24
Signs	Apathy on GD21 (related to low serum calcium)	0	0	2	2
	Vaginal hemorrhage	1 (GD14)	0	0	0

Autopsy F0
1LD (#115) that died on GD21 had hemorrhagic pulmonary edema, 1MD (#202) had acute cardiovascular failure. Death of LD animal on GD21 may be related to low serum Ca and dystocia, or drug-related systemic toxicity.

F0 findings
Body weight in HD females slightly (<5%) but statistically significantly (pairwise, and trend test in some cases) decreased compared to controls on GD 10, 12,13, 15, 16.

Litter data

Group F0 dams		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0 4	1.5	
Dams evaluated		24	24	20	24	
	Weight of male fetuses (gr)	5.4	5.4	5.3	5.2*	*

	Weight of female fetuses (gr)	5 1	5 0	5 0	4.9	ns

No effects on litter averages of : #implantation sites IS, % postimplantation loss/IS, % live fetuses/IS, % dead fetuses/IS, % sex ratio, weight of female fetuses, placental weight, uterine weight (full/empty).

F1 fetuses: Externally visible anomalies (GD21; all fetuses):
No treatment effects

F1 fetuses: Visceral anomalies (GD21; ca. 50% of fetuses):

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0 1	0 4	1 5	
N litters		24	24	20	23	
Variations	% (litter average)	64	62	68	67	ns
Malformation	% (litter average)	0.5	0	0	1 5	ns
N fetuses		191	205	169	192	
Variation	Urinary organs· RPU syndrome	67	81	74	84	Not tested

Even though no effect on average % variation was apparent, there appeared to be a slight increase in all treated groups of RPU (as seen in other ibandronate reprotox studies). This finding confirms the biological significance of the other study findings.

F1 fetuses: Skeletal anomalies (GD21; ca. 50% of fetuses):

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0 1	0.4	1 5	
N litters		24	24	20	24	
Retardations	% (litter average)	79	84	84	88	ns
Variations	% (litter average)	17	14	10	6	ns
Malformation	% (litter average)	3 8	3.1	1 9	1.1	ns
N fetuses		193	205	167	191	
Retardations	Sternum, 6th sternal center, asymmetry	3	9	12	11	
	Hindfoot, phalanges of toes, poorly ossified	1	4	4	7	
	Hindfoot, phalanges of toes, unossified	11	5	6	3	
	Fore foot, phalanges of fingers, poorly ossified	16	20	10	13	
	Fore foot, phalanges of fingers, unossified	8	2	7	16	
	Head, interparietal bone, poorly ossified	8 (4 2%)	28 (13 7%)	12 (7 2%)	29 (15 2%)	
	Head, interparietal bone, unossified	0	1	0	0	
Variation	Rudimentary rib	17 (8.6%)	17 (8 3%)	6 (3 6%)	9 (4 7%)	
Deformation (transitory)	Thoracic vertebrae, ribs, wavy ribs at thoracic vertebrae	2 (1 0%)	3 (1 5%)	1 (0 6%)	10 (5 2%)	

Even though no effect on average % retaradations or other anomalies was apparent, there appeared to be a dose-related increase in the fetal incidence of sternum asymmetry at the 6th

center (LD, MD, HD), poorly ossified hindfoot phalanges (LD, MD, HD), unossified fore foot phalanges (HD), and an increase in wavy ribs at thoracic vertebra (HD).
The retardation finding of sternal asymmetry at the 6th center was not seen at other centers (1st through 5th) and appears not biologically significant. Also there was no increase in the incidence of sternal 6th center asymmetry, classified as variation.
The hindfoot poorly ossified phalanges finding was accompanied by a decrease in hindfoot unossified phalanges and is of questionable significance.
The forefoot unossified phalanges finding in HD was not accompanied by an increase in fore foot poorly ossified phalanges. This finding may have some significance.
Taken together, the significance of the impaired ossification findings at the foot is unclear.
The head interparietal bone finding of poor ossification and the wavy rib finding may have been drug-related. In the oral Segment 2 (C-section) study (0, 10, 30 , 60, 100 mkd) the control incidence of the interparietal bone poor ossification was 15.7% and the incidence was reduced in treated groups. Thus, this finding in the IV study was probably not significant. Also, in this oral study the control wavy rib incidence was 5%, suggesting that the finding in the IV study was not significant.
There was no effect on the fetal incidence of rudimentary rib.

Serum chemistry F0 dams:

Group F0 dams		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.4	1 5	
Dams evaluated		24	24	20	24	
GD21	Serum calcium (mmol/L)	2.6 ±0.2	2 4 ±0.3	2.3 ±0.6	2.2 ±0.7*	*
	Serum P (mmol/L)	1 9	1.9	1.9	1.9	ns
	BUN (mmol/L)	7.1	6.7	7.1	7.3	ns
	Creatinine (umol/L)	53	48	56	56	*

Clear dose-related reduction in serum Ca (sign in HD) and dose-related increase in creatinine on GD21.

SUMMARY

Pregnant SD rats were dosed by IV injection, daily from GD6-GD15, with 0, 0.1, 0.4, 1.5 mg/kg/day. C-section was performed on GD21.

- Signs and possibly mortality related to drug-induced decrease in serum Ca on GD21
- Slight but significant decrease in maternal gestational body weight gain at 1.5 mkd.
- Decrease in fetal body weight in males and females at 1.5 mkd
- Slight increase in RPU (renal pelvis ureter) syndrome in all treatment groups
- Reduction in serum calcium on GD21 at all doses.

NOTE: IV dose 0.1 mg/kg ≈ oral dose of 10 mg/kg/day in rat ≈ 1.7 mg/kg/day oral dose in human (100 mg/day oral) (based on BSA comparison)

BM 21.0955.NA: EMBRYO-FETAL TOXICITY STUDY IN THE RAT (INTRAVENOUS INJECTION) SEGMENT II– SPONTANEOUS DELIVERY

Report No. K14
Study period 1992
Batch Nr. 780-462-59A (formulation for injection)
Segment II- Spontaneous Delivery

RAT STUDY (Segment II, IV dosing)

Species, strain	RAT, Crl: CD BR Sprague Dawley				
Route	IV injection (2x1ml/day)				
N	15 females/grp				
Dosing	GD6-GD15 (females)				
Dose selection	Based on MTD, ie dose at which dystocia was minimized with Ca substitution (Drf Study K13)				
Procedure	F0 females allowed to deliver				
	All F0 received s.c. Ca substitution from GD 18-PPD0 (32 mg/kg/day)				
	F0 dams sacrificed on PPD21, F1 pups sacrificed on PPD42 All F1 pups were evaluated (no culling)				
	F1 pups (1/s/litter) reared, mated and allowed to deliver, sacrificed with F2 pups on PPD7				
Group		Control	LD	MD	HD
Dosing	Doses (mg/kg/day)*	0	0.1	0.3	1.0
	Ca (mg/kg/day) Administered on GD18-PPD0, twice daily, sc injection	32	32	32	32

*Doses are free-acid equivalents

COMMENT: Calcium supplementation was based on results from drf studies, K11, K13, K12. In those studies, levels of 1.0 mg/kg/day that were not toxic in other ways lead to disturbances of birth causing maternal deaths. A dose-dependent decrease in serum calcium concentration was observed at the end of pregnancy, presumably due to inhibition of bone resorption. This is thought to affect parturition (dependent on extracellular calcium). The lack of maternal calcium could also affect fetal development at end of pregnancy or during parturition or lactation. Ca substitution of dams was found to overcome the dystocia at 1.0 mg/kg, but not at 1.5 mg/kg/day. Thus, 1.0 mg/kg was selected as HD. Calcium (approximately 13mg/day) was given as a 4- to 6-day daily sc supplement from GD18-PPD0.

Definitions

Postimplantation loss		# living pups at 1st check/#implantation sites
Postnatal loss (PPD0-PPD4)		#pups that died from PPD0-4/#living pups at PPD0
Breeding loss (PPD5-PPD21)		# pups that died from PPD5-21/# of living pups at PPD4
Live birth index (%)		(Number of pups born alive/Number of implantations)x100
Viability index 1 (%)		(Number of pups alive on PPD4/Number of pups born alive)x100
Weaning index (%)		(Number of pups alive on PPD21/Number of pups alive on PPD4)x100

RESULTS

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD

Dose (mg/kg/day)		0	0.1	0 3	1.0
F0 dams	Pregnant females	15	15	15	15
	Unscheduled kill	0	0	0	2 (#312 GD22, #313 PPD1)
	Females with signs of sedation	0	0	0	2 (#312,313)
	Females with delivery	15	15	15	14
	Females with live pups at parturition	15	15	15	13
	Females with total postnatal loss				1 (#313: all pups stillborn)
	Females with live pups on PPD21	15	15	15	13

Two HD dams were killed, one on GD22, the other on PPD1 after all pups were stillborn. Both dams had signs of sedation (no tremor or other signs of dystocia).

Autopsy findings in F0 dams;
Vaginal bleeding in 0-0-0-1(#312).

F0 findings
No effect on body weight in F0 dams during gestation or lactation

Litter data

Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.1	0.3	1.0
Litters evaluated		15	15	15	13-15
	Gestation duration	21 9	22 1	22 0	21.9
	No. implantations (# IMP) (mean)	16.1	16 3	16.1	16 8
	Postimplantation loss (% of IMP)	5.3	6.3	4.2	5.2

F0 dams (exclusive)	Live birth index (%)	92	97	98	92
	Viability index 1(%)	96	99	98	98
	Weaning index (%)	96	98	97	98

No effects on # live or dead pups, sex ratio in F0 litters

F1 pup findings

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0 05	0.15	0 50	
Litters evaluated		15	15	15	13	
Body weight (males)	BW PPD14 (gr)	32	29*	30*	29*	*
	BW PPD21 (gr)	53	49	49	47*	*
	BW PPD42 (gr)	224	214	216	214	
	AUC PPD7-42 (sum of diff's to initial BW)	2989	2810	2842	2780	
Body weight (females)	BW PPD14 (gr)	31	28	29*	27*	*
	BW PPD21 (gr)	51	47	48	45*	*

	BW PPD42 (gr)	177	171	170*	170	ns
	AUC PPD7-42 (sum of diff's to initial BW)	2615	2484	2495*	2437	ns
Neuromuscular coordination/refl exes	Air righting	0 97	0 83**	0.95	0.92*	ns

Nd=no data; ns = not significant

No effect on body weight at PPD0 (m,f) (7gr-6gr)
No effects on physical development parameters (pinna detachment, incisor eruption, full coat, eye opening, vaginal opening, testicular descent) in F1 pups
No effects on neuromuscular behavior (negative geotaxis, air righting, grip strength, pupillary reflexes, auditory startle) in F1 pups
Cliff avoidance test not performed
No effects on water maze behaviour tests in F1 pups.

Anomalies
Premature deaths: Visceral/skeletal anomalies in F1 pups that died prematurely

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0 05	0 15	0 50
N examined		26	9	6	3
Visceral					
Variation	Urinary organs (RPU syndrome)	5 (19%)	5 (56%)	1 (17%)	2 (67%)

No skeletal treatment effects in premature deaths

Surviving pups: Visceral anomalies in F1 pups that survived (autopsy findings)

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.05	0.15	0.50	
N litters		15	15	15	13	
N examined		169	188	187	173	
Individual Variation	Urinary organs (RPU syndrome)	5	9	5	23	
	% of fetuses with RPU	3%	5%	3%	13%	
Variations	% (litter average)	13 5	18.0	19.8	22.7**	*

*p<0.05

No visceral malformations, pathological findings
NOTE: Skeletons were not examined in surviving pups

The effect on variations was due to a high incidence of renal pelvis ureter (RPU) syndrome in HD animals. This finding is relatively common according to Sponsor (concurrent control incidence =3%; however, control incidence in oral Segment II C-section study No. K17; same SD rat strain: 29/116=25%, and in oral Segment II delivery Study K9: 9%).
The RPU syndrome includes various states of dilatation of the ureter and the renal pelvis without evidence of functional defects. According to Sponsor, it is a consequence of the nephrotoxicity of the class of compounds (bisphosphonates), and also seen with others in the class (???).

F1 generation mating and breeding performance
Female F1 performance

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.05	0.15	0.50
F1 dams	Mated females	15	15	15	13
	Pregnant females	15	13	14	13
	Females with live pups at parturition	15	13	13	13

No treatment related signs, no mortalities

F1 dams body weight

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.05	0.15	0 50	
N		15	13	14	13	
Body weight (females)						
Gestation	BW GD1 (gr)	285	262	268*	270	
	BW GD21 (gr)	502	471*	450***	**466	**
	AUC GD7-GD21 (sum of diff's to initial BW)	1495	1472	1288**	1375	*
Lactation	BW PPD0 (gr)	367	344	334**	339*	**
	BW PPD7 (gr)	397	375*	372**	365**	**

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

No treatment effects on litter data (#corpora lutea, #implantation sites, % preimplantation loss, % postimplantation loss, % live pups, % dead pups, % sex ratio, % live birth index, % viability index 1 and 2)

No effect at necropsy (autopsy) of F1 parents (m or f)

F2 pups
No effects in F2 pups on:

- Clinical findings or external anomalies
- BW development (BW in PPD0 and BW gain on PPD0-7)
- Visceral and skeletal anomalies in F2 pups that died prematurely. However, N was too small (10-4-4-14) to conclude anything regarding F2 anomalies.

SUMMARY
Pregnant SD rats were dosed by IV injection, daily from GD6-GD15, with 0, 0.1, 0.3, 1 mg/kg/day. Calcium (32 mg/kg/day) was administered s.c. from GD18-PPD0. Dams were allowed to deliver and raise the pups until weaning. F1 was evaluated for development and reproductive performance.

- Perinatal calcium administration reduced drug-induced dystocia and perinatal mortality (result from dose range finding study with 1 and 1.5 mg/kg and with/without 16-32 mg/kg/day sc Ca administration)
- Two HD dams with signs of dystocia were killed at the end of gestation or after delivery. One of those had all pups stillborn.
- Slight decrease in body weight gain in male and female F1 pups in first 2-3 weeks after delivery in all treatment groups with unclear dose relationship, resolved partially after 6 weeks.
- Increased incidence of fetuses with RPU (renal pelvis ureter) syndrome at 1 mkd.
- Decreased body weight in mated F1 female offspring in all treatment groups during F1 gestation and during lactation of F2 offspring. No clear dose relationship of this body weight finding.
- No effect on F1 reproductive performance.

NOTE: IV dose 0.1 mg/kg ≈ oral dose of 10 mg/kg/day in rat ≈ 1.7 mg/kg/day oral dose in human (100 mg/day oral) (based on BSA comparison)

BM 21.0955.NA: EMBRYO-FETAL TOXICITY STUDY (SEGMENT II) IN THE RABBIT (INTRAVENOUS ADMINISTRATION)
Report No. K7
Study period 1992
Batch Nr. 780-462-59A (formulation for injection)
Segment II- Caesarean Section GD29

RABBIT (Segment II, IV dosing)

Species, strain	RABBIT/CHbb:HM					
Route	IV injection ear vein					
N	17 females/grp					
Dosing	GD6-GD18					
Dose selection	Based on MTD determined in drf Studies K5, K6					
Procedure	F0 females C-sectioned and sacrificed on GD29					
Group		Control	LD	MD	HD	
Dosing	Doses (mg/kg/day)*	0	0.03	0.07	0.2	

*Doses are free-acid equivalents

Definitions

Postimplantation loss		# living pups at 1st check/#implantation sites

RESULTS

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.03	0.07	0 2
F0 dams	Inseminated	17	17	17	17
	Pregnant	17	17	15	16
	Prematurely killed			1 (broken paw) (GD6)	2 (#308,312; GD25)
	Dams with abortions				2 (#308, 312; GD23)
	Dams with delivery				1 (#302; GD29)
	Dams with total resorptions				1 (#311)
	Vaginal hemorrhage	0	1 (#104; GD22-26)	0	0
	Pregnant females evaluated	17	17	14-15	13-16

Autopsy F0

Dam with all resorptions (#311; 6 fetuses) had kidney hypertrophy

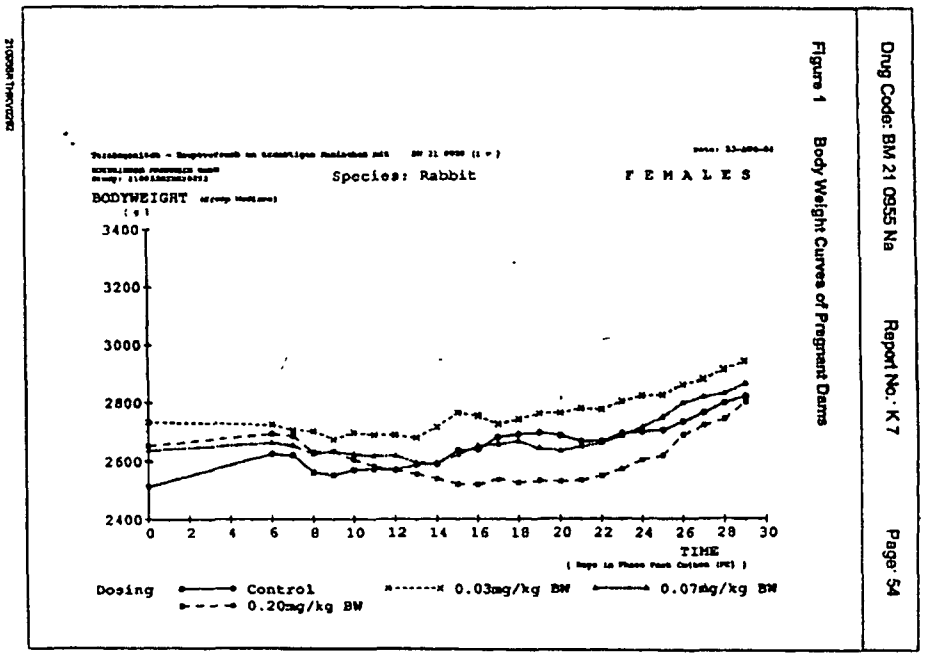
Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.03	0.07	0.2
Dams evaluated		17	17	17	17
Hypertrophy of gallbladder		0	2	3	1
Lung edema		0	0	1	0
Hindlimb fracture		0	0	1	0
Gallbladder missing		0	0	0	1
Hypertrophy of kidney		0	0	0	1 (#311)

F0 findings

Body weight

Group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0 07	0 2	
N litters		17	17	14-15	13-16	
F0 dams	BW GD0	2573	2704*	2658	2662	ns
	BW GD6	2619	2742	2656	2707	
	BW GD23	2701	2801	2744	2548	*
	BW GD29	2850	2940	2876	2783	
	AUC1	2078	1597	714	-559	

AUC1 = area-under-time curve for GD6-GD29 differences to initial body weight



Litter data:

No effects on litter averages of: #implantation sites IS, % sex ratio, uterine weight (full/empty).

Group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0.2	
Dams evaluated		17	17	14	12-16	
	Postimplantation loss/IS (%)	8.3	8.5	10.7	16.6	Ns increase in HD
	Live fetuses/IS (%)	92	92	89	83	Ns decrease in HD
	Weight of male fetuses (gr)	39	40	39	36	Ns decrease in HD
	Weight of female fetuses (gr)	39	39	39	37	Ns decrease in HD
	Placenta weight of all fetuses (gr)	5.1	5.3	4.9	4.5*	*
	Body weight gain since GD0 (minus uterine content)	-35	-86	-106*	-145*	**

	(gr)					

*p<0.05; **p<0.01; ns= non-significant

- Increased PI loss was due to an increased rate of resorptions (no dead fetuses)
- Resorption range: Ctrl 0-3, LD 0-3, MD 0-2, HD 0-6. Number of litters with late resorptions: ctrl 1/17- LD 3/17- MD 4/14- HD 5/12. LD dam with vaginal hemorrhage (#104) had 2/7 late resorptions. These events may have been related.
- #Live fetuses was reduced probably as a result of postimplantation loss
- The fetal weight reduction in HD m and f was not statistically significant. The effect was due to the fact that in some HD litters there were pups with lower body weights than the lowest observed in ctrl, LD, MD. Thus, there was a larger range of avg. body weights in the HD litters.
- Body weight gain: (Body weight – uterine weight: < 0) was significantly reduced in dose-related manner during gestation (all does lost body weight during gestation)

F1 fetuses:
No external anomalies

F1 fetuses: Visceral anomalies (GD29):

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0 2	
N litters		17	17	14	12	
Variations	% (litter average)	42	46	53	56*	*
N fetuses		111	112	88	77	
Variation	Gall bladder enlargement or underdevelopment	14	10	18	20	

The cause of the increase in average % variations was gallbladder anomalies

F1 fetuses: Skeletal anomalies (GD29):
No effect on average % retardations, variations or malformations. No clear effects on individual fetal findings either.

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0.2	
N litters						
Malformation	% (litter average)	3 5	3.5	2.7	5.7	Ns

Serum chemistry
(GD 0,20,29: Ca, P, BUN, creatinine) in F0 dams:

Group F0 dams		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0 03	0 07	0 2	
Dams evaluated		17	17	14	14	
GD29	Serum Ca (mmol/L)	3.1	3.1	3 2	3 0	
	Range of Ca	2.3-3 5	2.6-3.6	2 6-3 7	1.8-3.7	
	BUN (mmol/L)	6 6	6.2**	6 8	7.7	*
GD20	Creatinine (umol/L)	90	85	99	132***	***
GD29	Creatinine (umol/L)	88	91	93	96	
	Range of creat.	63-105	73-124	77-118	77-158	

- Serum Ca was significantly decreased in GD29 (not on GD20) in some HD dams, although no statistical overall effect was seen.
- No effect on serum P at any day
- Serum BUN increased on GD29 in HD

- Serum increased in most HD on GD20 (stat sign group effect), and increased in some HD dams on GD29 (ns group effect). This effect on kidney function markers (BUN, creatinine) was biologically significant.

SUMMARY

Pregnant Himalayan rabbits were dosed by IV injection, daily from GD6-GD18, with 0, 0.03, 0.07, 0.2 mg/kg/day. C-section was performed on GD29.

- Two (2/16) animals with abortions and one (1/16) with premature delivery in HD
- Reduction in gestational body weight gain in MD and HD
- One (1/16) HD animals with all resorptions. Related increase in postimplantation loss in HD.
- Increase in proportion of late resorptions at all doses.
- Slight decrease in fetal and placental weight in HD.
- Serum Ca decreased on GD29 in HD
- Serum creatinine increased on GD20 in HD

Conclusion: Maternal, embryo- and fetal toxicity observed in presence of maternal toxicity.

APPEARS THIS WAY
ON ORIGINAL

PERI- AND POSTNATAL STUDY WITH BM 21.0955-NA IN THE RAT (INTRAVENOUS INJECTION)

Report No. K18
— Project 381262
Study period 1994/1995
Batch Nr. 447-624-00
Segment III- Spontaneous Delivery

RAT STUDY (Segment III, IV dosing)

Species, strain	RAT, WIST Hanlbm: Wist					
Route	IV injection					
N	25 females/grp					
Dosing	GD17-PPD20 (females)					
Dose selection	Based on MTD (4-wk IV tox study with 0.1, 0.3, 1 mg/kg) (Study G2)					
Procedure	F0 dams delivered					
	F1 litters culled on PPD4 to N=8/group where possible					
	F1 pups (1/sex/litter) reared to maturity and mated					
	F1 dams delivered and F2 sacrificed at PPD4					
Group		Control	LD	MD	HD	
Dosing	Doses (mg/kg/day) (target doses)*	0	0 05	0 15	0.50	
	Real doses used (mg/kg/day)	0	0.0475	0.143	0.475	
Exposure	AUC					
	Human AUC Multiple					

*Doses are free-acid equivalents, and expressed as target doses. Since batch used was determined to have lower free acid content than initially assumed, the real free acid doses used are 0.95x intended (target) doses

Litters were culled on PPD4 to N=8/litter when possible

Definitions

Postimplanatation loss		# living pups at 1st check/#implantation sites
Postnatal loss (PPD0-PPD4)		#pups that died from PPD0-4/#living pups at PPD0
Breeding loss (PPD5-PPD21)		# pups that died from PPD5-21/# of living pups at PPD4
Birth index (%)		(Number of pups born alive/Number of implantations)x100
Viability index (%)		(Number of pups alive on PPD4/Number of pups born alive)x100
Weaning index (%)		Number of pups alive on PPD21/Number of pups alive on PPD4)x100

RESULTS

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0 05	0.15	0.50
F0 dams	Mated females	25	25	25	25
	Pregnant females	23	25	23	24

	Mortalities (total)	0	3	1	4
	Prenatal	0	GD22: 1 GD23: 2	GD 23: 1	GD21:1 GD23:1
	Postnatal				PPD2: 2 (#80, #100)
	Deaths with dystocia finding	0	3	1	3
	Deaths with no dystocia	0	0	0	1
	Females with dystocia that did not die (#, days with signs)		1 (#42, GD22-PPD7)	1 (#66, GD22)	
	Females with signs of dystocia	0	4	2	3
	Females with live pups at parturition	23	22	22	22
	Gestation duration (days)	21 4	21.5	21.5	21.4
	Females with total postnatal loss	1 (#10)	2 (#38, 42)	4 (#52, 56,65,66)	0
	Females with live pups on PPD21	22	20	18	20

Female #42 lost all pups (all dead at first litter check) on PP Day0/1, and female #62 also lost all pups (pups dead or missing on PPD1/2).

Signs of dystocia: tremor, ruffled fur, felt cold, dyspnea, bleeding from vagina, prostrate and lateral recumbency.
Dystocia was attributed to hypocalcemia resulting from inhibition of bone resorption and calcium mobilization. This has also been seen with other bisphosphonates. Sponsor mentions that lack of calcium is critical at the perinatal time when fetal demand and maternal demand are at its peak.

There were no remarkable treatment-related autopsy findings in F0 dams with dystocia

F0 findings

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.05	0.15	0.50
F0 dams	Food consumption (PPD1-PPD4)	28.9	25.5 (ns)	27.7	25.8 (ns)
	Body weight gain (PPD7-PPD14)	21	18	22	17 (ns)

No effects on maternal body weight or food consumption during gestation.

Litter/breeding data
Data EXCLUDING dams with total litter loss (1-2-4-0) and excluding dams which died postpartum (0-0-0-2)
This analysis did not include any animals with signs of dystocia
Data for dams excluding those with total postnatal loss

Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0 05	0.15	0.50
	Litters evaluated	22	20	18	20
	Gestation duration	21.4	21.6	21.4	21.4
	No. implantations (# IMP) (mean)	12.8	12.0	13.1	12.4
	Postimplantation loss (% of IMP)	10.7	15.8	17.4*	10.5
	Dead pups at first	0	0 4*	1.1*	0.5*

	litter check (N/litter)				
	Living pups at first litter check (N/litter)	11.4	10.1	10.8	11.1
	Postnatal loss (PPD0-4) (%)	0.8	7.9**	4 1*	6.8**
	# Litters affected with postnatal loss (PPD0-4)	2	5	4	5
	Breeding loss (PPD5-21) (%)	0	1.3	0	0
F0 dams (exclusive)	Birth index (%)	89	84	83*	90
	Viability index (%)	99	92**	96*	93**
	Weaning index (%)	100	99	100	100

Data INCLUDING dams with total litter loss (1-2-4-0) and excluding dams which died postpartum (0-0-0-2) showed larger significant effects in LD and MD groups on PostImplantation (PI) loss and PostNatal (PN) loss.

Data for dams including those with total postnatal loss

Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0 05	0.15	0.50
	Litters evaluated	23	22	22	20
	Gestation duration	21.4	21.5	21.5	21.4
	No. implantations (# IMP) (mean)	12.7	12.0	12.9	12.4
	Postimplantation loss (% of IMP)	10.6	19.2*	24.6*	10.5
	Dead pups at first litter check (N/litter)	0	0.9*	1.5**	0.5*
	Postnatal loss (PPD0-4) (%)	4 6	13.1**	12.1**	6 8
	# Litters affected with postnatal loss (PPD0-4)	3	7	8	5

Indices for dams including those with total postnatal loss

F0 dams (inclusive)	Birth index (%)	89	81**	75**	90
	Viability index (%)	95	87**	88**	93
	Weaning index (%)	100	99	99	100

Early postnatal loss (in litters with total or partial loss) occurred mostly on PPD1, 2, and 3

Females with PPD0-4 loss (excluding dams that died pre- or postpartum)

	ctrl	LD	MD	HD
Dams (#)	#6,14,10	#31,32,37,43,50,38,42	#53,54,62,75,52,56,65,66	#79,84,87,88,99
Dams with total loss	#10	#38,42	#52,56,65,66	none
Dams with dystocia	none	#42	#66	none
Dams with no signs of dystocia, but with partial postnatal loss	#6,14	#31,32,37,43,50	#53,54,62,75	#79,84,87,88,99
Dams with no signs of dystocia, but with total postnatal loss	#10	#38	#52,56,65	none

Serum chemistry/hematology

PPD21: No effect on albumin, serum calcium or differential blood counts in plasma of dams. Calcium was measured only in dams that did not die perinatally or postnatally.

Necropsy (macroscopic findings)
None treatment-related in F0 females

F1 findings

F1 group		Ctrl	LD	MD	HD	
Dose (mg/kg/day)		0	0 05	0.15	0.50	
Body weight	BW PPD1 (gr)	5 8	5.8	5.2*	5 3ns	
	BW PPD21 (gr)	42 1	43 4	41.4	37 9*	
	BW PPD35 (gr)	110	112	105 ns	-	
Behavior tests	Negative geotaxis, PPD21(%)	93	86	85*	87	Sponsor concludes this was incidental
	Water maze 3. learning (out of 6 trials), ppd35-43 (%)	68	63	52*	nd	Sponsor concludes this was incidental
Findings dunng lactation and rearing	Lost or malpositioned lower incisors			25pups/8 litters	All pups/all litters	Significant finding

nd=no data

After PPD21 (weaning) HD and some MD pups revealed abnormal odontogenesis: when beginning eating food pellets, lower incisors were lost or missing or malpositioned, and pups could not eat, starved and died. Thus, all pups in Grp 4 HD were sacrificed on PPD24-28. Some pups in MD (23 pups of 8 litters) had same abnormality causing perforation of the palate. These pups were also sacrificed on PPD25-29. Abnormality caused reduced group body weight mean in MD on PPD35. Effect was not seen in oral Segment 3 study, or other studies with delivery and F1 evaluation.

No effects on physical developmental indices and behavioral test parameters in F1 pups, including cliff avoidance on PPD21.

F1 generation mating and breeding performance
Female F1 performance

		Ctrl	LD	MD
Dose (mg/kg/day)		0	0.05	0.15
F1 dams	Mated females	25	25	25
	Pregnant females	24	25	23
	Females with live pups at parturition	24	25	23
	Females with total postnatal loss	0	0	0

No treatment related signs, no mortalities in F1 dams
No effects on FC and BW of F1 female parents during gestation and lactation.
No effects on parental reproduction parameters (% mating, fertility index, conception rate, gestation index, PI loss, PN loss, birth, viability, weaning indices)
No effect on necropsy of F1 parents

F2 pups: external examination

Group		Ctrl	LD	MD
Dose (mg/kg/day)		0	0.05	0.15
	No. litters	24	25	23

F2 pups	No. live pups	279	306	259
	No. dead pups	0	0	0
	Head multiple malformed	0	0	1

No effect on BW in PPD0 and BW gain on PPD0-4 in F2 pups

SUMMARY

Pregnant Wistar rats were dosed by IV injection, daily from GD17-PPD0, with target doses of 0, 0.05, 0.15, 0.5 mg/kg/day (actual doses 0, 0.048, 0.14, 0.48 mg/kg/day). Dams were allowed to deliver and raise the pups until weaning. F1 was evaluated for development and reproductive performance.

- Perinatal mortality and dystocia in F0 dams in all treatment groups (not dose-related)
- Increase in "postimplantation" loss due to increase in dead pups at first litter check at LD and MD (not dose-related)
- Increase in early postnatal loss (PPD0-PPD4) and in #litters affected with postnatal loss at all doses
- Postnatal loss (partial or total) also observed in dams with no signs of dystocia
- Reduced body weight at birth in MD and HD
- Reduced body weight gain during lactation in F1 pups at HD
- Abnormal odontogenesis in MD and HD apparent after weaning (lower incisors became lost or malpositioned, and palate perforated, upon start of eating food pellets)
- Reduced body weight gain and mortality due to dental abnormality in MD and HD
- No effect on reproductive performance of F1

NOTE: IV dose 0.1 mg/kg ≈ oral dose of 10 mg/kg/day in rat ≈ 1.7 mg/kg/day oral dose in human (100 mg/day oral) (based on BSA comparison)

Discussion of postimplanatation and postnatal loss due to periparturient toxicity

It appears that there was a treatment effect, although not always dose-related, on birth and viability index due to increased postimplantation loss and increased postnatal loss. This was an effect seen in all dams/litters including those who survived and had no signs of dystocia. A similar effect was seen in a Segment 3 oral study (K15) in rats. The Sponsor speculated in that study that the increased postimplantation loss was really due to undetected peri-postnatal loss. Sponsor also concluded that in the oral study that PI and PN loss only occurred in dams with dystocia and that there was no toxicity to pups unrelated to the maternal effect. Reviewer does not agree with that since in both this IV study and the oral Segment 3 study there was increased PI or PN loss in animals without signs of dystocia.

However, it appears that low serum calcium is responsible for the postnatal fetal loss effect in the Segment 3 studies (in which Ca is not supplemented), since the IV and oral Segment 2 studies with Ca-supplementation and delivery (K9, K14) showed no such postnatal loss, and in dams without dystocia in the oral Segment 3 study there was no increase in postnatal loss.

In a published study in SD rats with alendronate (oral dosing from 4 days prior to mating through GD20) (15 mg/kg/day), IV Ca supplementation (one 9.3 mg dose on GD21) prevented maternal dystocia/deaths, but some postnatal fetal deaths (on PPD1-2) were still observed and 1/155 pups was lost on PPD0 in alendronate-treated (REF·1). The PPD1-2 postnatal loss incidence in the treated group was 4%, and the authors argued that this was within historical control values for postnatal loss PPD1-2 (range 0-7%). Although in a different strain, the historical range for postnatal loss from PPD0-PPD4 in Wistar rats from Boehringer Mannheim is only 0-2.6% (studies from 1984-1992) (Report K18, p.212). The results from the oral and IV ibandronate Segment 2 studies showed that a 4-to 6-day sc supplementation with calcium (approximately 13mg/day) from GD18-PPD0 prevented maternal and fetal deaths to a large extent, although not completely.

REFERENCES

REF 1: Minsker DH, Manson, JM, and Peter CP (1993) Effects of the bisphosphonate, alendronate, on parturition in the rat. Toxicol. Appl. Pharmacol. 121, 217-223

RANGES OF HISTORICAL CONTROL DATA OF WISTAR RATS (SPF Quality)

Data from Peri- and Postnatal Studies

Parameter	Controls (treated with a vehicle, by gavage)	
	RANGES	
DATE	1984 - 1992	
	P	F1
DURATION OF GESTATION (days)	21.5 - 21.9	21.6 - 22.0
IMPLANTATION SITES (mean)	10.2 - 13.0	12.9 - 13.1
POST-IMPLANTATION LOSS (% of impl. sites)	3.0 - 13.9	4.3 - 9.0
MEAN NUMBER OF LIVING PUPS PER DAM:		
at first litter check	9.5 - 11.7	11.0 - 12.1
on day 4 p.p. before culling	9.3 - 11.4	11.0 - 12.1
on day 4 p.p. after culling	7.5 - 8.0	7.9 - 8.0
on day 21 p.p.	7.4 - 11.5	7.9 - 11.4
DEAD PUPS AT PARTURITION (mean)	0 - 0.4	0 - 0.1
SEX RATIO (% of male pups)	48.0 - 50.6	47.0 - 53.2
POSTNATAL LOSS BETWEEN DAYS		
0 AND 4 p.p. (%)	0.0 - 2.6	0.0 - 1.5
5 AND 21 p.p. (%)	0.0 - 2.0	0.0 - 1.5
BODY WEIGHT OF THE PUPS (grams):		
on day 1 p.p. (mean)	6.0 - 6.4	5.7 - 6.6
on day 4 p.p. (mean)	8.3 - 9.1	8.8 - 9.5
on day 7 p.p. (mean)	12.5 - 13.7	13.7 - 14.2
on day 14 p.p. (mean)	24.2 - 26.3	28.2 - 29.6
on day 21 p.p. (mean)	37.6 - 41.9	45.3 - 46.7
DEVELOPMENT INDICES: (group mean/day post partum)		
Pinna unfolding	2.9 - 4.0	
Incisor eruption	7.7 - 8.3	
Onset of coat development	7.0 - 10.1	
Eye opening	14.0 - 15.0	
Descensus of testes	22.5 - 24.0	
Opening of vagina	26.9 - 32.6	

REMARKS:

P = mated females breeding for F1 pups; F1 = F1 animals breeding for F2 pups

RANGES OF HISTORICAL CONTROL DATA OF WISTAR RATS (SPF Quality)

Data from Peri- and Postnatal Studies

Parameter	Controls (treated with a vehicle, by gavage)
	RANGES
DATE	1984 - 1992
RESULTS OF BEHAVIORAL TESTS OF PUPS: (% of positive behaviour)	
Righting reflex	100
Photo-phobotaxis	65.2 - 79.0
Pupillary reflex	100
Hearing ability	100
Palmar grasp ability	99.0 - 100
Activity test (mean)	1.2 - 2.2 (A)
Cliff avoidance	89.5 - 100
Negative geotaxis	60.0 - 97.0
WATER MAZE TEST (F1 pups): (% of positive behaviour)	
Water maze 1. learning	98.0 - 100
Water maze 2. learning	100
Water maze 3. learning	100
Water maze 4. learning	100
Water maze 5. learning	100
Water maze 6. learning	100
Water maze memory	76.0 - 100
Water maze 1. relearning	5.0 - 79.0
Water maze 2. relearning	35.0 - 92.0
Water maze 3. relearning	56.0 - 100
Water maze 4. relearning	62.0 - 100
Water maze 5. relearning	69.0 - 98.0
Water maze 6. relearning	94.0 - 100

(A) = Activity index (0 points = minimum = no activity; 3 points = maximum)

ADME STUDIES IN PREGNANT OR LACTATING RATS

Placenta transfer (Study I13)
In pregnant rats (single i.v. dose, 0.1 mg/kg) at 2-24h postdosing, dams retained 49%-35% of dose in carcass, and 2%-1% in kidney and liver. Spleen and sexual organ levels retained 0.5%-0.1% of dose. Fetuses retained 0.02% of dose after 2h, and 0.008% of dose after 24h. Placenta contained 0.07% of dose at 2h, 0.03% at 24h. Amniotic fluid contained 0.003% of dose at 2h only.

Transfer to milk (Study I14)
A single dose of radiolabeled ibandronate (0.08 mg/kg/day) administered by the i.v. route to lactating rats 12 days after delivery resulted in the appearance of compound in milk at 2, 6, 12 and 24h after dosing. The highest concentration in milk was seen at 2h after dosing (8.1 ng/mL). At 24h after dosing the milk concentration was 0.4 ng/mL. Higher concentrations of radioactivity in milk than in plasma (ca. 1.5-fold) may have been due to the higher calcium levels in milk.

Exposures and exposure multiples for reprotoxicity studies

Serum drug levels were not measured in the reprotoxicity studies, except the oral Segment 3 study in rats (K15). However, toxicokinetic data were collected in oral and IV repeated dose toxicity studies in rats. Data are also available from ADME studies. These data have been used to estimate exposure in the reprotoxicity studies. Exposure multiples were also calculated on basis of mg/m2 BSA dose comparison. In the latter calculation, oral bioavailability in humans and rats was assumed to be the same, e.g. 0.6%. All reprotoxicity studies were performed in fasted state except study K15 in which TK was measured.

TK data summary rat (female or sex-pooled data*) (repeat dose study data; Vol.1/103, p.308)

Species, strain	Study #	Duration	Study	Doses (mg/kg/day) (or mg/kg weekly or 2xmonthly) WDS=weighed drug substance FAE = free acid equivalents	Route	Feeding status	Mg/kg/day if not otherwise indicated	Cmax (ng/mL) for oral studies; Cpl right after injection for iv studies	AUC_{0-24} (ngxh/mL)	Time of TK
RAT, SD	G6	2-week	TK study	5,10 (FAE)	oral	Non-fasted; but no food from 0-5h post dose	5	3.2	18.6	2 wks
							10	11.8	22	2 wks
RAT, Wistar	H1	26-week	Tox	11.5, 34.3 WDS 10.1, 30.3 FAE	oral	Restricted feeding (ie o/n fast, and food from 2-10h post dose)	10.1	27f	79f	26 wks
							10.1	6.3m 24.4m 15.4avg	34.5m 142m 88avg	4 wks 26 wks avg
RAT, Wistar	H3	26-week	Tox	1.2, 3.4, 11.5 WDS 1.0, 3, 10 FAE	oral	Ad libitum	3	0.3	4.4	26 wks
							10	0.7	6.3	26 wks
RAT, SD	H4	12-month	Tox	3,10,20 FAE	oral	Ad libitum	10	5.8	48	50 wks
RAT, SD	H7	26-week	Tox	0.075, 0.15 weekly 0.3 twice monthly (FAE)	i.v.	N/A	0.15 weekly	487	464	26 wks
							0.3 twice monthly	1113	1069	26 wks
RAT, SD	H9	26-week	Tox	0.3, 0.9, 1.8, 2.7 weekly (FAE)	i.v. or s.c.	N/A	0.3 weekly i.v.	2573	1604	17-18wks
							0.9 weekly i.v.	3105	3553	17-18wks
							1.8 weekly s.c.			17-18 wks
							2.7 weekly s.c.			17-18 wks
							0.3 weekly i.v.	661	752	26-27 wks
							0.9 weekly s.c.	-	3259	26-27 wks
Rat, SD	I5	Single 14C dose	PK	0.1	iv		0.1 i.v.	No data	125	NA
Rat, Wistar	I11	Single ^{14}C dose	PK	0.03	sc		0.03 s.c.	39 6	38.1	NA
Human ptsts (F)	Mf7159	Single dose	PK	2.5 mg/day (0.04 mg/kg/day) (FAE)	oral	Overnight fast (12h), breakfast 2h after dosing	0.04 p.o.	0 6-0.8	1 8-3.3	Single dose data
Human volunteers	No ref study#	Single IV injection	PK	1 mg (0.017 mg/kg) (FAE)	i.v.	N/A	0.017 i.v.	125	131	Single dose data

*TK data from IV studies are pooled data for males and females

Oral studies:

Cmax and AUC (extrapolated values from 6-month oral toxicity study H1 with restricted feeding)

Species, strain	Study #	Duration of dosing	Study	Doses (mg/kg/day)	Route	Feeding status	Mg/kg/d	Cmax (ng/mL)	AUC_{0-24} (ngxh/mL)	TK values extrapolated from
RAT, SD	K2	6-9 wks (females)	Seg 1	1,4,16	oral	no food from 4h before until 4h after dosing	1	2.7	7.8	Data from 26 wk tox study (H1)
							4	10.7	31	"
							16	43	125	"
		10-12 wks (males)		1, 4, 16	oral	no food from 4h before until 4h after dosing	1	1.5	8.7	"
							4	6.1	35	"
							16	24	139	"
RAT, SD	K17	10 days	Seg 2	10,30,60,100	oral	no food from 4h before until 4h after dosing	10	27	77	"
							30	80	232	"
							60	160	463	"
							100	270	772	"
RAT, SD	K9	10 days	Seg 2	6,20,60	oral	no food from 4h before until 4h after dosing	6	16	46	"
							20	53	154	"
							60	160	463	"
RAT, Wistar	K15	4 days	Seg 3	1,5,20	oral	Fed ad libitum	1	0.03 (Cpl @1h)	Nd	TK data from reprotox study K15 - itself (PPD21)
							5	0.52 (C1h)	Nd	"
							20	2.72 (C1h)	Nd	"

Nd=not determined

IV studies

Cmax and AUC (extrapolated values from repeat dose IV toxicity studies)

Species, strain	Study #	Duration of dosing	Study	Doses (mg/kg/day)	Route	Feeding status	Mg/kg/d	AUC_{0-24} (ngxh/mL)	TK values extrapolated from
RAT, Wistar	K19	4 wks (f)	Seg 1	0.1, 0.3, 1.0	i.v.	—	0.1	345	Pooled (M,F) data from iv tox studies H7 and H9
							0.4	1380	"
							1.2	4140	"
		5 wks (m)		0.1, 0.4, 1.2	i.v.	—	0.1	345	"
							0.3	1035	"
							1.0	3450	"
RAT, SD	K12	10 days	Seg 2	0.1, 0.4, 1.5	i.v.	—	0.1	345	"
							0.4	1380	"
							1.5	5175	"
RAT, SD	K14	10 days	Seg 2	0.1, 0.3, 1.0	i.v.	—	0.1	345	"
							0.3	1035	"
							1.0	3450	"
RAT, Wistar	K18	4 days	Seg 3	0.048, 0.14, 0.48	i.v.	—	0.048	166	"
							0.14	483	"
							0.48	1656	"

Exposure multiples in reprotoxicity studies:

- Based on extrapolated TK data from repeat dose studies, and PK data for humans (data from 12-month study MF4348, 2.5 mg/day, orally, fasted: average AUC=2.5ngxh/mL)
- Based on mg/m^2 BSA comparison (oral bioavailability assumed to be same in human and rat, i.e., 0.6%)

Spec ies	Study #	Duration of dosing	Route	Mg/kg/d	Cmax (ng/mL) for oral studies	AUC_{0-24} (ngxh/mL)		Exposure multiple vs, humans at 2 5 mg/day orally (based on AUC data in rats and humans)	Multiple of human oral dose of 2 5 mg/day (based on mg/m^2 BSA comparison) (assuming oral BA in humans and rats is the same, eg 0.6%)
RAT	K2 Seg 1/2/3	6-9 wks (females)	oral	1	2.7	7 8		3 1x	4.1x
				4	10 7	31		12x	16x
				16	43	125		50x	66x
		10-12 wks (males)	oral	1	1 5	8 7		3 5x	4.1x
				4	6 1	35		14x	16x
				16	24	139		56x	66x
RAT	K17	10 days	oral	10	27	77		31x	41x
	Seg2			30	80	232		93x	123x
				60	160	463		185x	246x
				100	270	772		309x	410x
RAT	K9	10 days	oral	6	16	46		18x	25x
	Seg2			20	53	154		62x	82x
				60	160	463		185x	246x
RAT	K15	4 days (fed)	oral	0 95	0.03 (Cpl @1h)	(0.6)*		0.24x (fedl)	3.9x
	Seg3			4 75	0 52 (C1h)	(3 0)*		1 2x (fedl)	19x
				19	2 72 (C1h)	(12 0)*		4 8 (fedl)	78x
				10	0.7	6 3 (toxicity study H3)		2 5x (fedl)	41x
								Exposure multiple vs, humans at 2.5 mg/day orally (based on AUC data in rats and humans)	Multiple of human oral dose of 2.5 mg/day (based on mg/m2 BSA comparison) (assuming oral BA in humans is 0.6%)
RAT	K19	4 wks (females)	i.v.	0.1		345		138x	83x
	Seg1			0 4		1380		552x	333x
				1.2		4140		1656x	1000x
		5 wks (males)	i.v.	0.1		345		138x	83x
				0.3		1035		414x	250x
				1.0		3450		1380x	833x
RAT	K12	10 days	i.v.	0.1		345		138x	83x
	Seg2			0.4		1380		552x	333x
				1.5		5175		2070x	1250x
RAT	K14	10 days	i.v.	0.1		345		138x	83x
	Seg2			0 3		1035		414x	250x
				1.0		3450		1380x	833x
RAT	K18	4 days	i.v.	0.048		166		66x	40x
	Seg3			0.14		483		193x	116x
				0.48		1656		552x	398x

* extrapolated from Study H3

Human PK data.

Cmax (2.5 mg/day or 0 04 mg/kg/day, orally, fasted, 12 month Study MF4348) : 1 04 ng/mL

AUC (2.5 mg/day or 0 04 mg/kg/day, orally, fasted, 12 month Study MF4348): 2.5 ngxh/mL

For the oral studies, the multiples calculated based on AUC data are 1.5 to 2-fold lower than the ones based on mg/m2 comparison, assuming similar bioavailability in rats and humans (e.g. 0.6%). In the fed study (Segment 3, K1), the measured exposure multiples are much lower than those calculated on mg/m2 basis, since absorption is largely inhibited by food. Thus, calculation of exposure multiples on basis of mg/m2 comparison is appropriate when the compared species are dosed in the same feeding status.

For the IV studies multiples based on AUC data were ca. 1.7x larger than those based on mg/m2 comparison, assuming 0.6% BA in humans. This may be due to reduced clearance and increased AUC upon long term dosing in toxicity studies (6-months). It is not clear whether this reduction in clearance would also occur in the relatively short term reprotoxicity studies.

The AUC levels measured in the repeat dose IV toxicity studies are a reasonable basis for extrapolation to the short term reprotoxicity studies, and the multiples calculated using these levels are close to the ones based on mg/m2 dose comparison.

Conclusion:

- For the description of the results from oral or IV reprotoxicity studies in the label, exposure multiples can be based either on AUC or mg/m2 BSA comparison.
- For study K15 (oral Segment 3 study), exposure multiples can be calculated using AUC data.

REPRODUCTIVE TOXICOLOGY SUMMARY

In the oral Segment 1/2 study, male and female Sprague Dawley (SD) rats were dosed by oral gavage with 0, 1, 4, 16 mg/kg/day in the fasted state. Fertility was reduced at the high dose (HD). Gestational body weight gain was reduced significantly in LD, MD and HD. In the C-section group, there was a reduction in number of implantation sites (significant trend). The number of implantation sites was significantly reduced at the MD, possibly related to a significant increase in preimplantation loss in that group. Fetal weight (male and female) in the C-section group was reduced significantly in the HD group. In the fetuses delivered by C-section there were no treatment-related visceral or skeletal anomalies.
In the spontaneous delivery group, treatment impaired delivery and resulted in a dose-related incidence of maternal deaths in all treatment groups. Dams died around delivery time and in some HD dams that delivered all fetuses were nonviable. In this delivery group, the number of corpora lutea, number of implantation sites and number of live pups were significantly reduced at the HD, with dose-related significant trends for all three findings. Number of dead pups was increased, although not statistically significant, in HD. Postimplantation loss was significantly increased in LD and MD, with a dose-related significant trend.
In F1 pups, body weight at PPD0 or body weight gain over 7 weeks was not affected in a treatment-related manner. In the F1 offspring, there was no impairment of physical development or memory. However, among behavioral developmental parameters, cliff avoidance was significantly impaired in the HD pups. Weaned F1 pups had no visceral or skeletal anomalies and there was no effect on F1 reproductive performance. Sperm was not evaluated in treated males.

In the oral rat Segment 2 study with C-section, pregnant SD rats were dosed with 0, 10, 30, 60, 100 mg/kg/day from GD6-GD15, in the fasted state. Mortality was observed in 18/25 HD dams between GD 15 and GD19. Maternal toxicity (dose-related incidence of poor health) was observed at HMD and HD. Gestational body weight gain was reduced in HD from GD13-GD21.Fetal body weight (males and females) was slightly but not statistically significantly reduced in LMD, HMD and HD. In F1 fetuses there was a significant increase in total % visceral variations in LD, LMD, HMD and HD, due to an increased incidence of renal pelvis ureter syndrome (RPU) syndrome of ca. 2-fold in all treated groups as compared to controls. The syndrome is characterized by dilation of ureter and pelvis, and may be related to the nephrotoxicity of bisphosphonates. In F1 fetuses there was also a significant increase in total % skeletal variations in LD, LMD, HMD, due to an increased incidence of rudimentary rib of 3-4-fold in all affected treatment groups as compared to controls. No visceral or skeletal malformations were induced by treatment.
Evaluation of F0 dams showed decreased serum calcium levels on GD 21 (significant dose-related trend), and increased BUN and creatinine on GD 21 (significant dose-related trends, and significant effect in HMD). Autopsy findings in F0 dams included GI hemorrhage, spleen atrophy, kidney discoloration or edema, pulmonary edema w/wo hemorrhage, adrenal enlargement, liver congestion and hydrothorax, in HMD and/or HD. Gastric hemorrhage was also observed in 2/24 LMD dams.

In the oral rat Segment 2 study with spontaneous delivery, pregnant SD rats were dosed with 0, 6, 20, 60 mg/kg/day from GD6-GD15, in fasted state. Perinatal calcium was supplied s.c. from GD18-PPD0 at a 32 mg/kg total daily dose. Dystocia and periparturient mortality were observed in single females in all treatment groups. One of these females had GI hemorrhage. One additional HD female died on GD19. Maternal gestational or lactational body weight gain was not affected. There was a significant dose-related increase in postimplantation loss, significant in LD and MD but not in HD (due to large variation in HD), and a slight dose-related decrease in % live pups, significant in MD but not HD (again due to large variation in HD). There were no effects on body weight, or physical and neuromuscular development and behaviour (water maze performance) in F1 pups. In weaned F1 pups there was a non-statistically significantly increased incidence of RPU syndrome in the HD group. Skeletons were not examined in surviving weaned pups. F1 reproductive performance was not affected. No effects observed in F2 pups.

In the oral rabbit Segment 2 study, pregnant Himalayan rabbits were dosed with 0, 1, 4, 20 mg/kg/day from GD6-GD18, in the fasted state. Does were laparatomized on GD29. There was a dose-related increase in mortality in all treatment groups. Rabbits that died had pulmonary edema with pulmonary parenchymal hemorrhage. Mucoid enteritis was also observed in all groups (including controls) with increased incidence in MD and HD. No effect was observed on gestational body weight gain. There were no treatment-related skeletal findings in F1 offspring (retardations, variations, or malformations).
Based on mg/m2 comparison rabbit doses were 8.3, 33, 167 times the human dose of 2.5 mg/day, orally.

In the oral Segment 3 study in rats, pregnant Wistar rats were dosed with 0, 1, 5, 20 mg/kg/day (0.95, 4.75, 19 mg free acid equivalents/kg/day) from GD17 to PPD21 by oral gavage. Rats were fed *ad libitum*. Dose-related dystocia and periparturient mortality were observed at MD and HD. Dams died around delivery time with or without signs of dystocia, and some dams with dystocia did not die (although all of those had total postnatal loss). There was a dose-related increase in number of dams with total postnatal loss (PPD0-4) in MD and HD, and most of them had signs of dystocia. Postimplantation loss was increased significantly in HD dams (including and excluding those with total litter loss). Partly, this loss may have been undetected perinatal loss (cannibalization). There was a biologically significant increase in postnatal loss (PPD0-4) in dams including those with total postnatal loss, but no significant increase in postnatal loss (PPD0-4) in dams excluding those with total litter loss. Postimplantation (possibly perinatal) loss and postnatal loss of pups was apparently related to maternal dystocia induced by drug-related suppression of bone resorption and hypocalcemia. There was a statistically significant decrease in #living pups/litter and a non-significant increase in #dead pups/litter at MD and HD. Birth index was significantly decreased at HD. Autopsy showed dose-related red discoloration of the lung in MD and HD dams mainly in animals that had died. In F1 pups, there was no treatment effect on body weight gain, but there was a small but significant decrease in time to incisor eruption, and a significant reduction in number of days to onset of coat development in the HD group. There were no effects on F1 reproductive performance or early F2 pup development. Serum levels @1h postdose (Tmax) were lower than those available for fasted rats, probably due to feeding in the current study and/or inaccurate Tmax estimation. AUC multiples for rats in this study as compared to humans dosed with 2.5 mg/day, orally, based on extrapolated data from Study H3, are 0.24x, 1.2x, 4.8x.

ADME

In pregnant rats (single i.v. dose, 0.1 mg/kg) at 2-24h postdosing, dams retained 49%-35% of dose in carcass, and 2%-1% in kidney and liver. Spleen and sexual organ levels retained 0.5%-0.1% of dose. Fetuses retained 0.02% of dose after 2h, and 0.008% of dose after 24h. Placenta contained 0.07% of dose at 2h, 0.03% at 24h. Amniotic fluid contained 0.003% of dose at 2h only.

A single dose of radiolabeled ibandronate (0.08 mg/kg/day) administered by the i.v. route to lactating rats 12 days after delivery resulted in the appearance of compound in milk at 2, 6, 12 and 24h after dosing. The highest concentration in milk was seen at 2h after dosing (8.1 ng/mL). At 24h after dosing the milk concentration was 0.4 ng/mL. Higher concentrations of radioactivity in milk than in plasma (ca. 1.5-fold) may have been due to the higher calcium levels in milk.

VIII. SPECIAL TOXICOLOGY

Local tolerance studies evaluating i.v., s.c., p.v., and i.a. routes were carried out in rabbits and rats and skin tests were performed in rabbits and guinea pigs. These studies have not been reviewed in detail for this NDA, since the proposed clinical administration route is oral (2.5 mg tablet). The studies showed that ibandronate is well tolerated by the i.v. route in rabbits. However, ibandronate is generally not well tolerated in rats and rabbits after s.c. administration. Single p.v. (paravenous) or i.a. (intra-arterial) administration in rabbits also resulted in local intolerance. In the rabbit, ibandronate produced severe skin irritation, but there was no evidence of sensitization in the guinea pig skin sensitization test. Based on these results, i.v. injection in humans is the only recommended route of parenteral administration.

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IX. OVERALL SUMMARY AND EVALUATION

The current application (NDA #21-455) is for the use of ibandronate in the treatment and prevention of postmenopausal osteoporosis.

The mechanism of action of ibandronate, a nitrogen-containing bisphosphonate with high affinity for hydroxyapatite, is inhibition of osteoclastic bone resorption. This inhibition leads to the suppression of the coupled process of bone formation and thus to suppression of bone turnover. In postmenopausal women bone loss is accelerated due to increased activation of bone remodeling units (BRU) and a negative balance between bone formation and resorption in each remodeling cycle. Bisphosphonates prevent or reverse this bone loss by suppression of bone turnover leading to a closing down of the remodeling space and deceleration of the rate of bone loss both resulting in increased bone mass as reflected by increased bone mineral density (BMD). Additionally, bisphosphonates may increase bone mass by restoring the negative bone balance in each BRU through enhancement of osteoblastic bone mineral deposition.

In Phase 3 trial MF4411 in ca. 3000 postmenopausal women daily administration of 2.5 mg orally for 3 years reduced the relative risk of new vertebral fractures by 51% and increased lumbar spine BMD by 5.4%. Fracture efficacy and BMD increase were also achieved with a 20 mg intermittent oral dose regimen. In Phase 3 trial MF4380 with i.v. doses of 0.5 or 1 mg once every 3 months significant fracture efficacy was not obtained despite a significant increase in spine BMD of 3-4%. The cause of the lack of fracture efficacy in the i.v. trial is unclear. Possibly, the intermittent i.v. doses of 0.5 or 1 mg are suboptimal, or the intermittent dosing schedule lead to a loss of correlation between BMD and vertebral bone strength.
In clinical trials, ibandronate was well tolerated and only caused a slight increase in the incidence of dyspepsia and diarrhea as compared to placebo. Drug-related kidney, liver, cardiovascular, respiratory toxicity or anemia were not observed.

PHARMACOLOGY

Ibandronate is a potent inhibitor of bone resorption. Its mechanism of action includes direct and indirect inactivation of the osteoclast.

In vivo studies
In vivo pharmacology studies were carried out to investigate the effect of ibandronate in animal models of nonstimulated or stimulated bone turnover. In summary, the results showed that ibandronate inhibits bone resorption, and is approximately 10, 50, 500 more potent than alendronate, pamidornate and clodronate, respectively. In the rat s.c. ibandronate had a prolonged inhibitory effect and increased cancellous bone volume and density with optimal doses ≥0.001 mg/kg. Mineralization was not affected at a dose (1 mg P/kg= 5.14 mg/kg) in the young growing rat (Schenk assay). This dose is approximately 1000-5000x times higher than the lowest antiresorptive dose in the young growing male rat (0.0051 mg/kg/day) and the optimal dose inhibiting OVX-stimulated bone turnover in the aged female rat (0.001-0.005 mg/kg/day).

Long term pharmacology studies on the effects of ibandronate on bone quality in estrogen-deficient animals were the most relevant studies for the postmenopausal osteoporosis indication. Sponsor carried out two 5-month prevention studies in the OVX rat and a 12-month treatment study in the OVX rat, 4-week and 12-month studies in ovariohysterectomized dogs, and a 16-month intermittent i.v. study in OVX cynomolgus monkeys. OVX rat studies were done by s.c. route with daily or intermittent dosing, dog studies with (nearly) every day sc dosing, and the monkey study (16-mo) was carried out with monthly iv dosing.

Rat studies
In the 5-month studies, ibandronate (s.c.) prevented OVX-induced bone loss and trabecular separation optimally at 0.001 mg/kg/day. Comparing the effects of daily vs. intermittent dosing indicated that, within time limits, the efficacy was determined by the total cumulative dose.

In the 12-month study, in which treatment was started 4 weeks after OVX, ibandronate dose-dependently prevented the OVX-induced changes in bone mass and architecture in vertebrae and long bones. Doses of 0.001-0.005 mg/kg/day were optimal in the sense that they maintained bone parameters at concurrent sham levels. This dose range corresponds to 0.6-3 times the oral recommended human dose (RHD) of 2.5 mg/day. A 5 times higher dose of 0.025 mg/kg (15 times the RHD) was supraoptimal and had similar or superior effects as 0.005 mg/kg on most bone parameters.

In cancellous bone ibandronate efficacy was mainly due to a preservation of trabecular density and connectivity, in cortical bone to maintenance of cortical bone thickness and/or BMD. Femoral neck strength was not affected by OVX or treatment, but vertebral compression strength and femoral shaft bending strength were decreased by OVX, an effect that was inhibited by ibandronate at doses of 0.0002-0.001 mg/kg/d. Vertebral BMD and strength were strongly correlated, while femoral cortical BMD was not as good a predictor of cortical bone strength, particularly not in bone treated with a supra-optimal dose of ibandronate (0.025 mg/kg/d). The latter is probably due to the fact that cortical bone strength is more dependent on bone structure (distribution and geometry) than on volumetric bone mineral density. Effects of ibandronate were similar with optimal daily dosing as with 25-fold higher intermittent doses once every 25 days.

The data from rat studies indicate that dosing with ibandronate has a prolonged effect. At sufficiently high doses, reversal of efficacy does not occur until weeks or months after dose discontinuation. This is due to the retention of the compound in skeletal tissue as reflected by a half life in rat bone of >380 days.

Dog studies
Data from 4-week and 12-month s.c. treatment of OHX dogs showed that bone turnover was suppressed by ibandronate based on histomorphometric assessments. However, estrogen deficiency in the dog had no long term persistent effects on bone BMD, architecture or strength. Thus, the OHX dog model was not an adequate animal model for postmenopausal osteoporosis.

Monkey study
A long- term study was carried out in ovariectomized cynomolgus monkeys treated once monthly with i.v. doses of 0, 10, 30 or 150 ug/kg for 16 months. Sham controls were treated with vehicle only. The study was adequate and included assessments of BMD, bone architecture and bone strength. On a cumulative basis the monkey exposures were equivalent to 0.3x, 1.0x, 5.5x the clinical exposure at the recommended oral human dose of 2.5 mg/day. The optimal dose was 150 ug/kg. The results indicated efficacy of ibandronate to prevent impairment of bone quality due to estrogen deficiency.

Ovariectomy caused an increase in bone turnover, a decrease in cancellous and cortical BMD and a decrease in cortical thickness through expansion of the endocortical diameter. Ibandronate was partially effective at 30 ug/kg and fully effective at 150 ug/kg in preventing the effects in cancellous bone. In cortical bone ibandronate prevented the decrease in cortical BMD but the effects on bone geometry appeared to be less pronounced and/or site-specific. A dose of 10 ug/kg had minimal effects. Histomorphometry at various sites indicated an increase in the activation frequency of remodeling sites upon ovariectomy, which was suppressed by ibandronate at all doses. Data indicated greater efficacy on turnover at cancellous than at cortical bone sites. There was no evidence of mineralization defects or histologic abnormalities and the bone formed had a normal lamellar structure.

Compression tests of the vertebrae showed a decrease in ultimate load (Fu) upon OVX that could be prevented completely by ibandronate in parallel with its effects on BMD. Three point bending and shearing tests at the ulna diaphysis and femoral neck showed that the OVX-induced decreases in ultimate load (Fu) were only partially and not significantly prevented by ibandronate, while BMD at these sites was fully maintained. This apparent discrepancy at ulna and femoral neck was not due to a lack of effect on intrinsic cortical strength, but may have been the result of a lack of preservation of bone geometry (thickness, distribution) or the result of variability in the strength measurements. In the vertebra and femoral neck, strength was positively correlated to BMD. In the ulna, strength was strongly correlated to BMC but not to BMD.

The results of the monkey study indicate that ibandronate prevents the increase in bone turnover, the decrease in BMD and the change in skeletal microarchitecture that occurs upon ovariectomy. Effects of ibandronate on bone mass, geometry and strength are site-specific. In the vertebrae, ibandronate preserves BMD, bone mass, trabecular structure and compressive strength. Despite a protection of BMD, efficacy to preserve bone strength at other sites was not demonstrated.

In conclusion, the data from the long-term rat and monkey bone studies support the use of ibandronate for the indication of treatment and prevention of postmenopausal osteoporosis. The preclinical data predict a reduction in the risk of clinical vertebral fractures in association with increases in spine BMD. Increases in BMD at other skeletal sites may or may not be accompanied by fracture risk reductions. The data predict that with intermittent clinical dosing regimens the correlation between vertebral BMD and strength is maintained.

In vitro studies

In vitro studies showed that osteoclast function is inhibited in the presence of ibandronate or when cells are pretreated. The inhibitory effect on osteoclast resorption may be indirect through an action on osteoblasts. Ibandronate was more potent in inhibiting osteoclast function than pamidronate, clodronate or etidronate.

SAFETY PHARMACOLOGY

Safety pharmacology studies showed that single doses of ibandronate did not affect CNS, GI or cardiovascular function at doses of ca. 100-2000 times the oral RHD of 2.5 mg/day, based on mg/m^2 comparison. Renal function in dogs was not consistently or significantly affected. Ibandronate did not affect *in vitro* hERG K+ channel currents at concentrations >10,000x human C_{max}.

PHARMACOKINETICS/TOXICOKINETICS

In rats and dogs, ibandronate is poorly absorbed after oral administration (1% of dose or less) and food markedly suppresses oral bioavailability. After oral administration, Tmax is 0.5-1 h and compound is rapidly cleared (within hours) from plasma by uptake in bone and renal excretion. Upon repeated dosing, the AUC(0-4h) accounts for approximately 80% of the AUC for the dosing interval. $T_{1/2}$ (oral or i.v.) is approximately 56 hours in the dog. The bioavailability after s.c. administration is 100% in rats. Uptake in the bone compartment is reflected by a high volume of distribution (10L/kg in dogs). Approximately 40-50% of an absorbed dose is taken up and stored by bone, while approximately 50% is eliminated unchanged via the kidney. Uptake in bone is linear and related to total dose rather than treatment schedule. Bone levels attained after even a single dose remain high for several months, and $T_{1/2}$ for bone tissue in the rat is 400-500 days. Ibandronate is retained and accumulated in spleen, kidney and liver, but it virtually does not cross the blood-brain barrier. In pregnant rats, ibandronate is distributed to the placenta and the fetus, and in lactating rats it is excreted in the milk. Binding to plasma proteins is similar for rat, dog and human (80-99%). There is no evidence for metabolism in rats or dogs, and no evidence for hepatic or renal drug-drug interaction.

GENERAL TOXICOLOGY

Acute and repeat dose toxicity studies by the oral and IV routes were carried out in rats and dogs (APPENDIX). Acute toxicities in oral studies included GI hemorrhage, pulmonary edema and hemorrhage, liver and kidney discoloration in rats and mice at doses >500 times the oral recommended human dose (RHD), based on mg/m2 comparison.

In oral toxicity studies of up to 12 months duration in rats and dogs, target organs identified were kidney, liver, lung, esophagus, stomach, thymus and testes. Renal tubular integrity was particularly sensitive to ibandronate in rats and dogs. In 6- to 12-month studies kidney toxicity was evidenced by renal tubular changes, macroscopic changes, kidney weight increase, serum BUN and creatinine increases (rats and dogs), and glomerulopathy and interstitial fibrosis (dogs). Other target organs were GI tract and esophagus (dog), stomach (rat and dog), liver (rat and dog), and testis, thymus and lung (dog). Liver toxicity reflected by reversible serum chemistry changes was not accompanied by significant histological alterations. In dogs, GI toxicity was evidence by signs (vomiting, emaciation) and histologic lesions.

Safety margins were calculated as the ratio between the animal AUC at the NOAEL and the human AUC at the 2.5 mg oral dose. Safety margins for kidney toxicity were ≥4-7x and ≥27-50x based on data from rats and dogs, respectively. Safety margins for GI toxicity were 27-50x (vomiting, emaciation) and 100-200x (esophagitis and stomach irritation) based on data from dogs, and 34-62x based on data from rats (stomach irritation and hemorrhage). Liver (rat, dog), and lung, thymus and testicular (dog) toxicities were associated with safety margins ≥25x. Pharmacodynamic effects of ibandronate on bone were observed in all rat and dog toxicity studies at low human exposure multiples. This lead to secondary effects of decreased bone marrow space and increased extramedullary hematopoiesis and at higher doses to anemia in rats and dogs. Therapeutic margins calculated as the ratio between the AUC at the NOAEL for kidney toxicity and the AUC at the optimal pharmacologically effective dose were 8x-16x for rats and dogs.

Sponsor calculated NOAEL multiples ("safety margins") for kidney and liver toxicities for oral rat studies based on AUC values obtained in IV studies and assuming 1% oral bioavailability in non-fasted rats. This approach yielded safety margins (36-67x) that were overestimated by a factor of 1.5x-10x because oral bioavailability in non-fasted rats is << 1%.

GI toxicity (irritation, bleeding) was observed in acute and repeat dose IV toxicity studies in rats, mice and dogs, at higher exposures than those attained at orally GI toxic doses. This indicates that the presence of compound in the systemic circulation can lead to GI events that are usually ascribed to local GI irritation. GI effects have been observed in animals dosed by the i.v. route with other bisphosphonates and in humans treated with intermittent i.v. doses of ibandronate. The GI effects upon i.v. dosing may be due to exsorption (transport from interstitium to epithelial lumen) of the compound. This process might involve active transcellular transport mechanisms and may be related to the low oral bioavailability of bisphosphonates.

In conclusion, the results from preclinical toxicity studies support the use of a 2.5 mg daily oral dose for long term use in postmenopausal women.

CARCINOGENICITY

Three rodent carcinogenicity studies were carried out with ibandronate. One study was performed in Wistar rats, and two studies in NMRI mice. The rat study was a 104-week study by oral gavage, with doses of 0, 3, 7, 15 mg/kg/day. The first mouse study was an 18-month study by oral gavage with 0, 5, 20, 40 mg/kg/day. In that study increased mortality due to respiratory

distress was observed at the mid and high dose groups. A second 90-week mouse study was conducted by administering the compound in the drinking water at doses of 0, 5, 20, 80 mg/kg/day. Animals in the carcinogenicity studies were fed *ad libitum*.
A statistical review of the carcinogenicity study data was performed by the Statistical Reviewer, Cynthia Liu, MA (Review dated February 11, 2003). Data were presented by the Pharm/Tox Reviewer to the EXEC CAC on February 11, 2003 (Meeting Minutes, Appendix B).

Rat oral gavage study
The doses selected for the 104-week oral gavage rat study (3, 7, 15 mg/kg/day) were adequate based on data from a 14-week oral gavage dose range finding study (J7). There were no statistically significant tumor findings in the rat study. The small increase in incidence of skin histiocytoma in male rats at the high dose of 15 mg/kg/day (4%) was not considered biologically significant. Exposures in the rat study were 0.7x-12.2x (for males) and 1.3x-6.7x (for females) times human exposure at the recommended daily oral dose of 2.5 mg (1.8-3.3 ngxh/mL), based on AUC.

Mouse oral gavage study
The doses selected for the 18-month oral gavage mouse study (5, 20, 40 mg/kg/day) were adequate based on mortality in the high dose groups. There were no statistically significant tumor findings in the oral gavage mouse study. Exposures in the oral gavage mouse study were 1.1-476x (for males) and 7.9-71x (for females) times human exposure at the recommended daily oral dose of 2.5 mg (1.8-3.3 ngxh/mL), based on AUC.

Mouse drinking water study
The doses selected for the 90-week drinking water mouse study (5, 20, 80 mg/kg/day) were suboptimal and the maximum tolerated dose (MTD) was not reached at the high dose. However, the two mouse studies taken together are considered an adequate assessment of carcinogenic potential of ibandronate in the mouse (EXEC CAC, February 11, 2003). Exposures in the drinking water mouse study were 8.5-250x (for males) and 14-398x (for females) times human exposure at the recommended daily oral dose of 2.5 mg (1.8-3.3 ngxh/mL), based on AUC.
The drinking water mouse study was positive for adrenal subcapsular tumors. Statistically significant dose-related increases were observed in the incidences of subcapsular cell adenoma type A, subcapsular cell adenoma type A and type B combined, and subcapsular cell adenoma type A and type B and adenocarcinoma combined. Significance was shown by Sponsor's and by CDER's statistical review.

Adrenal subcapsular neoplasm incidence in NMRI mice treated with ibandronate in the drinking water for 90 weeks

Sex		Females					
Group		Control	LD	MD	HD	Trend test Sponsor	Trend test CDER
Dose (mg/kg/day)		0	5	20	80		
N		100	50	50	50		
Adrenal gland	Subcapsular cell adenoma, type A	0	1	1	3*	*	p=0.0228#
		(0%)	(2%)	(2%)	(6%)*		
	Subcapsular cell adenoma, type B	0	0	0	1	NS	NA
		(0%)	(0%)	(0%)	(2%)		
	Subcapsular cell adenocarcinoma	0	0	1	0	NA	NA
		(0%)	(0%)	(2%)	(0%)		
	Subcapsular cell adenoma type A and B	0	1	1	4	**	p=0.0069#
		(0%)	(2%)	(2%)	(8%)		
	Subcapsular cell, adenoma type A and B and adenocarcinoma	0	1	2	4	NA	p=0 0099#
		(0%)	(2%)	(4%)	(8%)		

*p<0.05, ** p<0.01(Sponsor's pairwise X^2 test; or Armitage's trend test)
significant at p≤0.025 for trend for rare tumor type
NS Not Significant; NA Not Applied

Sponsor dismissed the subcapsular cell tumor finding based on the lack of an effect in male mice and the relatively high combined control incidence of adrenal subcapsular tumors in the oral gavage study (8%). However, Reviewer and EXEC CAC concluded that the increased incidence of adrenal subcapsular adenoma type A appears to be a biologically significant finding. This conclusion was based on the dose-related effect and the assertion that subcapsular cell adenoma type A is a rare tumor in NMRI mice. The latter was based on concurrent control data from the drinking water study (0%) and the oral gavage mouse study (0.7%).

Adrenal subcapsular cell tumors (type A and type B) are not always identified as histologically distinct tumors from adrenal cortical cell tumors, and the different types of subcapsular adenoma are usually not distinguished. Historical control data provided for these tumors were not adequate to show that subcapsular cell adenoma type A is a common rather than a rare tumor in mice dosed via the drinking water. Adrenal subcapsular adenocarcinoma is a rare tumor based on both control data from the drinking water and gavage mouse studies and historical control data from studies in NMRI mice (—— database).

The adrenal subcapsular tumor finding in female mice was significant according to trend test, and the incidence appeared significantly increased at the high dose (80 mg/kg/day). At this dose exposure in female mice is 218-398, or approximately 200-400, times human exposure at the recommended daily oral dose of 2.5 mg/day (1.8-3.3 ngxh/mL), based on AUC comparison. Conversely, the oral mouse dose of 80 mg/kg/day is equivalent to 164x the daily oral human dose of 2.5 mg based on body surface area (BSA) comparison, mg/m^2. The mechanism underlying the mouse tumor finding and the relevance of the finding for humans is not known. Possibly, the tumors could result from stimulation of subcapsular endocrine mineralocorticoid-secreting cells upon renal toxicity and disturbances in mineral (Na, K) metabolism.

GENETIC TOXICOLOGY

Ibandronate was tested for mutagenicity *in vitro* in the *Salmonella typhimurium* assay (Ames test), the *Escherichia coli* assay, in a Chinese hamster V79 mammalian cell assay for gene mutations and in a human peripheral lymphocyte assay for chromosomal aberrations, with and without metabolic-activation. Two *in-vivo* mouse micronucleus tests for chromosomal damage were conducted by the oral and i.v. routes of administration. According to the results of these assays, ibandronate has no mutagenic or clastogenic potential.

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Reproductive toxicity studies were carried out in the rat and the rabbit by both oral and IV administration routes. The oral studies in the rat included a combined Segment 1/2/3 combined reproductive toxicity study, two Segment 2 embryofetal toxicity studies, and a Segment 3 peri- and postnatal study. In the Segment 1/2/3 study, part of the females were C-sectioned, while the rest was allowed to deliver. In one of the Segment 2 studies the dams were C-sectioned, and in the other study they were allowed to deliver the fetuses. In the Segment 2 delivery study dams were supplemented with calcium during the perinatal period to prevent dystocia and periparturient mortality. The latter is a common finding in reproductive toxicity studies with bisphosphonates and is believed to be due to drug-induced suppression of bone resorption and skeletal calcium mobilization, leading to hypocalcemia during delivery. Since uterine contraction in the rodent is largely dependent on extracellular calcium the presence of bisphosphonates in the circulation and/or skeleton leads to disruptions of delivery.

Both oral and IV reprotoxicity studies were reviewed in detail. However, the effects observed in oral studies are summarized here since they are relevant for the proposed dose regimen (2.5 mg/day, orally). Oral studies were carried out in rats and rabbits that were dosed in the fasted state, except the rat Segment 3 study in which animals were fed *ad libitum*. IV studies were evaluated to support the significant findings in the oral studies. Multiples of human exposure or human dose (mg/m2) achieved in the studies were determined and can be found in the reprotoxicity study section of this NDA review.

Oral Segment 1-3 study, rat

An oral Segment 1/2/3 study (0, 1, 4, 16 mg/kg/day; premating day -14 to GD21 or PPD21), was carried out in which females were either C-sectioned (GD21) or allowed to deliver and rear the litter until weaing (PPD21). A non-dose-related reduction in maternal body weight gain of <10% due to decreased full uterine weight was observed in all treated groups. Fertility index was reduced by ca. 15% at 16 mg/kg/day. The reduced fertility may have been due to male sperm effects or female effects.

In the C-section group there were non-dose-related increases in preimplantation loss and a reduced number of implantation sites in all groups. Fetal weight was reduced by <10% at 16 mg/kg/day.

In the delivery group, number of corpora lutea and implantation sites were reduced by ca. 10% at 16 mg/kg/day. Dose-related maternal deaths were observed at all doses around delivery time. There was a significant reduction in number of live pups at 16 mg/kg/day. Fetal body weight was slightly decreased in the C-section group at 16 mg/kg/day, but pup weight at birth was not affected. Increased postimplantation loss in the delivery group reflected perinatal pup loss. The deaths of dams and their pups are thought to be due to hypocalcemia-induced dystocia.

In the F1 offspring, there was a significant impairment in behavioral development based on the results of the cliff avoidance test at 16 mg/kg/day. There were no drug-related fetal anomalies or effects on fetal viability, physical or memory development. F1 reproductive performance was not affected.

The multiples of the human exposure attained in this study were 4x, 16x, 66x at 1, 4, 16 mg/kg/day, based on mg/m2 comparison. The LOAEL for maternal periparturient mortality (1 mg/kg/day) is the equivalent of 4x the oral recommended human dose (RHD) based on mg/m2 comparison. In the oral Segment 3 study periparturient mortality occurred at the 5 and 20 mg/kg/day doses, equivalent to 1x and 4x the RHD based on direct Cmax comparison. Thus, periparturient maternal deaths occur at very low human dose multiples when dosing is carried out around delivery time. Dystocia and maternal death at delivery were also seen in the oral and IV Segment 2 studies with delivery in animals supplemented with perinatal calcium, and the IV Segment 3 study. However, these deaths occurred at much higher multiples of the RHD (≥25x-40x) and the NOAEL was not determined. Reduced serum Ca on GD21 was observed in the oral Segment 2 Study with C-section at all doses of 10-100 mg/kg/day. The results from the oral and IV reprotoxicity studies showed that dosing before or during delivery causes dystocia and periparturient mortality due to hypocalcemia. A 4-to 6-day sc supplementation with calcium (approximately 13mg/day) from GD18-PPD0 prevented maternal and fetal deaths to a large extent, albeit not completely.

There were no studies to determine the effect of previous, e.g. premating, treatment on delivery disturbances.

In a parallel IV Segment 1 study in rats reductions in fertility, corpora lutea, increased preimplantation loss, and decreases implantation sites were observed at the high dose of 1-1.2 mg/kg/day. Male sperm parameters were altered at the mid dose of 0.3 and HD of 1 mg/kg/day. Female premating and gestational body weight were slightly decreased at the HD of 1 mg/kg/day but not at the LD of 0.1 mg/kg/day. The 0.1, 0.3 and 1-1.2 mg/kg/day doses represent multiples of 83, 250x and 833-1000x human exposure at RHD, much higher than the multiples attained in the oral study. The data from the oral and IV studies taken together suggest that at high doses ibandronate can reduce male and/or female fertility.

In conclusion, the significant drug-related findings in the oral Segment 1/2/3 study are the maternal periparturient mortality and perinatal pup loss at all doses (1, 4, 16 mg/kg/day), and the reduction in fertility, corpora lutea and implantation sites at 16 mg/kg/day.

Oral Segment 2 study (C-section), rat
An oral Segment 2 study was performed with relatively high doses (0, 10, 30, 60, 100 mg/kg/day; GD6-GD15; C-section on GD21). The NOAEL for maternal toxicity was 10 mg/kg/day. At 30, 60, 100 mg/kg/day there were maternal clinical signs, at 60-100 mg/kg/day there was dose-related mortality and at 100 mg/kg/day reduced gestational body weight gain. Maternal toxicity was clearly drug-related and included autopsy findings of GI hemorrhage, kidney and lung lesions at 30,60,100 mg/kg/day. On GD21 serum calcium was reduced dose-dependently in all dose groups. Despite severe maternal toxicity, there was no significant embryofetal toxicity in any dose group (postimplantation loss, fetal weight). A non-dose-related statistically significant increase in the incidence of a fetal variation, renal pelvis ureter (RPU) syndrome, was observed in all treatment groups. Skeletal variation incidence was increased due to increased rudimentary rib incidence in all treatment groups except the high dose group. The latter finding was not significant since the incidences were not dose-dependent, within historical control range, and not increased in Segment 2 IV studies at higher exposure multiples.

Oral Segment 2 study (delivery), rat
In the oral Segment 2 study (0, 6, 20, 60 mg/kg/day; GD6-GD15; delivery) dams were supplemented with perinatal Ca injections to prevent dystocia and periparturient mortality. Nevertheless, periparturient maternal deaths occurred in single animals in all dose groups. Concomitant decreases in # live pups and related increases in postimplantation loss were seen in those groups and was likely due to perinatal pup mortality. There was no effect on F1 body weight, physical, behavioral or memory development or reproductive performance. A non-significant increase in fetal RPU syndrome incidence was observed at 60 mg/kg/day.

Oral Segment 2 study, rabbit
In the oral rabbit Segment 2 study (0, 1, 4, 20 mg/kg/day; GD6-GD18, laparatomy GD29), dose-related mortality occurred in does associated with lung edema/hemorrhage and increased mucoide enteritis. There were no significant treatment-related visceral skeletal anomalies in the F1 offspring.

Oral Segment 3 study, rat
An oral Segment 3 study (0, 1, 5, 20 mg/kg/day; GD17-PPD21) was carried out without Ca supplementation and dosing in the fed state. At 5 and 20 mg/kg/day there was dose-related dystocia, periparturient mortality, decrease in #living pups and postnatal pup loss. Postimplantation losses in this study were likely to reflect undetected early perinatal pup loss. It was not clear if some perinatal pup loss occurred in dams that did not experience periparturient toxicity. However, postnatal loss was not increased in dams without dystocia, as has been observed with alendronate (Minsker et al, 1993). There was no increase in breeding loss. The periparturient mortality occurred at low human Cmax multiples of 1-5x as observed in the Segment 1-3 study. Red lung discoloration was observed in all animals that died. In F1 pups there were no significant drug-related effects on body weight, physical, behavioral or memory development, or reproductive performance.

IV studies in rats and rabbits
In the rat IV reprotoxicity studies the main finding was an increase in the incidence of fetuses with RPU syndrome in the Segment 2 rat studies.

ADME studies in pregnant/lactating rats
ADME studies showed that in pregnant rats ibandronate given as single IV injection (0.1 mg/kg) is transferred to the placenta and fetus with levels decreasing from 2-24h post dosing. In lactating rats given a single i.v. dose of 0.08 mg/kg ibandronate is present in milk at approximately 1.5 times the plasma concentrations with levels decreasing from 2-24h after dosing.

SPECIAL TOXICOLOGY

Local tolerance studies evaluating i.v., s.c., p.v., and i.a. routes were carried out in rabbits and rats and skin tests were performed in rabbits and guinea pigs. These studies have not been reviewed in detail for this NDA, since the proposed clinical administration route is oral (2.5 mg tablet). The studies showed that ibandronate is well tolerated by the i.v. route in rabbits. However, ibandronate is generally not well tolerated in rats and rabbits after s.c. administration. Single p.v. (paravenous) or i.a. (intra-arterial) administration in rabbits also resulted in local intolerance. In the rabbit, ibandronate produced severe skin irritation, but there was no evidence of sensitization in the guinea pig skin sensitization test. Based on these results, i.v. injection in humans is the only recommended route of parenteral administration.

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TOXICOLOGY STUDY LIST

Table 1 List of Toxicology Studies

Study Type/ Report No./Route of Administration	Species/Strain	Basis for Calculating the Dosages in the Study Report[1]	Dosages Expressed in Terms of Free Acid Equivalent	
Acute Toxicity - Oral			**mg/kg**	
[1000], p.o.	Mouse, NMRI	WDS	87 to 1391	1000: f3.pdf
[1001], p.o.	Rat, Sprague-Dawley	WDS	87 to 870	1001: f1.pdf
Acute Toxicity - Intravenous			**mg/kg**	
[1002], i.v.	Mouse, NMRI	WDS	8.7 to 70	1002: f4.pdf
[1003], i.v.	Rat, Sprague-Dawley	WDS	8.7 to 87	1003: f2.pdf
[1004], i.v.	Dog, beagle	FAE	5	1004: f5.pdf
Repeated Dose Toxicity - Oral			**mg/kg/day**	
[1005], 2 wk, p.o.	Rat, Sprague-Dawley	FAE	5, 10	1005: g6.pdf
[1006], 4 wk, p.o.	Rat, Sprague-Dawley	WDS	0.88, 2.65, 8.85	1006: g1.pdf
[1007], 26 wk, p.o.	Rat, Wistar	WDS	10.13, 30.34	1007: h1.pdf
[1008], 26 wk, p.o.	Rat, Wistar	WDS	1.01, 3.04, 10.13	1008: h3.pdf
[1009], 12 mo, p.o.	Rat, Sprague-Dawley	FAE	3, 10, 20	1009: h4.pdf
[1010], 7 day, p.o.	Dog, beagle	FAE	50 mg	1010: g5.pdf
[1011], 4 wk, p.o.	Dog, beagle	WDS	0.9, 2.69, 8.95	1011: g3.pdf
[1012], 26 wk, p.o.	Dog, beagle	WDS	2, 5, 13	1012: h2.pdf
[1013], 12 mo, p.o.	Dog, beagle	FAE	2, 5, 10	1013: h5.pdf
Repeated Dose Toxicity - Intravenous			**mg/kg/day**	
[1014], 9 day, i.v.	Rat, Sprague-Dawley	WDS	1.7, 2.5	1014: f113.pd
[1015], 4 wk, i.v., s.c.	Rat, Sprague-Dawley	WDS	0.09, 0.28, 0.9	1015: g2.pdf
[1016], 6 mo, i.v.	Rat, Sprague-Dawley	FAE	0.075, 0.15 (1/wk), 0.3 (2/mo)	1016: h7.pdf
[1017], 6 mo, i.v., s.c.	Rat, Sprague-Dawley	FAE	0.3, 0.9, 1.8, 2.7 (1/wk)	1017: h9.pdf
[1018], 4 wk, i.v , s.c.	Dog, beagle	WDS	0.09, 0.28, 0.9	1018: g4.pdf
[1019], 6 mo, i.v.	Dog, beagle	FAE	0.075, 0.15 (1/wk), 0.3 (2/mo)	1019: h6.pdf
[1020], 6 mo, i.v., s.c.	Dog, beagle	FAE	0.3, 0.9, 2.7 (1/wk)	1020: h8.pdf

[1]WDS = weighed drug substance (ibandronate $Na.H_2O$): FAE = free acid equivalent (ibandronate)

Table 1 List of Toxicology Studies (Cont.)

Study Type/ Report No./Route of Administration	Species/Strain	Basis for Calculating the Dosages in the Study Report[1]	Dosages Expressed in Terms of Free Acid Equivalent	
Carcinogenicity			**mg/kg/day**	
[1021], 3 mo, drinking water	Rat, Sprague-Dawley	WDS	8.9, 17.9, 26.8, 35.7, 44.6	1021: j17.pdf
[1022], 14 wk, gavage	Rat, Sprague-Dawley	WDS	8.9, 17.8, 26.7, 35.6, 44.4	1022: j7.pdf
[1023], 104 wk, gavage	Rat, Wistar	WDS	2.67, 6.22, 13.33	1023: j8.pdf
[1024], 3 mo, gavage	Mouse, NMRI	WDS	17.8, 35.6, 53.3, 71.1, 89	1024: j9.pdf
[1025], 3 mo, gavage	Mouse, NMRI	WDS	8.9, 13.3, 17.8, 26.7, 35.6	1025: j6.pdf
[1026], 18 mo, gavage	Mouse, NMRI	FAE	5, 20, 40	1026: j14.pdf
[1027], 3 mo, drinking water	Mouse, NMRI	WDS	8.9, 17.9, 26.8, 35.7, 44.6	1027: j12.pdf
[1028], 3 mo, drinking water	Mouse, NMRI	FAE	50, 100, 200, 300, 400, 800	1028: j13.pdf
[1029], 3 mo, drinking water	Mouse, NMRI	FAE	200, 400	1029: j11.pdf
[1030], 3 mo, drinking water (PK study)	Mouse, NMRI	WDS	8.9, 268, 44.6	1030: j16.pdf
[1031], 90 wk, drinking water	Mouse, NMRI	FAE	5, 20, 80	1031: j15.pdf
Reproductive Toxicity - Oral			**mg/kg/day**	
[1032], Seg. I, p.o.	Rat, Sprague-Dawley	FAE	1, 4, 16	1032: k2.pdf
[1033], Seg. II RF, p.o.	Rat, Sprague-Dawley	FAE	10, 20, 40	1033: k16.pdf
[1034], Seg. II RF, p.o.	Rat, Sprague-Dawley	FAE	50, 70	1034: k8.pdf
[1035], Seg. II, p.o.	Rat, Sprague-Dawley	FAE	10, 30, 60, 100	1035: k9.pdf
[1036], Seg. II, p.o.	Rat, Sprague-Dawley	FAE	6, 20, 60	1036: k9.pdf
[1037], Seg. III, p.o.	Rat, Sprague-Wistar	WDS	1, 5, 20	1037: k15.pdf
[1038], Seg. II RF, p.o.	Rabbit, CHbb:HM	FAE	1, 3, 10, 30	1038: k3.pdf
[1039], Seg. II RF, p.o.	Rabbit, CHbb:HM	FAE	3, 10, 30	1039: k4.pdf
[1040], Seg. II, p.o.	Rabbit, CHbb:HM	FAE	1, 4, 20	1040: k1.pdf
Reproductive Toxicity - Intravenous			**mg/kg/day**	
[1041], Seg. I RF, i.v.	Rat, Wistar	FAE	M/F: 0.6/0.8, 0.8/1, 1/1.2	1041: k10.pdf
[1042], Seg. I, i.v.	Rat, Wistar	FAE	M/F: 0.1/0.1, 0.3/0.4, 1/1..2	1042: k19.pdf
[1043], Seg. II RF, i.v.	Rat, Sprague-Dawley	FAE	1, 2, 5, 10	1043: k11.pdf
[1044], Seg. II RF, i.v.	Rat, Sprague-Dawley	FAE	1, 1.5	1044: k13.pdf
[1045], Seg. II, i.v.	Rat, Sprague-Dawley	FAE	0.1, 0.4, 1.5	1045: k12.pdf
[1046], Seg. II, i.v.	Rat, Sprague-Dawley	FAE	0.1, 0.3, 1	1046: k14.pdf

[1]WDS = weighed drug substance (ibandronate Na.H_2O); FAE = free acid equivalent (ibandronate)
RF = Range Finding studies

Table 1 List of Toxicology Studies (Cont.)

Study Type/ Report No./Route of Administration	Species/Strain	Basis for Calculating the Dosages in the Study Report[1]	Dosages Expressed in Terms of Free Acid Equivalent	
Reproductive Toxicity – Intravenous (Cont.)			**mg/kg/day**	
[1047], Seg. III, i.v.	Rat, Sprague-Dawley	FAE	0 05, 0.15, 0.5	1047: k18.pdf
[1048], Seg. II RF, i.v.	Rabbit, CHbb:HM	FAE	0.5, 1.5, 5	1048. k5.pdf
[1049], Seg. II RF, i.v.	Rabbit, CHbb:HM	FAE	0.01, 0.03, 0.1	1049: k6.pdf
[1050], Seg. II, i.v.	Rabbit, CHbb:HM	FAE	0.03, 0.07, 0.2	1050: k7.pdf
Mutagenicity			**Concentration/Dose**	
[1051], Ames test	*S. typhimurium*	WDS	44 to 4444 µg/plate	1051: j1.pdf
[1052], Ames test/E. coli	*S. typhimurium/E. coli*	WDS	44 to 4444 µg/plate	1052: j10.pdf
[1053], Gene mutation	V79 cells	WDS	34 to 1087 µg/mL	1053: j2.pdf
[1054], Chrom abs, in vitro	Human lymphocytes	WDS	26 to 261 µg/mL	1054: j4.pdf
[1055], Micronucleus, p.o.	Mouse, NMRI	WDS	435 mg/kg	1055: j3.pdf
[1056], Micronucleus, i.v.	Mouse, NMRI	WDS	9.4, 18.7, 37.4 mg/kg	1056: j5.pdf
Local Tolerance			**Dose: Formulation**	
[1057], i.v.	Rabbit, crossbred	FAE	1 mg/mL: Ga	1057: f103.pdf
[1058], i.v.	Rabbit, crossbred	FAE	1 mg/mL: L	1058: f106.pdf
[1059], s.c.	Rat, Sprague-Dawley	WDS	0.89, 1.8 mg/mL	1059: f101.pdf
[1060], s.c.	Rat, Sprague-Dawley	FAE	1 mg/mL: Mb	1060: f108.pdf
[1061], s.c.	Rabbit, crossbred	FAE	0.5, 1 mg/mL: G	1061: f102.pdf
[1062], s.c.	Rabbit, Russian	FAE	1 mg/mL: Mb	1062: f107.pdf
[1063], s.c.	Rabbit, NZW	FAE	0.05, 0.1, 0.2, 0.3 mg/ml: acetate buffer	1063. f114.pdf
[1064], s.c.	Rabbit, Chinchilla	FAE	0.25 mg/mL, polyoxy-gelatine or tween 80	1064: f115.pdf
[1065], s.c.	Rabbit, Chinchilla	FAE	0.76, 1.27 mg/mL, liposomes	1065: f116.pdf
[1066], s.c., i.m.	Rabbit, Chinchilla	FAE	0.2, 1 mg/mL; hydrogel with $CaCl_2$	1066: f117.pdf
[1067], s.c.	Rabbit, Chinchilla	FAE	0.2, 1 mg/mL, hydrogel	1067: f118.pdf
[1068], s.c.	Rabbit, Chinchilla	FAE	0.25 mg/mL, A/A, PEG	1068: f119.pdf
[1069], s.c.	Rabbit, Chinchilla	FAE	0.05, 0.08, 0.16, 0.25 mg/mL, .	1069: f120.pdf
[1070], s.c.	Rabbit, Chinchilla	WDS	0.796, 0.664 mg/mL, EPC liposomes	1070: f121.pdf
[1071], p.v.	Rabbit, crossbred	FAE	1 mg/mL: Ga	1071: f104.pdf
[1072], p.v.	Rabbit, Russian	FAE	1 mg/mL: Mb	1072: f109.pdf
[1073], i.a.	Rabbit, crossbred	FAE	1 mg/mL: Ga	1073: f105.pdf
[1074], i.a.	Rabbit, Russian	FAE	1 mg/mL: Mb	1074: f110.pdf
Special Studies - Skin			**Dose/Formula**	
[1075], patch	Rabbit, Himalayan	WDS	468 mg/patch	1075: f111.pdf
[1076], i.c., topical	Guinea pig, Pirbright	WDS	0.23, 0.28, 2.3% solution	1076: f112.pdf

[1]WDS = weighed drug substance (ibandronate $Na.H_2O$); FAE = free acid equivalent (ibandronate)
RF = Range Finding studies

19 page(s) of revised draft labeling has been redacted from this portion of the review.

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/s/

Gemma Kuijpers
4/16/03 02:50:39 PM
PHARMACOLOGIST

Please forward for signing. I CC'd Dave Morse.

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