EVALUATION OF REPEAT DOSE ORAL TOXICITY STUDIES

Calculation of multiples or safety margins

Multiples attained at various dose levels in rat and dog studies were calculated based on human PK and animal PK or TK data.

Human PK data

In humans, PK was linear at doses ranging from 2.5 to 100 mg orally in males and females and doses ranging from 0.25 to 5 mg for 12 months. Exposure (AUC_{0-6h}) after steady state (ca. 8 days) is ca. 1.5x-2x exposure on Day 1. This is consistent with a $T_{1/2}$ of ca. 16h. Human bioavailability is quite variable (<1%), and averages approximately 0.6%. PK data were from humans dosed in the fasted state.

Study MF7159 (0.5 mg i.v., and 2.5 and 20 mg oral, single doses) in healthy PM volunteers w or w/o HRT:

PK data from Trial MF7159

Dose		0 5 mg i v	2.5 mg oral	20 mg oral
Single dose	Cmax (ng/mL)	78	0.8	6.2
	AUC (ngh/mL)	95	3 5	23
	Tmax (h)	0 08	1	0.9
	T1/2 (h)	22	-	32
	f_e (% dose)	64	0.6	0 4

fe means the fraction of the dose that is excreted in urine

Study BP16304, a study on the effect of a meal on bioavailability. Doses were 2.5 and 5 mg, either 30 min or 60 min before a standard meal.

PK data from Trial BP16304

Dose		2.5 mg oral	
		60 min before meal	30 min before meal
Single dose	Cmax (ng/mL)	0 58	0.58
	AUC (ngh/mL)	1 8	1 63
	Tmax (h)	0.75	0 75
	f_e (% dose)	0 33 (± 0 16)	0 26 (± 0.089)

Study MF7131, a bioavailability study with 50 mg oral solution or tablet:

PK data from Trial MF7131

Dose		50 mg oral tablet	20 mg (extrapolated values)
Single dose	Cmax (ng/mL)	11	4 4
	AUC (ngxh/mL)	30	18
	Tmax (h)	1	–
	T1/2 (h)	2.3	–

In a12-month repeat dose PK study (MF4348) in patients with PMO, PK values showed that exposure was increased ca. 1.5-2x upon repeated dosing:

PK data from Trial MF4348

		1	2.5	5
First dose	Cmax (ng/mL)	0 2	0.6	1.3
	AUC (ngh/mL)	0.46	1.4	3.2
	Tmax (h)	0.7	1	1
After 12 months	Cmax (ng/mL)	0 28	1.04	1.5
	AUC (ngh/mL)	0.76	2.5	4.2
	Tmax (h)	1	0.7	1

Table 3 Toxicokinetic Parameters for Oral Administration

Study Description (Report No)	Dose levels mg/kg/day	Route	Sex	Time of Toxicokinetics	Dose Group mg/kg/day	C_{max} ng/mL	$AUC_{(0-24)}$ ng h/mL	Comment	
2 week rat, toxicokinetics [1005]	5, 10	Oral	Male	2 weeks	5	4 0	14 6	2 week toxicokinetic study	1005 g6.pdf
				2 weeks	10	18 3	47 8		
			Female	2 weeks	5	3 2	18.6		
				2 weeks	10	11 8	22		
26 week rat, restricted feeding [1007]	11 45, 34.28	Oral	Male	4 weeks	11 45	6 3	34 5	Kinetics assessed only at 11 45 4 wk F-insufficient sample	1007 h1 pdf
				26 weeks	11.45	24 4	142		
			Female	26 weeks	11 45	27.2	79		
26 week rat [1008]	1 15, 3.43, 11 45	Oral	Male	26 weeks	3.43	0 7	7 6	4 weeks-insufficient samples	1008 h3.pdf
				26 weeks	11 45	3 6	18 8		
			Female	26 weeks	3 43	0 3	4 4		
				26 weeks	11 45	0 7	6 3		
12 month rat [1009]	3, 10, 20	Oral	Male	50 weeks	10	5.9	36	Kinetics at week 50 only at 10 mg/kg	1009. h4 pdf
			Female	50 weeks	10	5 8	48		
26 week dog [1012]	2, 5, 13	Oral	Male	Day 1	2	34	60	The high-dose was toxic/lethal, thus there are no data at week 26.	1012 h2.pdf
				Day 1	5	67	91		
				Day 1	13	2511	5032		
			Female	Day 1	2	25	42		
				Day 1	5	271	602		
				Day 1	13	499	1004		
			Male	Week 26	2	34	99		
				Week 26	5	295	612		
			Female	Week 26	2	2 3	17		
				Week 26	5	6 3	94		
12 month dog [1013]	2, 5, 10	Oral	Pooled	Week 2	2	21	51	Kinetics were not done at the 10 mg/kg due to toxicity	1013. h5.pdf
				Week 2	5	88	256		
			Pooled	Week 51	2	15	89		
				Week 51	5	148	382		
Human patients [5015, 5023]	2.5 mg tablet	Oral			0.04	0 6-0 8	1.8-3.3	Single dose	5015. mf7159 pdf
	20 mg tablet	Oral			0 33	6 2	23	Single dose	5023. bp16304.pc
Human volunteers [5018]	20 mg tablet	Oral			0 33	4.2	14.1	Single dose	5018 mf7131 pdf

Table 4 Toxicokinetic Parameters for Intravenous Administration

Study Description (Report No.)	Dose levels mg/kg	Route	Sex	Time of Toxicokinetics	Dose Group mg/kg/day	Conc* ng/mL	$AUC_{(0-24)}$ ng h/mL	Comment	
26 weeks, rats	0.075, 0.15 weekly	i.v.	Pooled	Week 26	0.15	487	464	Low dose not done-expected low values	1016: h7 pdf
[1016]	0 3 twice monthly			Week 26	0 3	1113	1069		
26 week rat	0.3, 0 9, 1 8, 2 7	i.v	Pooled	Weeks 17-18	0.3	2573	1604	Due to local intolerance, the upper mid and high dose groups were injected s c . Data for weeks 17-18 are s c . No data listed for weeks 26-27 at these doses.	1017: h9 pdf
[1017]	Weekly			Weeks 17-18	0.9	3105	3553		
				Weeks 17-18	1 8 s.c.	-	8251		
				Weeks 17-18	2 7 s c.	-	15897		
			Pooled	Weeks 26-27	0 3	661	752		
				Weeks 26-27	0.9 s c	-	3259		
6 month dog	0.075, 0.15 weekly		Pooled	Week 24	0.075	205	263		1019: h6 pdf
[1019]	0.3 twice monthly			Week 24	0 15	563	633		
				Week 24	0.3	1187	1352		
6 month dog	0.3, 0.9, 2.7	i.v	Pooled	Day 1	0.3	1291	1214	Because of toxicity at the high dose, the kinetics were determined during week 16.	1020: h8.pdf
[1020]	Weekly			Day 1	0.9	6444	4994		
				Day 1	2.7	15890	14431		
				Week 23	0.3	1540	1364		
				Week 23	0 9	8682	5564		
				Week 16	2.7	15676	19360		
Human Volunteers	1 mg injection	i v			1	125	131	Single dose	

*Conc, Serum concentration at the end of the injection period. Value obtained by log arithmetic extrapolation of the concentrations after 0.5 and 1 hours.

Sponsor's calculations

Human exposure at the 2.5 mg oral daily dose is: Cmax (0.6-0.8 ng/mL) and AUC (1.8-3.3 ngxh/mL), based on data from studies MF7159 and BP16304. Exposure data for the 20 mg dose were available from Study MF7131. These values were used to calculate exposure multiples ("safety margins") attained at the NOAEL for kidney and liver toxicity in 6-mo and 12-mo rat and dog toxicity studies (Table 30). Safety margins were defined as $AUC_{(animal)}$/ $AUC_{(human)}$ for 2.5 mg and 20 mg tablet strengths.

For the oral rat toxicity studies, Sponsor calculated safety margins by extrapolation of exposure in 6-month repeat dose i.v. rat studies H7 and H9 and single dose i.v. rat study I5, assuming a rat oral BA of 1%, rather than using oral rat PK data. Reviewer feels this is not appropriate since IV and oral TK data indicate that BA in the rat is <<1% when animals are fed *ad libitum* (Studies H3, H4). Upon request, Sponsor provided data from ADME and TK studies to support that bioavailability is 1% in non-fasted rats. However, based on TK data Reviewer did not agree with the Sponsor's conclusions. In fact, Sponsor's own derivation of oral bioavailability in fed animals from TK data obtained in studies H1 and H3 was 0.13% (Sponsor's Summary; ADME conclusions).

For oral dog studies, Sponsor calculated safety margins using actual AUC values obtained in the oral dog studies.

Table 30 Summary of Safety Margins for 2.5 mg and 20 mg Tablets

Species/Study/Dose	Tablet Strength	Safety Margin (AUC_A/AUC_H)	Safety Margin ($Dose_A/Dose_H$)	
Rat / 6-month oral toxicity study [1008] NOAEL: 3 mg/kg/day	2.5 mg 20 mg	36 - 67 [1] 5 - 8 [2]	75 9	1008: h3.pdf
Rat / 12-month oral toxicity study [1009] NOAEL: 3 mg/kg/day	2.5 mg 20 mg	36 - 67 [1] 5 - 8 [2]	75 9	1009: h4.pdf
Dog / 6-month oral toxicity study [1012] NOAEL: 5 mg/kg/day	2.5 mg 20 mg	106 - 194 [3] 15 - 25 [4]	125 15	1012: h2 pdf
Dog / 12-month oral toxicity study [1013] NOAEL: 2 mg/kg/day	2.5 mg 20 mg	21 -39 [5] 3 - 5 [6]	50 6	1013: h5.pdf

References for exposure data:

A and H refer to animals and humans, respectively

[1] AUC_A extrapolated from IV-data [2000, 1016, 1017] assuming bioavailability of 1%; AUC_H = mean exposure data of [5015, 5023]. — 2000: I5.pdf

[2] AUC_A extrapolated from IV-data [2000, 1016, 1017] assuming bioavailability of 1%; AUC_H = mean exposure data of [5015, 5018]. — 1016. h7 pdf, 1017: h9.pdf

[3] AUC_A = Pooled median exposure data of [1012]. AUC_H = mean exposure data of [5015, 5023] — 5015. mf7159 pdf

[4] AUC_A = Pooled median exposure data of [1012]. AUC_H = mean exposure data of [5015, 5018] — 5023· bp16304 pdf

[5] AUC_A = Pooled median exposure data of [1013] AUC_H = mean exposure data of [5015, 5023] — 5018: mf7131.pdf

[6] AUC_A = Pooled median exposure data of [1013]. AUC_H = mean exposure data of [5015, 5018] — 1012: h2.pdf, 1013. h5.pdf

Taking into consideration that the safety margins for the 20 mg tablets are underestimated, it is concluded that the results of repeated dose toxicity studies clearly support the clinical use of 2.5 and 20 mg tablets.

Reviewer's calculations

Reviewer used available TK values to calculate exposure multiples for both rat and dog toxicity studies. The NOAEL value for rat study H3 used by Reviewer was 10.1 mkd (Sponsor: 3 mkd). Other NOAEL values used by Sponsor and Reviewer were the same.

For the rat, Reviewer calculated multiples for different NOAEL values, (A), (B), (C), based on different kidney lesions/toxicities. The first (A) was for reversible renal tubular hypertrophy, the only histologic kidney finding in Study H4, the second (B) for tubular nephrosis, swelling and basophilia and increased kidney weight, and the third (C) for renal histologic toxicity with functional changes.

For the dog, two NOAEL values were used (A), (B). Other organ toxicity NOAEL values were also included in the tables below.

RAT (oral studies)

Study Nr.			H1	H3	H4
Duration			26 week	26 week	12 month
Route (oral)			gavage	gavage	gavage
Feeding status			fasted	fed	fed
Doses (mg/kg/day)			10.1, 30.3	1.0, 3.0, 10.1	3, 10, 20
NOAEL (A)	Kidney toxicity (tubular hypertrophy) (H4)	Dose (mg/kg/day)		≥10 1	3
		AUC (ngxh/mL)		≥12.6	12.6
		Multiple of human oral dose (2.5 mg/day) based on AUC**		**≥4x-7x**	**4x-7x**
		Multiple of human 2 5 mg/day dose, based on mg/m2***		≥14x	4.1x
NOAEL (B)	Kidney toxicity: renal tubular nephrosis, swelling and basophilia; increased kidney weight (H1, H4) Liver toxicity increase in AST (H1)	Dose (mg/kg/day)	<10.1		10
		AUC (ngxh/mL)*	<111		42
		Multiple of human oral dose (2.5 mg/day) based on AUC**	**<34x-62x**		**13x-24x**
		Multiple of human 2.5 mg/day dose, based on mg/m2***	<41x		14x
NOAEL (C)	Kidney toxicity tubular dilation, vacuolation, necrosis; medullary reddening and congestion; increased serum creatinine (H1) Liver toxicity: increased ALT, decreased protein (H1) Stomach toxicity: irritation, hemorrhage (H1)	Dose (mg/kg/day)	10.1		20
		AUC (ngxh/mL)*	111		84
		Multiple of human oral dose (2.5 mg/day) based on AUC**	**34-62x**		**25x-47x**
		Multiple of human 2.5 mg/day dose, based on mg/m2***	41x		28x

*AUC values were (extrapolated) values from pooled M and F data (Sponsor's TK Table 3)
**Human AUC values: 1.8-3.3 ngxh/mL
***Human 2.5 mg dose: 0.04 mg/kg; Assumption. BA =0.6% (fasted), 0.2% (fed)

DOG (oral studies)

Study Nr.			H2	H5
Duration			26 weeks	12 months
Route (oral)			Gelatin capsule	Tablet
Doses (mg/kg/day)			2, 5, 13	2, 5, 10
NOAEL (A)	Kidney toxicity: tubulonephrosis, tubule basophilia, interstitial fibrosis (H5) GI toxicity: vomiting, appetite loss, emaciation (H5) Thymus toxicity: involution (H5)	Dose (mg/kg/day)	13	2
		AUC (ngxh/mL)*	3000	89
		Multiple of human oral dose (2.5 mg/day) based on AUC**	**900x-1666x**	**27x-50x**
		Multiple of human 2.5 mg/day dose, based on mg/m2***	163x	25x
NOAEL (B)	Kidney toxicity: tubule dilation, glomerulopathy, kidney discoloration; increased kidney weight; urine changes; increased serum BUN and creatinine; decreased serum Na, proteinuria, glucosuria (H2, H5) Liver: increase in enzymes and lipids, decrease in protein, vacuolation (H2, H5) Esophagus: esophagitis (H2) Stomach: irritation, cachexia (H5) Lung: pneumonia (H2) Testis: atrophy (H5)	Dose (mg/kg/day)	5	5
		AUC (ngxh/mL)*	350	382
		Multiple of human oral dose (2.5 mg/day) based on AUC**	**106x-194x**	**116x-212x**
		Multiple of human 2.5 mg/day dose, based on mg/m2***	63x	63x

*AUC values were (extrapolated) values from pooled M and F data (Sponsor's TK Table 3)
**Human AUC values: 1.8-3.3 ngxh/mL
***Human 2.5 mg dose: 0.04 mg/kg; Assumption: human BA = dog BA, i.e. 0.6%

Multiples of human exposure at NOAELs were calculated based on AUC comparison. In the rat, the lowest NOAEL multiple for kidney tubular hypertrophy is 4-7x. The NOAEL multiple for other histologic tubular lesions and increased kidney weight is 13-24x, and for renal lesions with functional changes is 25-47x. The NOAEL multiple for hepatic toxicity in the rat is 25-47x (based on Study H4). The NOAEL multiple for stomach toxicity in the rat is 34-62x (based on Study H1). In the dog the NOAEL multiple for histologic renal toxicity is 27-50x and for histologic and functional renal toxicity it is 106-194x. NOAEL multiples for liver and lung toxicity are 106x-194x, for GI tract toxicity (signs) 27-50x, for stomach and esophageal histopathology 106x-212x, for thymus involution 27-50x, and for testicular toxicity 116-212x.
Multiples were similar when based on AUC or mg/m2 comparison (correction for bioavailability included in latter case).

NOAEL multiples animal:human (AUC comparison)

Target organ	Kidney	Kidney	Liver	Stomach/ Esophagus	GI tract	Lung	Thymus	Testis
Effect	Histopath	Function	Function	Histopath	Function	Histopath	Histopath	Histopath
Rat	4x-7x	25x-47x	25x-47x	34x-62x	-	-	-	-
Dog	27x-50x	106x-194x	106x-194x	106-212x	27x-50x	106x-194x	27x-50x	116x-212x

Multiples calculated by the Reviewer for the kidney NOAEL's in the rat were about 10-fold, 3-fold and 1.5-fold lower than safety margins calculated by the Sponsor (4-7x, 13-24x, 25-47x, as compared to 36-67x). This is not surprising because the TK data used by Reviewer to calculate multiples reflect the relatively low bioavailability (<1%) in fed animals. Reviewer's NOAEL multiples for dog kidney and liver toxicities were similar to Sponsor's safety margins.

Alternative calculation of therapeutic margin

For ibandronate, multiples (safety margins, therapeutic margins) can also be calculated in a different way. In rat and dog, efficacy pharmacology studies were performed with sc dosing for up to 12 months. Determination of the optimal or efficacious dose (ED50, ED100) with regard to the compounds' pharmacologic bone effect can be compared to the NOAEL level and a therapeutic window can be estimated.
The estimates of therapeutic margins in the two animal models (rat, dog) suggest that the bone efficacious dose is a relative safe dose with regard to kidney and other target organ toxicity.

RAT: Effective dose (BMD or Cn-BV/TV of lumbar spine) and therapeutic margin (kidney toxicity)

Study				
D25				
Doses (sc) mg/kg/day	0.0002, 0.001, 0.005, 0.025			
Duration	12 months			
	Mg/kg/day	Exposure in animal (ngxh/mL) (based on Study I11)	Human exposure multiple*	Therapeutic margin
ED50	0 0002	0 25	0.1x	**40-70x**
ED75-100	0.001	6.3	0 5x	**8x-14x**
ED≥100	0.005	1.25	2.5x	**1.6x-2.8x**
NOAEL (A) (toxicity)	3 mkd (oral rat, study H4)	12.6 ngxh/mL	4x-7x	-

*At human oral dose of 2.5 mg/day (average AUC 2.5 ngxh/mL)

DOG: Effective dose (BV/TV and trabecular parameters of iliac crest) and therapeutic margin (kidney toxicity)

Study				
D9				
Doses (sc) mg/kg/day	0.0001, 0.0003, 0.001, 0.01, 0.1			
Duration	4 weeks			

	Mg/kg/day	Exposure in animal (ngxh/mL) (based on Study I12)	Human exposure multiple*	Therapeutic margin
ED100	0 001	7 9	3 2x	**8x-16x**
NOAEL (A) (toxicity)	2 mkd (oral dog, Study H5)	89 ngxh/mL	27-50x	-

*At human oral dose of 2.5 mg/day (average AUC 2.5 ngxh/mL)

Safety margin for 20 mg intermittent oral dose

The multiples attained in the daily repeat-dose studies for the 20 mg intermittent dose regimen (1 dose every other day, 12 times, every 3 months) are expected to be lower than for the 2.5 mg/day regimen. However, since dosing with the 20 mg human dose is intermittent, calculation of daily dose or exposure multiples yields underestimated values. For the 20 mg dose, multiples could be based on the cumulative weekly dose or an effective daily dose. However, the 20 mg treatment regimen is not currently recommended in the product label. Thus, the safety of the 20 mg intermittent clinical dose regimen based on animal data can be re-evaluated if Sponsor applies for marketing of this dose.

SUMMARY AND EVALUATION OF REPEAT DOSE IV TOXICITY STUDIES

Repeat dose IV studies of 4-week and 6-month duration were carried our in rats and dogs. Reviews of the 4-week IV toxicity studies (Rat Study G2, Dog Study G4) were performed when the IND ____ was submitted on September 30, 1994 (Review Date: December 16, 1994, Ron Steigerwalt).

Rat

The data from the 4-week rat study (G2; doses 0.1, 0.3, 1 mg/kg/day, WDS) showed poor local tolerance of IV administration route, at MD and HD. Main target organ was kidney, and moderate tubular nephrosis was observed at the HD. One 6-month IV rat study (H7; doses 0.075, 0.15, weekly or 0.3 mg/kg, biweekly) there were minimal effects on hematology parameters in all treated male groups, concomitant with new trabecular bone formation and spleen extramedullary hematopoiesis. In the other 6-month IV rat study (Study H9; doses 0.3, 0.9, 1.8, 2.7 mg/kg/wk) local tolerance was poor at MD and HD, and dosing was changed to s.c. dosing. Renal toxicity was observed reflected by changes in serum parameters (BUN, creatinine), increased kidney weight, and dose-related epithelial hypertrophy/hyperplasia and renal tubular changes in all dose groups. Bone changes (endochondral ossification, new trabecular bone formation), increased extramedullary hematopoiesis and related anemia and serum Ca and P decreases were observed in all dose groups.

Calculation of multiples of the HRD (2.5 mg/day) of IV doses was based on the assumption that a weekly dose of X mg/kg is equivalent to a daily dose of X/7 mg/kg: The rat IV NOAEL for kidney changes is 0.15 mg/kg/wk. This would be equivalent to 25 mg/kg/wk orally, or 3.5 mg/kg/day orally (15x HRD, mg/m2 comparison). The LOAEL is 0.3 mg/kg/wk IV, equivalent to 7 mg/kg/day orally (30x HRD, mg/m2 comparison).

Dog

Data from the 4-week IV dog study (G4; doses 0.1, 0.3, 1 mkd; WDS) showed toxicity in kidney, liver ad GI tract at MD and HD. Kidney damage included tubulonephrosis, necrosis, and papillary and pelvis pathology. Liver damage included fatty liver, icterus, necrosis. GI toxicity included diarrhea (all groups) and intestinal bleeding at HD. NOAEL < 0.1 mkd.

In the 6-month IV dog study (H6; doses 0.075, 0.15, weekly, or 0.3 mg/kg biweekly) there were no treatment related histopathologic findings.

In the other 6-month IV dog study (H8; doses 0.3, 0.9, 2.7 mg/kg/wk, WDS) there was renal toxicity at all doses reflected by a variety of histologic changes that were severe at the HD. Severe lung lesions were observed at the HD. GI irritation, testicular atrophy and thymus

involution were observed at HD. Liver toxicity judging from serum chemistry changes occurred at the HD. MTD in this study was 0.3 mg/kg/wk.
Bone effects included enlarged zone of endochondral ossification in all treated groups in all IV dog studies.

Calculation of multiples of the HRD (2.5 mg/day) of IV doses was based on the assumption that a weekly dose of X mg/kg is equivalent to a daily dose of X/7 mg/kg: The dog IV NOAEL for kidney changes is 0.15 mg/kg/wk. This would be equivalent to 15 mg/kg/wk orally, or 2 mg/kg/day orally (25x HRD, mg/m2 comparison). The LOAEL is 0.3 mg/kg/wk IV, equivalent to 4 mg/kg/day orally (50x HRD, mg/m2 comparison).

Multiples of kidney NOAEL's in 4-week IV studies G2 and G4 (0.3 mkd rat, 0.1 mkd dog) are equivalent to 200x and 800x the HRD (based on mg/m2 comparison, assuming human BA of 0.6%).

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.

CONCLUSION FROM ORAL TOXICITY STUDIES
Based on exposure-based NOAEL multiples ("safety margins") and therapeutic margins for kidney and other organ toxicities, Reviewer believes that the results from the chronic rat and dog toxicity studies support the use of a 2.5 mg daily oral dose for long term use in adult postmenopausal women.
In the Phase 3 clinical trials no major adverse events have surfaced except for an increased frequency of dyspepsia and diarrhea. This is expected for a bisphosphonate with potential GI irritative properties. Dog and rat studies predict renal toxicity at similar or higher exposures as GI toxicity, respectively.

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V. GENETIC TOXICOLOGY

Table 14 Mutagenicity Studies (Protocol Summary and Results)

Test Type	Study Description	Dose Levels (Batch No.)	Response	GLP Status	Rpt. No. & Date (Laboratory)	
Ames test	Two independent assays were conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence or absence of S9 metabolic activation.	50, 150, 500, 1500, 5000 µg/plate[a] (44, 133, 444, 1333, 4444 µg/plate)[b] (820 769 01A)	Ibandronate was not mutagenic at any dose level in any of the test strains, with or without metabolic activation.	Yes	[1051] 4/89 (BM)	1051: j1.pdf
Ames test and E. coli assay	Two independent assays were conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WPuvrA in the presence or absence of S9 metabolic activation.	50, 100, 500, 1000, 5000 µg/plate[a] (44,133, 444, 1333, 4444 µg/plate)[b] (454 555 00)	Ibandronate was not mutagenic at any dose level in any of the test strains, with or without metabolic activation.		[1052] 6/98 —	1052 j10.pdf
Gene mutation assay in Chinese hamster V79 cells	Two independent assays for mutation to 6-thioguanine resistance were conducted in the presence or absence of S9 metabolic activation.	39 to 1000 µg/mL[a] (34 to 870 µg/mL)[b] without S9, 78 to 1250 µg/mL[a] (68 to 1087 µg/mL)[b] with S9 (820 769 01A)	Ibandronate did not induce gene mutations, with or without metabolic activation.	Yes	[1053] 4/89 —	1053: j2.pdf
Chromosomal aberration test in human lymphocytes	Two independent assays: a 24-hr treatment period with no recovery and a 3-hr treatment with a 21-hr recovery period in either the presence or absence of S9 metabolic activation.	30, 150, 300 µg/mL[a] (26, 130, 261 µg/mL)[b] (820 769 01A)	There was no indication of a dose-dependent and reproducible chromosome-breaking effect with or without metabolic activation. Ibandronate was considered not clastogenic	Yes	[1054] 12/89 (BM)	1054: j4 pdf
Micronucleus test in NMRI mice Oral	Mice (5/sex/group/time point) treated with a single p.o. (gavage) dose of ibandronate; bone marrow sampled at 14, 24, and 48 hours (ibandronate) and 24 hours (control groups).	500 mg/kg[a] (435 mg/kg)[b] (820 769 01A)	Ibandronate p.o. did not induce micronuclei in NMRI mice in vivo. There was no indication of chromosome damage or of an effect on spindle function.	Yes	[1055] 11/89 (BM)	1055: j3 pdf
Micronucleus test NMRI mice i.v.	Mice (5/sex/group/time point) treated with a single i.v. dose of ibandronate, bone marrow sampled at 12, 24, and 48 hours (40 mg/kg group) or 24 hours (10 and 20 mg/kg, and both controls groups).	10, 20, 40 mg/kg[a] 9.4, 18.7, 37.4 mg/kg[b] (447 624 00)	Ibandronate i.v. did not induce micronuclei in NMRI mice in vivo. There was no indication of chromosome damage or of an effect on spindle function at any dose.	Yes	[1056] 10/93 (BM)	1056: j5.pdf

a Doses expressed as ibandronate $Na.H_2O$
b Doses expressed as the free acid equivalent

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. A detailed review of these studies was conducted when the original IND — was submitted (Ron Steigerwalt, December 16, 1994). Ibandronate tested negative in all assays. Thus, ibandronate appears to have no mutagenic or clastogenic potential.

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VI. CARCINOGENICITY

104-WEEK ORAL GAVAGE CARCINOGENICITY STUDY WITH BM 21.0955.NA IN THE RAT

GENERAL

Sponsor:	Boehringer Mannheim, GmbH, Germany
Study Number:	—— #068232
Study Report:	J8
NDA Volumes:	Vol. 68
Testing Facility:	————
Study Period:	January 1992-February 1994
QA Report:	Yes
GLP Statement:	Yes
Dose-range-finding study:	J7 (see Study Summary)

STUDY PROTOCOL

Species/strain:	Rat (Wistar, outbred, SPF), ——
Number of animals:	50/sex/dose group (Group 1-4)
Doses:	0, 3, 7, 15 mg/kg/day (oral gavage)
Age at start of study:	6 weeks
Weight at start of study:	142g (males), 115g (females)
Study Duration:	104 weeks
Animal housing:	5/cage
Animal diet:	Standard pelleted —— rat diet, and tap water, both *ad libitum*
Drug Name:	BM 21.0955.Na ("microfine"). This old name corresponds to the new name, BM 21.0955.Na.H_2O (ibandronate, monosodium salt, monohydrate), with 1.125 g BM.21.0955.Na microfine = 1.125 g BM 21.0955.Na.H_2O = 1.0 g free acid equivalent
Drug Lot Number(s):	447 624-00
Drug Analysis:	Dec 5, 1994
Drug Stability:	Stable (exp date Nov 1, 1994)
Dosage form:	Solution in 0.5% carboxymethyl cellulose in distilled water, prepared weekly (stable for >3 weeks)
Dosing route:	Oral gavage (animals fed *at libitum*)
Dosing frequency:	Once daily, 7 days/week
Dose volume:	5 mL/kg
Vehicle:	0.5% carboxymethyl cellulose

Dose Groups:

Group	Designation	N/sex/group	Dose Volume (ml/kg)	Dose (mg/kg/day)	Dose (free acid equivalents) (mg/kg/day
1	Control	50	5	0	0
2	LD	50	5	3	2.67
3	MD	50	5	7	6.22
4	HD	50	5	15	13.33

Relation to Clinical Use:	Recommended dose: 2.5 mg daily, orally (tablet)
CAC Concurrence:	Sponsor's dose selection was based on data obtained in 14-week study J7 (JZ2) and 26-week oral gavage toxicity studies H1 and H3. Concurrence of the Exec CAC with the dose selection was not obtained. However, the Division informed the Sponsor (March 29, 1996; FAX) that the dose selection for the 104-week

	study was adequate based on mortality and weight loss in study J7.
Interim Sacrifices:	None
Clinical Observations:	At least once daily, and recorded weekly. Also weekly palpation for tissue masses.
Mortality:	Twice daily
Body Weight:	Weekly
Water Consumption:	No quantitative determination
Food Consumption:	Weekly
Ophthalmoscopy:	Pretest (all), and Wks 52, 104 (control and HD)
Clinical Pathology:	In Wk 104, blood samples were obtained from all animals. Red cell morphology and differential WBC were determined for all animals, while a complete set of hematology parameters and clinical chemistry were determined for 10/s/g.
Toxicokinetics:	(TK Study # 367) Concentration of test substance was determined in serum [redacted] and urine [redacted] The [redacted] was carried out with standards prepared in plasma or serum samples, and the quantification limit was [redacted] The [redacted] method (validated for 2-25000 ng/mL) measures ibandronate after oxidation and esterification, and the quantification limit was [redacted]
Test substance in serum:	In Wk 52, at 3h after dosing, blood samples were obtained from all animals. In Wk 104, blood samples were obtained at predosing, and at 1h, 3h and 5h after dosing, from ¼ of remaining animals each.
Test substance in urine:	In Wk 104, 24h urine samples were collected from 5/s/g for TK evaluation.
Macroscopic examination:	All animals were necropsied, including those that died or were killed moribund, and macroscopic abnormalities were recorded. Scheduled necropsies were done in Weeks 104-106 for males, and Weeks 105-107 for females.
Organ weights:	Organs weighed at schedules sacrifice: adrenals, brain, heart, kidneys, liver, spleen, testes, ovaries.
Histopathology:	Tissue samples were processed from all animals (scheduled kill, moribund kill, found dead), from all organs and tissues listed in the histopathology inventory (appended). Tissues in parentheses were examined only if macroscopically abnormal. Larynx and rib (costochondral junction) were examined only for animals at scheduled necropsy. Femur and lumbar vertebrae L3-L5 were removed from first 10/s/g and frozen at –20°C for analysis by Sponsor. Findings were listed separately as nonneoplastic and neoplastic lesions. Histopathology summary tables were provided for all animals combined (decedents and survivors).
Peer Review:	A peer review of the histopathology findings was performed by the Sponsor.
Bone Analysis:	Right femur and vertebrae L3-L5 were preserved for analysis.
Statistical Evaluation:	In-life phase: Tests used for analysis of body weight, food consumption, hematology, clinical chemistry and organ weights: Dunnet-test, Steel-test, Fisher-test
Biostatistical evaluation:	Trend tests for increase in dose-response relationship were done for target endpoints (mortality and tumor findings) and concomitant endpoints (BW, FC, hematol, clin chem, org wt, pathol findings). Mortality was analyzed as crude proportions and as mortality-time relationship, tumors were analyzed as crude

proportions and by time adjusted analysis of fatal, incidental, clinically observable tumors.

Appendix: Sponsor's summary tables of neoplastic findings

Signficance levels

	Rare tumor (control incidence <1%)	Common tumor (control incidence >1%)
Trend test (Sponsor)	P<0 05	P<0.01

Rat Oral Carcinogenicity Study (J8)

Histopathology Inventory

Species: Rat	Pathology	
	Tissues examined in all animals (per protocol)	Tissues examined only when macroscopically abnormal
Adrenal glands	X	
Aorta	X	
Bone (sternum)	X	
Bone (femur)	X	
Bone (nb, longitudinal section)*	X	
Bone marrow (sternum)	X	
Bone marrow (femur)	X	
Brain	X	
Caecum	X	
Cervix		X
Clitoral gland		X
Colon	X	
Duodenum	X	
Epididymides	X	
Eyes with optic nerve	X	
Hardenan glands	X	
Heart	X	
Ileum	X	
Jejunum	X	
Kidneys	X	
Lacrimal gland, exorbital		X
Larynx*	X	
Liver	X	
Lungs	X	
Lymph nodes, mandibular	X	
Lymph nodes, mesenteric	X	
Mammary gland area	X	
Nasopharynx		X
Ovaries with fallopian tubes	X	
Pancreas	X	
Pituitary	X	
Preputial glands		X
Prostate gland	X	
Rectum	X	
Salivary gland, mandibular	X	
Salivary gland, sublingual	X	
Sciatic nerve	X	
Seminal vesicles	X	
Skeletal muscle	X	

Skin	X	
Spinal cord, cervical	X	
Spinal cord, midthoracic	X	
Spinal cord, lumbar	X	
Spleen	X	
Stomach	X	
Testes	X	
Thymus	X	
Thyroid and parathyroid	X	
Tongue		X
Trachea	X	
Urinary bladder	X	
Uterus	X	
Vagina		X
Zymbal gland		X
Gross lesions, tissue masses, tumors		

* Examined only for animals killed at scheduled necropsy

RESULTS

Mortality/Survival

SURVIVAL – Analysis of all animals at end of study

				MALES	FEMALES
Group		Dose (mg/kg/day)		Number (%)	Number (%)
1	Control	0	0	40/50 (80%) (=100%)	32/50 (64%) (=100%)
2	LD	3	2.67	39/50 (78%) (=98%)	39/50 (78%) (=122%)
3	MD	7	6.22	33/50 (66%) (=83%)	32/50 (64%) (=100%)
4	HD	15	13.33	33/50 (66%) (=83%)	37/50 (74%) (=116%)
Trend test				P<0.05	

Males: Significant positive trend in mortality (crude time-independent mortality rate) in males ($p<0.05$), for both spontaneous deaths and spontaneous deaths plus killed in extremis. Also, positive mortality-time relationship for males ($p<0.05$). Mortality in MD and HD males was possibly due to kidney damage and/or aspiration of test material and related respiratory toxicity. NOAEL for mortality in males was LD.
Females: No treatment effect

Clinical Observations

No treatment-related. Incidence of nodules and masses not related to treatment.

Body weight

Body weight gain reduced in males and females for the majority of the study.

Body weight (gr) at end of study (wk 105)

			MALES	FEMALES
Group		Dose (mg/kg/day)	Body weight (g)	Body weight (g)
1	Control	0	589	363
2	LD	3	538*	344
3	MD	7	540*	331*
4	HD	15	527*	331*
4 vs. 1			89%	91%

*p<0.05

Food consumption:
Absolute FC decreased in males and females, relative FC unaffected.

Ophthalmoscopy
Increase in anterior lens opacities in wk 104 without clear dose relationship.

Hematology
Small decrease in RBC (m,f) and Hb (m) in all dose groups. Increase in MCV and MCH (m,f) at all doses. Increase in platelets (m) in all treated.

Hematology at end of study (wk 104)

			MALES				FEMALES			
Group		Dose (mg/kg/day)	RBC (T/l)	Hb (mmol/l)	Hct (l/l)	Platelets (G/l)	RBC	Hb (mmol/l)	Hct (l/l)	Platelets (G/l)
1	Control	0	8.0	9.6	0.42	744	6.92	9 0	0.40	607
2	LD	3	7.2**	9.1*	0.41	565*	6.43*	8 8	0 39	553
3	MD	7	6 9**	8.9**	0.40*	494**	6.53	8.8	0.39	586
4	HD	15	7.1**	9.1*	0.41	586*	6.37**	8.8	0 39	623

*p<0.05

Clinical chemistry
Increase in serum Na in LD, MD, HD females. Decrease in inorganic P in MD, HD males. Decrease in creatine kinase in HD f.

Clinical chemistry at end of study (wk 104)

			MALES				FEMALES			
Group		Dose (mg/kg/day)	Na (mmol/l)	Ca (mmol/l)	P (mmol/l)	CK (ukat/l)	Na (mmol/l)	Ca (mmol/l)	P (mmol/l)	CK (ukat/l)
1	Control	0	142	2.59	1 61	13.1	140	2.46	1.48	16 5
2	LD	3	137	2.51	1 51	13.1	142*	2.56*	1.49	12 3
3	MD	7	143	2.57	1 45*	19.0	143**	2 55	1 43	11.2
4	HD	15	143	2.59	1.48*	13 4	145**	2.63**	1.54	7.9**

*p<0.05, ** p<0 01

Urinalysis:
No effects on volume

Toxicokinetics:
Dose related levels in week 52 and 104, higher in m than in f. Serum levels were highly variable at both times, and sometimes ranged from a fraction to up to 10-100 times the mean or median value.
Note: Animals were fed *ad libitum*

Week 52 Cplasma values (only 3h samples)

		Males				Females		
Group		LD	MD	HD		LD	MD	HD
Dose (mg/kg)		3	7	15		3	7	15
Cpl 3h (pg/mL)		216	1587	2518		110	396	1034

Week 104: amount excreted in urine (% of dose) (Ae ~ bioavailability)

		Males				Females		
Group		LD	MD	HD		LD	MD	HD
Dose (mg/kg)		3	7	15		3	7	15

Ae (%)*	Median	0.121	0.273	0 418		0 08	0.022	1.14
	Mean	0.162	0.421	0.882		0.13	0.038	1 01

*Values highly variable

Week 104: Cmax and AUC values

		Males				Females		
Group		LD	MD	HD		LD	MD	HD
Dose (mg/kg)		3	7	15		3	7	15
Tmax (h)		1	1	1		1	1	1
Cpl (3h) (pg/mL)		642	2752	36087		264	296	4071
Cmax (pg/mL)	Mean	5067	14213	333130		675	2944	4667
	Median	886	3604	7921		679	2274	4303
AUC (pgxh/mL)	Mean	9243	27763	577852		1773	5358	16599
(ngxh/mL)	Median	2 3	8.4	22		1 6	4.3	12

Organ Weights:
Slight increase in kidney in HD males, and increase in spleen in all treated.

Organ weights (Average for N=31-40)

	Males				Female s			
Group	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
Dose (mg/kg/day)	0	3	7	15	0	3	7	15
Body weight	589	523**	528**	524**	358	338	323**	326*
Kidney (rel wt)	0.49	0.53	0 53	0 54*	0.68	0.67	0.68	0.70
Spleen (rel wt)	0.19	0.37	0.34	0.29	0.29	0 35	0.33	0.37*

*p<0.05, ** p<0.01

Gross pathology:
No treatment related effects.

Histopathology

NON-NEOPLASTIC FINDINGS
Findings (Total incidence in all animals, N=50, unless indicated otherwise)

		Males					Femal es			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	3	7	15		0	3	7	15
N examined		50	50	50	50		50	50	50	50
Kidney	Hypertrophic tubular epithelium*	33	32	43	47		35	37	37	46
Spleen	Hematopoiesis*	40	46	47	48		38	48	46	46
	Hemosiderin deposits	41	26	30	20		40	42	37	31
Heart	Fibrosis of heart muscle	30	15	15	17		18	8	7	10
Lung	Interstitial pneumonia	4	4	6	11		1	6	4	1
Adrenal	Hemorrhages	0	0	1	4		0	0	0	5
Bone (sternum)	Trabecular extension*	0	50	50	50		0	50	49	50
Bone (nb)	Trabecular extension* (costochondral junction)	0	40/40	33/33	33/33		0	39/39	33/33	38/38
Bone (femur)	Trabecular extension*	0	50	50	50			50	49	50
Thyroid	C-cell hyperplasia	19	10	9	10		12	14	10	14
Uterus	Cystic gland hyperplasia	-	-	-	-		9	10	11	7

Cmax		1.1-1.5x	4.5-6x	10-13x		0.9-1.2x	2.9-3 8x	5.4-7.2x
AUC		0.7-1 3x	2.5-4 7x	6.7-12.2x		0 5-0 9x	1 3-2.4x	3 6-6.7x

*Human Cmax at 2.5 mg/day orally = 0.6-0.8 ng/mL; AUC = 1.8-3.3 ngxh/mL (Study MF7159).
*Multiples based on MEDIAN animal exposure values

Multiples based on BSA comparison*

Group	LD	MD	HD
Dose (mg/kgday)	3	7	15
Multiple of human oral dose (2.5 mg/day, 0.04 mg/kg) based on mg/m2 BSA	12.3x	29x	61x

*Since rats were fed ad libitum multiples based on BSA comparison are overestimated

Evaluation of tumor findings

Skin histiocytoma in males: incidence (0-0-0-2) (0%-0%-0%-4%):
According to Sponsor's trend analysis with a cut-off p-value of 0.05 for rare tumors (control incidence <1%), the finding in males of skin histiocytoma was statistically significant (p=0.023-0.026). However, the finding was dismissed by the Sponsor based on the following. The total incidence in ALL rats regardless of treatment (2/200=1%) was within the historical control rate for Wistar rats (0-5%, avg 0.6%; 41 studies) and this was considered unremarkable. Reviewer feels this argument is not valid. Additionally, Sponsor noted that combined benign and malignant skin histiocytomas were not statistically significant. Thus, Sponsor did not consider the finding of skin histiocytoma in males relevant.
According to CDER's statistical review, the skin histiocytoma finding in males was not statistically significant (p=0.0506). For a rare tumor the cut off p-value is 0.025.
The increased HD incidence for this tumor occurred at an exposure in rats of 6.7-12x the exposure in humans at 2.5 mg/day (orally), based on AUC.

Initially, Sponsor submitted historical control data with the NDA from the — database for Wistar rats (see Table below). Updated historical control data were submitted by Sponsor (February 21, 2003), from — (testing facility) and — database for Wistar rats (complete set and subset for breeder B). The data from — showed that in 3 out of 4 studies (N=50/grp) no skin histiocytoma were observed in males (or females). In 1 study there were 2/50 (4%). However, inspection of study identification numbers showed that the latter "control" data were from the current ibandronate study (Study # 068232, Study J8). Since in this study there were 2 tumors in the HD male group (N=50) but none in control, LD or MD groups data were probably provided incorrectly from the HD ibandronate group. Other control data for other tumor sites, however, appeared to agree with the control group data from study J8.

Updated historical control data from the — database submitted in February 2003 were similar as provided previously with the original NDA (shown below). The updated data indicated an avg. incidence of skin histiocytoma of 0.5%, range 0-5%, 66 studies. However, of the 66 studies, 15 studies were positive with range 0-2% and only 1 study with 1/20 (=4%). Data are provided in the Table below.
Additional historical control data were provided from the — database for the breeder — that supplied rats for the ibandronate study. In 300 males, there were 2 skin fibrous histiocytomas (incidence 0.7%).

Reviewer feels that the increased incidence of benign skin histiocytoma of 4% (2/50) in HD males, treated with 15 mg/kg/day for 104 weeks, although above historical control values of 0.7-2% is not a significant finding. The finding was not statistically significant according to CDER's statistical review and the incidence (2/50 rats) was too low to support biological significance.

C-cell adenoma in females: incidence (0-0-1-2) (0%-0%-2%-4%):
This finding was significant based on p<0.05 (p=0.036-0.040). However, for a common tumor the thyroid C-cell adenoma finding was not significant since the p-value was > 0.01 (cut off p-value

according to Sponsor). Also, the historical control incidence for this tumor in Wistar rats is 0-24% (avg 10%) (41 studies).
Reviewer agrees with the lack of significance due to the small extent of the incidence as compared to historical control values. In addition, the finding was not statistically significant according to analysis of the data by CDER's Biometrics Reviewer.

HISTORICAL CONTROL VALUES*

	Sex	Data	Number of studies	Number of animals	Number of animals with lesion	Average incidence (%)	Range of incidences (%)
Skin, histiocytoma, fibrous (B)	Males	July 2002, NDA	41	2345	13	0.6%	0%-5%
		February 2003	65	3579	17	0.47%	0%-2%
	Females	July 2002, NDA	41	2391	4	0.2%	0%-2%
Thyroid, C-cell adenoma	Males	July 2002, NDA	41	2341	220	9.4%	2%-24%
		February 2003	71	3640	386	10.6%	2%-24%
	Females	July 2002, NDA	41	2393	246	10.3%	0%-24%
		February 2003	71	3542	382	10.8%	0%-25%

*Database: [redacted] Wistar rats, study duration 24-31 months (Data Base: [redacted] rats). Data submitted with NDA (July 2002).

CONCLUSIONS

- Wistar rats were dosed with ibandronate for 104 weeks with 3, 7, 15 mg/kg/day (fed *ad libitum*). These doses resulted in exposures in the rat of approximately 0.7-12x (males) and 0.5-7x (females) times human exposure at the recommended daily oral dose of 2.5 mg/day, based on AUC comparison.
- No statistically significant dose-related increases in tumor incidences were noted in CDER's Statistical Review of the rat oral gavage study (Reviewer Cynthia Liu, MA, HFD-715).
- The skin histiocytoma finding in high dose males is not statistically or biologically significant.
- Based on the results of CDER's statistical review and evaluation of the skin histiocytoma finding, Reviewer agrees with the Sponsor that ibandronate did not cause tumors in Wistar rats dosed by oral gavage with 3, 7, 15 mg/kg/day for 104 weeks.

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APPENDIX I

Summary Tables Neoplastic Findings

BN 21.0955.Nb 0682

NUMBER OF ANIMALS WITH PRIMARY NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX

ORGAN/FINDING	SEX : MALE GROUP:	01	02	03	04
LIVER	- CHOLANGIOMA CYSTIC -	0/50	0/50	1/50	0/50
	- CARCINOMA/HEPATOC.	0/50	1/50	0/50	0/50
SPLEEN	- HEMANGIOSARCOMA	1/50	0/50	0/49	0/50
HEMOLYMPHORET. SYS.	- HISTIOCYTIC SARCOMA	0/50	1/50	2/50	0/50
MESENT. LYMPH NODE	- HEMANGIOMA	9/50	6/50	1/50	2/50
KIDNEYS	- LIPOMA	0/50	2/50	0/50	0/50
	- CARCINOMA	0/50	0/50	1/50	0/50
URINARY BLADDER	- TRANSIT.C.PAPILLOMA	0/50	2/50	0/50	0/50
STOMACH	- PAPILLOMA	1/49	0/50	0/49	0/49
DUODENUM	- ADENOMATOUS POLYP	0/49	1/50	0/49	0/49
	- LEIOMYOSARCOMA	0/49	0/50	0/49	1/49
JEJUNUM	- LEIOMYOMA	0/49	0/50	1/49	0/49
ILEUM	- LEIOMYOSARCOMA	0/49	1/50	0/49	0/49
THYMUS	- THYMOMA	0/50	2/50	0/50	1/50
	- THYMOMA SQUAM. DIFF.	0/50	1/50	0/50	0/50
TESTES	- LEYDIG CELL TUMOR	1/50	1/50	0/50	2/50
SALIVARY GLANDS	- SQUAMOUS C.CARCINOMA	1/50	0/50	0/50	0/50
PANCREAS	- ADENOMA/ISLET CELL	1/50	3/50	1/50	1/50
	- CARCINOMA/ISLET CELL	0/50	0/50	2/50	0/50
THYROID GLAND	- ADENOMA/C-CELL	2/50	0/50	0/50	2/50
	- CARCINOMA/C-CELL	1/50	3/50	0/50	2/50
	- ADENOMA/FOLLICULAR	0/50	0/50	1/50	0/50
ADRENAL GLANDS	- CORTICAL ADENOMA	0/50	1/50	2/50	0/50
	- CORTICAL CARCINOMA	0/50	0/50	1/50	0/50
	- PHAEOCHROMOCYTOMA	9/50	0/50	0/50	0/50
	- GANGLIONEUROMA	0/50	2/50	0/50	0/50
PITUITARY GLAND	- ADENOMA/P.INTERMEDIA	1/50	1/50	1/50	0/50
	- HEMORRHAGIC ADENOMA	9/50	6/50	6/50	6/50
	- SPONGIOCYTIC ADENOMA	0/50	0/50	1/50	0/50
	- PLEOMORPHIC ADENOMA	4/50	2/50	4/50	3/50
SKIN	- LIPOMA	0/50	0/50	1/50	0/50
	- SQUAMOUS C.PAPILLOMA	2/50	0/50	1/50	0/50
	- SQUAMOUS CARCINOMA	0/50	1/50	0/50	0/50
	- SEBACEOUS SQUAM CARC	0/50	1/50	0/50	0/50
	- BASALIOMA/MALIGNANT	0/50	0/50	0/50	1/50
	- HISTIOCYTOMA/BENIGN	0/50	0/50	0/50	2/50
	- OSTEOSARCOMA	0/50	0/50	1/50	0/50
	- MAL PLEO HISTIOCYT	2/50	0/50	0/50	1/50
	- FIBROSARCOMA	1/50	0/50	1/50	1/50
MAMMARY GLAND	- FIBROMA	1/50	4/50	1/50	0/50
	- FIBROADENOMA	1/50	0/50	0/50	1/50
BRAIN	- GRANULAR CELL TUMOR	0/50	0/50	0/50	1/50
	- OLIGODENDROGLIOMA	1/50	1/50	0/50	0/50
PROSTATE	- ADENOCARCINOMA	0/50	1/50	0/50	1/50
BODY CAVITIES	- LIPOMA	1/50	0/50	0/50	0/50
	- MESOTHELIOMA/MALIGN.	1/50	0/50	0/50	0/50

NUMBER OF ANIMALS WITH PRIMARY NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX

ORGAN/FINDING	SEX : FEMALE GROUP:	01	02	03	04
HEART	- ENDOCARDIAL SARCOMA	0/50	1/50	0/50	0/50
LIVER	- ADENOMA:HEPATOC.	2/50	1/50	0/50	0/50
	- CHOLANGIOMA CYSTIC	0/50	0/50	1/50	0/50
HEMOLYMPHORET. SYS.	- HISTIOCYTIC SARCOMA	0/50	0/50	1/50	0/50
MESENT. LYMPH NODE	- HEMANGIOMA	0/50	2/50	1/50	0/50
STOMACH	- LIPOMA	0/50	1/50	0/49	0/50
DUODENUM	- LEIOMYOSARCOMA	0/50	0/50	0/48	1/50
ILEUM	- LEIOMYOSARCOMA	0/50	1/50	0/48	0/49
THYMUS	- THYMOMA	1/50	5/50	3/50	1/50
OVARIES	- CYSTADENOMA	0/50	1/50	0/50	0/50
	- THECA/GRANUL.C.TUMOR	0/50	1/50	1/50	0/50
	- GRANULOSA CELL TUMOR	2/50	0/50	0/50	0/50
PANCREAS	- ADENOMA/ISLET CELL	0/50	4/50	0/50	0/50
THYROID GLAND	- ADENOMA/C-CELL	0/50	0/50	1/49	2/50
	- CARCINOMA/C-CELL	6/50	0/50	0/49	1/50
	- ADENOMA/FOLLICULAR	0/50	1/50	0/49	0/50
	- CARCINOMA/FOLLICULAR	2/50	1/50	0/49	2/50
ADRENAL GLANDS	- CORTICAL ADENOMA	0/50	1/50	0/50	0/50
PITUITARY GLAND	- ADENOMA/P.INTERMEDIA	0/50	1/50	0/50	0/50
	- HEMORRHAGIC ADENOMA	23/50	33/50	16/50	22/50
	- SPONGIOCYTIC ADENOMA	1/50	1/50	0/50	0/50
	- PLEOMORPHIC ADENOMA	7/50	1/50	12/50	9/50
SKIN	- SQUAMOUS CARCINOMA	1/50	0/50	0/50	0/50
MAMMARY GLAND	- FIBROMA	2/50	1/50	0/50	0/50
	- FIBROADENOMA	10/50	15/50	10/50	13/50
	- FIBROAD MALIGN AREA	1/50	0/50	0/50	0/50
	- ADENOMA CYSTIC	0/50	0/50	2/50	0/50
	- PAPILL. CYSTADENOMA	0/50	2/50	0/50	0/50
	- ADENOCARCINOMA	4/50	2/50	1/50	2/50
BRAIN	- OLIGODENDROGLIOMA	0/50	0/50	0/50	1/50
UTERUS	- POLYP	4/50	4/50	6/50	5/50
	- ADENOCARCINOMA	0/50	1/50	0/50	2/50
VAGINA	- PAPILLOMA	0/48	0/50	0/50	1/50
	- ADENOCARC. INVADING	0/48	1/50	0/50	0/50
BODY CAVITIES	- LIPOMA	0/50	1/50	1/50	0/50
	- LIPOSARCOMA	0/50	0/50	0/50	1/50
	- HEMANGIOSARCOMA	0/50	0/50	0/50	1/50
	- SQUAMOUS CELL CARC	0/50	1/50	0/50	0/50

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CARCINOGENICITY STUDY IN MICE WITH BM 21.0955.NA ADMINISTERED BY ORAL GAVAGE

GENERAL

Sponsor:	Roche
Study Number:	BM21.0955CAHVM01
Study Report:	J14
NDA Volumes:	Vols. 71-72
Testing Facility:	Boehringer Mannheim, GmbH (Hoffmann-LaRoche) (Germany)
Study Period:	June 1992-March 1994
QA Report:	Yes
GLP Statement:	Yes
Dose-range-finding study:	J9 and J6 (see Study Summary)

STUDY PROTOCOL

Species/strain:	Mouse (Crl:NMRI/BR, outbreed, SPF)
Number of animals:	Subset 0: 110/sex/dose group (Group 0, control) 55/sex/dose/group (Groups 2, 3, 4; LD, MD, HD) Subset 1: 30/sex/dose/group (Groups 1, 4; control, HD)
Doses:	0, 5, 20, 40 mg/kg/day (oral)
Age at start of study:	4 weeks (SUBSET 0); 12 weeks (SUBSET 1)
Weight at start of study:	31.7g (males), 26.7g (females)
Study Duration:	18-19 months (Groups 0,2,3: 568-581 days, ie, 18.8 months; Group 4: 542 days, ie, 17.8 months). Study was planned for ca. 18 months with little interference of (expected) local respiratory tract irritation at the selected HD of 40 mg/kg. However, gavage caused problems in HD animals, so that this group was terminated early (542 days, or 17.8 months). Also, additional control and HD animals (Subset 1) were added to the study after ca. 1 month due to mortality in the HD group.
Animal housing:	1/cage
Animal diet:	pellets, and tap water, both ad libitum.
Drug Name:	BM 21.0955.Na. This old name (without H_2O) corresponds to the new name, BM 21.0955.Na.H_2O (ibandronate, monosodium salt, monohydrate)
Drug Batch Number(s):	447 624-00 (1 mg free acid = 1.069 mg weighed BM 21.0955.Na.H_2O) 448 913-00 (1 mg free acid = 1.125 mg weighed BM 21.0955.Na.H_2O)
Drug Stability:	Stable (expiry dates: November 1994 and August 1995)
Dosage form:	Solution in 0.5% sodium carboxymethyl cellulose in distilled water
Dosing route:	Oral gavage
Dosing frequency:	Once daily, 7 days/week
Dose Volume:	10 mL/kg
Vehicle:	0.5% sodium carboxymethylcellulose (tylose)

Dose Groups:

SUBSET 0

Group	Designation	N/sex/group	Dose Volume (ml/kg)	Dose (mg/kg/day)	Dose (free acid equivalents) (mg/kg/day
0*	Control	110	10	0	0
2	LD	55	10	5	4.44

3	MD	55	10	20	17.8
4	HD	55	10	40	35.6

*double-sized group

SUBSET 1

Group	Designation	N/sex/group	Dose Volume (ml/kg)	Dose (mg/kg/day)	Dose (free acid equivalents) (mg/kg/day
0	Control	30	10	0	0
4	HD	30	10	40	35.6

POOLED SUBSET 0 + SUBSET 1

Group	Designation	N/sex/group	Dose Volume (ml/kg)	Dose (mg/kg/day)	Dose (free acid equivalents) (mg/kg/day
0	Control	140	10	0	0
2	LD	55	10	5	4.44
3	MD	55	10	20	17.8
4	HD	85	10	40	35.6

Relation to Clinical Use: Recommended dose: 2.5 mg daily, orally (tablet)

CAC Concurrence: Sponsor's dose selection was based on results from two 3-month studies (DRF Study J9 with doses 0, 20, 40, 60, 80, 100 mg/kg/day; DRF Study J6 with doses 0, 10, 15, 20, 30, 40 mg/kg/day). Concurrence of the Exec CAC with the dose selection was not obtained. However, the Division informed the Sponsor in a FAX communication (March 29, 1996) that the high dose in the then-ongoing oral gavage study was appropriate based on observed mortality.

Interim Sacrifices: None

Clinical Observations: At least once daily, and recorded weekly. Also weekly palpation for tissue masses.

Mortality: At least once daily

Body Weight: Twice weekly

Food Consumption: Twice monthly

Water Consumption: No quantitative determination

Clinical Pathology: At scheduled sacrifice, blood samples were obtained from all animals. Red cell morphology and differential WBC and a complete set of hematology parameters and clinical biochemistry were determined for all animals. Data are listed for Week 78, irrespective of real date of death.

Toxicokinetics: (TK Study #TV 370) Concentration of test substance was determined in serum [redacted] The [redacted] was carried out with standards prepared in serum, and the quantification limit was [redacted]

Test substance in serum: At scheduled sacrifice (18 months), blood samples were obtained from 3-6/sex/group/time point (0,1,3,5h postdosing), from animals in both subsets.

Macroscopic examination: All animals were necropsied, including those that died or were killed moribund, and macroscopic abnormalities were recorded. Scheduled necropsies were carried out on Days 568-581 for controls, LD, and MD groups, and Day 542 for HD groups. Tissues were collected and fixed as listed in the histopathology inventory.

Organ weights: Organs weighed at scheduled sacrifice: brain, heart, kidneys, liver, spleen.

Histopathology:	Tissue samples were processed from all animals (scheduled kill, moribund kill, found dead), from all organs and tissues listed in the histopathology inventory (appended). Findings were listed separately as nonneoplastic and neoplastic lesions. Histopathology summary tables were provided for all animals combined (early death and terminal sacrifice), and for early death and terminal sacrifice separately.
Statistical Evaluation:	In-life phase: Tests used for analysis of body weight, food consumption, hematology, clinical chemistry and organ weights: Kruskal-Wallis Anova and Mann-Whitney U-tests
Biostatistical evaluation:	For target endpoints, i.e., mortality and tumor findings, trend tests for an increase in dose-response relationship were carried out in accordance with EC, US, and FDA guidelines. Tests performed were for crude mortality and tumor rates (all animals, early deaths, and terminal sacrifices), and for time-mortality and time-tumor relationships for fatal, incidental and clinically observable tumors. Tests for tumor combinations and metastasing tumors were also performed. Pairwise tests of tumor incidences in control vs. dosed group were also carried out. Concomitant endpoints were analyzed by other trend tests.
Appendix:	Sponsor's summary tables of neoplastic findings

NOTE: For the purpose of biostatistical analyses [Subset 0] and [Subset 0 + Subset 1] are considered two possible entities.

Mouse Carcinogenicity Study (J14)

Histopathology Inventory

Species: NMRI Mouse	Pathology	
	Tissues retained and examined in all animals (per protocol)	Tissues collected but not examined
Gross lesion, masses, tumors	X	
Adrenals	X	
Aorta	X	
Bone (sternum)	X	
Bone (femur)	X	
Bone marrow (femur, sternum)	X	
Bone (vertebra)	X	
Brain	X	
Cecum	X	
Colon	X	
Duodenum	X	
Epididymides	X	
Eyes with optic nerves	X	
Gall bladder	X	
Harderian gland	X	
Heart	X	
Ileum	X	
Jejunum	X	
Kidneys	X	
Lacrimal gland	X	
Larynx	X	
Liver	X	
Lung	X	

Lymph nodes, mesenteric	X	
Oesophagus	X	
Ovaries	X	
Pancreas	X	
Pituitary gland	X	
Parathyroids	X	
Prostate gland	X	
Rectum	X	
Salivary gland (mandibular, parotid)	X	
Sciatic nerve	X	
Seminal vesicle	X	
Skeletal muscle (hindleg)	X	
Skin and mammary gland	X	
Spinal cord	X	
Spleen	X	
Stomach	X	
Testes	X	
Thymus	X	
Thyroid	X	
Tongue		X
Trachea	X	
Urinary bladder	X	
Uterus	X	
Vagina		X

RESULTS

Mortality/Survival

Due to increased mortality in the initial phase of the study in Subset 0 (month1), additional groups of N=30/sex were added to control and HD groups (Subset 1).
Increased mortality occurred in both subsets, and was statistically significant in males and females according to trend analysis of crude mortality rates as well as time-mortality relationships ($p<0.001$). Effects were significant in MD and HD, m and f ($p<0.01$). Mortality in MD and HD was mainly due to drug-related respiratory tract irritation. In animals that died lung lesions and distension of the GI tract were observed. Survival rates (Subset 0+Subset 1) for males and females were 41% and 20% in HD groups. Survival curves are for all pooled animals.

SURVIVAL – Analysis of all animals at end of study (Wk 78)
SUBSET 0

				MALES	FEMALES
Group		Dose (mg/kg/day)	N	Number (%)	Number (%)
0	Control	0	110	79%	76%
2	LD	5	55	80%	65%
3	MD	20	55	53%***	51%**
4	HD	40	55	36%***	11%***

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

SUBSET 1

				MALES	FEMALES
Group		Dose (mg/kg/day)	N	Number (%)	Number (%)
0	Control	0	30	77%	50%*
4	HD	40	30	63%	37%*

*p<0.05

SUBSET 0 + SUBSET 1

				MALES	FEMALES
Group		Dose	N	%	%

		(mg/kg/day)			
0	Control	0	140	79%	74%
2	LD	5	55	80%	65%
3	MD	20	55	53%***	51%**
4	HD	40	85	41%***	20%***
Trend test				***	***

** p<0 01; *** p <0.001

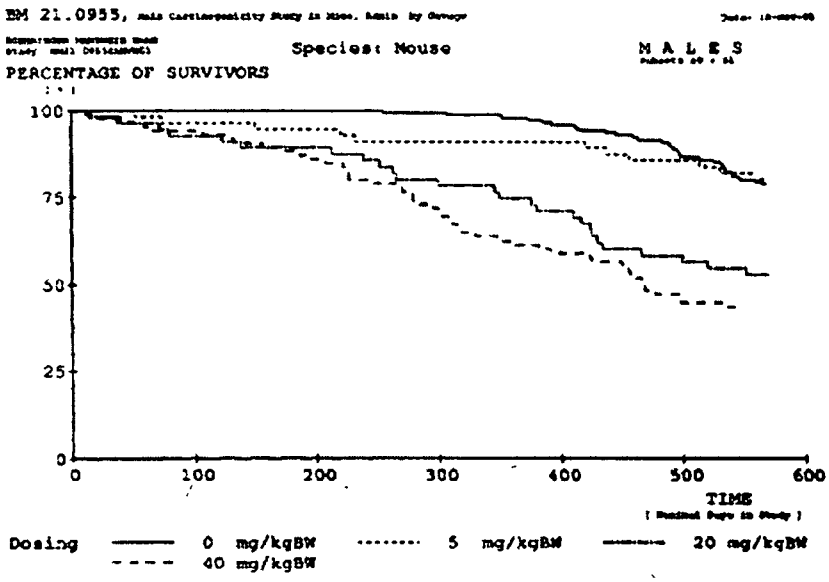

Fig. 1

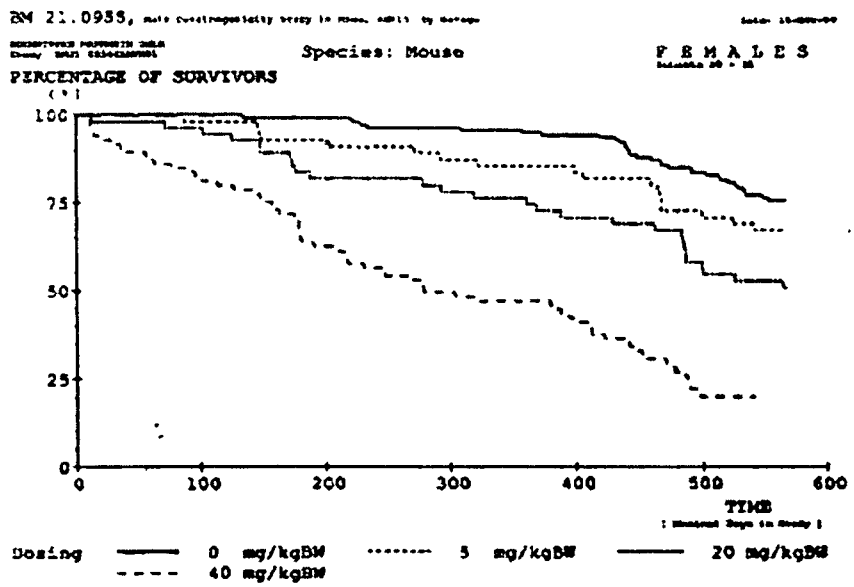

Fig. 2

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Clinical Observations
Dose-dependent increase in incidence and severity of animals with respiratory disturbances, in all dose groups. These included difficult breathing, wheezing, pumping, slow or rapid breathing, shortness of breath, abdominal respiration. These events lead to death or sacrifice in (ctrl-LD-MD-HD) 3-6-20-36 (3%-11%-36%-42%) males, and 8-3-20-51 (6%-5%-36%-60%) females.

Body weight
Decreased in LD, MD, HD males, and HD females

Body weight (gr) at end of study (Week 78)

			MALES	FEMALES
Group		Dose (mg/kg/day)	Body weight (g)	Body weight (g)
1	Control	0	46.9	40 0
2	LD	3	45.3*	40.3
3	MD	7	42.8***	38 9
4	HD	15	41 0***	37 2**

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

Food consumption
Trend towards dose-dependent reduction in food consumption

Hematology
No significant effects

Clinical chemistry
Small decrease in serum ALP, m and f, all dose groups. Increase in serum P and Ca in males, all dose groups. Ca and P values remained within normal range. Dose-dependent increase in creatinine and decrease in BUN in males, all dose groups.

Clinical chemistry at end of study (wk 78)

			MALES					FEM ALES				
Group		Dose (mg/kg /day)	Alk Phos (U/L)	Creat (umol/ L)	BUN (mmol/ l)	P (mmo l/l)	Ca (mmo l/l)	Alk Phos (U/L)	Creat (umol /L)	BUN (mmol/ l)	P (mmol/ l)	Ca (mmol/ l)
0	Ctrl	0	110	30	11.6	2.0	2.2	216	33	10	2 3	2.4
2	LD	3	88	35**	10.0	2.1	2.5***	126	31	10	1 8	2.5
3	MD	7	85	36***	9.1**	2.5***	2 6***	115	34	9.6	1.9	2 5
4	HD	15	90	48***	9.4**	2.4*	2.1	126	35	8 8	2.0	2.3

*p<0.05, ** p<0.01

Toxicokinetics:
Greater than proportional increase in Cmax with dose. Serum levels highly variable at all sampling times (0,1,3,5h postdose). Standard deviations of Cmax levels were 30%-25%-220% (LD-MD-HD males, N=3-3-6), and 40%-130%-80% (LD-MD-HD females, N=3-3-3)

Week 78: Cmax and AUC

	Males			Females		
Group	LD	MD	HD	LD	MD	HD
Dose (mg/kg)	5	20	40	5	20	40
Tmax (h)	3	5	3	1	5	1
Cmax (ng/mL)	1 1	3.2	95	2.4	4.3	24
AUC (ngxh/mL)	3 6	58	856	26	64	127

Multiples of human Cmax and AUC values (at 2.5 mg/day)*

	Males			Females		
Group	LD	MD	HD	LD	MD	HD

Dose (mg/kg)	5	20	40	5	20	40
Cmax	1.4x	4x	120x	3x	5.4x	30x
AUC	1.3x	21x	305x	9 4x	23x	45x

*Human Cmax at 2.5 mg/day orally = 0.8 ng/mL; AUC = 2 8 ngxh/mL (BM Report N7).

Macroscopic findings

Lung lesions (related to respiratory distress; edema and hemorrhage, emphysema), gaseous distension of stomach and/or intestine, mainly in MD and HD mice that died or were sacrificed prematurely. Gaseous distension of stomach and small intestine reached incidence of up to 30% in HD m,f. Respiratory finding of edema/hemorrhage was seen in up to 20-40% of HD m-f.

		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
N examined		140	55	55	85		140	55	55	85
Stomach	Tympania	0	2	5	9		2	1	2	5
Small intestine	Tympania	1	4	11	21		4	0	4	27
Lung	Edema and hemorrhage parenchymatous	1	0	5	16		2	2	9	35
	Emphysema	0	0	7	10		1	6	4	17

Organ Weights:

Decreases in heart, liver, kidney, and increase in brain (relative to body weight)

Organ weights (relative to body, %) (Average for N=17-110)

	Males				Females			
Group	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
Body weight	43 6	42*	40***	38***	38	39	36**	35*
Heart (rel wt)	0.66	0 64	0.62*	0 63*	0 66	0.51	0 53	0 57*
Liver (rel wt)	5.5	5.0***	5.0***	4.9***	5 8	5 4*	5 2*	5.8
Kidney (rel wt)	2.1	1.9***	1.9**	1 9**	1 4	1 3***	1 3*	1.4
Brain (rel wt)	1.2	1 1	1.2*	1 3***	1 4	1 3	1 4	1.5*

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

Histopathology

NON-NEOPLASTIC FINDINGS

Most prominent was local irritation/inflammation in the upper respiratory tract (including rhinitis/sinusitis, laryngitis, tracheitis) in all dose groups. Acute lung lesions ascribed to aspiration/regurgitation caused premature death in MD and HD. Lung lesions included emphysema, hemorrhage, edema. No atelectasis increase observed. Other effects included reduction of osteoporosis, increase in trabecular bone, and increase in extramedullary hematopoiesis in the spleen. All these effects were dose related in terms of incidence and/or severity, and statistically significant (trend test).

Findings (Total incidence in all animals)

		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
N examined										
Lung	Lesions causing death (edema, emphysema)	-	(+)	++	++		-	(+)	++	++
Larynx	Laryngitis	-	+	++	+++		-	+	++	+++
Nasal cavity	Rhinitis/sinusitis	-	+	++	+++		-	+	++	+++
Trachea	Tracheitis	-	+	++	+++		-	+	++	+++

+ = measure of incidence and/or severity

Lung and larynx findings

		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
N examined		140	55	55	85		140	55	55	85
Lung	Emphysema	2	1	5	22		2	7	11	32
	Edema	9	3	18	27		5	9	19	49
Larynx	Laryngitis	68	47	53	77		105	48	50	84

Bone and spleen findings with dose-dependent degree

		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
N examined		140	55	55	85		140	55	55	85
Bone	Osteoporosis									
	Minimal	21	0	0	0		5	0	0	0
	Slight	25	0	0	0		40	0	0	0
	Moderate	9	0	0	0		46	0	0	0
	Severe to extreme	2	0	0	0		18	0	0	0
	TOTAL	57	0	0	0		109	0	0	0
Bone	Trabecular extension									
	Minimal	0	7	1	0		0	1	1	0
	Slight	0	25	3	31		0	23	7	23
	Moderate	0	21	50	51		0	29	46	47
	Severe to extreme	0	0	0	1		0	2	0	11
	TOTAL	0	53	54	83		0	55	54	81
Spleen	Extramedullary hematopoiesis									
	Minimal	9	11	0	11		2	1	3	0
	Slight	4	21	9	18		11	19	24	17
	Moderate	4	0	0	1		1	11	3	6
	Severe	2	1	0	0		2	2	0	1
	TOTAL	19	33	9	30**		16	33	30	24***

**p<0 01, ***p<0.001 (trend test)

NEOPLASTIC FINDINGS (Appendix I: Summary Tables)

Animals and number of tumors

		Males					Females				
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD	
N examined		140	55	55	85		140	55	55	85	
Animals with tumors		95	41	19	27		102	33	23	21	
Number of tumors	Total	142	60	25	43		175	67	39	24	
	Benign	95	45	23	32		112	38	25	20	
	Malignant	47	15	2	11		62	29	14	4	

NOTE: In all tables below "*N at risk*" is the number of animals for which tissue could be evaluated, i.e., $N_{risk}=N_{grp}-N_{tissue\ missing}-N_{autolysis}-N_{not\ hit\ in\ section}-N_{died\ before\ first\ tumor}$ (Gart JJ et al, IARC Sci.Pubb. No.79, 1986, Lyon)

SINGLE NEOPLASMS

Crude rates of tumor-bearing animals (data pooled for Subsets 0 and 1)

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
Dose (mg/kg)		0	5	20	40		0	5	20	40	
N (total)		140	55	55	85		140	55	55	85	

N at risk		*140*	*54*	*53*	*76*		*137*	*54*	*51*	*62*	
Liver	Hepatocellular adenoma	0	2*	1	2	*					
		(0%)	(3.7%)*	(1 9%)	(2.6%)						
Harderian gland	Adenoma	11	10*	5	4	NS					
		(8%)	(19%)	(9%)	(5%)						
Lung	Bronchioalveolar adenoma						7	8*	4	7	NS
							(5%)	(15%)*	(8%)	(11%)	
	Bronchioalveolar carcinoma						14	8	3	0	NS
							(10%)	(15%)	(5 9%)	(0%)	
Uterus	Hemangiopercyt oma (B)						0	0	0	1	NS
							(0%)	(0%)	(0%)	(1.6%)	

*=p<0.05 (pairwise X^2 test; or Armitage's trend test)
(B) = benign; (M) = malignant

SINGLE NEOPLASMS
Early deaths only: crude rates of tumor-bearing animals (data pooled for Subsets 0 and 1)

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
N (total)		140	55	55	85		140	55	55	85	
N at risk							*33*	*17*	*26*	*68*	
Lung	Bronchioalveolar adenoma						1	1	1	5	NS
	Bronchioalveolar carcinoma						7	1	1	0	NS

SINGLE NEOPLASMS
Terminal sacrifice only: crude rates of tumor-bearing animals (data pooled for Subsets 0 and 1)

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
N (total)		140	55	55	85		140	55	55	85	
N at risk		*114*	*45*	*30*	*35*		*107*	*38*	*29*	*17*	
Harderian gland	Adenoma	11	9*	4	4	NS					
		(9.7%)	(20%)*	(13%)	(11%)						
Lung	Bronchioalveolar adenoma						6	7*	3	2	NS
							(5.6%)	(18%)	(10%)	(12%)	
	Bronchioalveolar carcinoma						7	7*	2	0	NS
							(6.5%)	(18%)*	(6.9%)	(0%)	
Uterus	Hemangiopericyt oma (B)						0	0	0	1	*
							(0%)	(0%)	(0%)	(5.9%)	

*=p<0.05 (pairwise X^2 test; or Armitage's trend test)
(B) = benign; (M) = malignant

Sponsor also analyzed crude tumor incidence rates - for terminally sacrificed animals - when groups of 20 and 40 were combined. This was done based on low survival. Reviewer feels that

this analysis did not add much information as the results were merely an average of the two higher dose groups.

NEOPLASM COMBINATIONS
Crude rates of tumor-bearing animals (data pooled for Subsets 0 and 1)

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
All animals											
Lung	Bronchioalveolar adenoma + carcinoma						21	16*	7	7	NS
							(15%)	(30%)*	(14%)	(11%)	
	N at risk						*137*	*54*	*51*	*62*	
Early deaths											
Lung	Bronchioalveolar adenoma + carcinoma						8	2	2	5	NS
							(24%)	(12%)	(8%)	(7%)	
	N at risk						*33*	*17*	*26*	*68*	
Terminal sacrifice											
Lung	Bronchioalveolar adenoma + carcinoma						13	14**	5	2	NS
							(12%)	(37%)**	(17%)	(12%)	
	N at risk						*107*	*38*	*29*	*17*	

*=p<0.05, **p<0 01 (pairwise X^2 test; or Armitage's trend test)

A separate analysis was performed for adrenal subcapsular adenomas (Type A, type B, or mixed) since this appeared to be a finding in the drinking water study. None of these tumors were significantly increased, either single or as combinations, in either all animals or early deaths or terminally sacrificed (pooled subsets).

Single adrenal neoplasms: Crude rates of tumor-bearing animals (data pooled for Subsets 0 and 1) (all animals)

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
Dose (mg/kg)		0	5	20	40		0	5	20	40	
N (total)		140	55	55	85		140	55	55	85	
N at risk		*140*	*53*	*52*	*76*		*137*	*54*	*51*	*62*	
Adrenal	Subcapsular cell adenoma type A	0	0	0	0		1	2	0	0	Ns
							(0.7%)	(3.7%)			
	Subcapsular cell adenoma type B	15	8	1	4		3	1	0	0	Ns
		(11%)	(15%)	(2%)	5%)		(2.1%)	1.9%)			
	Subcapsular cell adenoma mixed	13	7	3	2		4	0	2	0	ns
		(9.3%)	(13%)	(6%)	(3%)		(2.9%)		(3.9%)		

Ns = not significant

Time-adjusted analysis of tumor rates (fatal, incidental, and selected combined fatal and incidental tumor; pooled subsets) did not reveal any statistical significance. There were also no

statistically significant findings in time-adjusted analysis of clinically observable and metastasizing tumors (pooled subsets).

SUMMARY AND EVALUATION

Findings:

Treatment of NMRI mice for 18 months with 0, 5, 20, 40 mg/kg/day ibandronate by oral gavage caused:

- mortality in MD and HD males and LD, MD, HD females
- fatal respiratory disturbances and lung/trachea/larynx lesions in MD and HD males and females
- slight dose-related reduction in body weight in MD and HD males and females
- decrease in serum ALKP in all treated, and increase in serum P in MD, HD males
- slight decreases in heart, liver, kidney weights, and increase in brain weight in MD, HD or all treated
- distension of stomach and GI tract in MD and HD
- pulmonary edema, hemorrhage, atelectasis, emphysema in MD and HD
- bone decrease in osteoporosis and increased trabecular extension due to inhibition of bone resorption (pharmacodynamic effect of drug) in all treated males and females
- secondary effects to bone extension spleen extramedullary hematopoiesis in all treated

Dose selection

The selection of does was based on two 3-month studies at doses up to 40 and 100 mg/kg/day. Sponsor concluded that the MTD had been exceeded at 20 and 40 mg/kg/day in the main study because of the irritant effect of the dosing solution and resulting mortality, and that 20 mg/kg/day would have been a better HD. Reviewer agrees with this conclusion. However, the current oral gavage study in conjunction with the drinking water study is adequate as carcinogenic assessment.

The current oral gavage study (J14) was carried out with 0, 5, 20, 40 mg/kg/day. An other carcinogenicity study (J15) was done by dosing mice in the drinking water, at 0, 5, 20, 80 mg/kg/day. Comparative data on exposure and respiratory tract irritation from these studies suggest that the respiratory tract toxicity in the gavage study was at least partly due to local irritation/aspiration of test article. However, comparative data on acute oral and iv toxicity in mice suggest that the lung toxicity (edema, hemorrhage) can also represent systemic toxicity.

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Exposure

Serum levels (Week 78) were highly variable and calculated AUC multiples are thus fairly inaccurate. The following table shows values for both oral gavage and drinking water studies.

Comparison of survival and exposure in oral gavage and drinking water study

	Oral gavage study				Drinking water study			
Group	LD	MD	HD		LD	MD		HD
Doses	5	20	40		5	20	(50)	80
Survival*	100%-88%	67%-69%	52%-27%		118%-128%	112%-120%	-	109%-132%
Concentration of drug in dosing solution	0.05%	0.2%	0 4%		0.001%-0.0025%	0.0035%-0.01%	-	0.02%-0.04%
AUC (ngxh/ml)	3 6m-26f	58m-64f	856m-127f				(281m-449f)**	
Multiple of human AUC (@ 2.5 mg/day)***	1.3x-9.4x	21x-23x	305x-45x				(100x-160x)	

* relative to controls (avg, m and f)
**AUC @ 50 mg/kg/day (3-mo study)
*** Human AUC at 2.5 mg/day: 2.8 ngxh/mL (BM Report N7)

Oral gavage study exposure multiples

Multiples of human exposure based on more recent clinical data (MF 7159, BP16304) show the following. Calculations are based on AUC (human) = 1.8-33 ngxh/mL. Exposure multiples based on AUC are similar to those based on BSA comparison for all groups except HD males. Since animals were fed ad libitum and bioavailability is reduced by food, one would expect that AUC multiples would be lower than those based on BSA comparison.

Exposure in oral gavage study

Group	LD	MD	HD
Doses (mg/kg/day)	5	20	40
AUC (ngxh/ml)	M 3 6 F: 26	M: 58 F: 64	M: 856 F: 127
Multiple (in m-f) of human AUC at 2.5 mg/day*	M: 1 1-2.0x F: 7.9-14 4x	M: 18-32x F: 19-36x	M: 259-476x F: 39-71x
Multiple of human oral dose (2.5 mg/day, 0.04 mg/kg) based on mg/m2 BSA	10x	41x	82x

* Human AUC value at 2.5 mg/day: 1 8-3.3 ngxh/mL

Evaluation of tumor findings

Sponsor discussed the tumor incidence in the pooled Subsets, under the assumption that there was an adequate number of survivors in the Subsets taken together. In some instances Sponsor also considered combined incidences in MD and HD groups since there was a sufficient number of animals for analysis in these combined groups.

The statistically significantly increased incidence of hepatocellular adenoma in males (0-2-1-2) (0%-3.7%-1.9%-2.6%) (trend test and LD pairwise test) was dismissed as unrelated to treatment because there was no significant increase in combined adenoma and adenocarcinoma (7-4-1-4). This finding was not statistically significant according to analysis of the data by CDER's Biometrics Reviewer (Cynthia Liu, MA). Historical control data for NMRI mice submitted by Sponsor (average %'s: BM database/2 studies 9%; RITA database/6 studies 8%; published data/18 studies 4%) confirm the lack of significance.

The statistically significant increase in Harderian gland adenoma in LD males (all animals and terminally sacrificed) was dismissed as unrelated to treatment because there was no increase in MD and HD and no significant trend. Reviewer agrees with this conclusion.

The statistically significant increase in lung bronchioalveolar adenoma and/or carcinoma and/or combined lung tumors in the LD females (all and terminally sacrificed) (incidence 15-18%) was dismissed as unrelated to treatment since there was no increase in MD or HD or (MD+HD)-combined females and no significant trend. This finding was not statistically significant according to analysis by CDER's Biometrics Reviewer. Control data from Study J15 (e.g. control incidence of 18% for pulmonary adenoma in all animals and of 39% for combined adenoma/carcinoma in terminally sacrificed) confirm the lack of significance. Additional historical control data submitted by Sponsor included 13%-12%-17% (BM, 3 studies), 10%-10%-12%-14% — database, 4 studies), 8% (published data from 18 studies; range 0-14%). These data indicate no cause for concern.

The uterine hemangiopericytoma in the HD females (0-0-0-1) was statistically significant for terminal animals (trend test). Sponsor dismissed the finding as not relevant biologically because a pairwise test (control-HD) and a trend test with the pooled MD+HD group were not significant. This finding was not statistically significant according to analysis of the data by CDER's Biometrics Reviewer. Historical control data show that this uterine tumor is extremely rare in NMRI mice and has not been diagnosed in historical studies (BM; — database; published data, Bomhard,1993).

Since the significant tumor findings were mainly in the LD groups rather than a dose-related trend, it could be argued that reduced survival in MD and HD group precluded detection of tumors in the higher dose groups. However, the limited difference in survival between LD and MD or HD groups (males) or LD and MD groups (females) does not appear sufficient to explain the absence of an increase in the incidence of the tumors in the MD or HD males or MD females. However, tumors may have been missed in HD females.

In the 90-week drinking water study in mice (J15), a statistically significant positive trend in adrenal subcapsular cell adenoma/carcinoma in female mice was found. The effect was biologically significant at 80 mg/kg/day. The absence of this finding in the current oral gavage study is not incompatible with this observation. The incidence of adrenal subcapsular adenoma type A in the female LD group in this oral gavage study was 3.7%. Although the historical control data are somewhat equivocal, this type of tumor is likely to be rare (see Review of Study J15). Thus, the LD incidence of 3.7% was high. Pairwise statistical test of control vs. LD, however, was negative according to Sponsor.

CONCLUSIONS

- NMRI mice were dosed by oral gavage with ibandronate for 18 months with 5, 20, 40 mg/kg/day (fed *ad libitum*). Dosing resulted in highly variable exposures. At 40 mg/kg/day, average exposure multiples were 259-476x in males and 39-71x in females, based on AUC comparison.
- Sponsor concluded that the test article did not demonstrate oncogenic potential in NMRI mice, dosed by oral gavage with 5, 20, 40 mg/kg/day.
- No statistically significant dose-related increases in tumor incidences were noted in CDER's Statistical Review of the mouse oral gavage study (Reviewer Cynthia Liu, MA, HFD-715).
- Based on historical control data for liver, Harderian gland, lung and uterine tumors from the testing facility (Boehringer Mannheim), the — database and published data for NMRI mice (Bomhard, 1993) and based on the results of CDER's statistical review, Reviewer agrees with the Sponsor that ibandronate did not cause tumors in NMRI mice dosed by oral gavage with 5, 20, 40 mg/kg/day for 18 months.

APPENDIX I

Summary Tables Neoplastic Findings

MALES

Table 8: Pooled data (Subset 0 + Subset 1) - Crude rates of tumor-bearin animals - Males

Global number of tumor-bearing animals

Organ	Neoplastic lesion	C	5 mg/kg	20 mg/kg	40 mg/kg	Trend test
Liver	Hepatocellular adenoma	0	2	1	2	
			*	-	-	*
	Hepatocellular carcinoma	7	2	0	2	
			-	-	-	-
	Hemangiosarcoma	2	0	0	0	
			-	-	-	-
	# animals at risk	149	54	53	76	
Kidneys	Carcinoma	1	0	0	0	
			-	-	-	*
	# animals at risk	149	54	53	76	
Adrenal glands	Subcap. cell adenoma type b	15	8	1	4	
			*	-	-	-
	Subcap. cell adenoma mixed	13	7	3	2	
			-	-	-	-
	Cortical adenoma	19	6	2	6	
			-	-	-	-
	Medullary malignant pheochromocytoma	2	0	0	0	
			-	-	-	-
	Medullary benign pheochromocytoma	2	1	0	0	
			-	-	-	-
	# animals at risk	149	53	53	76	
Lungs	Bronchiolo-alveolar adenoma	23	10	11	13	
			-	-	-	-
	Bronchiolo-alveolar carcinoma	15	9	1	2	
			-	-	-	-
	# animals at risk	149	54	53	76	
Urinary bladder	Transitional cell carcinoma	7	2	0	2	
			-	-	-	-
	# animals at risk	139	54	53	74	

One-sided exact test for an increase (p = 0.05) * p < 0.05 ** p < 0.01 p ≥ 0.05

Whereas multiple tumors (see Table 10) glioneural and multiple tumors of one type and one breast are counted as a single tumor

Global number of tumor-bearing animals

Organ	Neoplastic lesion	C	5 mg/kg	20 mg/kg	40 mg/kg	Trend test
Brain	Benign meningioma	1	0	0	0	
			-	-	-	-
	# animals at risk	139	54	53	76	
Bone	Osteosarcoma	1	0	0	0	
			-	-	-	-
	# animals at risk	149	54	53	76	
Thyroid glands	Follicular cell adenoma	1	1	0	0	
			-	-	-	-
	# animals at risk	140	52	53	73	
Pituitary gland	Adenoma, pars distalis	4	0	0	1	
			-	-	-	-
	Adenoma, pars intermedia	3	0	0	0	
			-	-	-	-
	# animals at risk	134	52	51	75	
Duodenum	Adenocarcinoma	0	0	0	1	
			-	-	-	
	# animals at risk	140	54	53	76	
Harderian glands	Adenoma	11	10	5	4	
			*	-	-	-
	Adenocarcinoma	2	0	0	0	
			-	-	-	-
	# animals at risk	140	54	53	76	
Pancreas	Benign islet cell tumor	1	0	0	0	
			-	-	-	-
	# animals at risk	140	54	53	76	
Prostate gland	Adenoma	1	0	0	0	
			-	-	-	-
	# animals at risk	140	54	53	76	
Seminal vesicles	Granular cell tumor, malignant	1	0	0	0	
			-	-	-	-
	Adenocarcinoma	0	0	0	1	
			-	-	-	-
	# animals at risk	140	54	53	76	

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Global number of tumor-bearing animals

Organ	Neoplastic lesion	C	5 mg/kg	20 mg/kg	40 mg/kg	Trend test
Abdominal cavity	Hemangiosarcoma	1	0	0	0	
			-	-	-	-
	# animals at risk	140	54	53	76	
Epididymis	Benign granular cell tumor	1	0	0	0	
			-	-	-	-
	# animals at risk	140	54	53	76	
Lymphoreticular/ Hematopoietic system	Malignant lymphoma	7	2	0	3	
			-	-	-	-
	# animals at risk[2]	140	54	53	76	
Phagocytic system	Histocytic sarcoma	1	0	1	0	
			-	-	-	-
	# animals at risk[2]	140	54	53	76	

FEMALES

Table 11: Pooled data (Subset 0 + Subset 1) - Crude rates of tumor-bearing animals - Females

Global number of tumor-bearing animals

Organ	Neoplastic lesion	C	5 mg/kg	20 mg/kg	40 mg/kg	Trend test
Liver	Hepatocellular carcinoma	3	0	0	0	
			-	-	-	-
	Hepatoblastoma	0	1	0	0	
			-	-	-	-
	# animals at risk	137	54	51	62	
Pancreas	Ductal cell adenoma	0	0	0	1	
			-	-	-	-
	# animals at risk	137	53	51	62	
Adrenal glands	Subcap. cell adenoma mixed	4	0	2	0	
			-	-	-	-
	Subcap. cell adenoma type a	1	2	0	0	
			-	-	-	-
	Subcap. cell adenoma type b	3	1	0	0	
			-	-	-	-
	Cortical adenoma	1	1	0	1	
			-	-	-	-
	Benign medullary tumor	1	0	0	0	
			-	-	-	-
	# animals at risk	136	54	51	62	
Lungs	Bronchiolo-alveolar adenoma	7	3	4	7	
			-	-	-	-
	Bronchiolo-alveolar carcinoma	14	3	3	0	
			-	-	-	-
	Benign mesothelioma	1	0	0	0	
			-	-	-	-
	# animals at risk	137	54	51	62	
Thymus	Benign thymoma	1	0	0	0	
			-	-	-	-
	Malignant thymoma	2	1	0	0	
			-	-	-	-
	# animals at risk	133	54	50	58	
Stomach	Squamous cell papilloma	0	0	1	0	
			-	-	-	-
	# animals at risk	137	54	51	62	

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Global number of tumor-bearing animals

Organ	Neoplastic lesion	C	5 mg/kg	20 mg/kg	40 mg/kg	Trend test
Colon	Adenocarcinoma	0	1	0	0	
			-	-	-	-
	# animals at risk	137	54	51	62	
Brain	Malignant meningioma	1	0	0	0	
			-	-	-	-
	# animals at risk	137	54	51	62	
Skin	Basal cell carcinoma	0	0	1	0	
			-	-	-	-
	Malignant schwannoma	0	1	0	0	
			-	-	-	-
	# animals at risk	136	54	51	62	
Thyroid gland	Follicular cell adenoma	4	1	0	0	
			-	-	-	-
	# animals at risk	136	54	51	62	
Ovaries	Tubulostromal adenoma	25	7	4	3	
			-	-	-	-
	Tubulostromal carcinoma	5	3	0	0	
			-	-	-	-
	Benign granulosa cell tumor	9	1	0	0	
			-	-	-	-
	Malignant granulosa cell tumor	2	2	1	1	
			-	-	-	-
	Benign luteoma	4	2	0	0	
			-	-	-	-
	Benign sertoli cell tumor	2	1	0	1	
			-	-	-	-
	Malignant sertoli cell tumor	0	1	0	0	
			-	-	-	-
	Cystadenocarcinoma	1	1	0	0	
			-	-	-	-
	Cystadenoma	4	0	0	0	
			-	-	-	-
	# animals at risk	136	53	50	61	

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Global number of tumor-bearing animals

Organ	Neoplastic lesion	C	5 mg/kg	20 mg/kg	40 mg/kg	Trend test
Mammary gland	Adenocarcinoma	1	0	0	0	
			-	-	-	-
	# animals at risk	136	54	51	62	
Uterus	Hemangiosarcoma	0	0	1	0	
			-	-	-	-
	Benign hemangiopericytoma	0	0	0	1	
			-	-	-	-
	Benign granular cell tumor	0	0	0	1	
			-	-	-	-
	Leiomyoma	1	0	0	0	
			-	-	-	-
	Stromal polyp	4	4	2	2	
			-	-	-	-
	# animals at risk	137	54	51	62	
Clitoral gland	Adenoma	1	0	0	0	
			-	-	-	-
	# animals at risk[a]	137	54	51	62	
Pituitary gland	Adenoma, pars distalis	28	9	10	1	
			-	-	-	-
	# animals at risk	135	53	49	59	
Harderian gland	Adenoma	11	1	2	2	
			-	-	-	-
	Adenocarcinoma	1	0	0	0	
			-	-	-	-
	# animals at risk	137	54	50	62	
Lymphoreticular/ Hematopoietic system	Malignant lymphoma	25	8	6	2	
			-	-	-	-
	# animals at risk[a]	137	54	51	62	
Phagocytic system	Histiocytic sarcoma	7	2	2	1	
			-	-	-	-
	# animals at risk[a]	137	54	51	62	

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CARCINOGENICITY STUDY IN MICE WITH BM 21.0955.Na IN DRINKING WATER

GENERAL

Sponsor:	Roche
Study Number:	BM21.0955CAHVM02
Study Report:	J15
NDA Volumes:	Vols. 77-782
Testing Facility:	Boehringer Mannheim, GmbH (Hoffmann-LaRoche) (Germany)
Study Period:	September 1993-July 1995
QA Report:	Yes
GLP Statement:	Yes
Dose-range-finding studies:	J12,13,11,16 (see Study Summary)

STUDY PROTOCOL

Species/strain:	Mouse (Crl:NMRI/BR, outbreed, SPF)
Number of animals:	100/sex/dose group (Group 0, control) 50/sex/dose/group (Groups 2, 3, 4; LD, MD, HD)
Doses:	0, 5, 20, 80 mg/kg/day (drinking water)
Age at start of study:	6 weeks
Weight at start of study:	32.1g (males), 26.5g (females)
Study Duration:	609-631 days (87-90 weeks). Study was planned for 90 weeks.
Animal housing:	1/cage
Animal diet:	[redacted] pellets, and deionized water, both ad libitum.
Drug Name:	BM 21.0955.Na. This old name (without H_2O) corresponds to the new name, BM 21.0955.Na.H_2O (ibandronate, monosodium salt, monohydrate)
Drug Batch Number(s):	450 459-00 (1 mg free acid = 1.125 mg weighed BM 21.0955.Na.H_2O)
Drug Stability:	Stable (expiry date: August 1996)
Dosage form:	Solution in distilled water
Dosing route:	Drinking water (amount and concentration in drinking water canister was determined based on water consumption in previous week)
Dosing frequency:	Continuously, 7 days/week, solution given once weekly
Dose Volume:	N/A
Vehicle:	Deionized water

Dose Groups:

Group	Designation	N/sex/group	Dose (mg/kg/day)	Dose (free acid equivalents) (mg/kg/day
0*	Control	100	0	0
2	LD	50	5.6	5
3	MD	50	22.5	20
4	HD	50	90	80

*double-sized group

Relation to Clinical Use:	Recommended dose: 2.5 mg daily, orally (tablet)
CAC Concurrence:	Sponsor's dose selection was based on results from four 3-month studies (DRF Study J12 with doses 0, 10, 20, 30, 40, 50; DRF Study J13 with doses 50, 100, 200, 300, 400, 800 mg/kg/day; pH Study J11 with doses 0, 200, 400 mg/kg/day; TK Study J16 with doses 0, 10, 30, 50 mg/kg/day). Concurrence of the Exec CAC with the dose selection was not obtained.

	However, the Division told the Sponsor in a telephone conversation (Sept 13, 1996) that there would probably be enough information from the gavage and drinking water mouse studies together (which were both ongoing at the time) to allow a reasonable evaluation of carcinogenic potential.
Interim Sacrifices:	None
Clinical Observations:	At least once daily, and recorded weekly. Also weekly palpation for tissue masses.
Mortality:	At least once daily
Body Weight:	Weekly
Food Consumption:	Weekly
Water Consumption:	Weekly
Clinical Pathology:	At scheduled sacrifice, blood samples were obtained from all animals. Red cell morphology and differential WBC and a complete set of hematology parameters and clinical biochemistry were determined for all animals. Data are listed for Week 88, irrespective of real date of death. Serum Na, K, Cl were not determined.
Toxicokinetics:	(TK Study #TV 371) Concentration of test substance was determined in serum — . The [redacted] was carried out with standards prepared in serum, and the quantification limit was [redacted]
Test substance in serum:	At scheduled sacrifice, blood samples were obtained from 12/sex/group/time point between hours 0800 and 1100 ("point samples") from non-fasted animals. Values do not represent Cmax (animals peak at different times several times daily depending on when they drink water). AUC was not determined.
Macroscopic examination:	All animals were necropsied, including those that died or were killed moribund, and macroscopic abnormalities were recorded. Scheduled necropsies were carried out on Days 609-631 for controls, LD, MD, HD. Tissues were collected and fixed as listed in the histopathology inventory.
Organ weights:	Organs weighed at scheduled sacrifice: brain, heart, kidneys, liver, spleen.
Histopathology:	Tissue samples were processed from all animals (scheduled kill, moribund kill, found dead), from all organs and tissues listed in the histopathology inventory (appended). Findings were listed separately as nonneoplastic and neoplastic lesions. Histopathology summary tables were provided for all animals combined (early death and terminal sacrifice).
Statistical Evaluation:	In-life phase: Tests used for analysis of body weight, food consumption, hematology, clinical chemistry and organ weights: Kruskal-Wallis Anova and Mann-Whitney U-tests
Biostatistical evaluation:	For target endpoints, i.e., mortality and tumor findings, trend tests for an increase in dose-response relationship were carried out in accordance with EC, US, and FDA guidelines. Tests performed were for crude tumor rates (all animals, early deaths, and terminal sacrifices), time-adjusted analysis of fatal, incidental and clinically observable tumors, and tests for tumor combinations and metastasing tumors. Pairwise tests of tumor incidences in control vs. dosed group were also carried out. Concomitant endpoints were analyzed by other trend tests.
CDER statistical evaluation:	See CDER Biometrics Review. There was no statistical evaluation of clinical pathology or non-neoplastic findings.

Mouse Carcinogenicity Study
Drinking water (J15)

Histopathology Inventory

Species: Rat	Pathology	
	Tissues retained and examined in all animals (per protocol)	Tissues collected but not examined
Gross lesion, masses, tumors	X	
Adrenals	X	
Aorta	X	
Bone (sternum)	X	
Bone (femur)	X	
Bone (vertebra)	X	
Bone marrow	X	
Brain	X	
Cecum	X	
Colon	X	
Duodenum	X	
Epididymides	X	
Eyes with optic nerves	X	
Gall bladder	X	
Harderian gland	X	
Head	X	
Heart	X	
Ileum	X	
Jejunum	X	
Kidneys	X	
Lacrimal gland	X	
Larynx	X	
Liver	X	
Lung and bronchus	X	
Lymph nodes, mesenteric	X	
Lymph nodes, mandibular	X	
Oesophagus	X	
Ovaries	X	
Pancreas	X	
Pituitary gland	X	
Parathyroids	X	
Prostate gland	X	
Rectum	X	
Salivary gland (mandibular, parotid)	X	
Sciatic nerve	X	
Seminal vesicle	X	
Skeletal muscle (hindleg)	X	
Skin and mammary gland	X	
Spinal cord	X	
Spleen	X	
Stomach	X	
Testes	X	
Thymus	X	
Thyroid	X	
Tongue	X	
Trachea	X	
Urinary bladder	X	
Uterus	X	
Vagina	X	

RESULTS

Drug intake

Drug intake varied upon amount water consumed, which could not be exactly predicted.

Actual drug intake (average over 88 weeks):

				MALES	FEMALES
Group		Intended dose (mg/kg/day)	N	Actual dose (mg/kg/day)	Actual dose (mg/kg/day)
0	Control	0	100	-	-
2	LD	5	50	5.0 (3 7-5 9)	5.1 (3 9-7 0)
3	MD	20	50	19.9 (15 3-27 1)	20.3 (15.6-25.8)
4	HD	80	50	78.8 (40-111)	81.1 (56-117)

Drug concentrations in the drinking water:

				MALES	FEMALES
Group		Intended dose (mg/kg/day)	N	Concentration in drinking water	Concentration in drinking water
0	Control	0	100	-	-
2	LD	5	50	0 01-0 025 mg/ml	0 01-0.017 mg/ml
3	MD	20	50	0.05-0.1 mg/ml	0 035-0.065mg/ml
4	HD	80	50	0 2-0.4 mg/ml (0.02%-0.04%)	0 2 mg/ml (0 02 %)

Mortality/Survival

Decreased mortality occurred in all treated. However, effect was not statistically significant in m or f.

SURVIVAL – Analysis of all animals at end of study (Wk 88)

				MALES	FEMALES
Group		Dose (mg/kg/day)		Number (%)	Number (%)
0	Control	0	100	73 (73%)	50 (50%)
2	LD	5	50	43 (86%)	32 (64%)
3	MD	20	50	41 (82%)	30 (60%)
4	HD	80	80	40 (80%)	33 (66%)

Clinical observations

No drug-related effects

Body weight

No drug-related effects

Body weight (gr) at end of study (Week 88)

			MALES	FEMALES
Group		Dose (mg/kg/day)	Body weight (g)	Body weight (g)
1	Control	0	47.3	41.0
2	LD	3	47.1	42.8
3	MD	7	47.3	41 7
4	HD	15	46.9	41.0

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

Food consumption:

In some weeks, values slightly lower in LD, MD, or HD males; no effect in females

Water consumption:
Significantly increased up to ca. 30% during several weeks in the first part of the study (wks 1-33), in males and females; effect declining as study progressed

Hematology
No significant effects

Clinical chemistry
Small decrease in serum ALP, m and f, all dose groups. Decrease in serum Ca in females. Ca and P values remained within normal range. No data on serum Na, K, Cl.

Clinical chemistry at end of study (wk 88)

			MALES			FEMALES		
Group		Dose (mg/kg /day)	Alk Phos (U/L)	P (mmol/l)	Ca (mmol/l)	Alk Phos (U/L)	P (mmol/l)	Ca (mmol/l)
0	Ctrl	0	120	3 2	2.4	252	2.8	2 6
2	LD	3	110	3.1	2.4	102	2.7	2.5
3	MD	7	87	3.1	2.5	76*	3 0	2.3**
4	HD	15	91	3.1	2.4	68*	2 6	2 4*

*p<0.05, ** p<0.01

Toxicokinetics:
Data from a 3-month drinking water study, at 50 mg/kg/day (J16):

Cmax and AUC values (Day 99)

	Males	Females
Dose (mg/kg)	50 mkd	50 mkd
Cmax (ng/mL)	15.5	24.6
AUC (ngxh/ml)	281	449

Multiples of human Cmax and AUC values (at 2.5 mg/day)* in 3-mo study

	Males	Females
Dose (mg/kg)	50	50
Cmax multiple	19 4x	30 8x
AUC multiple	100x	160x

*Human Cmax at 2.5 mg/day orally = 0.8 ng/mL (BM Report N7) (Tmax=0 9h)
*Human AUC at 2.5 mg/day orally = 2.8 ngxh/ml (BM Report N7).

Data from this carcinogenicity study:
Plasma concentrations Cpl @ 8-11h am (Wk 88) ("point levels")

		Males			Females		
Group		LD	MD	HD	LD	MD	HD
Dose (mg/kg)		5	20	80	5	20	80
Cpl (ng/mL) (N=12/s/g)	Mean	0.2	0.8	2.3	0.1	1.2	3.6
	SD	0.4	0.7	1.6	0.3	1.4	2.8
Cpl (ng/mL) (N=12/s/g)	Range						

Multiples of human Cmax values (at 2.5 mg/day)* of Cpl values in 90-wk study

	Males			Females		
Group	LD	MD	HD	LD	MD	HD
Dose (mg/kg)	5	20	80	5	20	80
Mean Cpl multiple	0.23x	0 98x	2.83x	0.14x	1.45x	4 55x
Highest Cpl	1.2x	2.8x	8.3x	1.2x	6.8x	19x

multiple						

*Human Cmax at 2.5 mg/day orally = 0.8 ng/mL (BM Report N7) (Tmax=0.9h)
*Human AUC at 2.5 mg/day orally = 2.8 ngxh/ml (BM Report N7).

The Cmax levels and multiples achieved in the drinking water study are unclear, but were probably much higher than those of the mean CpI "point levels", and in the range of those attained in animals with the highest CpI point levels. The AUC multiples achieved are not clear either. Based on highest CpI multiples they were probably in the order of those attained in the 3-month study with 50 mg/kg/day (100-160x), i.e., 160-250x at the HD of 80 mg/kg/day (based on human AUC of 2.8 ngxh/mL). In both studies male levels were lower than females.

Macroscopic findings
Slight increase in gaseous distension of small intestine in HD males, and lung edema/hemorrhage in MD and HD males. Minimal increase in kidney enlargement in HD males and females. No other significant treatment-related effects.

		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
Dose		0	5	20	80		0	5	20	80
N examined		100	50	50	50		100	50	50	50
Small intestine	Tympania (distension)	3	0	0	4		4	0	0	2
Lung	Edema and hemorrhage parenchymatous	2	1	2	2		7	3	3	0
Kidney	Enlargement	1	1	0	2		1	0	0	2

Organ Weights:
No treatment effects

Histopathology

NON-NEOPLASTIC FINDINGS
Changes in: Bone and hematopoietic system (all treated, both sexes), and larynx/trachea (HD males).
Effects included reduction of fibrous osteodystrophy/osteoporosis (increased osteoclast activity, bone thinning, and collagen deposition), increase in trabecular bone within marrow cavity, increase in extramedullary hematopoiesis in the spleen and liver (and lymph nodes), and irritation of the larynx and trachea. All these effects were dose related in terms of incidence and/or severity, and statistically significant (trend test).

Non-neoplastic findings with dose-dependent degree

		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
Dose		0	5	20	80		0	5	20	80
N examined		100	50	50	50		100	50	50	50
Bone	Trabecular extension									
	Minimal	1	26	17	0		0	0	0	0
	Slight	0	5	27	18		2	4	0	0
	Moderate	0	0	4	30		0	15	2	0
	Marked	0	0	0	2		0	21	33	14
	Severe	0	0	0	0		0	6	15	36
	TOTAL	1	31	48	50		2	46	50	50
	Fibrous osteodystrophy/osteoporosis*									
	TOTAL	13	4	1	0		63	19	10	0
Spleen	Extramedullary									

	hematopoiesis									
	Minimal	13	7	2	1		12	4	2	0
	Slight-Moderate	66	21	32	25		55	21	19	18
	Marked-Severe	18	11	16	22		21	22	26	32
	TOTAL	97	49	50	48		88	47	47	50
Larynx	Ulceration, focal	0	0	0	1					
	Inflammatory cells in lumen	0	0	0	1					
	Laryngitis	0	0	0	2					
Trachea	Tracheitis	1	0	2	8		0	0	0	1
	Submucosal fibrosis	0	0	0	4					
	Squamous metaplasia	0	0	0	1					
	Fluid in lumen	0	0	0	1					
	Inflammatory cells, lumen	0	0	0	0		0	0	1	2
Lung	Inhalation pneumonia	0	0	0	1					

* Finding of higher seventy in controls than in treated

Adrenal subcapsular cell hyperplasia was observed at similar incidences and severity in control and treated male groups. However, there was a small increase in incidence and severity of the finding in treated females (% incidence of marked plus severe hyperplasia:2%-2%-6%-6%)

Adrenal subcapsular cell hyperplasia

		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
Dose		0	5	20	80		0	5	20	80
N examined		100	50	50	50		100	50	50	50
Adrenal	Subcapsular cell hyperplasia									
	Minimal	9	3	6	3		3	3	7	3
	Slight	21	17	10	10		38	20	17	13
	Moderate	25	13	10	15		50	26	22	30
	Marked	10	4	6	2		2	1	2	3
	Severe	6	6	1	2		0	0	1	0
	TOTAL	71	43	33	32		93	50	49	49

NEOPLASTIC FINDINGS (Appendix I: Summary Tables)

NOTE: In all tables below "*N at risk*" is the number of animals for which tissue could be evaluated, i.e., $N_{risk}=N_{grp}-N_{tissue\ missing}-N_{autolysis}-N_{not\ hit\ in\ section}-N_{died\ before\ first\ tumor}$ (Gart JJ et al, IARC Sci.Pubb. No.79, 1986, Lyon)

SINGLE NEOPLAMS
Early deaths + terminal sacrifice: crude rates of tumor-bearing animals

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
Dose (mg/kg)		0	5	20	80		0	5	20	80	
N (total)		100	50	50	50		100	50	50	50	
Pituitary	Adenoma, pars intermedia	0	0	0	2	*					
		(0%)	(0%)	(0%)	(4.1%)						
Adrenal gland	Subcapsular cell adenoma, type A						0	1	1	3*	*
							(0%)	(2%)	(2%)	(6%)*	
	Subcapsular cell adenoma, type B						0	0	0	1	
							(0%)	(0%)	(0%)	(2%)	
	Subcapsular cell adenocarcinoma						0	0	1	0	

							(0%)	(0%)	(2%)	(0%)	
Lungs	Pulmonary adenoma						18	11	6	12	
							(18%)	(22%)	(12%)	(24%)	
	N at risk	*99*	*50*	*50*	*49*		*99*	*50*	*50*	*50*	

*=p<0.05 (pairwise X^2 test; or Armitage's trend test)
(B) = benign; (M) = malignant

SINGLE NEOPLASMS
Early deaths only: crude rates of tumor-bearing animals

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
N (total)		100	50	50	50		100	50	50	50	
Adrenal gland	Subcapsular cell adenoma, type A						0	1	0	0	
								(5.6%)			
Lungs	Pulmonary adenoma						2	0	1	3	*
							(4%)	(0%)	(5%)	(18%)	
	N at risk						*50*	*18*	*20*	*17*	

SINGLE NEOPLASMS
Terminal sacrifice only: crude rates of tumor-bearing animals

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
N (total)		100	50	50	50		100	50	50	50	
Pituitary	Adenoma, pars intermedia	0	0	0	2	*					
		(0%)	(0%)	(0%)	(5%)						
Adrenal gland	Subcapsular cell adenoma, type A						0	0	1	3*	**
							(0%)	(0%)	(3.3%)	(9%)*	
	Subcapsular cell adenoma, type B						0	0	0	1	
							(0%)	(0%)	(0%)	(3%)	
	Cortical adenoma						1	0	0	0	
Lungs	Pulmonary adenoma						16	11	5	9	
							(32%)	(34%)	(17%)	(27%)	
	N at risk	*73*	*43*	*41*	*40*		*50*	*32*	*30*	*33*	

NEOPLASM COMBINATIONS
Combined analysis of similar tumor sites (crude rates in early deaths and terminally sacrificed)

		Males					Females				
Group		Ctrl	LD	MD	HD	Trend test	Ctrl	LD	MD	HD	Trend test
All animals											
Adrenal gland	Subcapsular cell adenoma type A and B						0	1	1	4	**
							(0%)	(2%)	(2%)	(8%)	
	Subcapsular cell, adenoma type A and B and adenocarcinoma						0	1	2	4	NA