							(0%)	(2%)	(4%)	(8%)	
	N at risk						*99*	*50*	*50*	*50*	

*<p<0.05, **p<0.01
NA = not analyzed

Time-adjusted analysis of tumor rates (fatal, incidental, and selected combined fatal and incidental tumor) revealed statistical significance for adrenal gland subcapsular adenoma type A in females, and for pituitary gland adenoma, pars intermedia, in males. There were no statistically significant findings in time-adjusted analysis of clinically observable and metastasizing tumors.

SUMMARY AND EVALUATION

This drinking water study was performed as an additional study to avoid respiratory irritation as observed in the oral gavage study. Treatment for 90 weeks of NMRI mice with ibandronate (5, 20, 80 mg/kg/day) in the drinking water caused

- decreases in serum ALKP in all treated and decrease in serum Ca in MD, HD females
- small increase in gaseous distension of small intestine in HD males
- small increase in lung edema/hemorrhage in MD and HD males
- minimal increase in kidney enlargement in HD males and females.
- bone trabecular extension within marrow cavity due to inhibition of bone resorption (pharmacodynamic effect of drug), in all treated males and females
- secondary effect to bone extension: spleen increased extramedullary hematopoiesis
- slight incidence of laryngitis in HD males, and of tracheitis and tracheal fibrosis in MD and HD (mainly males)

Dose selection

The HD was selected based on four dose finding studies, including two 3-month toxicity studies (doses up to 50, 800 mkd) (J12,J13). Based on respiratory tract findings in these studies (lung hemorrhage/edema) and GI toxicity, a dose of 80 mg/kg/day was selected as high dose for the main study. Adjustment of pH of the drinking water to 6-6.5 had not proved helpful to prevent respiratory irritation (J11). A TK study (50 mg/kg/day) (J16) was performed to demonstrate exposure to test compound.

The data on drug levels and respiratory tract toxicity in gavage and drinking water studies indicate that a dose higher than 80 mg/kg/day (levels > 0.04% in the dosing solution) would probably have led to irritation of the respiratory tract possible with associated mortality. By comparison, drug concentration in the oral gavage LD solution was 0.05%. Reviewer agrees with the Sponsor with regard to this speculation. However, the minimal drug-related toxicities (serum chemistry in m and f, and GI and respiratory tract lesions in males) in the current drinking water study indicate that the MTD had not been reached and a higher HD (by a factor of e.g 2x) would have been more appropriate. Based on exposure multiples, however, the high dose in the drinking water study is acceptable (see below).
In the 3-month dose range finding study J13 (50, 100, 200, 400, 800 mg/kg) the NOAEL for mortality was 200 mg/kg for males and 300 mg/kg for females. NOAEL for respiratory disturbances was 50 mg/kg for males, and 100 mg/kg for females. These findings were the basis of the 80 mg/kg/day high dose selection for the main study.

It was concluded in 1996 by the Pharm/Tox reviewer that although the mouse studies individually were suboptimal, sufficient information would probably be obtained from both mouse studies taken together to allow a reasonable evaluation of carcinogenic potential. The Sponsor was informed of this conclusion (Sept 13, 1996). Sponsor was also informed that for the drinking water study to be considered valid, appropriate exposure or toxicity must be demonstrated (April 25, 1996; FAX).

Exposure

Cmax and AUC data from a previous 3-mo study with 50 mg/kg/day and the values of the highest Cpl levels measured in the current 90-wk study were used to calculate AUC multiples.

Comparison of exposure and toxicity data from the gavage and drinking water carcinogenicity studies indicates that the respiratory tract toxicity observed in the gavage study (lung edema/hemorrhage) at 20 and 40 mg/kg/day was probably due to drug aspiration rather than systemic toxicity. Similar exposure in the drinking water study did not cause these types of severe lung lesions and death. It should be noted, however, that serum levels were highly variable and there is a fair amount of inaccuracy in the exposures and exposure multiples.

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

	Oral gavage study				Drinking water study			
Group	LD	MD	HD		LD	MD	(HD, Study J16)	HD
Doses (mg/kg/day)	5	20	40		5	20	(50)**	80
Concentration of drug in dosing solution	0 05%	0.2%	0.4%		0.001%-0.0025%	0.0035%-0.01%	-	0.02%-0 04%
Survival (m-f)*	100%-88%	67%-69%	52%-27%		118%-128%	112%-120%	-	109%-132%
AUC (ngxh/ml)	3 6m-26f	58m-64f	856m-127f				(281m-449f)**	
Multiple (in m-f) of human AUC at 2.5 mg/day***	1.3x-9.4x	21x-23x	305x-45x		(10x-16x)**	(40x-64x)**	(100x-160x)**	(160x-250x)**
Multiple (in m-f) of human oral dose (2.5 mg/day, 0.04 mg/kg) based on mg/m2 BSA	10x	41x	82x		10x	41x	-	164x

* survival relative to controls
**Data from 3-month study @ 50 mg/kg/day (J16), or extrapolated from this study
*** Human AUC value at 2.5 mg/day used for this calculation: 2 8 ngxh/mL (BM report N7)

NOTE: Comparison on the basis of mg/m2 BSA comparison is surprisingly close to comparison based on AUC Since the mice in both the oral gavage and drinking water studies were fed ad libitum and the human AUC data are for the fasted state, one would expect calculations based on BSA comparison to have yielded higher multiples than those based on AUC. Possibly, bioavailability in the mice is larger than in humans, or in mice feeding does not interfere to a large extent with absorption.

Drinking water study exposure multiples

For the drinking water study, 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 in drinking water study (extrapolated values)

Group	LD	MD	HD	HD, Study J16**
Doses (mg/kg/day)	5	20	80	50**
AUC (ngxh/ml)	-	-	-	M: 281 F: 449
AUC (ngxh/mL) Extrapolated	M: 28 F: 45	M: 113 F: 180	M: 450 F: 718	-
Multiple (in m-f) of human AUC*	M: 8.5-16x F: 14-25x	M: 34-62x F: 54-100x	M:136-250x F: 218-398x	M: 85-156x F: 136-249x
Multiple (in m-f) of human oral dose (2.5 mg/day, 0.04 mg/kg) based on mg/m2 BSA	10x	41x	164x	103x

*Based on extrapolated animal AUC, and human AUC value of 1.8-3.3 ngxh/mL (@2 5 mg/day)

Evaluation of tumor findings

According to Sponsor's analysis, two tumor findings appeared to be significant when considering all animals (early death and terminal sacrifice): adenoma of the pars intermedia of the pituitary gland in males (incidence 0-0-0-2), and adrenal subcapsular adenomas (type A and type A/B combined) in females (Type A: incidence 0-1-1-3; Type A/B combined 0-1-1-4). Pulmonary adenomas were significant in females (incidence 2-0-1-3) in the early death group only. Historical control data are appended to this study review.

Pituitary adenoma

Analysis of the data by CDER's Biometrics Reviewer showed that the pituitary adenoma finding in males was not statistically significant. Sponsor dismissed the pituitary tumors in males as unrelated to treatment since this type of tumor was observed in control males in the oral gavage study with ibandronate (Study J14; Incidence 3/134, or 2.2%). Historical control data for the pars intermedia from Boehringer Mannheim (BM) and — databases (see below) showed that the incidence of 4% in the HD males was above the historical incidence of 0%. However, in studies in which no distinction was made between pars intermedia and pars distalis, adenoma incidence ranged from 0-8% (BM and published data, Bombard 1993). Taken together, Reviewer feels that the finding is not significant.

Adrenal subcapsular tumors

Sponsor dismissed the adrenal subcapsular adenomas in females based on the lack of an effect in male mice and the fact that in the gavage study the incidence of adrenal subcapsular adenomas (Type A, B, and "mixed" combined) in female terminal sacrifice groups was significantly lower in mid and high dose groups than in controls (8/103-3/38-1/29-0/17; i.e. 8%-7.9%-3.6%-0%) (Study J14). Reviewer feels this argument is not relevant.

According to the data analysis by CDER's Biometrics Reviewer the adrenal subcapsular cell adenomas (type A, type A/B combined and typeA/B/adenocarcinoma combined) were statistically significant findings based on the concurrent control incidences of both types of adenoma and adenocarcinoma of 0/100 (i.e., assuming these are rare tumors). The control incidences in the current drinking water study J15 and the concomitant oral gavage study J14 (0.7% for type A, 2.1% for type B) suggest that type A is a rare tumor, while type B a common one. The slightly increased incidence and severity of adrenal subcortical cell hyperplasia in females supports the significance of the adrenal tumor finding in the current study (J15).

Historical data were submitted by the Sponsor (February 21, 2003) from Boehringer Mannheim (BM), the — data base, and published data on NMRI mice (Bomhard, 1993). Data from the — database were for subcapsular tumors without a distinction between type A or type B —. Data from BM and Bomhard were for adrenal cortical tumors, because the distinction between cortical and subcapsular is not always made. However, Sponsor provided information that subcapsular cell adenoma (or adenocarcinoma) is histologically distinct from cortical adenoma ("International Classification of Rodent Tumors, The Mouse" (2001). The distinction was made by BM in the current drinking water study J15 and in gavage Study J14, but apparently not in previous studies. There was no information whether the historical control data were from gavage or drinking water or other dose routes.

The most relevant historical control data are those for subcapsular cell adenoma and adenocarcinoma from the — database. Also relevant are the data from the mouse carcinogenicity studies carried out by the Sponsor (J14, J15). Although the — data suggest that adrenal subcapsular adenoma is not a rare tumor (average incidence 3.3%, range 1.7-8.2%, 4 studies) there was no distinction in that database between type A and type B. Reviewer believes that Sponsor's concurrent control data from the drinking water and oral gavage studies are most important (J14, J15). These data suggest that subcapsular adenoma type B is common, while subcapsular adenoma type A is rare. Control incidences in study J15 and J14 for adrenal subcapsular cell adenoma type A were 0% and 0.7%.

Thus, in accordance with the conclusion from CDER Biometrics' statistical review, Reviewer feels that the adrenal subcapsular cell tumor findings (resulting from an increased incidence of subcapsular cell adenoma type A) in the mouse drinking water study are biologically significant.

Pulmonary adenoma

The significant trend for pulmonary adenomas in the early death group was dismissed by Sponsor as unrelated to treatment, because of lack of significance upon pairwise comparison, and lack of significance of the pulmonary adenoma and carcinoma combination (considered most appropriate comparison). Reviewer agrees that the pulmonary adenoma does not seem to be a biologically significant finding due to the size of the effect and the occurrence in the early death group only.

CONCLUSIONS

- NMRI mice were dosed with ibandronate in the drinking water for 90 weeks with 5, 20, 80 mg/kg/day (fed *ad libitum*). These doses resulted in highly variable exposures in the mice of approximately 10-400 times human exposure at the recommended daily oral dose of 2.5 mg/day (AUC 1.8-3.3 ngxh/mL).
- Sponsor concluded that there was no oncogenic effect of ibandronate in mice when administered in the drinking water at doses up to 80 mg/kg/day.
- According to the CDER's Biometrics Review the adrenal subcapsular adenomas (type A, type A/B combined and typeA/B/adenocarcinoma combined) were statistically significant findings based on the concurrent control incidences of subcapsular adenoma and adenocarcinoma of 0/100 (0%) each.
- Based on historical and concurrent control data and CDER Biometrics' statistical analysis, Reviewer concludes that in the female NMRI mouse dosed in the drinking water for 90 weeks, ibandronate induces a biologically significant increase in the incidence of adrenal subcapsular adenoma (type A), adrenal subcapsular adenoma type A and type B (combined), and adrenal subcapsular adenoma type A and type B and adenocarcinoma (combined).
- The adrenal subcapsular cell tumor finding needs to be included in the label. The finding is significant for the HD group (80 mg/kg/day). At this dose average exposure in female mice is approximately 718 ngxh/mL, i.e. 218x-398x times human exposure at the recommended daily oral dose of 2.5 mg/day (1.8-3.3 ngxh/mL), based on AUC comparison, and 164x human exposure based on mg/m^2 BSA comparison.

HISTORICAL CONTROL DATA

Pituitary gland, adenoma, pars intermedia (MALES)

		Incidence	Incidence (average) (%)	Incidence (range) (%)
BOEHRINGER MANNHEIM				
Pituitary gland, adenoma, pars intermedia	1 study	0/50 (0%)	0%	0%
Pituitary gland (no anatomic distinction)	2 studies	6/100 (6%) 2/60 (3.3%)	5%	3.3%-6%
NMRI MICE DATABASE (Dec 2002)				
Pituitary gland, adenoma, pars intermedia	6 studies (1989-1996)	0/329	0%	0%-0%
PUBLISHED DATA ON NMRI MICE (Bomhard E, Exp. Toxic Pathol 45, 1993) (breeder who provided mice)				
Pituitary gland (no anatomic distinction)	18 studies (21-month duration)	8/839	1%	0%-8%

Adrenal gland, subcapsular or cortical* adenoma (FEMALES)

		Incidence	Incidence (average) (%)	Incidence (range) (%)
BOEHRINGER MANNHEIM				
Adenoma, cortical*	3 studies	1/100 (1%) 1/48 (2.1%) 0/60 (0%)	0.96%	0%-2.1%
Adenoma, subcapsular cell, type A	Study J14	1/136	0.7%	0.7%
Adenoma, subcapsular cell, type B	Study J14	3/136	2.2%	2.2%
Adenoma, subcapsular cell, mixed	Study J14	4/136	4 4%	4.4%
Adenoma, cortical*	Study J14	1/136	0.7%	0.7%
NMRI MICE DATABASE (Dec 2002)				
Adenoma, subcapsular cell	4 studies (1985-1990)	1/60 (1.7%) 1/50 (2%) 1/50 (2%) 4/49 (8.2%)	3.3%	1.7-8.2%
PUBLISHED DATA ON NMRI MICE (Bomhard E, Exp. Toxic Pathol 45, 1993) (breeder who provided mice)				
Adenoma, cortical*	18 studies	11/866 (1.2%) (5 pos. studies)	1.2%	0%-10%

*Cortical adenoma (or carcinoma) is histologically distinct from subcapsular cell adenoma, but in historical BM studies and in published data (Bomhard, 1993) the distinction was not made. The distinction was made in the ——— database.

Adrenal gland, subcapsular or cortical* adenocarcinoma (FEMALES)

		Incidence	Incidence (average) (%)	Incidence (range) (%)
BOEHRINGER MANNHEIM				
Adenocarcinoma, cortical*	3 studies	1/100 (1%) 0/48 (0%) 0/60 (0%)	0.48%	0%-1%
Adenocarcinoma, subcapsular cell	Study J14	0/136	0%	0%
		0/136	0%	0%
NMRI MICE DATABASE (Dec 2002)				
Adenocarcinoma, subcapsular cell	4 studies (1985-1990)	0/60 (1.7%) 0/50 (2%) 0/50 (2%) 0/49 (8.2%)	0%	0%-0%
PUBLISHED DATA ON NMRI MICE (Bomhard E, Exp. Toxic Pathol 45, 1993) (breeder who provided mice)				

Adenocarcinoma, cortical*	18 studies	3/866 (0.35%) (2 pos studies)	0.35%	0%-4.2%

*Cortical adenoma (or carcinoma) is histologically distinct from subcapsular cell adenoma, but in historical BM studies and in published data (Bomhard, 1993) the distinction was not made. The distinction was made in the ___ database.

Adrenal subcapsular tumor finding in current Study J15

		Incidence (N) Ctrl-LD-MD-HD	Incidence (%) Ctrl-LD-MD-HD	P-value, trend test (CDER)
Adenoma, subcapsular cell, type A	J15	0-1-1-3	0%-2%-2%-6%	0.0228*
Adenoma, subcapsular cell, type B	J15	0-0-0-1	0%-0%-0%-2%	Not sign
Adenocarcinoma, subcapsular cell (no type)	J15	0-0-1-0	0%-0%-2%-0%	Not sign
Combined adenoma type A and type B	J15	0-1-1-4	0%-2%-2%-8%	0 0069*
Combined adenoma type A and B and adenocarcinoma	J15	0-1-2-4	0%-2%-4%-8%	0.0099*
Adenoma, cortical	J15	1-0-1-0		Not sign
Combined adenoma, type A, B, cortical	J15	1-1-2-3		Not tested

* significant according to criterion p<0 025 for rare tumor
NOTE: Cortical adenoma (or carcinoma) is histologically distinct from subcapsular cell adenoma

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APPENDIX I

Summary Tables Neoplastic Findings

MALES

90 WEEK DRINKING WATER CARCINOGENICITY STUDY WITH BM 21.0955.Na H

Table 5: Crude rates of tumor-bearing animals - males

Global number of tumor-bearing animals

Organ	*Neoplastic lesion*	*Control*	*Low dose*	*Mid dose*	*High dose*	*Trend test*
Liver	Hepatocellular adenoma	9	3	6	5	-
	Hepatocellular carcinoma	4	1	1	1	-
	Ito cell Liposarcoma	0	0	0	1	-
	Cholangiocarcinoma	1	0	0	1	-
	Hemangiosarcoma	2	2	1	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Kidneys	Adenoma	0	0	1	1	-
	# animals at risk	*100*	*50*	*50*	*50*	
Adrenal glands	Subcap. cell adenoma type B	9	6	2	1	-
	Subcap. cell adenoma mixed	8	4	6	2	-
	Cortical adenocarcinoma	1	0	0	1	-
	Subcap. cell adenocarcinoma	1	1	0	0	-
	Pheochromocytoma	1	0	0	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Lungs and bronchi	Pulmonary adenoma	33	20	23	16	-
	Pulmonary adenocarcinoma	12	8	5	6	-
	# animals at risk	*100*	*50*	*50*	*50*	
Thymus	Thymoma	0	0	0	1	-
	# animals at risk	*82*	*44*	*37*	*39*	
Stomach, non-glandular	Squamous papilloma	1	0	1	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Ileum	Adenocarcinoma	0	0	1	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Urinary bladder	Transitional cell papilloma	3	0	0	1	-
	Benign mesenchymal tumor	6	4	7	2	-
	Malignant mesenchymal tumor	2	0	1	1	-
	# animals at risk	*100*	*50*	*50*	*50*	

Global number of tumor-bearing animals

Organ	*Neoplastic lesion*	*Control*	*Low dose*	*Mid dose*	*High dose*	*Trend test*
Thyroid glands	Follicular cell adenocarcinoma	1	0	0	0	-
	# animals at risk	*99*	*50*	*50*	*50*	
Pituitary gland	Adenoma, pars distalis	1	1	1	0	-
	Adenoma, pars intermedia	0	0	0	2	+
	Pairwise tests 'control versus doses'		-	-	+	
	# animals at risk	*99*	*50*	*50*	*49*	
Head	Osteosarcoma	1	0	0	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Harderian glands	Adenoma	9	8	6	5	-
	Adenocarcinoma	1	0	0	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Femur with marrow	Hemangioma	1	0	0	0	-
	Hemangiosarcoma	0	1	0	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Epididymides	Interstitial cell adenoma	1	1	0	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Prostate gland	Adenoma	1	1	2	1	-
	# animals at risk	*100*	*49*	*50*	*50*	
Seminal vesicles	Adenoma	0	0	1	0	-
	Adenocarcinoma	1	0	0	0	-
	# animals at risk	*99*	*50*	*50*	*50*	
Hematopoietic tissue	Lymphoma	6	8	3	5	-
	Histiocytic sarcoma	0	0	1	1	-
	Malignant mast cell tumor	1	2	0	0	-
	# animals at risk	*100*	*50*	*50*	*50*	
Abdominal cavity	Hemangiosarcoma	0	1	0	0	?
Preputial gland	Adenoma	0	1	0	0	?
Ear	Sarcoma, NOS	1	0	0	0	?

Cochran-Armitage test for an increase (or a decrease) for p < 0.05

** p < 0.05 ** p < 0.01 p > 0.05*

Without metastases from Table 21

Sarcomas and malignant tumors of one type and one tissue are counted as a single tumor

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FEMALES

Table 8: Crude rates of tumor-bearing animals - females

Global number of tumor-bearing animals

Organ	Neoplastic lesion	Control	Low dose	Mid dose	High dose	Trend test
Liver	Hepatocellular adenoma	2	0	0	0	-
	Hepatocellular carcinoma	0	0	0	1	-
	Hemangioma	1	0	0	0	-
	Hemangiosarcoma	1	0	0	1	-
	# animals at risk	99	50	50	50	
Spleen	Fibroma	1	0	1	0	-
	# animals at risk	97	50	50	50	
Pancreas	Exocrine adenoma	0	1	0	0	-
	# animals at risk	98	50	49	50	
Adrenal glands	Subcap. cell adenoma type A	0	1	1	3	*
	Pairwise test 'control versus doses'		-	-	*	
	Subcap. cell adenoma type B	0	0	0	1	-
	Subcap. cell adenocarcinoma	0	0	1	0	-
	Cortical adenoma	1	0	1	0	
	Pheochromocytoma	1	0	0	2	-
	# animals at risk	99	50	50	50	
Lungs and bronchi	Pulmonary adenoma	18	11	6	12	-
	Pulmonary adenocarcinoma	5	1	1	3	-
	# animals at risk	99	50	50	50	
Thymus	Undifferentiated sarcoma	1	0	0	0	-
	# animals at risk	93	50	46	46	
Stomach, non-glandular	Squamous papilloma	0	1	0	1	-
	# animals at risk	98	50	49	50	
Stomach, glandular	Adenocarcinoma	1	0	0	0	-
	# animals at risk	99	50	49	50	-
Colon	Adenomatous polyp	1	0	0	0	-
	Adenocarcinoma	0	0	0	1	-
	# animals at risk	99	50	50	50	
Urinary bladder	Benign mesenchymal tumor	1	1	0	0	-
	Malignant mesenchymal tumor	0	1	0	0	-
	# animals at risk	97	50	46	50	

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Global number of tumor-bearing animals

Organ	Neoplastic lesion	Control	Low dose	Mid dose	High dose	Trend test
Brain	Astrocytoma	0	0	1	0	-
	# animals at risk	99	50	50	50	
Pituitary gland	Adenoma, pars distalis	31	19	11	18	-
	Adenocarcinoma, pars distalis	1	0	1	0	-
	Adenoma, pars intermedia	0	1	1	0	-
	Neurofibroma	0	0	1	0	-
	# animals at risk	99	50	49	50	
Head	Neuroepithelial carcinoma	1	0	0	0	-
	# animals at risk	99	50	50	50	
Harderian glands	Adenoma	3	4	1	2	-
	Adenocarcinoma	0	0	1	0	-
	# animals at risk	99	50	50	49	
Hematopoietic tissue	Lymphoma	37	18	12	20	-
	Mast cell tumor	0	0	0	1	-
	# animals at risk	99	50	50	50	

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One-sided test for an increase (p = 0.05)

* p < 0.05 ** p < 0.01 - p ≥ 0.05

Without taxonomy (see Table 2.8)

Bilateral and multiple tumors of one type and one tissue are counted as a single tumor

NOTE: ECAC meeting minutes have been appended to this NDA Review

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

BM 21.0955 Na
MALE AND FEMALE FERTILITY STUDY (SEGMENT I) IN THE RAT (ORAL GAVAGE)

Report Nr.	K2 (Segment 1-3)
NDA Vols	79-80
Drug	BM 21.0955.Na (monosodium salt, monohydrate)
Batch Nr	443.848-00 (1 mg free acid =1.145 mg weighed BM21.0955.Na monohydrate).
Test facility	Boehringer Mannheim, GmbH (Hoffmann-LaRoche) (Germany)
Study period	1991
QA/GLP	Yes/Yes
Species/strain	SD rats (Crl:CD BR Sprague Dawley)
N	30/sex/grp at start
Doses	0, 1, 4, 16 mg/kg/day (free acid equivalents)
Route	Oral gavage
Dosing period	PMD-14 to PPD21
Dosage form	Solution in 0.5% carboxymethylcellulose
Dose volume	10 ml/kg
Food/water	pellets, and tap water. Comment: Food was withheld for 4h before and 4h after dosing
Dose selection	Based on oral gavage 6-mo toxicity study with restricted feeding (10, 30 mkd) (Study H1), in which 30 mkd caused mortality and 10 mkd reduced body weight.

	Males	Females
Initial age	6 wks	6 wks
Initial weight	210-240g	160-220g
Dosing period	60 days before start of mating period until confirmed copulation	14 days before mating, throughout mating and gestation until scheduled sacrifice on GD21 (C-section grp), or throughout lactation until PPD21 (spontaneous delivery grp)
Mating period	Up to 21 days	Up to 21 days

Group	I	II	III	IV
Doses (mg/kg/day)	0	1	4	16
N/sex/grp (study start)	30	30	30	30

PROTOCOL

Male and female SD rats (F0 generation), were treated daily, by oral gavage, throughout premating, mating and gestation/lactation. At the end of gestation approximately half of the females (15/grp) were designated for C-section (C-section subgroup). The remaining animals were allowed to give birth (littering subgroup). F0 dams from the C-section group were evaluated (autopsied) on GD21. F1 fetuses from the C-section group were evaluated, with ca. 50% of fetuses examined for skeletal and soft tissue anomalies. F0 dams of littering groups were killed and evaluated after lactation (PPD21). F1 pups from the littering groups were evaluated for development. Of these, 1 male and 1 female F1 animal per litter were selected to be raised to maturity, and mated at ca. 9 weeks of age. The F1 dams were allowed to deliver and their F2 pups were examined and killed 7 days after birth. F1 dams were killed together with F2 pups. All other F1 animals were sacrificed after PPD42 and internal organs examined. Serum levels of drug were not determined.

Definitions:

First day of treatment	Day 0 premating	Day0 p.m , or PMD0
Day with sperm-positive vaginal smear	Day 0 post conception	Day0 p.c., or GD0
Day on which litter found	Day 0 postpartum	Day0 p p., or PPD0

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

RESULTS

F0 MALES

Mortality

F0 males mortality

Group		Ctrl	LD	MD	HD
Doses (mg/kg/day)		0	1	4	16
Mortality due to:	Gavage accident	3	3		1
	Unclear cause			1	
	Total	3	3	1	1

F0 males: body weight premating

Group		Ctrl	LD	MD	HD
Doses (mg/kg/day)		0	1	4	16
Body weight (gr)	At PMD0	340	346	350	345
	At PMD59	553	558	562	556

F0 males clinical signs

In F0 males, there were no clinical signs due to treatment

F0 males autopsy

Animals with gavage accidents had lung emphysema.

F0 FEMALES

Mortality and loss of animals

Group		Ctrl	LD	MD	HD
N		30	30	30	30
Doses (mg/kg/day)		0	1	4	16
Loss of mating partner*		3	3	1	1
Mortality	Death at GD17				1 (no signs)
	Death at GD21-23 (around delivery time)		1	1	3
	Killed in extremis				1 (during delivery, GD22)
Total loss of F0		3	4	2	6

*females were sacrificed when mating partner died

Also, in the littering group, there was one surviving HD dam (#328) with all fetuses not breathing, and one dam (#311) with all pups stillborn. No evidence of other drug-related clinical signs. One HD animal had vaginal discharge on PPD1-2. These effects are believed to be due to

hypocalcemia resulting from suppressed bone resorption and Ca mobilization leading to dystocia and periparturient mortality.

Premating body weight

Group		Ctrl	LD	MD	HD	Trend test
N		30	30	30	30	
Doses (mg/kg/day)		0	1	4	16	
Body weight	At PMD0	245	245	241	250	
	At PMD13	273	274	265	273	

Statistically significant, *p<0.05, **p<0.01

Mating performance:

No effect on days to successful mating

Number of non-pregnant F0-females

Group		Ctrl	LD	MD	HD	
Doses (mg/kg/day)		0	1	4	16	
N	Total	27	27	29	29	
	Pregnant	27	27	27	25	
	Not pregnant	0	0	2	4	**

The significant trend in non-pregnant animals suggests significantly decreased fertility in the HD females.

The pregnant animals were divided between two groups: a C-section and a littering group

Number of animals in two groups

Group		Ctrl	LD	MD	HD	
Doses (mg/kg/day)		0	1	4	16	
Total pregnant	N	27	27	27	25	
Total deaths during gestation	N		1	1	5	
Remaining evaluable	N	27	26-27	26-27	20-25	
C-section group	N assigned	13	14	15	15	
	N evaluated	13	14	15	11-13	
Littering group	N assigned	14	13	14	14	
	N evaluated	14	12-13	11-12	8-12	

Body weight

F0 females body weight during pregnancy

Group		Ctrl	LD	MD	HD	Trend test
N		27	27	27	21-24	
Doses (mg/kg/day)		0	1	4	16	
Body weight	At GD0	281	279	272	278	
	At GD21	465	450	437***	449*	*
	AUC weight gain (GD0-GD21)	1383	1274*	1226**	1272	*
	BW Gain (GD21-GD0)	184	171 (-7%)	165 (-10%)	171 (-7%)	

Statistically significant, * p<0.05, **p<0 01, ***P<0.001

AUC weight gain is the "area-under-the-time-curve" for PMD1 to PMD13 differences to initial body weight. In other words, it is the cumulative body weight difference between PMD1 through PMD13, and PMD0. This is an unusual way of expressing body weight gain and overestimates any effect occurring in the earlier days

(1) C-section group:

Litter data

Treatment effect on: implantation sites (all doses), preimplantation loss (all doses), fetal body weight (HD).

F0 dams (C-section group) : reproductive parameters

Group		Ctrl	LD	MD	HD	Trend test
Doses (mg/kg/day)		0	1	4	16	
N pregnant (evaluated)		13	14	15	10-11	
Corpora lutea		19.8	19 9	19.9	19.8	
Preimplantation lossX100/CL (%)		12.1	20.9	27**	18.4	
Implantation sites (IS)		17 2	15.7	14 5**	16.0	*
Resorptions/IS (%) (postimplantation loss, %)		1.72	1.68	6.83[&]	0.0	
Live fetuses/IS (%)		98	98	93	100	
Fetal weight (m) (gr)		5.4	5.4	5.2	5.0**	**
Fetal weight (f) (gr)		5.1	5.1	4.9	4.8*	*
Uterus weight full (g)		119	110	98**	106**	**

Statistically significant, * p<0.05, **p<0.01
[&]one animal with 60% resorption rate

Comment: Reduced uterine weight reflected reduced fetal weight and #implantation sites.

Fetal anomalies

F1-fetuses parameters: anomalies
(C-section group)

Group		Ctrl	LD	MD	HD	Trend test
N evaluated		13	14	15	11	
Doses (mg/kg/day)		0	1	4	16	
VISCERAL						
Variations (%)		77	81	84	84	
Malformations (%)		0	0.9	8.3*	1.5	
Pathological findings (%)		1.1	3.7	1.0	4.0	
SKELETAL						
Retardation (%)		84	86	81	92	
Variation (%)		24	12	13	14	
Malformation (%)		1 8	1 6	4.5	1.0	

*Due to one animal with a 100% malformation rate (one fetus with enlarged brain ventricle)

F0 Autopsy

F0-females autopsy (N=13-14-15-11)
Lung parenchymic hemorrhage: incidence 0-0-0-2, trend test positive
Supplementary liver lobes increased (incidence 1-4-7-6), trend test positive.

(2) Littering group:

Litter data

Treatment effect on: # corpora lutea (HD), #implantation sites (HD), postimplantation loss (all doses), #live pups and #dead pups (HD), live birth index (pups alive on Day0x100/pups born). These effects were in dams that did not include the ones with total litter loss (#311,#328).

F0 dams (spontaneous delivery group): reproductive parameters (surviving dams)

Group		Ctrl	LD	MD	HD	
N evaluated		14	12-13	11-12	8-12	
Doses (mg/kg/day)		0	1	4	16	
Length of pregnancy	D21	2	3	1	1	
	D22	12	9	10	5	
	D23	0	0	0	2	
Mortality		0/30	1/30	1/30	4/30	

Corpora lutea		19.4	19.3	18	17 1*	*
Preimplantation lossX100/CL (%)		10.6	6 4	9.1	13.6	
Implantation sites (IS)		17.1	18	16 5	14 8*	*
Postimplantation lossX100/IS (%)		4.2	8 9*	7.0	9.1**	*
Live pups/IS (%)		93	89	87	70***	**
Dead pups /IS (%)		2.4	2 4	5 6	19.6	
Live birth index		97 5	97 3	94	79	
F0 mortality at GD21-23 (N/30)		0	1	1	4	

Statistically significant, * p<0 05, **p<0.01, ***P<0 001

Reproductive indices

No effects on viability indexes (pups alive on Day 4,7,14,21x100/pups alive on Day 0,4,7,14) or weaning indexes (pups alive on Day21x100/pups alive on Day0)

Body weight in F0 dams (lactation)

F0 females body weight during lactation

Group		Ctrl	LD	MD	HD	Trend test
N		14	12	11	9-8	
Doses (mg/kg/day)		0	1	4	16	
Body weight	At PPD1	330	329	311	299*	**
	At PPD21	357	356	353	338	
	AUC weight gain (PPD1-21)	451	583	574	608	

Statistically significant, * p<0.05, **p<0.01, ***P<0.001

F0 autopsy

F0 dams autopsy (N=14-12-11-9):
Supplementary liver lobes increased (2-1-6-5), trend test positive
This finding was dismissed by Sponsor as a common variation occurring in up to 50% of (historical) controls

F1 pups

No treatment related externally visible malformations or clinical signs

Body weight gain PPD0-PPD42:
Apparently, body weight after delivery was reduced in LD m and f pups and remained lower than controls throughout the observation period. Body weight gain was reduced in LD pups, and increased in HD pups. The reason for this is unclear.

F1 males body weight during lactation

Group		Ctrl	LD	MD	HD	Trend test
N		14	12	11	9-8	
Doses (mg/kg/day)		0	1	4	16	
Body weight	At PPD0	6 5	6 1*	6.2	6.3	
	At PPD42	211	204	217	224	
	AUC weight gain (PPD7-42)	2745	2623	2838	2988	*

Statistically significant, * p<0.05, **p<0.01, ***P<0.001

F1 females body weight during lactation

Group		Ctrl	LD	MD	HD	Trend test
N		14	12	11	9-8	
Doses		0	1	4	16	

(mg/kg/day)						
Body weight	At PPD0	6.1	5 8*	5 9	6 0	
	At PPD42	171	165	172	180	
	AUC weight gain (PPD7-42)	2448	2321*	2481	2677*	

Statistically significant, * p<0 05, **p<0 01, ***P<0.001

F1 pups observations

Physical development: No treatment effects on pinna detachment, incisor eruption, hair coat development, eye opening, vaginal opening, testicular descent

Neuromuscular (behavior) development: No treatment effects on air righting, grip strength, auditory startle, pupillary reflex. Cliff avoidance was impaired in F1 HD pups on Day 5. This effect appeared to be due to impaired performance in most litters (including one litter with 0% success rate) and seemed to be biologically significant.

		Ctrl	LD	MD	HD	Trend test
N (litters) evaluated		14	12	11	8	
Doses (mg/kg/day)		0	1	4	16	
Cliff avoidance (% of animals reaching criterion)		92.6	95 1	93 8	69 3*	*

*p<0.05

Water maze test: This test included measurement of 3 parameters (training trials, memory test, test for relearning capacity). The relearning capacity was impaired in treated MD, HD animals, with a significant dose-related trend. Finding deemed not biologically significant since effect was not significant as compared to control in any dose group, and since other 2 parameters were normal.

Water maze performance

		Ctrl	LD	MD	HD	Trend test
N (litters) evaluated		14	12	11	8	
Doses (mg/kg/day)		0	1	4	16	
Test for relearning capacity (%) (#negative trials/#total trialsx100 - of all pups per litter)		23 6	23.3	28.2	31 8	*

*p<0.05

This effect appeared due to a slight average increase but there was no change in the range of litter relearning values. It appeared not biologically relevant.

Weaned F1 pups autopsy and evaluation for anomalies: No effects on variations, malformations or pathological findings of internal (visceral) organs, and no effects on variations, retardations and malformations of the skeleton.

F1 reproductive performance data (N=14-12-11-8/9/sex)
No significant effects on:
Mean time until successful mating
Clinical findings in F1 females
Body weight gain during pregnancy of F1 females
Length of pregnancy in F1 dams (21-23 days)
Litter data (corpora lutea, pre- and postimplantation loss, implantation sites, sex ratio, live pus, dead pups)
Reproductive indices: live birth or viability indices
Body weight (gain) during lactation (Day 0-7) in F1 dams
Body weight of F2 male and female pups (PPD 0-7)

Externally visible anomalities and clinical findings in F2 pups
Visceral and skeletal findings in prematurely dying F2 fetuses
Autopsy findings in F1 parental males and females

Toxicokinetics
No data from this study

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

SUMMARY

SD rats were dosed orally with 0, 1, 4, 16 mg/kg/day. Rats were fasted from 4h before until 4h after dosing. Females were dosed from Day14 prior to mating until C-section at GD21 (C-section group) or through delivery and lactation on PPD21 (littering group). Males were dosed from Day 60 before mating

- Number of non-pregnant females significantly increased in HD group. Decreased fertility due to male sperm effects (see Segment 1 IV study) and/or female effects.
- Mortality of F0 dams around delivery time in all treatment groups. Also one animal dosed with 16 mkd died before delivery time on GD17. In littering group, one litter stillborn and one litter not breathing at 16 mkd.
- Body weight gain of F0 females not significantly affected during 14-day premating period at 16 mkd
- Body weight gain of F0 dams slightly but significantly decreased during gestation in all treatment groups (by 7%-10%). This body weight decrease appeared to be wholly due to a reduction in full uterine weight. Since in the Segment 1 IV study there were no effects on gestational body weight at higher dose multiples than in this oral study, Reviewer concluded that this non-dose-related effect was not biologically significant and did not reflect maternal toxicity.
- C-section group: Increase in preimplantation loss and decrease in # implantation sites (#IS) in all treated, significant at 4 mkd, with the reduction in # IS significant according to trend analysis. This effect was not clearly drug-dose-related and not seen in the littering group. Reviewer feels it was not biologically significant. Fetal weight (m and f) was reduced dose-dependently and significantly at 16 mkd (<10%). No effect on fetal anomalies in fetuses delivered by C-section.
- Littering group: Significant decreases in #corpora lutea and #implantation sites at 16 mkd. Increase in postimplantation loss (ca. 2x), significant at 1 and 16 mkd (not at 4 mkd), and significant according to trend analysis. Increase in #dead pups/IS (not significant). Decrease in #live pups/IS (significant) at 16 mkd, and 20% decrease in live birth index at 16 mkd. No effect on viability or weaning indices. No treatment effects on F0 body weight, F1 pup weight at PPD0 or F1 pup body weight increase during lactation. No effects on F0 dams or F1 pups at autopsy.
- Statistically significant impairment in cliff avoidance test at 16 mkd (performance 70% of control), apparently occurring in all litters. Other physical and behavioral development parameters of F1 pups not significantly affected.
- F1 reproductive performance not affected.
- Sperm (concentration, morphology, motility) not investigated in treated males.

Segment 1, oral rat study (K2)

Duration of dosing	Route	Mg/kg/d	Cmax* (ng/mL)	AUC_{0-24}* (ngxh/mL)		Exposure multiple vs. humans at 2.5 mg/day orally (based on AUC data)**	Multiple of human oral dose of 2.5 mg/day (based on mg/m^2 BSA comparison)
6-9 wks (females)	oral	1	2.7	7.8		3.1x	4.1x
		4	10.7	31		12x	16x
		16	43	125		50x	66x

*TK data extrapolated from 6-month oral, fasted rat toxicity study H1
**Assumption: Human AUC at 2.5 mg/day = 2.5 ngxh/mL

BM 21.0955.Na
EMBRYO-FETAL TOXICITY STUDY IN THE RAT PER OS (GAVAGE) (Caesarean Section)

Report Nr.	K17 (Segment 2)
NDA Vols	82
Drug	BM 21.0955.Na (monosodium salt, monohydrate)
Batch Nr	443.848-00 (1 mg free acid =1.145 mg weighed BM21.0955.Na)
Test facility	Boehringer Mannheim, GmbH (Hoffmann-LaRoche) (Germany)
Study period	1992
QA/GLP	Yes/Yes
Species/strain	SD rats (Crl:CD BR Sprague Dawley)
Groups	1,2,3,4,5
Doses	0, 10, 30, 100, 60 mg/kg/day (free acid equivalents). Group 5 (60 mg/kg) was added when treatment of Group 4 (100 mg/kg) proved to be severely toxic.
Route	Oral gavage
Dosing period	GD6-GD15
Dosage form	Solution in 0.5% carboxymethylcellulose
Dose volume	10 ml/kg
Food/water	pellets, and tap water. Comment: Food was withheld for 4h before and 4h after dosing
Dose selection	Based on drf study with restricted feeding in pregnant rats (0, 10, 20, 40 mkd) with no signs of toxicity (Study K16), and data from 4-wk and 6-mo oral toxicity studies (Study G1,H1)

	Males	Females
Initial age	4 months	6 wks
Initial weight	>500g	181-210g
Dosing period	Not dosed	Day 6p.c.-Day15 p.c. (GD6-GD15) Scheduled sacrifice on GD21 (C-section)
Mating period	N/A	Up to 2 weeks, until N=99 sperm positive females out of N=120 (Grps 1,2,3,4) Up to 3 weeks, until 25 positive matings out of N=50 (Grp 5)

Group	1	2	3	5	4
	Control	LD	LMD	HMD	HD
Doses (mg/kg/day)	0	10	30	60	100
N/sex/grp (study start)	25	25	24	25	25

PROTOCOL

Pregnant female SD rats were treated daily, by oral gavage, from GD6 through GD15. Blood samples were taken from F0 females on GD15 for serum Ca and P determination. On GD 21 F0 dams were subjected to C-section. Blood samples were taken on GD21 for determination of serum Ca, P, BUN, creatinine. F0 dams were evaluated (autopsied) on GD21. Fetuses were examined. Skeletons of ca. 50% of fetuses were examined for malformations and 50% for soft tissue anomalies. Serum levels of drug were not determined.

Definitions:

First day of experiment= day with sperm-positive vaginal smear	Day 0 post conception, or Day 0 of gestation	Day 0 p.c., or GD0

RESULTS

Mortality F0 females

F0 females mortality

		1	2	3	5	4
Group		Ctrl	LD	LMD	HMD	HD
Dose		0	10	30	60	100
N		25	25	24	25	25
Mortality	Gavage accident	1	2			
	Died at GD16, 19, 15, 19, 18, 19, 16, 16, 19, 18, 18, 15, 19, 18, 15, 16, 18					17 Rat #302,303,305, 306,307,309,311, 312,313,314,316, 317,318,319,320, 321,324
	Killed in extremis GD 20				1 #422	1 #325
	Total	1	2		1	18

Clinical signs in pregnant F0
LD: no signs
LMD: Stained pelt in 4/24 @ 30 mkd
HMD: Poor health in 4/18 @ 60 mkd (1 killed)
HD: Anogenital and snout region stained in 6/23 to 7/23, poor health in 11/23, other signs in single animals @100 mkd (17 died, 1 killed)

It is unlikely that the deaths were due to inhibition of Ca mobilization needed for delivery and hypocalcemia resulting from drug-induced suppression of bone resorption, since they occurred before delivery time (GD15,16,18,19; gestation time is usually 21-23 days). Based on results of acute and chronic oral toxicity studies, it is most likely that the dose of 100 mg/kg/day caused systemic toxicity and mortality unrelated to pregnancy after 10 days of dosing.

Mating performance:
No effect on days to successful mating
Number of non-pregnant F0-females

Group		Ctrl	LD	LMD	HMD	HD
Dose		0	10	30	60	100
N	Total	25	25	24	25	25
	Pregnant	21	19	24	18	23
	Not pregnant	4	6	0	7	2

Body weight F0 females

F0 females body weight during pregnancy (N=21-19-24-18-23 at start, 20-17-24-17-7 at GD21)

Group		Ctrl	LD	LMD	HMD	HD	Trend test
Dose		0	10	30	60	100	
Body weight	At GD0	232	233	234	221**	233	*
	At GD6	267	272	268	266	269	
	At GD18	354	366	355	351	307**	#
	At GD21	405	420	406	405	376	
	AUC (period GD6-GD21)$	1249	1405	1260	1466**	1183	*

$ AUC=area under the time curve for differences to initial body weight on GD6 through GD21 (Δbody weight, grxd)
Statistically significant, * p<0.05, **p<0 01, ***p<0.001 excluding 100 mkd group, # significant including 100 mkd group

- Body weight gain impaired in HD from GD13 through GD21. AUC did not indicate this effect, since premature decedents from HD group were not considered in AUC calculation.

- Increased body weight gain (reflected by AUC of Δbody weight,grxd) in 60 mkd HMD group was due to lower initial weight on GD0.

Litter data/Reproductive parameters F0 females

F0 females: litter data

		Ctrl	LD	LMD	HMD	HD	Trend test
N		19-21	16-19	24	16-18	6-7	
Corpora lutea		ND					
Preimplantation lossX100/CL (%)		ND					
Implantation sites (IS)		12.9	13 2	12 3	14 7	13.7 (N=23)	
Postimplantation lossX100/IS (%)		6.9	4.0	4.3	4.6 (SD5.8)	17.3 (SD37)	
Live fetuses/IS (%)		93	96	96	95 (SD7)	83 (SD37)	
Dead fetuses /IS (%)		0	0	0	0.6	0	
Fetal weight (m) (gr)		5.6	5 5	5.4	5.2	5.2	
Fetal weight (f) (gr)		5.3	5.3	5.1	4 9	4.7	
Uterus weight full (g)		87	91	90	92	80	
BW gain – uterus content (GD0-21)		91	101	87	99	69*	

ND = not determined
Statistically significant, * p<0.05, **p<0 01, ***P<0.001

Treatment effects:

- Increase in % postimplantation loss and decrease in % live fetuses. The increased postimplantation loss and decrease in % live fetuses in HD was due to 1/7 dams/litters with all resorptions (18 fetuses resorbed). Lack of statistical significance of these effects was due to large SD values.
- Slight reduction in fetal BW (m,f), and uterine weight, not statistically significant.
- Reduction in (body - uterus) weight gain, significant in HD.

(Trend tests all negative)

F1 fetuses: anomalies

F1-fetuses parameters: visceral anomalies

		Ctrl	LD	LMD	HMD	HD	Trend test
N (F0 females)		19-20	16-17	24	17	6	
VISCERAL							
N fetuses examined		116	105	149	117	37	
Variations (%)		57	76*	70	81*	66	*,#
RPU syndrome (N)		29	59	60	56	18	
(% of fetuses)		25%	56%	40%	48%	49%	
Malformations (%)		2.1	1 3	1.3	3.5	0.0	

Statistically significant, * p<0.05, **p<0.01, ***P<0.001 excluding 100 mkd group, # significant including 100 mkd group

		Ctrl	LD	LMD	HMD	HD	
N (F0 females)		19-20	16-17	24	17	6	
SKELETAL							
N fetuses examined		121	107	153	120	39	
Retardation (%)		80	78	80	71	63	
Head, interparietal bone, poorly ossified		19	5	8	5	2	
(% of fetuses)		15.7%	4.7%	5.2%	4.2%	5.1%	
Variation (%)		6.2	15.8*	15.1*	18.6*	2.1	
Rudimentary rib (N)		3	13	10	14	0	
(% of fetuses)		2.5%	12%	6.5%	12%	0%	
Malformation (%)		0.7	0.7	2.3	1.0	0.0	

Deformation							
Thoracic verebrae, wavy nbs (N)		6	4	1	0	0	
% of fetuses		5%	3 7%	0 7%	0%	0%	

Statistically significant, * p<0 05, **p<0 01, ***P<0 001 excluding 100 mkd group, # significant including 100 mkd group

Comments:
Increased % of visceral variations in treated (significant in 10 and 60 mkd groups) was due to increased incidence of renal pelvis ureter (RPU) syndrome in the fetuses in all treated groups.

The RPU syndrome is characterized by various states of dilatation of the ureter and renal pelvis without functional defect. Finding is common and control incidence in this study was 29/116=25%.
This anomaly was not observed in an IV Segment 2 study in rats (K12), but it was also observed in the 60 mkd HD group of the other oral rat Segment 2 study (offspring investigation). It may be related to the nephrotoxicity of bisphosphonates, including ibandronate.

Increased % of skeletal variations in 10, 30, 60 mkd groups was due to increased incidence of rudimentary thoracic ribs. The finding was not dose-dependent, did not occur in 100 mkd group, and Sponsor considered it incidental.

Historical control data (Submitted March 28, 2003) rudimentary rib

Strain: Crl CD Sprague-Dawley

Laboratory: Boehringer Mannheim

Incidence of Rudimentary Ribs in Fetuses. Caesarean Section (1980 – 1992)[1)]

Year of Study	Number of evaluated litters	Number of evaluated fetuses	Rudimentary ribs	
			N	%
1980	21	198	7	3 5
1982	26	236	25	10.6
1983	22	149	7	4 7
1983	20	149	10	6.7
1984	24	170	0	0 0
1986	11	80	3	3.8
1986	20	126	0	0.0
1988	21	127	3	2.4
1991	27	267	51	19 1
1992	24	193	0	0 0

[1)] Only those studies were taken into account which were conducted under GLP and in which the finding "rudimentary rib" was explicitly listed

Average: 5.1%
Range: 0.0-19.1%

Sponsor concluded that the incidences of rudimentary ribs in the oral segment II study with ibandronate in rats (K17) were independent of the dose and within the range of historical control data of the laboratory performing the study. Reviewer agrees.

There were no effects on skeletal retardations.

F0 females biochemistry

Calcium, phosphorus, BUN, creatinine concentrations in serum (GD15 and GD21)

		Ctrl	LD	LMD	HMD	HD	Trend test
N (F0 females)		20	17	24	18	GD15: 21 GD21: 7	
Ca (serum) (mmol/L)	GD15	2.70	2.78	2.77	2.84	2.65	

	GD21	2.32	2.22	2 19	2.11	1.94	#
Inorganic P serum (mmol/L)	GD15	2 52	2 45	2.73	2 32	2.96	
	GD21	2.10	1 92	1 96	1 98*	2.64	*
BUN (mmol/L)	GD21	6 2	6 3	6.1	8.7*	14.0	#
Creatinine (umol/L)	GD21	47	45	47	68*	108	*,#

Statistically significant, * p<0.05, **p<0.01, ***P<0 001 excluding 100 mkd group, # sign including 100 mkd group

Serum Ca (GD21) was decreased, and serum P, BUN and creatinine (GD21) were increased in HMD and HD group. Effect was not statistically significant in HD group due to large variation. Trend test however revealed significant effect on all these parameters on GD21.

F0-females autopsy (all pregnant animals: died, killed, or scheduled sacrifice)

Incidence of autopsy findings at incidence >1 (one animal can have more than 1 finding)

Group		Ctrl	LD	LMD	HMD	HD
Dose (mg/kg)		0	10	30	60	100
N pregnant		21	19	24	18	23
N that died		0	0	0	1	18
N survived		21	19	24	17	5
Gastric hemorrhage				2	3	11
GI hemorrhage						3
Spleen atrophy					3	1
Aqueous/bloody feces					2	1
Kidneys discolored					3	8
Nephrotic edema						7
Adrenal gland enlarged						4
Pulmonary edema w/wo hemorrhage						9
Liver congestion						2
Hydrothorax						3

The findings in the HD group were for the most part in animals that died (GI, kidney, lung). Animals that survived had a few findings (2/5 hemorrhage, 2/5 kidney discolored or enlarged, and some single events). No pulmonary events seen in survivors.

Toxicokinetics

No data from this study

SUMMARY

Pregnant SD rats were dosed orally with 0, 10, 30, 60, 100 mg/kg/day, from GD6-GD15. C-section performed on GD21. Rats were fasted from 4h before until 4h after dosing.

- Maternal toxicity (signs) at 30, 60 and 100 mkd. Severe drug-related maternal toxicity (signs, decrease in gestational body weight gain, and marked mortality) at 100 mkd. NOAEL for maternal toxicity was 10 mkd.
- One of surviving 100 mkd dams had all resorptions
- Slightly reduced fetal weight (not statistically significant) at 30, 60 and 100 mkd
- Significant increase in % fetal visceral and skeletal variations in all treated groups (10, 30, 60, 100mkd)
- Increased incidence of renal pelvis ureter (RPU) syndrome of ca. 2-fold in all treated groups. Finding occurred in groups without (10 mkd) or with maternal toxicity (≥30mkd).
- Increased incidence of rudimentary ribs (skeletal variation) (3- to 5-fold) in 10, 30, 60 mkd groups was not dose-dependent and not significant. Historical control data indicated that the incidences in treated groups were within historical control range (average 5.1%, range 0.0%-19.1%, 11 studies). Conclusion was confirmed by the lack of this finding in the IV Segment 2 studies at higher exposure multiples.
- No effect on visceral or skeletal malformations.
- Decrease in maternal serum Ca on GD21 but not GD15 at 60 and 100 mkd.
- Increase in maternal serum BUN and creatinine on GD21, significant at 60 and 100 mkd.

- Autopsy findings in dams that died at 100 mkd (GI hemorrhage, spleen atrophy, kidney lesions, pulmonary edema w/wo hemorrhage, adrenal and liver findings). In some surviving dams at 60 mkd there was gastric hemorrhage, spleen atrophy, aqueous/bloody feces, kidney discoloration and in a few dams treated with 30 mkd gastric hemorrhage was observed.

Segment 2, oral rat study (K17)

Duration of dosing	Route	Mg/kg/d	Cmax* (ng/mL)	AUC_{0-24}* (ngxh/mL)		Exposure multiple vs. humans at 2.5 mg/day orally (based on AUC data)**	Multiple of human oral dose of 2.5 mg/day (based on mg/m^2 BSA comparison)
10 days	oral	10	27	77		31x	41x
		30	80	232		93x	123x
		60	160	463		185x	246x
		100	270	772		309x	410x

*TK data extrapolated from 6-month oral, fasted rat toxicity study H1
**Assumption: Human AUC at 2.5 mg/day = 2.8 ngxh/mL

APPEARS THIS WAY
ON ORIGINAL

BM 21.0955.Na

EMBRYO-FETAL TOXICITY STUDY (SEGMENT II) IN THE RAT PER OS (GAVAGE)

(Offspring Investigation)

Report Nr.	K9 (Segment 2)
NDA Vols	83
Drug	BM 21.0955.Na (monosodium salt, monohydrate)
Batch Nr	443.848-00 (1 mg free acid =1.145 mg weighed BM21.0955.Na)
Test facility	Boehringer Mannheim, GmbH (Hoffmann-LaRoche) (Germany)
Study period	1992
QA/GLP	Yes/Yes
Species/strain	SD rats (Crl:CD BR Sprague Dawley)
Groups	1,2,3,4
Doses	0, 6, 20, 60 mg/kg/day (free acid equivalents)
Dosing period	GD6-GD15
Route	Oral gavage
Dosage form	Solution in 0.5% aqueous carboxymethylcellulose
Dose volume	10 ml/kg
Food/water	pellets, and tap water. Comment: Food was withheld for 4h before and 4h after dosing Animals were Ca-supplemented around delivery time
Dose selection	Based on dose range finding study with calcium substitution regimen of 16 mg/kg Ca given s.c. twice daily (daily dose 32 mg/kg) from GD18-PPD0 (Study K8). In that study a 32 mg/kg Ca regimen reduced dystocia seen with oral treatment from GD6-GD15 with 70 mg/kg. Lower Ca dose (16 mg/kg) did not prevent dystocia after 50 mg/kg.

	Males	Females
Initial age	10 months	2 months
Initial weight	>500g	185-241g
Dosing period	Not dosed	Day 6p.c.-Day15 p.c. (GD6-GD15)
Mating period	N/A	Up to 3 weeks, until N=60 sperm-positive females were obtained (Grps 1,2,3,4)

Group	1	2	3	4
Doses (mg/kg/day)	0	6	20	60
N/sex/grp (study start)	15	15	15	15

PROTOCOL

Pregnant female SD rats were treated daily, at the same time every day, by oral gavage, from GD6 through GD15. Ca was administered daily by two s.c. injections of 2x1 ml calcium borogluconate (2x16 mg Ca/kg) from GD18 to PPD0 (early morning and afternoon). Animals were allowed to deliver, and F1 pups were reared to PPD42. F0 dams were sacrificed after lactation, and F1 pups were sacrificed on PPD42 or 43. All F1 pups were examined for internal organ anomalies, but skeletal anomalies were only evaluated in prematurely dying F1 pups. One male and one female per litter were reared to maturity and mated for maximal 3 weeks. F1 dams were allowed to deliver and were killed on PPD7 together with F2 pups. Prematurely dying F2 pups were investigated (internal organs and skeleton). Serum drug and serum calcium levels were not determined.

Definitions:

First day of experiment= day with sperm-positive vaginal smear	Day 0 post conception, or Day 0 of gestation	Day 0 p.c., or GD0
Day of birth	Day 0 post partum	Day 0 pp., or PPD0

RESULTS

Mortality F0 females

F0 females mortality

		1	2	3	4
Group		Ctrl	LD	MD	HD
Dose		0	6	20	60
N		15	15	15	15
Mortality	Gavage accident				1 (#315)
	Died on GD 22 or 23		1 (#107)	1 (#206)	1 (#309)
	Killed in extremis				1 (GD19) #314
N not pregnant					
	Total	0	1	1	3

Clinical signs in pregnant F0

Dystocia leading to death in 1 LD, 1MD, 1HD, on GD 22 or GD23. One additional HD was killed in extremis on GD19 following intrauterine death of all fetuses. HD animal #309 (death on GD23) had blood in gut. Signs in dying animals not described in more detail.
Death may have been due to hypocalcemia resulting from suppressed bone resorption and Ca mobilization needed for delivery. However, it is unclear if this is case for all animals, since one died before delivery time (GD19)

Mating performance:

Number of non-pregnant F0-females

Group		Ctrl	LD	MD	HD
Dose		0	6	20	60
N	Total	15	15	15	15
	Pregnant	15	11	15	14
	Not pregnant	0	4	0	1

No effect on length of pregnancy (21, 22, or 23 days)

Body weight during pregnancy in F0 females

F0 females body weight dunng pregnancy (N=21-19-24-18-23 at start, 20-17-24-17-7 at GD21)

Group		Ctrl	LD	MD	HD	Trend test
Dose		0	6	20	60	
Body weight	At GD0	235	235	241	241	
	At GD21	427	438	432	418	

No sign effects

Litter data/Reproductive parameters F0 females

F0 females: litter data

Group		Ctrl	LD	MD	HD	Trend test
N		15	10-11	14-15	11-13	
Corpora lutea		Nd				
Preimplantation lossX100/CL (%)		Nd				
Implantation sites (IS)		16.3	17.5	16.3	15 6	

Postimplantation lossX100/IS (%)		4.0	6.8*	7.9*	11.2	*
Live pups/IS (%)		93.9	90.0	90.1*	87.8	
Dead pups /IS (%)		2.1	3.2	2.0	1.1	
Live birth index (%)		97.7	96 5	97.9	98 9	
Viability index (PPD0-4) (%)		98.9	97.1	99.1	97 8	
Weaning index (PPD0-21) (%)		97	97	98	97	

Statistically significant, * p<0.05, **p<0.01, ***P<0.001
NOTE: Viability Index (PPD0-4) = 100%- Postnatal Loss (PPD0-4) (pups alive on PPD4/pups alive on PPD0)

Treatment effects:
Increase in % postimplantation loss and decrease in % live pups, related to each other. Lack of statistical significance of effect in HD group was due to relatively large SD values.
The increased postimplantation loss was due to an average small increase in this parameter in LD, MD and HD, and in addition to one animal with a largely increased value (47%) in HD. Part of this loss was associated with the decrease in #live pups at first litter check.

Body weight during lactation in F0 females

F0 females body weight

Group		Ctrl	LD	MD	HD	Trend test
Dose		0	6	20	60	
N		15	10	14	11	
Body weight	At PPD0	315	312	320	324	
	At PPD21	330	342	338	344	

No sign effects

F0-females autopsy (all pregnant animals)
1 HD with intestinal hemorrhage (#309: animal that died on GD23 with blood in gut). Other animals that died or were killed in extremis had no autopsy findings.

Clinical findings in F1 animals
No treatment related effects

Body weight development in F1 animals
No treatment effects in males or females

Physical development in F1 pups
No treatment effects on:
Pinna detachment, hair coat development, incisor eruption, eye opening, testicular descent, vaginal opening;

Neuromuscular development in F1 pups
No treatment effects on:
Negative geotaxis PPD5, air righting PPD15, grip strength PPD15, auditory startle PPD21, pupillary reflexes PPD21
Cliff avoidance test was not done.

Behaviour F1 pups
No effect on water maze test results for behavior and memory

F1 pups: anomalies
Premature F1 pup decedents:
One (1/6) MD pup had sense organ malformation. No other visceral malformations observed.
RPU syndrome variation was seen in 1/7 HD pup.

F1 fetuses parameters: visceral and skeletal anomalies (premature deaths)

N (F0 females)		15	10	14	11	

N fetuses examined	6	10	6	7	
VISCERAL					
Variation: RPU syndrome	0	0	0	1 (14%)	
Malformation: sense organ, eye agenesis, ear malposition	0	0	1 (17%)	0	
SKELETAL					
Variation Sternum asymmetry (6th sternal center)	1	1	1	2	
Variation: Sternum thickening (6th sternal center)	0	0	0	1	

No other treatment effects on internal organ variations, malformations, pathological findings, or skeletal retardations, variations or malformations in premature F1 decedents.
Variation of sternum asymmetry (at 6th center) was not accompanied by increased incidences of sternal asymmetry at the 4th or 5th center (latter two anomalies classified as retardations).

Weaned F1-pups:

F1-fetuses parameters: visceral anomalies

VISCERAL	Ctrl	LD	MD	HD	Trend test
N (F0 females)	15	10	14	11	
N fetuses examined	193	133	175	116	
Variation: RPU syndrome (% of fetuses)	18 (9%)	3 (2%)	10 (6%)	23 (20%)	
Variations (%)	39	28	26	34	
Malformations (%)	0.4	0	0	0	
Pathological findings (%)	8.6	7.2	3,7	12.7	

No treatment effects other then increased % of individual visceral variation (RPU). This was not reflected in the average variation %!
NOTE: Skeletons not examined in surviving pups

Reproductive performance of F1 generation
No effects on:
Mating success, days until successful mating, clinical findings in F1-females during pregnancy and lactation, body weight development in F1 females during pregnancy, length of pregnancy in F1 females, litter data (#corpora lutea, #implants, % preimplantation and % postimplantation loss, % live and dead pups, sex ratio), reproductive indices (live birth and viability indices), body weight development during lactation in F1-females, and autopsy findings in F1-males and females.

F2 pups data

Clinical findings: no treatment related

Body weight: Dose-related increase in body weight in F2 male pups, significant in pups from HD group (10-20%), on PPD0 and PPD7. Trend significant on both days. Effect not seen in females, and significance unclear.

F2 pups: anomalies
Premature decedents: (N=4-11-1-9)
Visceral: No treatment effect on visceral malformations, variations or findings
Skeletal: 1/9 HD pups had tail malformation (vertebral fusion). No other treatment effects on skeletal anomalies.
Numbers too small to draw conclusions on F2 pups.

Toxicokinetics
No data from this study

SUMMARY

Pregnant female SD rats were treated daily, by oral gavage, from GD6-GD15, with 0, 6, 20, 60 mg/kg/day (N=15/grp). Ca was administered s.c. (32 mg/kg/day) from GD18 to PPD0. Rats were fasted from 4h before until 4h after dosing.

- Despite Ca supplementation, dystocia and peripartum mortality in 1/15 females in all treatment groups (≥6 mkd).
- Dose-dependent increase in postimplantation loss (2- to 3-fold) in all treated group (postimplantation loss not further defined).
- Decrease in #live pups/IS (but not in #dead pups/IS) in all treated groups
- Decreased # live pups possibly due to undetected perinatal pup loss rather than resorptions or other types of postimplantation loss, since there was no significant effect on postimplantation loss in the oral Segment 2 study with C-section at doses up to 60 mkd and higher (K17). Perinatal pup loss was likely related to perinatal maternal toxicity
- No effect on physical development (pinna detachment, hair coat development, incisor eruption, eye opening, testicular descent, vaginal opening) or neuromuscular development (negative geotaxis, air righting, grip strength, auditory startle, pupillary reflexes) or memory (water maze test) in F1 pups.
- Increased incidence of RPU syndrome in weaned pups at 60 mg/kg/day (20%), although not statistically significant.
- No effect on reproductive performance of F1 pups.
- No significant treatment effects on F2 signs, body weight, or on anomalies in premature F2 decedents.

Segment 2, oral rat study (K9)

Duration of dosing	Route	Mg/kg/d	Cmax* (ng/mL)	AUC_{0-24}* (ngxh/mL)		Exposure multiple vs. humans at 2.5 mg/day orally (based on AUC data)**	Multiple of human oral dose of 2.5 mg/day (based on mg/m^2 BSA comparison)
10 days	oral	6	16	46		16x	25x
		20	53	154		55x	82x
		60	160	463		165x	246x

*TK data extrapolated from 6-month oral, fasted rat toxicity study H1

**Assumption: Human AUC at 2 5 mg/day = 2.8 ngxh/mL

BM 21.0955.Na

EMBRYO-FETAL TOXICITY STUDY IN THE RABBIT / ORAL ADMINISTRATION OF TEST ARTICLE (GAVAGE)

Report Nr.	K1 (segment 2)
NDA Vols	88
Drug	BM 21.0955.Na (monosodium salt, monohydrate)
Batch Nr	443.848-00 (1 mg free acid =1.145 mg weighed BM21.0955.Na)
Test facility	Boehringer Mannheim, GmbH (Hoffmann-LaRoche) (Germany)
Study period	1990
QA/GLP	Yes/Yes
Species/strain	Himalayan Rabbit (CHbb:HM)
Groups	1,2,3,4
Doses	0, 1, 4, 20 mg/kg/day (free acid equivalents)
Route	Oral gavage
Dosing period	GD6-GD18
Dosage form	Solution in 0.5% aqueous carboxymethylcellulose
Dose volume	5 ml/kg
Food/water	[redacted] diet, and tap water. Comment: Food was withheld for 4h before and 4h after dosing
Dose selection	In a dose range finding study in non-pregnant rabbits with daily oral doses of 1, 3, 10, 30 mg/kg (Day7-11 and Day14-18) (fasted) (N=5/grp), there were 1,2,1 deaths in the1,10,30 mkd groups. Animals died from pulmonary edema, hemorrhage or atelectasis. (Study K3). Sponsor suggested that drug-related toxicity was enhanced by pre-existing infection as evidenced by mucoidal enteritis in animals from all groups. In another dose range finding study in pregnant rabbits with daily oral doses of 1, 3, 10, 30 mg/kg (GD6-GD18) (5/grp), there were 0,1,0,1 deaths in the 1,3,10,30 mkd groups, reduced maternal body weight and an increased resorption rate in HD, but no teratogenicity at any dose (Study K4).

	Females
Initial age	3-4 months
Initial weight	2.8-3 kg
Dosing period	Day 6p.c.-Day18 p.c. (GD6-GD18)
Mating	Artificial insemination with pooled sperm

Group	1	2	3	4
Doses (mg/kg/day)	0	1	4	20
N/sex/grp (study start)	17	17	17	17

PROTOCOL

Pregnant female rabbits were treated daily, by oral gavage, from GD6 through GD18 in the morning. All dams were laparotomized on GD29 and fetuses and uteri were examined. Fetuses were evaluated for internal organ and skeletal anomalities, the latter by X-ray.

Definitions:

First day of experiment= day with sperm-positive vaginal smear	Day 0 post conception, or Day 0 of gestation	Day 0 p.c., or GD0

RESULTS

Mortality F0 females

F0 females mortality

		1	2	3	4
Group		Ctrl	LD	MD	HD
Doses (mkd)		0	1	4	20
N		17	17	17	17
Mortality		0	2	4	6
N not pregnant		0	1	0	1
N evaluable		17	14	13	10

Time of death (Dosing: GD6-GD18)

		1	2	3	4
Group		Ctrl	LD	MD	HD
N at start		17	17	17	17
Mortality (total)		0	2 (12%)	4 (24%)	6 (35%)
Day 8					1
Day 9			1	2	
Day 10				1	2
Day 12			1		1
Day 15					2
Day 27				1	

Clinical signs

Increased incidence of dark red eyes, grey conjunctiva, wheezing respiration (trend tests all positive)

All animals that died (LD-MD-HD: 2-4-6) had pulmonary edema with pulmonary parenchymal hemorrhages.

Sponsor considered the deaths not a result of drug toxicity, and speculated they were either gavage accidents or the result of "endotoxemia" as evidenced by enteritis (observed in both controls and treated, see autopsy data). It was suggested that the animals in this study were in poor general condition and had disturbed gut flora. Reviewer does not agree with this evaluation. The deaths occurred in the treated only, with dose-dependent incidence and associated lung findings. Although the health status of the animals may have been suboptimal and contributing to the mortality, it per se does not explain the mortality finding, and the mortality was clearly drug-related. Also, death occurred mainly on GD8-15 (dosing period: GD6-18).

Sponsor partly based its conclusion on the results from a dose range finding study with 10 and 30 mkd (N=5/grp), in which only 1/5 (20%) of animals died at 30 mkd. However, in that study 1/5 also died at 3 mkd. In the other range finding study 1/5 (20%) died at 3 mkd and at 30 mkd each, too. It appears that these were all drug-related deaths, since they occurred only in treated groups.

Body weight during pregnancy

No treatment effect (all gained about 100g) (N=17-14-13-10)

C-section findings

No significant treatment effects on: uterus weight (full or empty), #implantation sites, % resorptions, %live fetuses (per #IS), % dead fetuses, %male fetuses (per total #fetuses), mean body weight of male and female fetuses, placenta weight.

Litter data

Group		Ctrl	LD	MD	HD	Trend test
Doses (mkd)		0	1	4	20	
N evaluated		17-16	14-13	13-12	10-9	
Implantation sites (#)		6.35	6 13	5.38	5 93	
Resorptions (resorptions/#IS x 100) (%)		10.9	14.9	12.7	19.5	NS

Non-statistically significant increase in resorptions in HD. In 2 HD animals there were 3 resorptions. In Ctrl, LD, and MD animals maximal 2 resorptions were seen.

F0-females autopsy (all pregnant animals)

Autopsy findings

Group		Ctrl	LD	MD	HD	Trend test
Doses (mkd)		0	1	4	20	
N evaluated		17	16	17	16	
Liver, mottled surface		0	2	1	3*	*
Gallbladder, hypertrophy		0	4*	0	0	
Mucoide enteritis		5	5	6*	10*	**
Lung, edema and hemorrhage[a]		0	2	4*	6**	**

[a] only seen in animals that died prematurely

The lung finding clearly appeared drug-related and associated with death. This effect was also seen in rats that died at a dose of 100 mkd in oral Segment 2 reprotoxicity study K17 (study with C-section) and appears to be direct drug-related toxicity.

Anomalies in F1 fetuses

Externally visible anomalies
No treatment-related effects

Visceral findings: per-litter analysis

Group		Ctrl	LD	MD	HD	Trend test
Doses (mkd)		0	1	4	20	
N (F0 females)		17	14	12	10	
N fetuses examined		96	75	66	51	
Variations (%)		36	60**	40	57**	NS
Malformations (%)		1.0	0 0	1.2	0 0	NS

Visceral findings: per-fetus analysis

Group		Ctrl	LD	MD	HD	Trend test
Doses (mkd)		0	1	4	20	
N fetuses examined		96	75	66	51	
Variation	Supplementary vessels	12	27***	16*	10	NS
	Gallbladder reduced	6	22***	7	7	NS
	Gallbladder enlarged	5	2	6	12	***
	Gallbladder variated	11	24	13	19	**
Malformation	Exencephaly	0	0	1	0	NS

These visceral findings show dose-dependent increase in gallbladder enlargement. The significance of this finding is uncertain, particularly because there was increased gall bladder reduction in the LD only.

Skeletal findings: per-litter analysis

Group		Ctrl	LD	MD	HD	Trend test
Doses (mkd)		0	1	4	20	
N (F0 females)		17	14	12	10	
N fetuses examined		96	75	66	51	
Retardation (%)		61	73	65	62	NS
Variations (%)		12	22	15	23	NS
Malformations (%)		7.6	6.9	7.6	3.1	NS

Skeletal findings: per-fetus analysis

Group		Ctrl	LD	MD	HD	Trend test
Doses (mkd)		0	1	4	20	
N fetuses examined		96	75	66	51	
Retardation	Cervical vertebrae: 1st cervical vertebral center, underdevelopment, poorly ossified	6	15*	5	5	NS
	Head: Parietal bone, underdevelopment, poorly ossified	4	9	11*	4	NS
	Head: Frontal bone, underdevelopment, poorly ossified	0	2	1	1	NS
	Head: Presphenoid, underdevelopment, poorly ossified	0	0	1	0	NS
Variation	Cervical or thoracic vertebrae: additional structure (nb)	0	5*	0	0	NS
	Sternum: 3rd sternal centre, fusions	0	0	1	0	NS
	Sternum: 4rd sternal centre, fusions	1	2	0	0	NS
	Sternum: 5rd sternal centre, fusions	0	0	1	2	NS

These skeletal findings did not suggest a treatment-related effect.

Toxicokinetics
No data from this study

SUMMARY

Pregnant female rabbits were treated daily, by oral gavage, from GD6-GD18, with 0, 1, 4, 20 mg/kg/day. All dams were laparotomized on GD29. Rabbits were fasted from 4h before until 4h after dosing.

- Dose-related mortality in all treatment groups associated with lung edema and hemorrhage, and increased incidence of mucoide enteritis
- No drug-related effects on F1 fetal skeletal parameters
- Increased incidence of gallbladder enlargement in fetuses of the HD group (significant dose-related trend).
- Significant increase in incidence of gall bladder reduction in fetuses of the LD group.
- Biological significance of the gall bladder effects is questionable.
- No treatment-related skeletal findings in F1 offspring (retardations, variations, or malformations).

Rabbit, oral dose multiples

Rabbit Study K1	Dose duration	Mg/kg/d	Multiple of human oral dose of 2.5 mg/day (0.04 mg/kg/day), based on mg/m^2 BSA comparison
Segment 2	12 days	1	8.3x
		4	33x
		20	167x

PERI- AND POSTNATAL STUDY WITH BM 21.0955-Na IN THE RAT (ORAL GAVAGE)

Report Nr.	K15 (Project Nr. 381251) (Segment 3)
NDA Vols	84-86
Drug	BM 21.0955.Na (monosodium salt, monohydrate)
Batch Nr	447.624-00 (1 mg free acid =1.125 mg weighed BM21.0955-Na monohydrate). Since initial batch analysis indicated that 1 mg free acid=1.069 mg monohydrate, doses received were 0.95x intended doses.
Test facility	[redacted]
Study period	1995
QA/GLP	Yes/Yes
Species/strain	Rat WIST Hanlbm: WIST [redacted]
Doses	0, 1, 5, 20 mg/kg/day (0, 0.95, 4.75, 19 mg/kg) (free acid equivalents)
Route.	Oral gavage
Dosing period	GD17-PPD21
Dosage form	Solution in 0.5% carboxymethylcellulose
Dose volume	10 ml/kg
Food/water	[redacted] rat/mouse diet, and tap water, both *ad libitum* (NO FASTING)
Dose selection	Based on 6-mo study with restricted feeding (10, 30 mkd) (Study H1) in which 30 mg/kg was lethal and 10 mg/kg had body weight toxicity.

	Males	Females
Initial age	?	10 wks
Initial weight	?	174-239g
Dosing period	Not dosed	GD17-PPD21(end of gestation and throughout lactation)

Group	1	2	3	4
Doses (mg/kg/day)	0	1*	5*	20*
N/sex/grp (study start)	25	25	25	25

*Intended doses; Real doses were: 0.95, 4.75, 19 mg/kg

PROTOCOL

Mated females were treated orally, by gavage, once daily, from GD17 through to PPD21 (through lactation). Females were allowed to deliver and rear F1 pups. No Ca supplementation was given. Litters were culled on PPD4 to 8 pups/litter when possible. F0 dams were killed and autopsied on PPD21. After weaning and developmental/behavior tests, selected F1 pups (1/sex/litter) were reared to maturity, and mated at ca. 10 weeks of age. All other F1 animals were sacrificed after test completion. The F1 mated dams were allowed to deliver and rear their F2 pups up to PPD4. F1 animals were not dosed. F1 dams were killed together with F2 pups on PPD4. On PPD21, one hour after dosing, blood samples were obtained from F0 females for measurement of plasma albumin, calcium, differential blood count, and concentration of BM 21.0955-Na in serum.

Definitions:

Day with sperm-positive vaginal smear	Day 0 post conception	Day0 p.c., or GD0
Day on which pups born	Day 0 postpartum	Day0 p.p., or PPD0

Birth index (%)		(Number of pups born alive/Number of implantations)x100
Viability index (%)		(Number of pups alive on PPD4/Number of pups born alive)x100

Weaning index (%)		Number of pups alive on PPD21/Number of pups alive on PPD4)x100

Percentage mating		(Females mated/Females paired)x100
Fertility index (%)		(Females achieving pregnancy/Females paired)x100
Conception rate (%)		(Females achieving pregnancy/Females mated)x100
Gestation index (%)		(Females achieving pregnancy/Females paired)x100

RESULTS

F0 females

Mating performance

		Ctrl	LD	MD	HD
Dose (mg/kg/d)		0	1	5	20
# mated females		25	25	25	25
# pregnant females		25	25	25	23
# females with signs of dystocia		0	0	2 (#62,64)	3 (#80, 94,99)
# mortalities		0	0	2 (#59,64) (#64 w dystocia)	5 (#80,87,92,97,100) (#80 w dystocia)
#females with live pups at parturition		25	25	23	18
#females with total postnatal loss (pups dead or cannibalized)		0	0	1 (#62) (#62 w dystocia)	3 (#78,94,99) (#94,99 w dystocia)
#females with live pups on PPD21		25	25	22	15

Mortality and clinical signs

F0 females clinical signs in MD and HD

Group		LD	MD	HD
Dose (mkd)		1	5	20
Clinical Signs				
Poor condition, dystocia, not nursing	GD22, PPD1		1 (#62)	
Severe dystocia, tremor	PPD0			1 (#94)
Dystocia, cold to touch, poor condition, ruffled fur	PPD0-6			1 (#99)
Dystocia (tremor, exophtalmia, died during delivery)	GD22		1 (#64)	
Poor condition, dystocia, tremor, found dead	PPD0 and/or 1			1 (#80)
Found dead	GD22			3 (#87,92,97)
Poor condition, chromodarcryorrhea, found dead	GD21-22			1 (#100)
No signs			(#59)	
Total deaths			2 (#59,64)	5 (#80,87,92,97,100)

Food consumption F0

Small reduction (5-10%) in FC during lactation in Grps 3 and 4 (MD and HD).

Body weight F0

No treatment effects in F0 dams

Reproductive parameters

INCLUDING females with total litter loss (0-0-1-3), and excluding dams that died

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	1	5	20
N pregnant		25	25	23	18
N evaluated		25	25	23	18
Implantation sites (IS)		12 7	13 4	11.8	12.3
Postimplantation loss/IS (%)		6.9	6.3	10 7	26 2(*)
Postnatal loss (PPD0-PPD4) (% of living pups at PPD0)		1.7	3.2	3 7	14.1(*)
Breeding loss PPD5-PPD21 (% of living pups at PPD4)		0	0	0	0

Result: Increased postimplantation loss, and increased postnatal loss in MD and HD (no statistical test results mentioned in report). However, clear and significant effect in HD for both parameters.

(*Note*: Postimplantation loss = # living pups at 1st check/#implantation sites)

EXCLUDING females with total litter loss (0-0-1-3), and excluding dams that died

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	1	5	20
N pregnant		25	25	23	18
N evaluated		25	25	22	15
Implantation sites (IS)		12.7	13.4	11.8	12.1
Postimplantation loss (% of IS)		6.9	6.3	10.0	19.3**
Postnatal loss (PPD0-PPD4) (%)		1.7	3 2	0 4	4.1(ns)
Breeding loss (PPD5-PPD21) (%)		0	0	0	0
Dead pups at 1st check (N/litter)		0.1	0.1	0.2	0.5
Living pups at 1st check (N/litter)		11 8	12 5	10 6*	9 7*
Living pups PPD 4 (N/litter)		8	7 8	8	7.4
Living pups PPD21 (N/litter)		8	7 8	7 9	7.4
Birth index (%)		93	93	90	81**
Viability index (%)		98	97	99 6	96
Weaning index (%)		99.5	99.5	99.4	100

Statistically significant, * p<0.05, **p<0.01

NOTE:
Postimplantation loss = # living pups at 1st check/#implantation sites
Postnatal loss (PPD0-PPD4) = #pups that died from PPD0-4/#living pups at PPD0
Breeding loss PPD5-PPD21 = # pups that died from PPD5-21/# off living pups at PPD4

Result: In females excluding those with total postnatal loss there were still increased postimplantation losses in MD and HD. Also, postnatal loss was increased in LD and HD, but not clearly treatment-related. Sponsor argues that increases in postimplantation and postnatal loss were possibly due to undetected perinatal loss of pups and not due to loss of fetuses in utero since the body weight of dams on GD1 and GD21 and the # implantation sites were similar for all groups. Data are lacking to confirm this hypothesis.

Sponsor concluded that only in dams with distinct signs of dystocia (#80,94,99) increased PI and PN losses were noted. Thus, it was assumed that treatment of dams did not cause toxicity in pups independent of maternal toxicity. However, in females excluding those with total litter loss (a group in which there were NO dams with dystocia) there was still a significant increase in postimplantation loss and # live pups at 1st litter check in the HD. This could be due to perinatal pup loss in litters/dams with no external signs of dystocia. Or, there could be loss of pups due to drug treatment unrelated to dystocia. This has also been an issue with alendronate (Minsker DH,

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

Hematology and biochemistry of F0
No effect on differential WBC count, or plasma Ca or albumin in F0 females on PPD21.

Autopsy of F0 females (PPD21)

	Ctrl	LD	MD	HD
Dose (mg/kg/day)	0	1	5	20
Mortality	0	0	2	5
Jejunum, mucosa dark red discoloration			1	
Lung, dark red discoloration or dark red foci			2 (both animals who died)	4 (included 3 animals who died)
Kidney, pelvic dilatation				2

F1 pups
No treatment effects on external examination PPD0, sex ratio, body weight (gain) from PPD0-PPD21 or PPD21-PPD35.

Developmental indices (days pp)

		Ctrl	LD	MD	HD
Dose (mg/kg/d)		0	1	5	20
N (litters) evaluated		25	25	22	15
Incisor eruption (days)		8.1	7 7	7.7	7 3*
Coat development, onset (days)		9.7	9 3	9.4	9.0*

Statistically significant, * p<0.05
No effects on pinna unfolding, eye opening, testicular descent, prepuce or vagina opening.

Behavior tests
No effects on righting reflex PPD14, photo-phobotaxis PPD21, pupillary reflex PPD21, hearing PPD21, grasp ability PPD16, activity PPD21, cliff avoidance PPD21, negative geotaxis PPD21.

		Ctrl	LD	MD	HD
Dose (mg/kg/d)		0	1	5	20
N (litters) evaluated		25	25	22	15
Water maze 3. relearning		56%	36%**	38%**	31%**
Water maze 4. relearning		64%	48%*	59%	46%*

There were no significant dose-related effects in other water maze learning or relearning trials on PPD36-43 (6 learning and 6 relearning sessions; 2/sex/litter in water maze test;). Reviewer concluded that the decrease in relearning performance was not a significant finding because it was not dose-dependent, occurred in trials 3 and 4 only, and was not seen in the Segment 3 IV study at higher exposure multiples in Wistar rats.

Signs
No treatment effects on general clinical findings during F1 pup rearing through PPD42

Necropsy
No significant effects on necropsy findings in F1 pups.

F1 parental generation

Mating performance

		Ctrl	LD	MD	HD
Dose (mg/kg/d)		0	1	5	20
# mated females		25	25	25	25
# pregnant females		25	24	25	25
#females with live pups at parturition		25	24	25	25
#females with total postnatal loss (pups dead or cannibalized)		0	0	0	1 (#376)
#females with live pups on PPD4		25	24	25	24

Mortality
No deaths occurred in F1 females.

Clinical signs of F1 parental animals
In HD group, one dam cannibalized its single pup by PPD1.

Body weight
F1 parental females: No treatment effects during gestation and lactation

Food consumption
F1 parental females: No treatment effects during gestation and lactation

Reproduction
No effect on % mating (100% for all groups), fertility index (=females achieving pregnancy/females paired; 100-96-100-100%), and gestation index (number of females with living pups/number of pregnant females; 100-100-100-96%).

Breeding (litter) data

		ctrl	LD	MD	HD
Dose (mg/kg/d)		0	1	5	20
N pregnant		25	24	25	25
N evaluated		25	24	25	24
Duration of gestation		21.6	21 8	21 2*	21.5
Implantation sites (IS)		12.0	12.3	11 6	12.7
Postimplantation loss/IS (%)		13	6.8	8.7	8 9
Postnatal loss (PPD0-PPD4) (% of living pups at PPD0)		0.8	1.1	0.4	0.7
Birth index (%)		87	93**	91.3	91.1
Viability index (%)		99 2	98 9	99.6	99.3

Statistically significant, * p<0.05, **p<0.01

Necropsy of F1 parents
No findings

F2 pups

No effects on external examination @PPD0, sex ratio, body weight and body weight gain from PPD0-PPD4, findings during rearing, or necropsy findings at PPD4.

Toxicokinetics (Internal Study Nr. TV406)

Serum levels of BM21.0955-Na were determined by [redacted] Standard were prepared in human pooled serum. 1 hour after dosing (gavage) of non-fasted F0 females, on PPD21. This time is the expected Tmax in rats.

Doses and multiples

Doses (mg/kg) (free acid equivalent)		0 95	4 75	19 0
N		25	22	18
Serum concentration				
BLQ [redacted]		N=24	N=11	
0.5-1 ng/ml		N=1	N=9	
1 1-3 ng/ml			N=1	N=14
3 1-5 7 ng/ml			N=1	N=4
Cpl (1h) values	Mean (ng/ml)	0.03	0.52	2.72
	Median (ng/ml)	0	0.29	2.20
Multiple of human dose (Cmax basis)		0.04-0.05x	0.65-0.87x	3.4-4 5x
Multiple of human dose (mg/m2)		3.9x	19.5x	78x

Human Cmax = 0 6-0.8 ng/mL @ 2.5 mg/day oral dose (0.04 mg/kg) (MF7159)

Apparently, there is an enormous difference when Cmax multiples in the rat are calculated based on real serum values, or based on mg/m2. Partly this may be due to the fed state of the rats (causing serum levels to be relatively low). Also, Tmax may be later than 1h when rats are fed, and thus multiples are underestimated. Or mg/m2 is not a good basis of multiple calculation. However, data on p.308 (Vol2/103) show that feeding can make a huge difference in Cmax and AUC (10-fold or more). Also those data suggest that using mg/m2 for calculating multiples is adequate (as long as the species that are compared are in the same feeding status).

SUMMARY

Pregnant Wistar rats were treated orally, by gavage, once daily, from GD17 through PPD21 (through lactation), with 0, 1, 5, 20 mg/kg/day (0.95, 4.75, 19 mg/kg/day free acid equivalents). Rats were not fasted. TK assay was performed on postpartum Day 21 in F0 females.

- Dose-related dystocia and peripartum mortality at MD and HD
- Dystocia included tremor, ruffled fur, cold to touch, exophtalmia
- Dams with dystocia either died or had total postnatal loss
- Increase in dams with total postnatal loss (PPD0-4) related to dystocia in MD and HD
- Significant increase in postimplantation loss at HD in all dams (excluding or including those with total postnatal loss). Partly, this loss may have been undetected perinatal loss (cannibalization)
- Increased postnatal loss (PPD0-4) at HD in dams including those with total litter loss(not statistically significant), but not in those excluding those with total litter loss
- Postimplantation (possibly perinatal) loss and postnatal loss of pups apparently related to maternal dystocia resulting from hypocalcemia.
- Statistically significant decrease in #living pups/litter and non-significant increase in #dead pups/litter at MD and HD
- Significantly decreased birth index at HD
- At autopsy, dose-related red lung discoloration at MD and HD in F0 females, mainly in dams that died
- No effect on body weight gain in F1 pups
- In F1 pups, small but significant decrease in time to incisor eruption, and time to onset of coat development in F1 pups at HD
- No effects on F1 reproductive performance or early F2 pup physical development
- Serum levels @1h postdose (Tmax) lower than expected (compared to data available for fasted rats), probably due to feeding in current study and/or inaccurate Tmax estimation.

- C_{max} multiples for rats in this study as compared to humans dosed with 2.5 mg/day, orally, were 0.04-0.05x (LD), 0.65-0.87x (MD), and 3.4-4.5x (HD). This is based on human Cmax levels of 0.6-0.8 ng/mL (MF7159; BP16304).
- AUC multiples based on extrapolated data from Study H3 are 0.24x, 1.2x, 4.8x (based on human AUC of 2.5 ngxh/mL, Study MF4348).

Segment 3, oral study, rat

Duration of dosing	Route	Mg/kg/d (WDS)	Mg/kg/day (FAE)	Cmax (ng/mL)	AUC_{0-24} (ngxh/mL)		Exposure multiple vs. humans at 2.5 mg/day orally (based on AUC data extrapolated from H3)**	Multiple of human oral dose of 2.5 mg/day (based on mg/m^2 BSA comparison)
4 days (fed)	oral	1	0 95	0.03 (Cpl @1h)	(0 6)*		0 24x (fedl)	3.9x
		5	4 75	0.52 (C1h)	(3.0)*		1.2x (fedl)	19x
		20	19	2.72 (C1h)	(12.0)*		4 8x (fedl)	78x
		10	10	0.7	6.3 (toxicity study H3)		2.5x (fedl)	41x

* extrapolated from Study H3
**Assumption: Human AUC at 2.5 mg/day = 2 5 ngxh/mL

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STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION WITH BM 21.0955-Na IN THE RAT (IV INJECTION)

Report No. K19
[redacted] Project Nr. 379438
Study period 1994/1995
Batch Nr. 447-624-01
Segment I (Fertility): C-section (on GD14)

RAT STUDY (SEGMENT I, IV dosing)

Species, strain	RAT, Wist Hanlbm: WIST (SPF)					
Route	IV injection					
N	24/sex/grp					
Dosing	Males: 28 days before pairing, during pairing (max 14 days), and up to 7 days p.c.					
	Females: 14 days before pairing, during mating, and until GD7					
Dose selection	Based on MTD (dose range finding Study K10)					
Procedure	C-section on GD14					
Group			Control	LD	MD	HD
Dosing	Doses (mg/kg/day)	Males	0	0.1	0.3	1.0
		Females	0	0.1	0 4	1.2

Doses are free-acid equivalents

RESULTS

Group			Control	LD	MD	HD
Doses M/F (mg/kg/day)			0/0	0 1/0 1	0 3/0.4	1.0/1.2
Reproduction Findings	Females with plug	N	24	24	24	23
	Pregnant females	N	24	24	23	21
	Evaluated pregnant females*	N	23	23	22	20
	Precoital time	# days	3	2	3	4
	Mating index	%	100	100	100	96
	Fertility index	%	100	100	96	88
	Conception rate	%	100	100	96	91
	Corpora lutea	N (mean)	14 2	13 8	14 2	11 3*
	Preimplantation loss	% of CL	2 1	4.1	1 9	8.0*
	Implantation sites (IS)	N (mean)	13.9	13.2	13 9	10.4*
	Postimplantation loss	% of IS	5.3	5.6	6 5	4.8
	Embryos (all alive)	Total N	302	287	286	197
		N (mean)	13.1	12.5	13.0	9.9
		% of IS	95	94	94	95

* Females not evaluated in which mating was not detected
*p<0.05, ** p<0.01

Group			Control	LD	MD	HD	
Doses M/F (mg/kg/day)			0/0	0.1/0.1	0.3/0.4	1.0/1.2	

Mortality						1M (D28)	
Body weight	Males	D1	272	268	270	271	
		D28	315	305	309	288**	*decrease
		D53 (D10 after pairng)	337	332	333	292*	*decrease
	Females	D1	201	200	205	203	
		GD14	264	263	268	255	
Body weight gain	Males	D1-D28 prepairing	43	37	39	17	No stat analysis
		D1-D14 pairng	23	22	16	6	
		D1-D10 after pairng	3	8	9	3	
		Total D1-D53	65	64	63	21	
	Females	D1-D14 prepairing	11	11	12	4	
		GD1-GD8	25	25	23	20	
Food consumption (g/day) *.	Males	D1-D28 prepairing	22	22	22	21	* decrease
		D22-28 prepairng	22	21	22	19**	
		D1-D7 after pairing	23	22	21**	18**	* decrease
	Females	D1-D14 prepairing	No sign effects				
Sperm analysis		Motility	No effects				
		Conc (x10^6/mL), left vas deferens	8.4	7 5	4.7**	3 2**	* decrease
		Conc (x10^6/mL), right vas deferens	8.1	6 8	4.8**	3.0**	* decrease
		Morphology	See below				

*p<0 05, ** p<0 01

Other findings:
One male died at D28. From D20, this animal had body weight loss, sedation, ruffled fur, stiff gait, poor condition. Sponsor considered the death incidental. Reviewer feels it may have been drug-related.

Clinical signs:
Males: Increased incidence of reddened tails, encrusted injection sites in HD.

Sperm morphology:
Significant effect: Increased % of sperm with abnormal hook (D) or reversed head (E) in MD and HD males

Morphology		Ctrl	LD	MD	HD	T-test
	A (sperm) (%)	97	97	97	97	
	D (%)	0.85	0 75	1.05	1 45	* increase
	E (%)	0	0.01	0 02	0 09	* increase

Hematology (prior to necropsy):
Significant effects:
Males: slight RBC decrease in HD; slight decrease in Hb and Hct in MD, HD; slight increase in platelet count in HD; slight increase in WBC in HD; slightly altered differential WBC count in MD, HD. All were drug-dose-related effects.
Females: slight RBC increase in HD; slight decrease in platelet count in HD.

Necropsy (macroscopic findings):
No obvious treatment effects

SUMMARY

Wistar rats were dosed by IV injection, once daily, with (males/females) 0/0, 0.1/0.1, 0.3/0.4, 1.0/1.2 mg/kg/day. Males were treated from 28 days before pairing, females from 14 days before pairing. C-section was performed on GD14.

- Mortality (1/24) in HD males.
- Decreased fertility in males and females. Increase in precoital time and decrease in number of pregnant females at HD. Decreased fertility indices in HD.
- Males: significant effects on sperm parameters in MD and/or HD (decrease in sperm count in MD (40%) and HD (60%), and altered sperm morphology in HD).
- Females: decrease in corpora lutea at HD
- Increase in preimplantation loss at HD, and decrease in # implantation sites and #embryos at HD.
- Decreased food consumption and decreased body weight gain in males during prepairing and pairing periods at HD. Slight (non-significant) decrease in body weight gain in females during prepairing and gestation in HD.
- Effect on hematology parameters in males at MD and HD, indicating anemia and inflammation

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