CORRELATIONS BMD-STRENGTH:

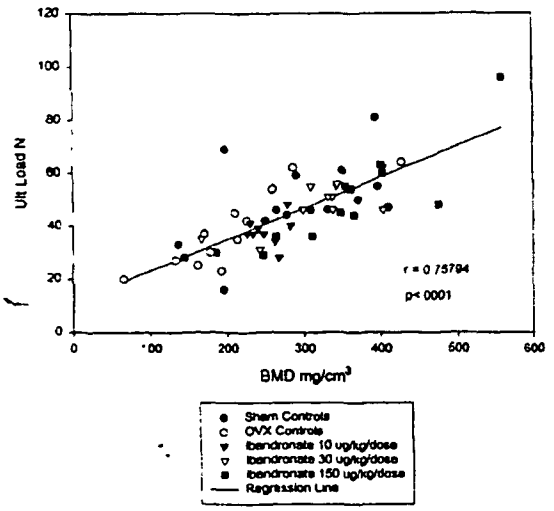
Vertebrae:
For vertebra there was a reasonable correlation between BMD and ultimate load (L5 cores: r=0.75794; L1 vertebrae r=0.6573). The correlations appeared similar for the different dose groups, and data points for sham and HD bone were similarly distributed. The data for vertebral core and whole vertebrae confirm the biomechanical test data that an increase in BDM is accompanied by an increase in strength.

Ulna:
For cortical ulna, there was no correlation between cortical BMD and ultimate load (r=0.23384) The correlation between BMD and intrinsic strength (N/mm2) was also not very strong (r=0.51196). This was probably because there were other factors affecting strength, most likely bone size (pQCT area) which was lower in OVX and treated groups. A better analysis would be to correlate BMC to ultimate load (data requested) as was reported for vertebrae from this study (Muller et al. 2001, and report D31). However, the individual data points support the notion that an increase in cortical BMD is associated with an increase in intrinsic cortical strength.
Upon request Sponsor carried out a correlation analysis for BMC and ultimate load for the ulna (Amendment date April 8, 2003; Submission April 9, 2003). The correlation between BMC and load was very good (r=0.96408). This suggests that BMC is the main determinant of bone strength at this cortical bone site. Note, however, that BMC and strength were not significantly increased as compared to OVX even at the HD.

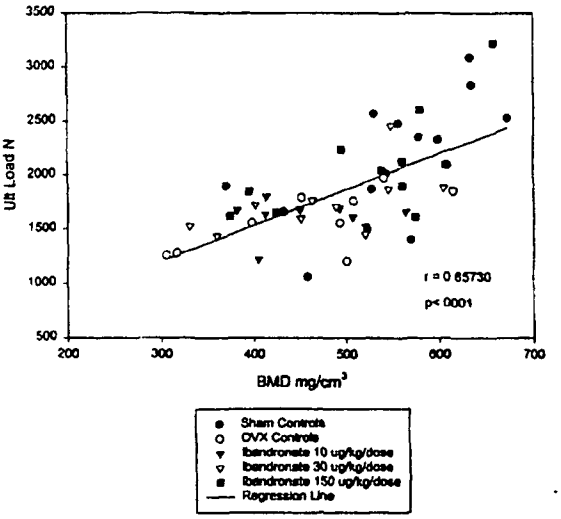
Proximal femur:
The data on correlation between BMD (at proximal femur) and strength (at femoral neck) suggest that the correlation is fairly strong for this site (r=0.70878). However, the graph illustrates that correlating the data for all groups may not be appropriate. The individual data suggest that in the HD group BMD is differently correlated to strength than in sham controls (most sham points are above regression line, while HD points are underneath it). This confirms the results of femoral neck biomechanical testing that at the HD BMD preserved at sham levels while strength is not significantly different from OVX (or sham). The cause of this is unclear.

The lack of efficacy at the femoral neck is somewhat disconcerting. The lack of efficacy was not paralleled by lack of effect on bone geometry as determined by pQCT at cancellous/cortical sites (cortical thickness of tibia) or lack of effect on bone dynamics reflected by histomorphometry parameters (periosteal or endocortical remodeling rate at femoral neck). Also, BMC at femoral neck - which reflects bone size and density - appeared protected to full extent. The anomalous result may have been due to a lack of effect on cortical BMD (since Haversian remodeling was not significantly suppressed), masked by the predominant contribution of trabecular bone to overall BMD. It could also be that structural bone parameter(s) that were not measured were inefficiently protected by treatment. Although Sponsor argues otherwise, it is not clear to Reviewer that small sample size and variability were the cause of the lack of a significant effect of treatment on femoral neck strength. Thus, the data for femoral neck do not show significant mechanical protection in conjunction with BMD. However, they do not indicate that bone strength is deteriorated with treatment.

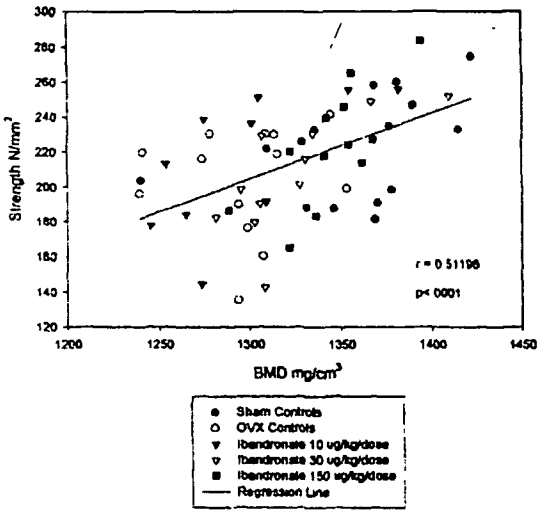
Correlation of Density and Ultimate Load - L5 Vertebral Cores



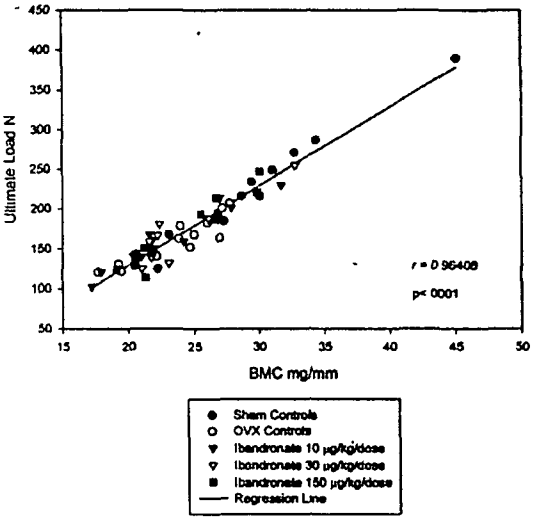
Correlation of Density and Ultimate Load - L1 Whole Vertebrae



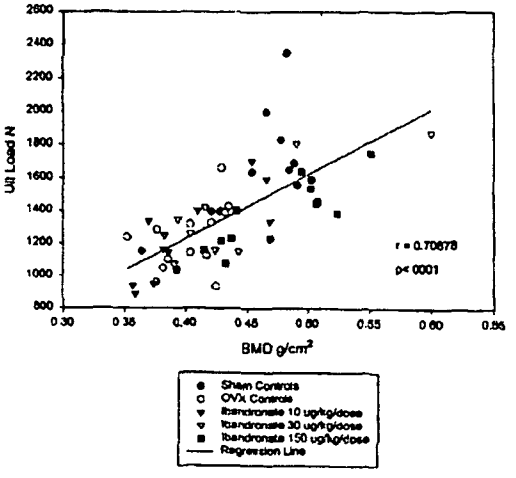
Correlation of Density and Strength - Ulna



Correlation of Bone Mineral Content and Ultimate Load - Ulna



Correlation of Density and Ultimate Load - Proximal Femur



PK data

Determination of ibandronate in monkey serum (Month 16)

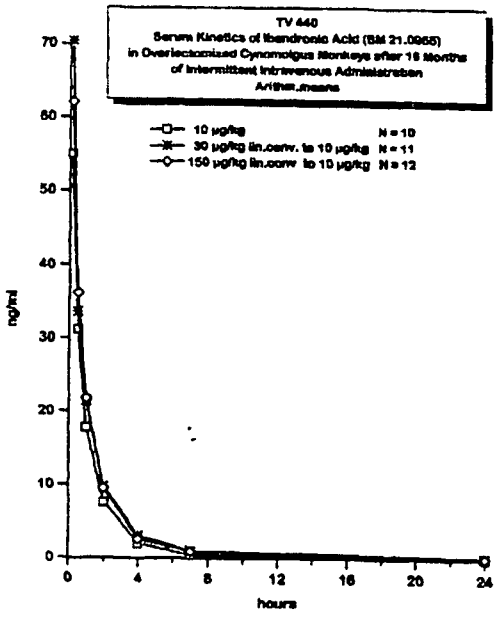
Monkey PK data (serum, [redacted]): (Appendix 31, pp 1549-1550, Report L16)

Group	Dose (ug/kg)	Cmax (ng/mL) median and range	AUC (ngxh/mL) median and range	Multiple of AUC at human 0.5 mg IV dose	Multiple of AUC at human 1 mg IV dose	Multiple of AUC at human 2 5 mg oral daily dose (cumulative over 30 days)	Multiple of AUC at human 2.5 mg oral daily dose (cumulative over 90 days)
LD	10	55 7 [redacted]	62 2 [redacted]	0 65x	0 33x	0 6-1.2x	0.2-0.4x
MD	30	200.0 [redacted]	198 [redacted]	2.1x	1.1x	2.1-3.6x	0.7-1.2x
HD	150	877.3 [redacted]	1115 [redacted]	11.7x	5.9x	11 1-21x	3.7-7 0x

Data for human 0 5 mg iv dose (8.3 ug/kg) (Study MF7159)
AUC = 95 ngxh/mL
Cmax: 78 ng/mL
T1/2= 22h
CL = 90 mL/min
F (oral bioavalilability) 0.63% (based on data with oral 20 mg dose)

Conclusions:

- Monkey doses of 10, 30, 150 ug/kg are equivalent to 0.65x, 2.1x, and 11.7x the human 0.5 mg IV dose, based on AUC comparison.
- Monkey doses of 10, 30, 150 ug/kg once monthly are equivalent to approximately 0.9x, 3x, 15x the cumulative human dose of 2.5 mg/day over 1 month, based on AUC comparison.
- Doses of 10, 30, 150 ug/kg are equivalent to approximately 0.3x, 1.0x, 5.5x the cumulative human oral dose of 2.5mg/day given over a period of 3 months, based on comparison of monkey $AUC_{0\text{-}1mo}$ with human $AUC_{0\text{-}3mo}$. The rationale for this comparison is the difference in the length of a remodeling cycle (1 month in monkey, 3 months in human).



Thus, the 30 ug/kg dose is equivalent to the human IV dose of ca. 1 mg and the cumulative oral human dose of 2.5 mg/day over 3 months.
The 1-mg IV dose (once every 3 months) was tested in clinical trial MF4380. At this dose lumbar spine BMD was increased by 3.9% as compared to pbo-treated, but new vertebral Fx incidence (9.2% vs. 10.7% in pbo) was not significantly reduced. The 2.5 mg dose (once daily, p.o) was tested in trial MF 4411 and increased BMD significantly by ca. 5.4% as compared to placebo while new vertebral fracture incidence was reduced by ca. 50%. The monkey data show that the MD of 30 ug/kg given once monthly (≈human 1 mg IV dose every 3 months) was also suboptimal for several bone parameters. However, the correlation between vertebral BMD and strength in this study was reasonably good. There are no data on effects of daily oral dosing in monkeys to compare with.

Mechano-structure relationship assessed by micro-tomographic imaging and biomechanics
(Report D31) (and Muller R et al, 2001)

Introduction: Strength of bone is determined by its material properties in combination with its architecture (how it is built). Bone density is not the sole determinant of bone strength and structural/trabecular microarchitecture plays an important role.

Assessments: *Ex vivo* vertebral BMC, BV/TV, and trabecular bone architectural parameters by micro-CT, bone strength by compression testing, and relationship between these parameters in sham, OVX and treated (LD, MD, HD) monkeys.
SMI=structure-model-index=model type of bone (ie rod-like vs. plate-like),
TBf=trabecular bone pattern factor=connectivity measure
DA=degree of anisotropy

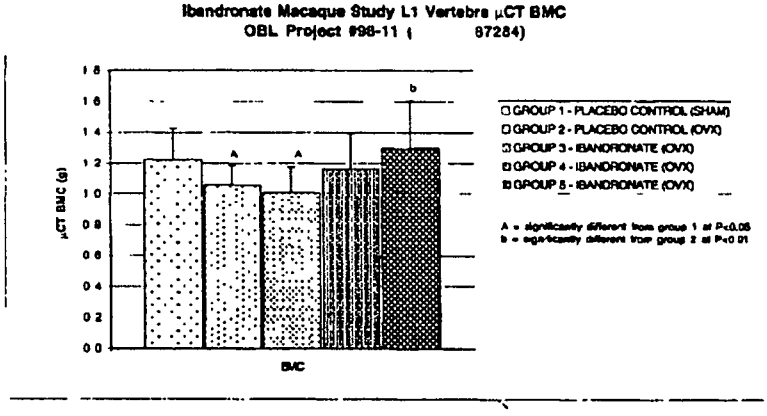


Figure 1: L1 Vertebra µCT: Bone Mineral Content (BMC)

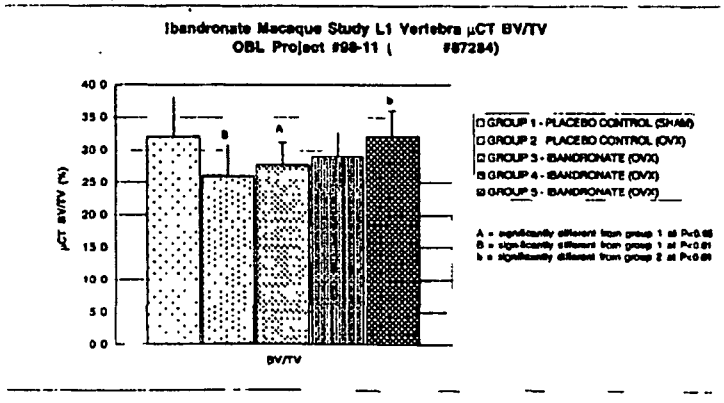


Figure 2: L1 Vertebra µCT: Bone Volume Density (BV/TV)

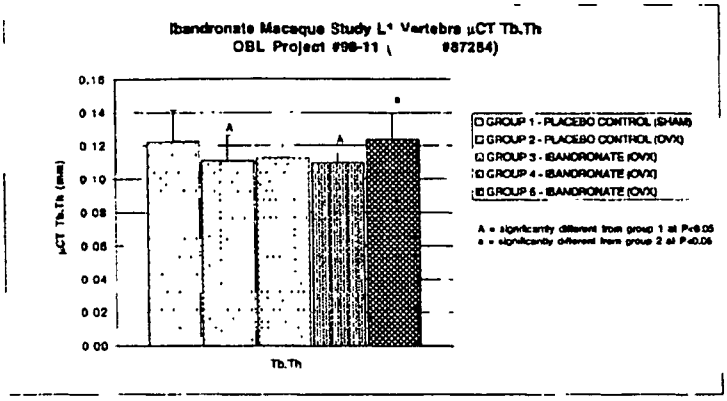


Figure 6 L1 Vertebra µCT: Trabecular Thickness (Tb.Th)

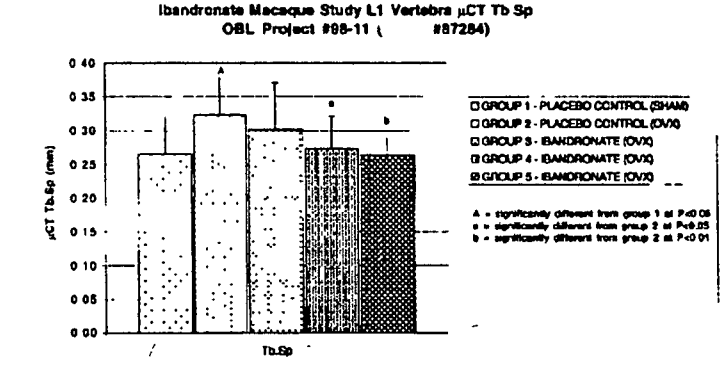


Figure 7: L1 Vertebra µCT: Trabecular Spacing (Tb.Sp)

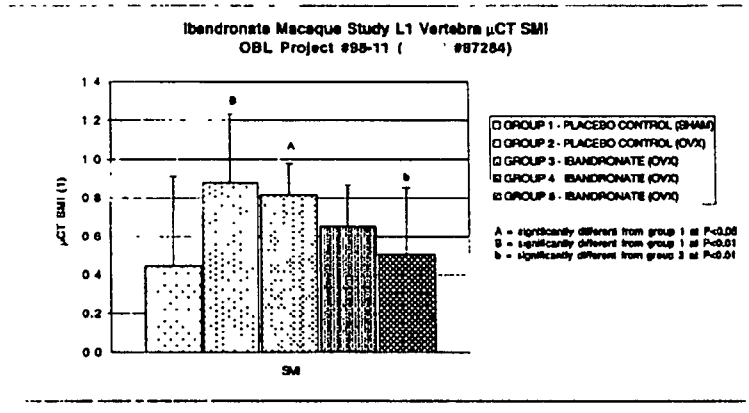


Figure 8: L1 Vertebra µCT: Structure Model Index (SMI)

Results:

OVX caused vertebral body bone loss (BMC, BV/TV), and architectural deterioration (reflected by changes in BS/TV, Tb.N, Tb.Th, Tb.Sp, SMI TBf, DA). MD and HD were not significantly different from Sham. HD was significantly different from OVX and fully effective in preserving BMC and BV/TV. MD was partially effective.

Predictive factors for bone strength

Overall, BMC was the most important predictor of ultimate load (r2=0.67) followed by SMI (r2=0.56) and BS/BV (r2=0.55). Tb.Sp and SMI contributed additional 18% independently of BMC (67%+18%=85%).

Interestingly, there was a difference between the groups with regard to these correlations:

Values for r2 (describing relationship between variable X and bone strength)

Variable X		BMC	TbSp	SMI
Sham	Vehicle	72	26	41
OVX	Vehicle	44	55	10
	LD	75-79	1-13	5
	MD	75-79	1-13	17
	HD	75-79	1-13	63

Morphology (CT)

- Trabecular structure was affected by OVX. In Sham animals trabeculae were more plate-like and in OVX more rod-like.
- Loss in bone mass upon OVX is caused by thinning of trabecular network, leading to perforations and as a result increased trabecular spacing
- However, trabecular spacing and thickness were highly variable within individual vertebrae
- Ibandronate (HD) fully prevented the trabecular thinning and perforations and preserved trabecular spacing
- Ibandronate (MD) did not prevent trabecular thinning but did preserve spacing

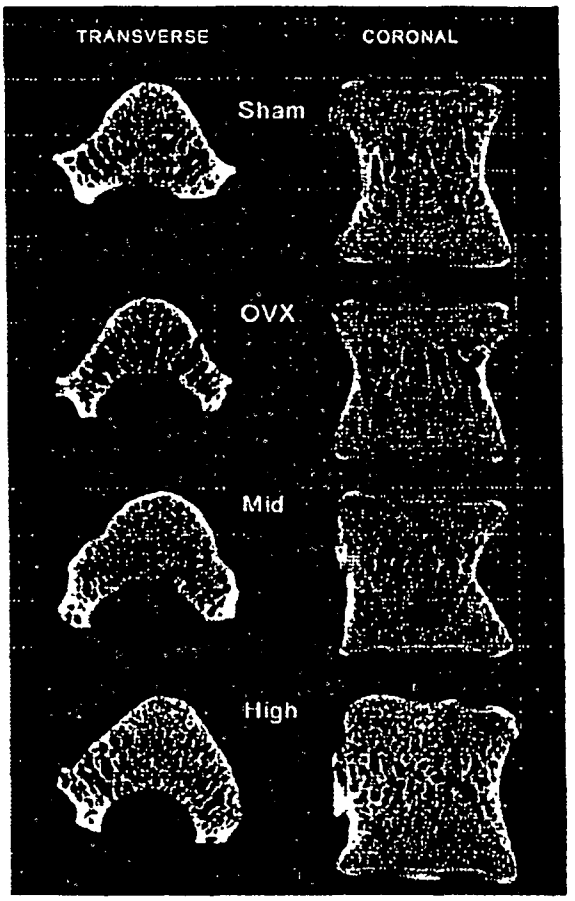


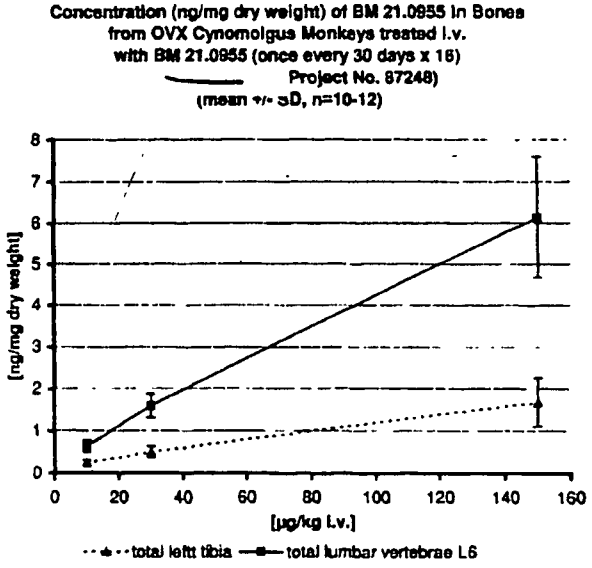
Figure 11. Two-dimensional trabecular bone architecture of four lumbar vertebrae (L1). Median animals with respect to BV/TV are illustrated for the Sham (first row), OVX (second row), Mid (third row) and High (fourth row) dose group. The left panel shows mid-transverse sections and the right panel the corresponding mid-coronal sections. The vertebrae were measured noninvasively using three-dimensional micro-tomographic imaging providing a nominal isotropic resolution of 34 µm

Conclusions:

- OVX impaired bone mass, architecture and strength
- Effects of treatment were dose-related. Either MD or HD were optimal in preserving various parameters
- BMC is single most important predictor for ultimate load ie bone strength
- Other structural parameters improve the prediction
- OVX bone is structurally different from and mechanically inferior to sham bone
- In vertebrae, ibandronate preserves normal bone mass, microarchitecture and strength as well as their relationships

Concentrations of ibandronate in bone

- In tibia ibandronate was concentated in proximal > distal metaphysis and diaphysis.
- In L6 mean bone concentration of drug was higher than in tibia. This was probably due to higher surface/volume ratio (more trabecular bone).
- Concentration was dose-dependent in non-linear manner.



In all doses, the concentration in vertebrae is significantly higher than those in tibiae ($p \leq 0.001$, Mann-Whitney rank sum test, one-tailed)

MONKEY STUDY SUMMARY AND EVALUATION

SUMMARY

Cynomolgus monkeys (N=15/grp or 12/grp), age 12-14 yrs, OVX'ed, treated for 16 months with IV injections of 0, 10, 30, 150 ug/kg, once every 30 days. Sham monkeys treated with 0 ug/kg only. Assessments: bone turnover markers, BMD, (DXA, QCT), histomorphometry, biomechanical testing.

Significant effects (unless indicated otherwise):

Bone markers

OVX caused increase in bone resorption and formation (markers: 2-5-fold). IBN prevented effect dose-dependently, partially at LD and MD and completely at HD.

BMD

Vertebra, femur:

- OVX decreased BMD at lumbar spine and femoral neck (DXA) by ca. 10%. IBN inhibited effect dose-dependently, completely at HD (spine), partially at LD and MD. HD was partially effective at femoral neck.

Proximal tibia:

- OVX decreased metaphyseal trabecular-BMD by 25%. IBN dose-dependently inhibited effect at all doses, completely at HD.
- OVX decreased metaphyseal cortical-BMD by 5%. IBN inhibited effect dose-dependently at all doses, for 85% at HD.
- OVX decreased outer bone perimeter and bone area (2-4%). IBN prevented this effect dose-dependently, completely at HD.
- OVX decreased cortical thickness by 20%. IBN inhibited effect dose-dependently at all doses, for 95% at HD
- IBN was more efficacious to prevent trabecular than cortical bone loss.

Histomorphometry

Cancellous bone (iliac crest, prox femur, dist radius, L4):

- OVX decreased BV/TV and Tb.Th, and IBN inhibited effects. Effects were not statistically significant. Effects on Tb.N. and Tb.Sp were minimal.
- OVX increased Ac.F, MS/BS, BFR (i.e. bone turnover). IBN inhibited effect dose-dependently, completely at LD or MD, and below sham levels (1/3-1/10) at HD
- OVX increased ES/BS and OcS/BS (erosion and osteoclast surface). IBN caused further increases in ES/BS at LD, MD and/or HD

Cortical bone (rib, femoral neck, central radius):

- OVX increased AcF as reflected by area and % labeled Haversian systems. IBN prevented the effect dose-dependently in rib and radius. IBN effect was not statistically significant at femoral neck
- OVX increased Ec.MS/BS, and IBN prevented the effect dose-dependently, completely at HD

No evidence of increased osteoid volume or thickness and no evidence of mineralization defects.
No effects on microscopic bone appearance and normal lamellar bone structure.

Biomechanics

Vertebrae:

- *Vertebral cores*: OVX reduced BMD (28%, pQCT) and ultimate load (N) by 20%. IBN prevented effects dose-dependently with complete protection at the HD to above-sham levels. Large variability caused lack of statistical significance of effects.
- *Whole vertebrae*: OVX significantly reduced BMD (18%) and ultimate load (25%). IBN prevented the effects dose-dependently, completely and significantly at the HD to sham levels.

Humerus (machined specimens)

- OVX reduced intrinsic strength (N/mm2) by 11%. IBN appeared to prevent effects completely. Effects of OVX and IBN were not statistically significant.

Ulna

- OVX reduced bone area (14%) and diameter (7%). IBN did not prevent effects. Effects were not statistically significant.
- OVX decreased BMC (17%) and BMD (4%) (significantly), and moment of inertia (MI) by 25% (not significantly). IBN prevented effect on BMD dose-dependently, with complete and statistically significant efficacy at HD. IBN did not prevent decrease in BMC or MI at any dose.
- OVX decreased maximum load significantly (24%). IBN prevented the decrease partially (for 25%) at MD and HD but effects were not significant at any dose. Strength (N/mm2) was not significantly affected by OVX or ibandronate.

Femoral neck

- OVX decreased BMD significantly at proximal femur (11%). BMC was similarly decreased but effect was not always significant. IBN prevented the BMD and BMC decreases dose-dependently, with complete and significant efficacy for BMD and/or BMC at HD.
- OVX significantly decreased ultimate load by 24%. IBN prevented effect partially (for 33%) at MD and HD, but effects were not significant at any dose.

Correlations BMD-strength

BMD was correlated to ultimate load (N) in vertebral cores (r=0.76), whole vertebrae (r=0.66) and femoral neck (r=0.71). At the ulna, BMD was not correlated to ultimate load (r=0.23), BMC was correlated to ultimate load (r=0.96), and BMD was correlated to ultimate strength (N/mm2) (r=0.51).

EVALUATION

Study design and dose selection

The design of the 16-month monkey study was adequate. OVX clearly reduced bone mass and strength and increased bone turnover, and ibandronate treatment prevented these effect. Overall, BMD was positively correlated to bone strength. Parameters measured were appropriate, a large amount of information was obtained and significant effects were observed.

The mid dose of 30 ug/kg was not the "optimal" dose when optimal is defined as having a maximal effect. In most instances, 30 ug/kg had a partial effect while 150 prevented the effect of OVX completely. The doses were selected based on data from other species rather than data from a dose range finding study in the monkey. Thus, a dose of 5x the optimal dose (as recommended in the draft guidelines for the evaluation of drugs for osteoporosis) was strictly speaking not tested. However, the study appears to address the issue of bone safety to an acceptable extent. First, the dose-dependence of the effects of ibandronate on vertebral BMD suggests that the optimal dose was somewhere in the range of 60-90 ug/kg. This means that the HD of 150 was 1.7-2.5x the optimal dose. Also, according to PK data the multiples of the LD, MD, HD as compared to the human dose were 0.3x, 1x, 6x, based on cumulative exposure over 1 month (monkey) or 3 months (human) at the 2.5 mg/day clinical oral dose. It should be noted that for cortical bone ibandronate was less effective on several parameters (BMD, AcF) and supraoptimal doses were not tested.

Reviewer concludes that the dose selection was not ideal but the study provided valuable information on cancellous and cortical bone quality.

Cancellous bone effects

Ibandronate had a protective effect on cancellous BMD at tibia and spine, and was able to maintain strength at the lumbar spine. The effects on cancellous bone appeared to be due to a preservation of trabecular bone volume and structure (thickness, spacing, connectivity, shape). The effects were at least partially related to a reduction in trabecular bone resorption (and formation, i.e., turnover), as expected of a bisphosphonate. Comparison of dynamic histomorphometry parameters and bone mass data suggested that effects of ibandronate on osteoclast and/or osteoblast function - other then merely a suppression of bone turnover- were also involved in the preservation of bone mass. The correlation between vertebral BMD and strength indicates that for this bone site BMD is a reasonable surrogate parameter for strength.

Cortical and mixed cortical/cancellous bone effects
Ibandronate had a protective effect on cortical BMD and thickness at proximal tibia and radius. Effects on BMD were smaller than in cancellous bone and were due to suppression of haversian system remodeling and cortical porosity. Effects on cortical thickness in tibia were due to suppression of endocortical and/or periosteal bone remodeling with consequent prevention of cortical thinning.

In ulna, bending strength reduction due to ovariectomy was not significantly reversed. This was due to lack of significant preservation of bone size and BMC rather than lack of effect on cortical BMD and intrinsic cortical bone strength.
In proximal femur, ibandronate prevented the decrease in BMD and BMC due to ovariectomy, but did not significantly protect femoral neck breaking strength. It is unclear why the compound did not prevent the strength reduction.

Sponsor attributed the lack of effect on biomechanical competence at ulna and femoral neck even at the high dose to various factors: Either doses were too low, or effect on cortical bone and sample size (i.e. power of study) were too small, or variances were too high. Reviewer feels that the lack of effect was not due to the small extent of the cortical effect since cortical strength was reduced by ovariectomy as much as it was in vertebrae (ca. 25%). Also variability was unlikely to be the cause of the lack of significance. Variability in strength data for ulna (23-25%) was slightly larger than for vertebrae (10-27%), but that appeared related to the size of the bone. Variability in femoral neck strength (17%-21%) was similar as for vertebrae (10-27%). It is, however, possible that higher dose would have been efficacious.

In Reviewer's opinion, the lack of effect on ulna strength was related to a decrease in bone thickness and BMC in the OVX and ibandronate-treated groups. This may have revealed a reduced ability of ibandronate to prevent an OVX-induced reduction in bone size. This conclusion is supported by the strong correlation between ulna BMC and ultimate load. However, the cause of the lack of effect on femoral neck strength was unclear. Histomorphometry data showed that haversian remodeling was not significantly suppressed in the femoral neck at any dose. By contrast, endocortical remodeling at the femoral neck appeared to be efficiently suppressed by ibandronate. Thus, it is possible that intracortical remodeling or bone distribution were not significantly protected in the treated groups and lead to reduced strength despite overall (cortical plus cancellous) BMD and BMC preservation.

Taken together, the efficacy of ibandronate to prevent cortical bone loss with respect to BMD (tibia), bone turnover (histomorphometry parameters), cortical thickness (tibia, ulna) and strength (ulna, femoral neck) appeared to be less than for cancellous bone loss. For these parameters the dose response curved seemed to be shifted to the right and even the high dose was not always effective. This suggests that doses that are optimal for cancellous bone may not be optimal for cortical bone.

The data also suggest that an increase in BMD in non-vertebral bone is not necessarily accompanied by an increase in bone strength, and BMD at those sites is only partially responsible for bone strength. Since pQCT data for the proximal tibia (meta- and diaphysis) suggest that ibandronate protected bone geometry changes while data from the cortical ulna showed that bone thickness was not significantly protected, effects of treatment on BMD and strength and the relationship between these two parameters appear to be site-specific. Importantly, the data support the notion that at non-vertebral sites treatment with ibandronate does not impair bone strength. This is in accordance with the finding that bone structure and mineralization were normal and with the finding in rats that ibandronate at high doses does not impair mineralization (D8, Muhlbauer et al, 1991).

Clinical relevance
The monkey data predict efficacy on BMD and bone strength at the spine. However, they a potential lack of efficacy at cortical/mixed bone due to relative low potency or inability of the compound to prevent adverse structural or geometric changes in cortical bone. This appears to be the opposite seen with the anabolic agent, PTH. In a monkey study with PTH1-34 an increase in cortical porosity was observed but was accompanied with an increase in bone size that compensated for the loss in terms of bone performance. Similar findings at the distal radius were obtained in humans with PTH.

The data from the monkey study with intermittent (once monthly) IV dosing do not provide an explanation for the lack of vertebral fracture efficacy observed in the clinical trial with IV injections of 0.5 or 1 mg once every 3 months (MF4380). In this trial lumbar spine BMD was significantly increased in the treated groups.

In the current clinical trials with oral drug (2.5 mg/day or 20 mg intermittent), the incidence of new vertebral fractures was reduced significantly by ca. 50% and BMD was increased at the spine (by ca. 5%). Hip and femoral neck BMD were increased by 2-3%, but total non-vertebral fracture incidence was not affected by treatment 8.2% placebo, 9.1% 2.5 mg/day). These clinical data are in accordance with the prediction based on preclinical data from the monkey study that significant fracture efficacy at the hip or other partially cortical bone sites may not occur. Thus, the apparent correlation between vertebral BMD and vertebral fracture efficacy can not be generalized to other skeletal sites.

Conclusions
In conclusion, ibandronate effectively prevented bone loss and strength in cancellous bone in the ovariectomized monkey. At the vertebrae, complete protection of BMD and strength was observed. At cortical or partly cortical sites (ulna, femoral neck) BMD was preserved but significant effects on strength were not obtained. The relative lack of cortical efficacy may have been due to a lack of efficacy on geometric or other structural skeletal effects of estrogen deficiency. Thus, BMD is a good predictor for cancellous (vertebral) bone strength but may not be a good predictor for bone strength at cortical or mixed cortical/cancellous sites. There were no adverse effects of ibandronate on osteoid mineralization or microscopically evaluated bone structure.

In vitro studies

Results from in vitro studies have been published over the last 15 years. As appears to be the case with other bisphosphonates, the inhibitory effect of ibandronate on osteoclast resorption may be indirect through an action on osteoblasts, possibly by induction of the synthesis of an osteoclast inhibitor. Ibandronate inhibited pit formation on ivory slices by pretreated rabbit and human cells. Inhibition of rabbit osteoclast activity by ibandronate was accompanied by inhibition of the production of actin rings upon cell activation. Ibandronate was more potent than pamidronate, clodronate or etidronate. Ibandronate also potently inhibited the adhesion and spread of human mammary carcinoma cells to bovine cortical bone and mouse trabecular bone.

Table 3 Primary Pharmacology Studies (Protocol Summary and Results) (Cont.)

Species/Sex/Model	Test Article (Batch No.)	Route	Dose/Administration/Duration	Results	GLP Status	Rpt/Pub. No. & Date (Laboratory)
***In vitro* studies**						
Osteoclasts from Wistar rats and osteoblastic cell line CRP 10.30	NS	*in vitro*	10^{-12} to 10^{-4} M ibandronate, pamidronate, clodronate, etidronate, alendronate	There was a high correlation of relative potencies between *in vivo* results in rats and humans and these *in vitro* results. The inhibitory effect of ibandronate on osteoclast resorption may be partly the result of an action on osteoblasts rather than on osteoclasts.	NR	[2522] 2522 pdf [2503] 2503 pdf 93, 97
Osteoblastic cell line CRP 10.30	NS	*in vitro*	10^{-7} M ibandronate or alendronate	Ibandronate and alendronate induced osteoblasts to synthesize an osteoclast inhibitor that resulted in >50% inhibition of bone resorption.	NR	[2529] 2529.pdf [2503] 2503.pdf 96 97
Human mammary carcinoma cell line MDA-MB-231	NS	*in vitro*	10^{-6}, 10^{-5}, or 10^{-4} M ibandronate, etidronate, clodronate pamidronate, olpadronate, or alendronate	Ibandronate, pamidronate, olpadronate, and alendronate caused a concentration-related inhibition of the adhesion and spreading of breast cancer cells to bovine cortical bone and to mouse trabecular bone. Ibandronate was the most potent inhibitor	NR	[2528] 2528 pdf [2503] 2503.pdf 96, 97
Osteoclasts (OC) from rabbits and human giant cell tumor-derived OC-like cells	ibandronate (454 555 00) clodronate (454 237 00) etidronate (J279)	*in vitro*	10^{-12} to 10^{-4} M ibandronate 10^{-6} to 10^{-4} M clodronate 10^{-6} to 10^{-4} M etidronate	Ibandronate inhibited osteoclastic pit formation on ivory slices concentration-dependently in pre-treated cells. clodronate and etidronate were equipotent. In co-cultures, clodronate and etidronate were 10-times less potent. Pit formation of human tumor-derived osteoclast-like cells was also inhibited by ibandronate (10^{-4} to 10^{-8} M). Production of actin rings in activated osteoclasts was inhibited by ibandronate with etidronate being 100 to 1,000 times less potent.	NR	[1729] d24.pdf 06/98

NR = Not required NS = Not stated. BM = Boehringer Mannheim GmbH. U of B = University of Berne, Switzerland;

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PHARMACOLOGY SUMMARY AND EVALUATION

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

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

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

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

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

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

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

Dog studies
Data from 4-week and 12-month s.c. treatment of OHX dogs showed that bone turnover was suppressed by ibandronate based on histomorphometric assessments. However, estrogen

deficiency in the dog had no long term persistent effects on bone BMD, architecture or strength. Thus, the OHX dog model was not an adequate animal model for postmenopausal osteoporosis.

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

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

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

The results of the monkey study indicate that ibandronate suppresses the increase in bone turnover, prevents the decrease in BMD and maintains skeletal microarchitecture upon ovariectomy. Effects of ibandronate on bone mass, geometry and strength are site-specific. Ibandronate has no deleterious effects on bone strength or mineralization.

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

In vitro studies
In vitro studies showed that the inhibitory effect of ibandronate on osteoclast resorption may be indirect through an action on osteoblasts, possibly by induction of the synthesis of an osteoclast inhibitor. Ibandronate inhibited pit formation, i.e., bone resorption by osteoclasts *in vitro* also when cells were pretreated rather then incubated with bone in the presence of inhibitor. Ibandronate was more potent in inhibiting osteoclast function than pamidronate, clodronate or etidronate.

II. SAFETY PHARMACOLOGY

Several studies were reviewed when submitted as non-GLP data to the original IND review (Ron Steigerwalt, December 16, 1994, Addendum).

Table 4 Other Pharmacology Studies (Protocol Summary and Results)

Species/Sex/Model	Test Article (Batch No.)	Route	Dose/Administration/Duration	Results	GLP Status	Rpt/Pub. No. & Date (Laboratory)
Other Pharmacologic Activities						
Central Nervous System						
Mouse female NMRI	ibandronate (90017-88-8)	i.p.	1 and 3 mg/kg[a] single dose	Ibandronate was negative in the Irwin behavioral test.	NR	[1730] e2 pdf 7/88 (BM)
Mouse, anesthetized (urethane) female NMRI	ibandronate (90017-88-8)	i.p.	ibandronate: 3 and 10 mg/kg[a] diazepam. 2 mg/kg; single dose	Ibandronate did not potentiate the anesthetic effect of urethane, whereas diazepam resulted in 80% potentiation of the anesthetic effect	NR	[1731] e10 pdf 10/88 (BM)
Mouse, female NMRI	ibandronate (90017-88-8)	i.p.	ibandronate: 1 and 3 mg/kg[a] diazepam. 2 mg/kg (p.o.) pervitin. 2 mg/kg (p.o.), single dose	Ibandronate had no effect on spontaneous locomotor activity at 1 mg/kg. At 3 mg/kg, ibandronate caused decreased motility comparable to that caused by 2 mg/kg of diazepam. Motility was potentiated by pervitin.	NR	[1732] e6 pdf 9/88 (BM)
Mouse, conscious female NMRI	ibandronate (90017-88-8)	i.p.	ibandronate: 1 and 3 mg/kg[a] diazepam. 2 mg/kg Dopram® 100 mg/kg, single dose	Ibandronate had no effect on pentetrazole-induced cramps. In comparison, diazepam almost completely inhibited cramps and Dopram® almost doubled the intensity of cramps.	NR	[1733] e7 pdf 9/88 (BM)
Mouse, male SWISS	ibandronate (454-555-00)	s.c.	ibandronate: 0.01, 0.1, and 1 mg/kg[a] single dose indomethacin 3 mg/kg single dose	Ibandronate had no effect in the phenyl-p-benzoquinone writhing test in the mouse while indomethacin had a marked analgesic effect characterized by a decrease in the number of writhing responses.	YES	[1734] e16 pdf 2/98
Gastrointestinal System						
Mouse, female NMRI	ibandronate (90017-88-8)	i.p.	ibandronate: 1 and 3 mg/kg[a] atropine: 2 mg/kg single dose	Ibandronate had no effect on intestinal motility, whereas atropine decreased.	NR	[1735] e1 pdf 7/88 (BM)
Rat, anesthetized male Sprague-Dawley	ibandronate (90017-88-8)	i.v.	ibandronate: 1 mg/kg[a] cimetidine: 8 mg/kg carbachol: 9 µg/kg, single 15-min infusion	Ibandronate had no effect on gastric acid secretion The production of gastric acid was promoted by carbachol and reduced by cimetidine.	NR	[1736] e4 pdf 8/88 (BM)

NR = Not required, BM = Boehringer Mannheim GmbH,
a. Doses expressed as the weighed drug substance

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Table 4 Other Pharmacology Studies (Protocol Summary and Results) (Cont.)

Species/Sex/Model	Test Article (Batch No.)	Route	Dose/Administration/Duration	Results	GLP Status	Rpt/Pub. No. & Date (Laboratory)
Cardiovascular System						
Rat, conscious, normotensive male Wistar	ibandronate (820 687 01)	i v	0 01 to 3 mg/kg[a] in increasing doses at 15-min intervals	Ibandronate had no effect on blood pressure or heart rate when administered by i v or s.c routes.	NR	[1737] e12 pdf 7/89 (BM)
		s.c	3 mg/kg[a], single dose daily for 3 consecutive days			
Dog, conscious, normotensive mongrel	ibandronate (90017-88-8)	i v	Cumulative dose of 1 mg/kg (0 1, 0.2, and 0 7 mg/kg given at 10-min intervals)[a]	Ibandronate had no relevant effects on hemodynamic parameters (blood pressure, heart rate, cardiac output, stroke volume, total peripheral resistance, and ECG)	NR	[1738] e13 pdf 7 91 (BM)
Dog, conscious beagle	ibandronate (90017-88-8)	i v	Cumulative dose of 1 mg/kg (0 1, 0.2, and 0 7 mg/kg[a] given at 15-min intervals)	Protracted administration of ibandronate had no effect on hemodynamic parameters (blood pressure, heart rate, and ECG) or blood chemistry parameters indicative of respiratory function (pH, pCO_2, pO_2, HCO_3, and BE).	NR	[1739] e5 pdf 10/88 (BM)
hERG K^+ channel, expressed in CHO cells	ibandronate (MH00047007)	*In vitro*	30 µM ibandronate (corresponding to approximately >10,000 ng/mL), and 10 µM of E-4031 (hERG K^+ channel blocker, used as a positive control)	Ibandronate, tested for its pro-arrhythmic potential in the hERG K^+ channel assay at a concentration of approximately 10 times the C_{max} in humans had no influence on in inward and outward K^+ currents.	NR	[1751] 1006141 pdf 09/01
Renal Function						
Dog, conscious, episiotomized female beagle	ibandronate (90017-88-8)	i v	0 1 and 1 mg/kg[a], single dose	Ibandronate had no effect on urine volume, electrolyte excretion, or Na^+/K^+ ratio	NR	[1740] e8.pdf 10/88 (BM)

pCO_2 = Carbon dioxide partial pressure; pO_2 = Oxygen partial pressure, HCO_3 = Actual bicarbonate concentration, BE = Base excess, NR = Not required; hERG K^+ = human ether-a-gogo related gene potassium channel, (CHO) cells = Chinese hamster ovary cells· BM = Boehringer Mannheim GmbH, R = F Hoffmann-La Roche Ltd.
a. Doses expressed as the weighed drug substance

Table 4 Other Pharmacology Studies (Protocol Summary and Results) (Cont.)

Species/Sex/Model	Test Article (Batch No.)	Route	Dose/Administration/Duration	Results	GLP Status	Rpt/Pub. No. & Date (Laboratory)
Renal Function (Cont.)						
Dog, conscious, episiotomized female beagle	ibandronate (90017-88-8)	p.o.	5 and 10 mg/kg[a], single dose	The 5 mg/kg group had a significant ($p < 0.05$) increase in K^+ excretion in the 2- to 6-hr sampling period, whereas the 10 mg/kg group experienced no change At 10 mg/kg, urine volume and Na^+/K^+ ratio were significantly decreased. No significant differences from controls were noted in the 0 to 2- or 0 to 6-hr ibandronate samples at either dose.	NR	[1741] e3.pdf 8/88 (BM)
Other Investigations						
Rabbit, conscious, crossbred	ibandronate (90017-88-8)	i v.	1 mg/kg[a] via infusion pump (1 mg/mL, 0 5 mL/min)	Ibandronate had no effect on body temperature or blood glucose concentration.	NR	[1742] e9 pdf 10/88 (BM)
Global screening	ibandronate (NS)	Various	Various *in vivo* and *in vitro*	A minimal increase in urinary elimination of Na^+ and K^+ occurred in rats given a single p o. dose of 20 mg/kg. No other effects were noted.	NR	[1743] e11 pdf 1 89
Guinea pig, Dunkin-Hartley	ibandronate (487 624-01)	Various	Various	Ibandronate was determined not to be antigenic in the following anngenicity assays. ASA, PCA, PHA	NR	[1744] e14 pdf 6/96 (BM)
Peripheral human leukocytes, enzyme immunoassay	ibandronate (45 480-02) Clodronate, NS Alendronate, NS Pamidronate, NS	*In vitro*	0 0001 to 0 01 mg/mL	None of the bisphosphonates at concentration 0 0001 to 0 01 mg/mL had an effect on the synthesis of TNFα, IL-1β, IL-1ra, or IL-6 by LPS-stimulated human peripheral mononuclear cells	NR	[1745] e15 pdf 7/97 (BM)

NR = Not required, NS = Not specified; ASA = Active systemic anaphylactic reaction, PCA = Passive cutaneous anaphylaxis test, PHA = Passive hemagglutination anaphylaxis test, BM = Boehringer Mannheim GmbH; P =
a· Doses expressed as the weighed drug substance

Ibandronate was not found to have any effect on gastrointestinal motility or secretion, general behavior, electrolyte excretion, cardiovascular parameters, blood chemistry, pentetrazole-induced cramps, body temperature, blood glucose or potentiation of urethane anesthetic effect. Irwin's behavioral test was negative

SAEFTY PHARMACOLOGY SUMMARY

Safety pharmacology studies showed that single doses of ibandronate did not affect CNS, GI or cardiovascular function at doses of ca. 100-2000 times the intended human oral dose of 2.5 mg/day (0.04 mg/kg/day p.o. ≈ 0.00025 mg/kg i.v.), based on mg/m^2 comparison. Ibandronate did not affect *in vitro* hERG K+ channel currents at concentrations >10,000 ng/mL, which is >10,000x human Cmax of 0.6-0.8 ng/mL at the 2.5 mg/day oral dose, and >10x the Cmax of approximately

900 ng/mL at a human i.v. bolus dose of 3 mg/kg. Renal function in dogs at a single p.o. doses of 5-10 mg/kg (63-125x human oral dose, mg/m2 basis) appeared to be affected in that urine volume and Na/K ratio were decreased at 10 mg/kg and K+ excretion increased at 5 mg/kg but not at 10 mg/kg. However, equivalent and 10-fold higher IV doses did not have such effects.

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III. PHARMACOKINETICS/TOXICOKINETICS

Ibandronate is a highly polar substance and, like other bisphosphonates, is poorly absorbed via the oral route. For that reason, several nonclinical ADME studies were done using the s.c. or i.v. dosing route. Single dose pharmacokinetic studies were performed in the rat (i.v., s.c.), and the dog (i.v., p.o., s.c.). Clearance after repeated dosing was determined in the rat and dog (i.v), and PK upon repeated i.v. dosing was determined in the monkey. Tissue distribution studies are relevant because the compound is preferentially taken up by bone (and some other organs), which is the target organ for the pharmacologic action of the drug. Compound is eliminated slowly from the bone tissue. Accordingly, a single drug dose continues to be pharmacologically active for an extended time. In early studies, radioactive ibandronate was used while [redacted] were developed later for determination of drug in plasma/serum and urine. [redacted] methods were also developed, and this method was used for determination of compound in bone. Some of the early PK and ADME studies were reviewed in the original IND review (GLP and non-GLP data) by Ron Steigerwalt (December 16, 1994). Sponsor's tabular summaries of most of the ADME studies are appended to this section of the Review. TK data from multiple dose toxicity studies are evaluated in the Toxicity Study section of this NDA Review.

Pharmacokinetics and bioavailability

Data on Cmax, AUC, CL, T1/2 and excretion in single dose studies are summarized in the Table below. Elimination half life was similar after and i.v. and p.o. dosing.

In the dog, upon s.c. dosing, there is rapid absorption (T_{max} 0.5h). In the dog, although absorption based on AUC (i.v. vs p.o.) appeared to be 4.6%, excretion data indicated that oral bioavailability was 0.89%. For the purpose of the review, Reviewer assumed oral bioavailability in the dog of 1%. Oral bioavailability is further suppressed by food.

PK and single dose oral bioavailability in the rat was not established due to extremely large individual variability in serum levels. However, multiple dose kinetic data are available from oral rat toxicity studies. Based on single dose iv and sc PK data and multiple dose oral and IV TK data Reviewer concluded that rat oral bioavailability is 0.6% in fasted condition. This is the same value as in humans. After i.v. administration to rats, there were no substantial differences between male and females.

Monkey PK data from a repeat i.v. dose study (10, 30, 150 ug/kg, once monthly) indicated linear, dose-dependent increases in C_{max} and AUC_{0-24}. Data on ibandronate concentrations in bone are also available for this study.

Single dose data

Study Nr.	Dose (mg/kg)	Route	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-\infty}$ (ngh/mL	CL or Cl/f (mL/min kg)	L_z (h^{-1})	$T_{1/2}$ (h)	V_z (L/kg)	Excretion, recovery			CL_R (mL/min kg)	Other data
										% in urine (0-24h)	% in urine (0-96h)	% in feces (0-96h)		
RAT														
I5	0.1	i.v.	-	-	125	13.7	0.18	7.1	7.6	22	24	16	3.3	Appr. 36% found in bone
I11	0.03	s.c.	39.6	0.5	38.1	13.1	0.13	5 4	6.1	33 5	-	-	4 4	-
DOG														
I4	0.1	i v.	-	-	746	2 3	0.016	49	9.2	62	69	0.4	1.7	
I4	1	p.o.	33	0.7	323	53	0.011	64	-	1.66	2.3	89	-	4 6% absorption of radioactivity
I12	0 01	s.c.	20	0.5	79	2.4	0.02	42	-	42	-	-	1.7	

CL = total body clearance
CL/f = total body apparent clearance
L_z = elimination rate constant
V_z = apparent volume of distribution

CL_R = renal clearance

Multiple dose PK data (toxicokinetics)
Toxicokinetic studies were performed for the repeated dose oral and IV toxicity studies in rats and dogs and for the oral carcinogenicity studies in rats and mice. Toxicokinetic parameters exhibit a wide variation in individual values. Multiple dose rat studies showed that fasting before dosing increases the oral availability several-fold (5-10x). Data on clearance indicated reduced clearance and increased AUC after repeated (weekly) dosing in the rat (I5, H7). The AUC(0-4h) accounts for approximately 80% of the AUC for the dosing interval due to rapid uptake into bone and renal excretion.

Tissue distribution
Upon single i.v. injections in the rat, radioactivity was retained at 2h, 24h, and 96h post dose in bone >> liver> kidney > spleen (order of % of dose retained) (I2, I3, I15). Level in bone after 96h was >240 ng/g. Plasma levels were BLQ within 24h. At 2 and 24h, 50% of dose was found in calcified tissue, and 2% in noncalcified tissue. Bisphosphonates are known to have very high affinity for bone and accumulate particularly in metaphyseal cancellous bone. The compounds are eliminated slowly from the bone with elimination half lives of months to years.

In a study with radiolabeled ibandronate (I15), about 50% of the administered dose was found in calcified tissue at 2 and 24h, 10% of which was in the lumbar vertebrae. In addition, virtually no radioactivity was found in the brain, which means that ibandronate does not readily cross the blood-brain barrier.

In one study with single dose administration by i.v. route in rats, tissue levels were determined up to 1 year after dosing (Study I8). Compound was detected in bone (femur), carcass, femur joint, kidney, liver, spleen. After 1 year, 18% of administered radioactivity remained in the animals, with 16% of dose in carcass, and 0.005% in kidney and 0.01 % in spleen. Elimination half-lives from bone, carcass and femur joint were ca. 500, 380, 440 days.

TISSUE	Level	Day 1	Day 21	Day 90	Day 365	T1/2 (days)
Plasma	Mcg/L	0 3	BDL	BDL	BDL	-
Bone (femur)	mcg/kg	196	265	195	182	
	% of dose	0.7	1.2	1.4	0.9	Ca 500
Carcass	mcg/kg	41	24	18	7.1	
	% of dose	33	28	28	16	Ca. 380
Joint (femur)	mcg/kg	365	369	216	165	
	% of dose	3.2	4.1	1.9	1.3	Ca. 440
Kidney	mcg/kg	24	11	2.2	0.3	
	% of dose	0 3	0.15	0.02	0.005	24
Liver	mcg/kg	27	7	1	BDL	
	% of dose	1 5	0.5	0.07	BDL	,22
Spleen	mcg/kg	73	79	35	2.9	
	% of dose	0 25	0.24	0.10	0 01	77

BDL below detection limit

Evaluation of tissue distribution after repeated administration (7 days, 0.1 mg/kg i.v.) in rats showed that compound distributed similarly as after a single dose (bone>>kidney cortex>liver>spleen) (I22). However, after 7 doses concentrations in calcified tissue (femur shaft and joint, lumbar vertebra, carcass) and kidney were 5-7 fold higher as compared to after a single dose, while accumulation in liver and spleen was ca. 2-fold (I25).

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

The uptake of ibandronate in bone was determined in several pharmacology and toxicology studies. Uptake was dose-dependent and essentially linear in femur, vertebra, tibia. In male bones, after 104 weeks of dosing (J8), drug concentration in femur (24ng/mg, at 15 mg/kg/day, orally, fed) was twice that in females (I18). In retired breeder rats (D28), bone concentrations after 20 weeks of treatment in rats was nearly unchanged after a subsequent 20-week treatment-free period (I21). In OVX Wistar rats (D25), bone levels in femur and vertebra varied between 1 and 10 ng/mg in the s.c. dose range that had optimal effects on bone mass (0.001-0.005 mg/kg/day) (I20).
In rats, uptake in bone is linear and related to total dose rather than treatment schedule in vertebra and tibia (within limits of experimental designs, e.g., once-daily dosing compared with a 25-fold higher dose every 25 days, Study D25) (I20).
In monkeys, dosed with 10, 30, 150 ug/kg/mo, i.v., for 16 months, bone levels at end of study were dose-dependent and varied between 0.6-6.1 ng/mg in lumbar vertebrae, and between 0.24 and 1.7 ng/mg in tibia (Monkey study D30, ADME Study I17). Relating the pharmacologic action of the compound to bone levels (if available) may thus be more relevant than relating it to plasma levels.

Metabolism
There is no evidence of metabolism after entrance of compound in the systemic circulation (I7, I6).

Drug-drug interaction
In the rat, in vivo (0.1 mg/kg, i.v., 7 days), ibandronate induced no increase in liver cytochrome P450, b5, c-reductase, or other metabolizing liver enzymes (I24). In human liver microsomes, ibandronate did not inhibit the major cytochrome P450 iso-enzymes, suggesting that hepatic drug-drug interaction is unlikely (I26). Based on results of equilibrium dialysis and ultrafiltration experiments, protein binding interactions are considered unlikely to occur with ibandronate (I9). Renal excretion of ibandronate in rats was not affected by inhibitors of ionic renal transport mechanisms, indicating that the compound is not secreted by ionic renal tubular transport (I27).

Excretion
When dosed by the oral route, most of the dose is eliminated/recovered unchanged in the feces. Upon i.v. dosing, renal excretion is the main route of elimination. At 96 hours after i.v. administration, the recovery of radioactivity in urine and feces of rats and dogs was 40% and 68% of the administered dose, respectively (Table for single dose data). These incomplete recoveries are due to the retention of ibandronate in bone (the target organ for pharmacology) and to a lesser extent in liver and kidney (the target organs for toxicity). However, the small fraction of the dose absorbed upon oral dosing is partly excreted by the kidney, and partly taken up by bone, as it is with i.v. dosing. Fecal, i.e., biliary excretion upon i.v. dosing in dogs and rats is negligible (0.035% of dose at 6h after 0.1 mg/kg iv dosing in rats) (I23).

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

Protein binding
Ibandronate binds to serum proteins independent of concentration in the range of [redacted] At higher concentrations, such as those attained in animal toxicity studies protein binding in rats and dogs is slightly reduced. In human serum, binding is reduced at very high concentrations (>1000 ng/mL). At the effective oral clinical dose, human plasma levels are not likely to not exceed ca. 10 ng/mL. Thus, although protein binding in rats and dogs appears to be slightly lower than in humans (and more so with increasing animal plasma concentration), the difference does not appear large enough (0.8-0.9x) to warrant correction of total plasma levels

when comparing plasma concentrations between species. If correction were made, multiples based on plasma levels would become slightly higher.

% of bound ibandronate in serum (human) or plasma (dog,rat)

Study	N1	N1	N1		N16
Concentration	0 002 mg/L	1 mg/L	50 mg/L		0.0005-0 01 mg/L
	2 ng/mL	1000 ng/mL	50,000 ng/mL		0.5-10 ng/mL
Human	99%	85	50%		99-85
Dog	-	75	-		80
Rat	-	76	-		86

PHARMACOKINETICS/TOXICOKINETICS SUMMARY

Ibandronate is poorly absorbed after oral administration (1% of dose or less). After oral administration, Tmax is 0.5-1 h and compound is rapidly cleared (within hours) from plasma by uptake in bone and renal excretion. Uptake in the bone compartment is reflected by a high volume of distribution. Fasting greatly enhances oral bioavailability. Approximately 40-50% of an absorbed dose (injected or oral) is taken up and stored by bone, while approximately 50% of the absorbed dose is eliminated unchanged via the kidney. Upon repeated dosing, the AUC(0-4h) accounts for approximately 80% of the AUC for the dosing interval. Bone levels attained after even a single dose remain high for several months. There is some retention and accumulation of ibandronate in spleen, kidney, and liver. Uptake in bone is linear and related to total dose rather than treatment schedule. In pregnant rats, ibandronate is transferred to the fetus and in lactating rats it is excreted in the milk. In the relevant concentration range, binding to plasma proteins is similar for rat, dog and human [redacted]. There is no evidence for metabolism in rats or dogs, and no evidence for hepatic or renal drug-drug interaction.

Table 2 Ibandronate Related ADME Studies

Species/Test Type	Kinetic	Tissue Distribution	Metabolism	Excretion	
Rat:					2000. I5.pdf 2001. I11.pdf
Single i v. dose of ^{14}C-ibandronate	[2000, 2004, 2001]	[2007]	[2000, 2020]	---	2002: I4.pdf 2004: I10.pdf
Single i v. dose of ^{14}C-ibandronate	---	[2007, 2008]	---	[2007, 2008]	2007: I3.pdf
Repeated i v. dose of ^{14}C-ibandronate	---	[2011, 2012]	[2021]	---	2008 I15 pdf 2011: I22.pdf
Dog:					2012: I25.pdf 2020: I6 pdf
Single i.v. and p.o. dose ^{14}C-ibandronate	[2002, 2004]	---	[2020]	---	2021: I24.pdf

Table 3 ADME Studies (Protocol Summary and Study Results)

Study Description	Test Article (Batch No.)	Kinetic Parameters		Other	GLP Status	Report. No. & Date (Laboratory)	
Kinetics and Bioavailability							
Rat, Sprague-Dawley, 6 males and 5 females, single i.v. injection of 0 1 mg/kg ^{14}C-ibandronate	^{14}C-ibandronate (1500)		i.v.	Renal excretion was predominant route of elimination; also apparent fecal recovery recorded in this study was far higher than that found subsequently [2010, 2008, 2025]. indicating possible contamination with urine during the sample collection No metabolite was detected in urine, which suggests that ibandronate is not metabolized in rats after a single i.v injection. Plasma radioactivity, therefore, represented the concentration of the parent compound. * Although the study report recorded this parameter as $AUC_{(0\text{-}\infty)_{obs}}$ from 24h onwards all plasma levels were below the limit of quantification ([redacted] As the C_{24h} values were close to the detection limit., the elimination rate constant (λ_z) reported, is probably overestimated. Consequently, $AUC_{(0\text{-}\infty)_{obs}}$, $t_{1/2z}$, V_z, and Cl are also influenced.	NR	[2000] 11/91 (BM)	2000: I5.pdf 2010. I13.pdf 2008 I15 pdf 2025. I23.pdf
		$AUC_{(0\text{-}24h)}$ (ng/h/mL)*	124				
		$AUC_{(0\text{-}\infty)}$ (ng/h/mL)	125				
		Cl (mL/min/kg)	13 7				
		λ_z (h)	0 1796				
		$t_{1/2}$ (h)	7 1				
		V_z (L/kg)	7 6				
		Excretion, recovery					
		% in urine (0-24 h)	21 9				
		% in urine (0-96 h)	24 2				
		% in feces (0-96 h)	15 6				
		Cl_R (mL/min/kg)	3.25				

BM = Boehringer Mannheim GmbH; NR = Not required

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters		Other	GLP Status	Report. No. & Date (Laboratory)	
Kinetics and Bioavailability (Cont.)							
Rat, Wistar, 40 females; single s.c. injection of 0 03 mg/kg ibandronate	Ibandronate (451 326 00)	Plasma		Comparing the dose-normalized AUC of this study, with the above values [2000], indicates complete absorption/bioavailability for s.c. administration The limitations described above for calculation of the elimination rate constant and derived parameters also apply to that study. * This value is included in the summary tabulation of PK parameters in the report, but there is no mention in the Methods of how it was measured, nor and corresponding data in the Results. The value is, however, consistent with the first protein binding study [2027] *[these statements rely upon the questionable PK parameter values]*	NR	[2001] 8/95 (BM)	2001: I11 pdf 2000: I5 pdf
		C_{max} (ng/mL)	39 6				
		T_{max} (h)	0.5				
		$AUC_{(0-24h)}$ (ng/h/mL)	37.8				
		$AUC_{(0-\infty)}$ (ng/h/mL)	38 1				
		Cl/f (mL/min/kg)	13 1				
		λ_z (h)	3				
		$t_{1/2}$ (h)	0 12				
		Protein binding*	9				
		(% at 100 ng/mL)	5 4				
			77.2				
		Excretion, recovery	*				
		% in urine (0-24 h)					
		Cl_R (mL/min/kg)					
			33 5				2027: n1.pdf
			4 4				

BM = Boehringer Mannheim GmbH; NR = Not required

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters	i.v.	p.o.	Other	GLP Status	Report. No. & Date (Laboratory)	
Kinetics and Bioavailability (Cont.)								
Dog, beagle, 3 sex, 0 1 mg/kg ^{14}C-ibandronate by single i v. injection and 1 mg/kg solution p o.	^{14}C-ibandronate (1500)				In contrast to the rat [2000], the concentration-time profile in dogs was more completely defined, with quantifiable plasma levels of radioactivity to 96 hours for both the i v. and p o dose, thus derived kinetic parameters can be considered reliable After i v administration, no metabolite was found in urine, although renal excretion was the predominant route of elimination This suggests that dogs do not metabolize ibandronate once it enters the systemic circulation, thus, the kinetics of radioactivity after i v injection represent those of the parent compound. The high V_z indicated penetration into a deep compartment After p o. administration, radioactivity recovered from urine was 2.3%, that in feces was 89%. About 25% of radioactivity in urine was attributed to the parent drug, with the remaining radioactivity attributed to a compound (M1), which was identical to a synthetic impurity in the radiolabeled ibandronate. Based on both plasma AUCs and urinary recoveries, the mean absorption of radioactivity after p o administration was <5%. Bioavailability of the parent compound after p o administration, assessed from its excretion in the urine, was 0 89%.	NR	[2002] 7 91 (BM)	2000. I5.pdf 2002: I4.pdf
		Plasma						
		C_{max} (µg/L)	-	33 0				
		T_{max} (h)	-	0 7				
		$AUC_{(0-96h)}$ (µg/h/L)	682	237				
		$AUC_{(0-\infty)}$ (µg/h/L)	746	323				
		Cl/f (mL/min/kg)	2.30	52 7				
		λ_z (h)	0 0155	0 01				
		$t_{1/2}$ (h)	48 9	12				
		V_z (mL/kg)	9.2	63.5				
				-				
		Excretion, recovery						
		% in urine (0-24 h)	62.0					
		% in urine (0-96 h)	69 0	1 66				
		% in feces (0-96 h)	0 4	2.30				
		Cl_R (mL/min/kg)	1 74	88 9				
				-				

BM = Boehringer Mannheim GmbH; NR = Not required

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters		Other	GLP Status	Report. No. & Date (Laboratory)	
Kinetics and Bioavailability (Cont.)							
Dog, beagle, 8 females; single s.c. injection of 0.01 mg/kg ibandronate	Ibandronate (451 326 00)	Plasma		Comparing dose-normalized AUC for this study, with the above values [2002], indicates complete absorption/ bioavailability for s.c. administration. A similar conclusion is also reached, based on a comparison with steady-state concentrations [2004]	NR	[2003] 8/95 (BM)	2003 I12 pdf
		C_{max} (ng/mL)	20.0				2002: I4.pdf
		T_{max} (min)	30				
		$AUC_{(0-max)}$ (ng/h/mL)	54.7				
		$AUC_{(0-\infty)}$ (ng/h/mL)	79.1				
		Cl/f (mL/min/kg)	2.36				
		λ_1 (h)	1.040				
		$t_{1/2}$ (h)	0.67				2004 I10.pdf
		λ_z (h)	0.0193	Apparent distribution volume (Cl/f $/\lambda_z$) was 7.3 L/kg, indicating penetration into a deep compartment. Terminal half-life of 42 h depended on rate of redistribution into the circulation rather than on clearance from the central compartment			
		$t_{1/2}$ (h)	41.8				
		Protein binding* (% at 500 ng/mL)	86.4*				
		Excretion, recovery					
		% in urine (0-24 h)	41.9				
		Cl_R (mL/min/kg)	1.68				
				* This value is included in the summary of PK parameters in the report, but there is no mention in the Methods of how it was measured, or corresponding data in the Results. The value is, however, consistent with the equilibrium dialysis data in the ex vivo dog protein binding study [2030]			2030: I9 pdf

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters				Other	GLP Status	Report. No. & Date (Laboratory)	
Kinetics and Bioavailability (Cont.)									
Dog, beagle, data from a single i.v. injection [2002] compared to multiple doses of 0.075 or 0.15 mg/kg/wk ibandronate for 6 months [1019], see Toxicology Summary page 158	^{14}C-ibandronate (NS)	Dog				In dogs, there was no appreciable difference between Cl after a single dose and during repeated administration.	NR	[2004] 8/95 (BM)	2004: I10.pdf
		Cl (mL/min/kg), 0.1 mg/kg single dose	2.30 [2002]						2002: I4 pdf
		Cl (mL/min/kg), 0.75 mg/kg/wk for 6 months	2.86 [1019] 3.29						1019: h6.pdf
		Cl (mL/min/kg), 0.15 mg/kg/wk for 6 months	[1019] 2.89						
		Weighted mean							
Rat, Sprague-Dawley, data from a single i.v. injection of 0.1 mg/kg ^{14}C-ibandronate [2000] compared to multiple doses of 0.15 or 0.3 mg/kg/wk ibandronate for 6 months [1016]; see Toxicology Summary page 158	^{14}C-ibandronate (NS)	Rat				In rats, there was a large difference between the apparent Cl after a single dose and during repeated administration. This is likely due (at least in part) to the plasma concentration-time profile for the single dose not being completely defined.			2000. I5 pdf
		Cl (mL/min/kg), 0.1 mg/kg single dose	13.74 [2000]						1016: h7 pdf
		Cl (mL/min/kg), 0.15 mg/kg/wk for 6 months	5.38 [1016]						
		Cl (mL/min/kg), 0.3 mg/kg/wk for 6 months	4.32 [1016]						
Monkey, Cynomolgus, 12-15 ovariectomized females; i.v. administration of 0.01, 0.03, and 0.15 mg/kg every 30 days for 16 months	ibandronate (871 449-59-Mb)		Dose (mg/kg) 0.01	0.03	0.15	Serum kinetics of ibandronate were linear and dose-dependent in the tested dose range.	Yes	[2005] 09/99 (BM,	2005: I16.pdf
		Serum values (median)							
		$C_{0.167h}$ (ng/mL)	55.69	200.02	877.25				
		C_{2h} (ng/mL)	0.10	0.60	3.08				
		AUC_{24h} (ng/h/mL)	62.17	197.85	1114.91				
		Dose normalized AUC	62.17	65.95	74.33				

BM = Boehringer Mannheim GmbH:

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters	Other	GLP Status	Report. No. & Date (Laboratory)	
Tissue Distribution after Single Administration						
Rat, Sprague-Dawley, 4/sex; single i v injection of 0.1 mg/kg ^{14}C-ibandronate, studied by whole body autoradiography	^{14}C-ibandronate (1471)		Distribution was essentially confined to four tissues - in order of decreasing concentration after 24 h in male rats: bone >> kidney > spleen, > liver; after 24 h in female rats, bone >> spleen >> kidney > liver; after 48, 72, and 96 h in male and female rats: bone >> spleen > kidney > liver	NR	[2006] 5/90 (BM)	2006: I2.pdf
Rat, Sprague-Dawley, 6 males and 5 females, single i v injection of 0.1 mg/kg ^{14}C-ibandronate, terminal study in animals used to measure 0-96 hour plasma levels and urine/fecal excretion [2000]	^{14}C-ibandronate (1500)		Mean tissue concentrations after 96 h: bone > 240 ng/g (varies with site), spleen 93 ng/g, kidney 43 ng/g, carcass 42 ng/g (due to residual bones), liver 29 ng/g; all other tissues <4 ng/g. Plasma levels below detection limit (<1.05 ng/mL) after 24 h. [2000]	NR	[2007] 12/90 (BM)	2007: I3 pdf 2000: I5.pdf
Rat, Sprague-Dawley, 3/sex, single i v injection of 0.1 mg/kg ^{14}C-ibandronate	^{14}C-ibandronate (2444)		Distribution (as % dose) at 2, and 24 hours, compared with previous 96 hour data [2007] : bone >> liver > kidneys > spleen. Approx. 10% administered dose recovered in urine and feces, of which renal elimination accounted for 9.4%. At 2 and 24 h, 50% of dose found in calcified tissue and 2% in noncalcified tissue.	NR	[2008] 8/97 (BM)	2008: I15.pdf 2007: I3.pdf

BM = Boehringer Mannheim GmbH; NR = Not required

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters	Other	GLP Status	Report. No. & Date (Laboratory)	
Tissue Distribution after Single Administration (Cont.)						
Rat, Sprague-Dawley, 12/sex, single i v injection of 0.1 mg/kg ^{14}C-ibandronate	^{14}C-ibandronate (NS)	Time (days after administration)	Distribution - concentrations of radioactivity per gram tissue in order of decreasing concentration femur joint >> femur >> spleen > kidney > carcass > liver. After 365 days, 18% of administered radioactivity was found in the animals, with 1.3% of the dose in the joints. To allow for the continued growth of the rats, the elimination half-lives were calculated from the % dose in tissues, rather than the concentrations	NR	[2009] 3/92 (BM)	2009: I8 pdf

	1	2]	9	365	$t_{1/2}$ (Days)
Plasma (µg/L)	[0.3]	[BDL]	[BDL]	[BDL]	--
Bone (femur)	[195 7]	[264 8]	[194.7]	[181 5]	--
	(0 7)	(1.2)	(1 4)	(0 9)	ca 500*
Carcass	[40.7]	[24 4]	[18.1]	[7 1]	--
	(32.9)	(28 4)	(27 5)	(16 1)	ca 380
Joint (femur)**	[365 4]	[369 0]	[215.8]	[164.8]	--
	(3.2)	(4 1)	(1 9)	(1 3)	ca 440*
Kidney	[24.2]	[11.3]	[2.2]	[0 3]	--
	(0.30)	(0.15)	(0.02)	(0 005)	24
Liver	[26.7]	[6 9]	[1 0]	BDL	--
	(1.5)	(0.5)	(0.07)	BDL	22
Spleen	[73.4]	[78 7]	[35 2]	[2.9]	--
	(0.253)	(0.24)	(0.10)	(0 01)	77

Results shown as mean [µg/kg] and (% of dose)
* Value estimated from the last two time points
** Joints are defined as proximal and distal metaphyses plus diaphyses

BM = Boehringer Mannheim GmbH; NR = Not required; NS = Not stated; BDL = Below detection limit

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters			Other	GLP Status	Report. No. & Date (Laboratory)	
Tissue Distribution after Single Administration (Cont.)								
Rat, Sprague-Dawley; 12 pregnant females, single i.v. injection of 0.1 mg/kg ^{14}C-ibandronate, 5 at each time-point for tissue distribution and 1 studied by whole body autoradiography	^{14}C-ibandronate (2444)	Total dam rats (including fetuses)	% of dose (mean) 2 hours	24 hours	Distribution Concentration of ibandronate in fetuses was approx 0 02% of dose after 2 h and 0 008% after 24 h. Placenta accounted for 0 07% of dose after 2 h and 0 03% after 24 h. Amniotic fluid accounted for 0 003% of dose after 2 h and was below quantification limit after 24 h	NR	[2010] 8/98 (BM)	2010: I13.pdf
		Calcified tissue						
		Non-calcified tissue	48 5	34 7				
		- liver	2.32	1 254				
		- kidneys	1 7	0 89				
		- spleen	0.454	0 29				
		Sexual organs	0 13	0 05				
			0.54	0 24				
Tissue Distribution after Repeated Administration								
Rat, Sprague-Dawley, 1 sex, i.v. injection of 0 1 mg kg ^{14}C-ibandronate for 7 consecutive days studied by whole body autoradiography	^{14}C-ibandronate (2444)				Distribution - the relative distribution at 24 hours, bone >>> kidneys (cortex) > liver > feces and spleen A darker shading of cortical bone versus trabecular bone suggests an inhomogeneous uptake of ibandronate by both types of bone. Also kidney levels appeared relatively higher than for a single dose	NR	[2011] 10/98 (BM)	2011: I22.pdf

BM = Boehringer Mannheim GmbH; NR = Not required

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters					Other	GLP Status	Report. No. & Date (Laboratory)	
Tissue Distribution after Repeated Administration (Cont.)										
Rat, Sprague-Dawley, 12/sex, i.v. injection of 0 1 mg kg ^{14}C-ibandronate for 7 consecutive days	^{14}C-ibandronate (2444)		Median concentrations (µg/kg)				Distribution - The highest concentration was found in decreasing order in carcass, femur joint (metaphyses plus diaphyses) and lumbar vertebrae, femur shafts, kidney, liver and spleen Compared to the single dose data (n=3/sex) [2008], calcified tissue and kidney, concentrations were 5-7 fold higher. Accumulation in other tissues was no more than 2-fold.	NR	[2012] 11/98 (BM)	2012: I25.pdf
		Organs	2 h after admin.		24 h after admin.					
			1 dose	7 doses	1 dose	7 doses				
		Calcified tissue								
		- femur shaft	202 0	1,406 0	233 0	1,201 0				
		- femur joint	477.1	3,090 0	433 9	2,504 0				
		- lumbar vertebrae	940 8	5,221 0	1,075 0	4,641 0				
		- carcass	51.5	320 1	51 0	249 4				
		Non-calcified tissue								
		- liver	37.5	15 94	15 95	16.06				2008: I15.pdf
		- kidneys	54 7	251 4	47 5	263.1				
		- spleen	187 9	23 9	32.8	29 0				
Long-term Disposition and Drug Concentration in Bone										
Rat, Wistar, 9-10/sex, daily p.o. administration of 2 67, 6.22, and 13.33 mg/kg once daily for 2 years (samples from carcinogenicity study; see Toxicology Summary page 158)	ibandronate (447 624-00)		Mean concentration in proximal femur (ng/mg):				Ibandronate expressed dose-dependent and linear concentrations in rat bone. Male bones had about twice the concentration than female bones, which is consistent with the stronger growth rate of male rats.	NR	[2013] 8/98 (BM)	2013: I18.pdf
		Dose (mg/kg)	Male rats		Female rats					
		2 67	4 34		2.21					
		6.22	10.97		4.89					
		13 33	23 58		12.25					

BM = Boehringer Mannheim GmbH; NR = Not required

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters			Other	GLP Status	Report. No. & Date (Laboratory)	
Long-term Disposition and Drug Concentration in Bone (Cont.)								
Rat, Wistar, 6 matures/sex, 0 1 mg/kg s c once daily for 7 consecutive days	ibandronate (455 892 00)		Mean concentration (ng mg))		Ibandronate incorporation in long bones and vertebrae is equivalent between male and female adult rats.	NR	[2014] 8/98 (BM)	2014· I19 pdf
			Male rats	Female rats				
		Lumbar vertebra (L5)	6 65	6 15				
		Left femur, total	4 16	4 38				
		Right femur, total	4 13	4 14				
		- proximal	3 77	3 03				
		- distal	5 13	6 15				
		- midshaft cortex	2 61	1 72				
Rat, Wistar, 12-15 female retired breeders, daily s.c. administration of 0 001, 0 003, 0 01 and 0 03 mg/kg once daily for 20 weeks, ± a 20 weeks recovery period without treatment	ibandronate (451 326-00)		Mean concentration (ng mg) in femur (F) and vertebrae L1-L2 (V).		Ibandronate was incorporated in a dose-dependent manner in long bones and vertebrae. The drug-concentration is nearly unchanged after an administration-free recovery period of 20 weeks.	NR	[2015] 10/98 (BM)	2015 I21.pdf
			Without recovery		With recovery			
		Dose (mg/kg)	F	V	F	V		
		0 001	0 42	0 6	0.39	0 6		
		0.003	0 99	1.2	0 93	1 1		
		0 01	3 17	4 0	2 87	3 5		
		0 03	8 31	10 2	7 47	9 9		

BM = Boehringer Mannheim GmbH; NR = Not required

Table 3 ADME Studies (Protocol Summary and Study Results) (Cont.)

Study Description	Test Article (Batch No.)	Kinetic Parameters			Other	GLP Status	Report. No. & Date (Laboratory)	
Long-term Disposition and Drug Concentration in Bone (Cont.)								
Rat, Wistar, 9-13 ovariectomized females, daily s c administration of 0 0002, 0.001, 0.005, 0.025 mg/kg or 25 and 125 mg/kg every 25 days for 12 months starting 10 weeks after ovariectomy (doses cover full range for preventing bone loss in rats; i e sub- to supra-optimal)	ibandronate (451 326-00)		Mean concentration (ng/mg)		Concentration of ibandronate in tibiae and vertebrae was dose-dependent and linear suggesting that linear kinetics apply in the tested dose range Drug uptake was related to the total dose administered irrespective of the treatment schedule.	NR	[2016] 9/98 (BM)	2016. I20.pdf
		Dose (mg/kg)	Vertebrae (L1)	Tibia (right)				
		0 0002 daily	0.35	0 23				
		0 001 daily	1.58	0 77				
		0 005 daily	6 76	3 38				
		0 025 daily	31 23	16.22				
		0.025 every 25 days	1 97	0 95				
		0 125 every 25 days	7.30	3 97				
Monkey, Cynomolgus, 12-15 ovariectomized females; i.v. administration of 0.01, 0 03, and 0 15 mg/kg every 30 days for 16 months	ibandronate (871 449-59-Mb)		Doses (mg/kg)		Ibandronate expressed higher concentrations in vertebrae than in tibiae. In tibiae, higher values occurred in proximal metaphysis than in distal metaphysis for both trabecular and cortical bone Non-linear dose-dependency is consistent with inhibition of bone turnover.	Yes	[2017] 11/98 (BM,	2017: I17 pdf
			0.01	0.03	0.15			
		Mean concentration (ng/mg)k						
		Tibia, total	0.24	0 50	1 68			
		- proximal trabeculae	0.33	0 89	2 40			
		- distal trabeculae	0.23	0 47	1.29			
		- proximal cortex	0.29	0 62	2 11			
		- midshaft cortex	0.24	0.32	1 08			
		- distal cortex	0.16	0 35	1 14			
		Lumbar vertebra (L6)	0 64	1 60	6 14			

BM = Boehringer Mannheim GmbH; NR = Not required

IV. GENERAL TOXICOLOGY

The toxicology program was conducted according to GLP conditions. Acute, single dose toxicity studies were performed in rats and mice by the oral route and in rats, mice and dogs by the IV route. Repeated dose toxicity studies were carried out in rats (SD or Wistar) and dogs (beagle) by oral and IV routes. Studies were identified by letter and number. Toxicokinetic data were collected in several of the oral and IV repeated dose toxicity studies. PK data are also available from ADME studies. For the purpose of the review of this NDA for osteoporosis (recommended dose of 2.5 mg/day, orally), the oral toxicity studies were considered most relevant and the data from these studies have been reviewed in more detail. A complete list of all toxicology studies has been appended to this review (Sponsor's Table 1).

The doses of ibandronate in the toxicity studies are expressed either as ibandronate monosodium salt (weighed drug substance, WDS), or free acid equivalent (FAE). The conversion factor is 1.125 (range , with 1 g FAE = 1.125 g monosodium salt. No major impurities were identified in the test substance. Ibandronate content was >99%, and impurities comprised mainly

Results of acute and repeat dose toxicity studies have been tabulated with LOAEL values. In the acute toxicity study tables NOAEL levels were converted to multiples of the oral recommended human dose (RHD) of 2.5 mg/day based on mg/m^2 body surface area (BSA) comparison. In the repeat dose studies multiples of the NOAEL levels were calculated in two different ways, based either TK data from the toxicity studies or on mg/m^2 comparison. The data from a 2-week repeat dose oral gavage study in rats (G2) have not been included. This study was a TK study (0, 5, 10mg/kg/day) and there were no toxicities.

A detailed review of the 12-month oral dog study (H5) was performed for this NDA review. Reviews of other oral toxicity studies (4-week rat G1, 26-week rat H1, 26-week rat H3, 4-week dog G3, 26-week dog H2) were carried out when IND' was originally submitted on September 30, 1994 (Review by Ron Steigerwalt, Ph.D., December 16, 1994). The original IND review also included a summary review of multiple non-GLP or published data submitted with the original IND (pharmacology, ADME, acute rat, mouse, and dog toxicity studies, 1-week dog toxicity study G5, local tolerance studies).

SUMMARY OF ACUTE STUDIES

Acute oral toxicity studies

RAT, acute study: LOAEL levels

Strain			SD
Study Nr			F1
Duration			Acute (14 d)
N			5/s/g
Route			Oral gavage
Doses (mg/kg)			100, 200, 400,640, 1000 WDS
			87, 174, 348, 557, 870 FAE
Feeding			Ad libitum
TK			No
Mortality			640 (2M, 1F) 1000 (4M, 3F)
Signs		Sedation	640
Necropsy	Stomach	Gastic mucosa hemorrhage	640
	Intestine	Intestinal dilation, watery content	640
	Lung	Pulmonary edema and hemorrhage	640
LD50	Dose		811 mg/kg
NOAEL	Dose		400 mg/kg
	Multiple of human 2.5 mg oral dose (mg/m2 basis)*		546x

*Assumption: human BA = 0.6%, rat BA = 0.2% (fasted)

MOUSE, acute study: LOAEL levels

Strain			NMRI
Study Nr			F3
Duration			Acute (14 d)
N			5/s/g
Route			Oral gavage
Doses (mg/kg)			100, 200, 800, 1200, 1600 WDS
			87, 174, 696, 1043, 1391 FAE
Feeding			Ad libitum
TK			No
Mortality			
Signs		Sedation	1200
		Rough pelt, ptosis, hunched posture	1600
Necropsy	Stomach	Mucosal hemorrhage	1200
	Intestine	Dilation, watery content	1200
		Mucosal hemorrhage	1200
	Lung	Pulmonary edema	1200
	Liver	Discoloration	1600
	Renal cortex	Discoloration	1600
LD50	Dose		1494 mg/kg
NOAEL	Dose		800 mg/kg
	Multiple of human 2.5 mg oral dose (mg/m2 basis)*		546x

*Assumption human BA = 0.6%; mouse BA = 0.2% (fasted)

Acute IV toxicity studies

In an acute IV mouse study (10, 20, 40, 50, 64, 80 mg/kg WDS) the acute IV LD50 was 47.8 mg/kg. Toxicity was observed at doses >20mg/kg including sedation, motor activity impairment and paralysis. In mice that died there were pulmonary edema, liver congestion and GI hemorrhage. The NOAEL of 20 mg/kg (i.v.) represents a multiple of 6850x the human oral 2.5 mg dose (0.04 mg/kg), based on mg/m2 comparison and assuming 0.6% bioavailability (BA) in humans.

Findings in the acute IV rat study (10, 20, 25, 28, 32, 40, 64, 100 mg/kg WDS) were LD50 of 30 mg/kg, toxicity (signs) at doses > 20 mg/kg, necropsy findings of hydrothorax, pulmonary edema and hepatic congestion, GI hemorrhage. The NOAEL of 20 mg/kg (i.v.) represents a multiple of 13,700x the human oral 2.5 mg dose, based on mg/m^2 comparison and assuming 0.6% bioavailability (BA) in humans.

In dogs, an i.v. dose of 5 mg/kg (10,400x human 2.5 mg dose, mg/m2 basis, 0.6% human BA) was toxic but not lethal, and there was histologic evidence of renal tubular damage.

In conclusion, acute toxicity studies showed NOAEL values of >500x (oral) or >6000x (i.v.) fold the recommended human dose of 2.5 mg/d, based on mg/m2 comparison. Target organs were kidney, liver, lung, and CNS in all test species. Notably, GI toxicity was observed in rodents in i.v. studies, although at doses leading to higher exposure multiples than oral doses causing GI toxicity. GI effects associated with IV administration have been observed with other bisphosphonates and may be due to exsorption (transport from interstitium to epithelial lumen) of the compounds.

Table 4 Repeated Dose Toxicity Studies – Oral (Protocol Summary)

Species/Strain	No./Group	Route	Test Article (Batch No.)	Dose (mg/kg/day)	Duration	GLP Status	Rpt. No. & Date (Laboratory)	
Rat, Sprague-Dawley	12/sex	p.o. (gavage)	ibandronate (447 624-01)	5, 10[b]	2 wk	Yes	[1005] 4/99 (BM)	1005 g6 pdf
Rat, Sprague-Dawley	10/sex	p.o. (gavage)	ibandronate (90017-88/8)	1, 3, 10[a] 0.88, 2.65, 8.85[b]	4 wk	Yes	[1006] 2/89 (BM)	1006 g1.pdf
Rat, Wistar	30/sex (up to 10/sex, observation)	p.o. (gavage)	ibandronate (821 339-01)	11.45, 34.28[a]; 10.13, 30.34[b] (restricted feeding)	26 wk 13-wk observation	Yes	[1007] 2/92	1007: h1.pdf
Rat, Wistar	30/sex (10/sex, observation)	p.o. (gavage)	ibandronate (821 339-01)	1.15, 3.43, 11.45[a] 1.01, 3.04, 10.13[b] (fed ad libitum)	26 wk 13-wk observation	Yes	[1008] 2/92	1008: h3 pdf
Rat, Sprague-Dawley	40/sex (5/sex, observation)	p.o. (gavage)	ibandronate (447 624-00)	3, 10, 20[b]	12 mo 6-mo observation	Yes	[1009] 9/95 (BM)	1009: h4 pdf
Dog, beagle	2/sex	p.o. (enteric-coated tablet)	ibandronate (780 65426Bb)	50[b]	7-day treatment 7-day observation	Yes	[1010] 9/92 (BM)	1010 g5.pdf
Dog, beagle	4/sex	p.o. (gelatin capsule)	ibandronate (820687 01)	1, 3, 10[a] 0.9, 2.69, 8.95[b]	4 wk	Yes	[1011] 2/89 (BM)	1011: g3 pdf
Dog, beagle	5/sex (1/sex, observation)	p.o. (gelatin capsule)	ibandronate (821 339-01)	2.26, 5.65, 14.69[a]; 2, 5, 13[b]	26 wk 13-wk observation	Yes	[1012] 8/96	1012: h2.pdf
Dog, beagle	6 M, 5-6 F (2/sex, observation)	p.o. (tablet)	ibandronate (447 624-00)	2, 5, 10[b]	12 mo 6-month observation	Yes	[1013] 7/95 (BM)	1013: h5.pdf

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

Table 6 Repeated Dose Toxicity Studies – Intravenous (Protocol Summary)

Species/Strain	Animals/Group	Route	Test Article (Batch No.)	Dose (mg/kg) [Volume]	Duration	GLP Status	Rpt. No. & Date (Laboratory)	
Rat, Sprague-Dawley	5/sex	i.v.	ibandronate (447 624-00)	1.8, 2.7 mg/kg[a] 1.7, 2.5 mg/kg[b] [2 mL/kg]	9 days	Yes	[1014] 9/96 (BM)	1014 f113 pdf
Rat, Sprague-Dawley	10/sex	i.v., s.c.	ibandronate (90017-88/8)	0.1, 0.3, 1[a] 0.09, 0.28, 0.9[b] [2 mL/kg]	4 wk	Yes	[1015] 12/88 (BM)	1015 g2 pdf
Rat, Sprague-Dawley	10/sex	i.v.	ibandronate (447 624-00)	0.075, 0.15 (1/wk) or 0.3 (2/mo)[b] [2 mL/kg]	6 mo	Yes	[1016] 7/95 (BM)	1016. h7.pdf
Rat, Sprague-Dawley	10/sex	i.v., s.c.	ibandronate (447 624-00)	0.3, 0.9, 1.8, 2.7 (1/wk)[b] [2 mL/kg]	6 mo	Yes	[1017] 2/96 (BM)	1017: h9 pdf
Dog, beagle	4/sex	i.v., s.c.	ibandronate (90017-88/8)	0.1, 0.3, 1[a] 0.09, 0.28, 0.9[b] [1 mL/kg]	4 wk	Yes	[1018] 11/88 (BM)	1018: g4 pdf
Dog, beagle	4/sex	i.v.	ibandronate (447 624-00)	0.075, 0.15 (1/wk) or 0.3 (2/mo)[b] [1 mL/kg]	6 mo	Yes	[1019] 7/95 (BM)	1019 h6 pdf
Dog, beagle	4/sex	i.v., s.c.	ibandronate (447 624-00)	0.3, 0.9, 2.7 (1/wk)[b] [1 mL/kg]	6 mo	Yes	[1020] 12/95 (BM)	1020 h8.pdf

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

12-MONTH ORAL TOXICITY STUDY OF BM 21.0955·Na IN DOGS

IND Vol.:	11.7-11.11
Sponsor Study No.:	BM 21.0955TOCHH01
Report No.:	H5
Performing Laboratory:	Boehringer Mannheim GmbH, Mannheim, Germany
Study period:	April 1992 - April 1993
Batch Nr.:	447 624-00 (BM 21.0955 Na H_2O microfine)
Description:	White powder
Particle size:	48% 12-200 µm, and 52% <12µm
Gram equivalents:	1.125 g BM 21.0955.Na.H_2O corresponds to 1.0g BM 21.0955 free acid
Purity:	99.6 area-%
Water content:	4.9%
Formulation:	Tablets
Control:	Placebo tablets, undefined.
Assay:	(serum); (urine)
GLP:	GLP compliance statement, GLP certificate and QA statement included
Review date:	November 18, 1999

METHODS

Pure-bred beagle dogs (6/sex/dose group, except 5/sex/group in MDf), average age at start 6 months (5/sex/group), or 21 months (1/sex/group except MDf), mean body weights 7.4 kg (males) and 7.0 kg (females), were used in the study. Unlike the 6-month old dogs, the 21-month old animals had closed femoral/sternal epiphyses. Animals were dosed orally, by tablets, with **0, 2, 5, 10 mg/kg/day** (free acid equivalents), daily for 12 months.

Group	Control	LD	MD	HD
Dose (mg/kg/day)	0	2	5	10
N/group				
Start age 6-mo	6m, 6f	6m, 6f	6m, 5f	6m, 6f
Start age 21-mo	1m, 1f	1m, 1f	1m, 1f	1m, 1f

Animals were given the tablets between 7 and 8 am, with 20ml distilled water, and fed immediately after dosing from study Day 8. Feed (300 gram/day) was available to animals from 10 am to 3 pm. Water was available ad libitum. All 6/sex/group were observed during the 12-mo treatment period, 4/sex/group were scheduled to be sacrificed after 12 months, and 2/sex/group were continued for a recovery period of 6 months. Blood samples were taken in weeks 3,6,9,14,26,39,48,52, and in weeks 56, 65, 78 of the recovery period. Bones examined in histopathology evaluation were rib, sternum and femur.

Blood and urine samples to determine levels of test compound were taken from 3/sex/group, in week 2 and week 51. Sampling times were 0,1,2,4,7,24h after dose for serum, and 0-24h postdose for urine. In HD groups, only urine but not blood was collected. Test compound assay methods used were (serum) and (urine). Toxicokinetic parameters were calculated using the VAX system.

Dose groups in 12 -month oral dog toxicity study

Group		Dose	Males		Females	
			N	Numbers	N	Numbers
0	control	0 mg/kg/day	6	956 ® 957 ® 987 953 998 804*	6	475® 385®* 414 416 482 429
1	LD	2 mg/kg/day	6	986 ® 993 ® 703 958 990 774*	6	470® 476® 403 469 424 386*
2	MD	5 mg/kg/day	6	814 ® 984 ® 976 994 702 814*	5	458® 465® 425 405 428
3	HD	10 mg/kg/day	6	988 ® 816 ®*†wk53 971†wk38 989 991 985	6	436 ® 417 ® 413†wk46 415 473 379*†wk47

† animal died or was sacrificed prematurely
*animal was 21-22 months old at start of treatment
® animal continued after 12-mo treatment for 6-mo recovery

Observation times:

Clinical signs	Twice daily
Body weights	Once weekly
Food consumption	Every day (remainder of 300g was weighed)
Ophthalmoscopy	Predosing (Baseline/Week 0), at 6 months, and at end of study
Heart rate and ECG	Predosing, on Days 1, 3, 8, 15, in Weeks 4, 13, 26, 39, 52, and in Week 77 of recovery period
Reflex tests:	Predosing, in Weeks 3, 6, 9, 14, 26, 39, 48, 52, and in Weeks 56, 65, 78 of recovery period
Hematology	Predosing, in Weeks 3, 6, 9, 14, 26, 39, 48, 52, and in Weeks 56, 65, 78 of recovery period
Clinical chemistry	Predosing, in Weeks 3, 6, 9, 14, 26, 39, 48, 52, and in Weeks 56, 65, 78 of recovery period
Urinalysis	Predosing, in Weeks 3, 6, 9, 14, 26, 39, 48, 52, and in Weeks 56, 65, 78 of recovery period
Fecal blood	Predosing, and in Months 3, 6, 12, 18
Gross pathology	At sacrifice (see Histopathology Inventory in Appendix I)
Organs weighed	At sacrifice; Organs listed in Histopathology Inventory (Appendix I)
Histopathology	At sacrifice (see Histopathology Inventory in Appendix I)
Toxicokinetics	Week 2 and Week 51

RESULTS

Mortality -

Males: 1/6 HDm (young) sacrificed prematurely in extremis in wk 38, 1 HDm (21-month old) died 1 week after start of recovery in wk 53.

Females: 2/6HDf sacrificed prematurely in extremis in wk 46 and wk 47 (one young, one 21-month old).

Clinical signs - HDm,f: Vomiting. In premature deaths: no appetite, apathy, vomiting, emaciation, alopecia.

BW - BW at wk52 reduced in HDm, not HDf. BW gain reduced in HDm and HDf (wk0-wk52). In animals that died, there were BW losses.
FC - Slightly reduced in HDf at end of treatment period. FC severely reduced in premature deaths.
ECG - No changes
Heart rate - No effects
Reflex test - No effects
Ophtalmology - No effects
Hematology -
Hb, RBC, PCV: Slightly decreased in 1/6HDm
ESR (erythrocyte sedimentation rate): Moderately increased in HDm. ESR tended to be normalized upon recovery.
Clinical chemistry -
AST: Minimally increased in HDm
Creatinine, BUN: Increased in 1/6HDm in wk38, and in 1/6HDf in wk47 (both occurred before premature death of these animals). Creatinine slightly increased in 1/6HDm in wks 13, 38, 51 (male died in wk53), and BUN increased in 1/6HDm in wk 51.
Glucose: Slightly increased in 1/6 HDm in wk52.
Cholesterol: Slightly increased in 2/6HDm in wk52, wk39.
Ca, P: Slightly decreased in LD,MD,HDm,f animals. Ca and P still reduced in LD, MD,HDm at end of recovery. P markedly increased in 1HDm and 1HDf (before their premature deaths due to renal failure).
CK (creatinine kinase): Spurious increases at various times and doses.
Urinalysis - Increased urinary glucose from wk26-wk52 in 1/6 HDm, and increased urinary ketone from wk48-wk52 in same HDm animal.
Occult fecal blood - No treatment effect
Toxicokinetics -

Parameters:

Cminss	mean of concentrations at time point 0 and T (T= dosage interval = 24h)
Cmaxss	maximum measured concentration with dosage interval T
tmaxss	time of maximum concentration
tfss	time of last concentration >= LOQ within interval T
AUCtfss	area under the concentration-time curve up to fss
AUCss	area under the concentration-time curve within dosage interval T
tregss	starting point for calculating $L_z$ss
$L_z$ss	terminal rate constant
t1/2ss	terminal half-life corresponding to $L_z$ss
CL/fss	apparent oral total clearance
Aess	amount excreted in urine over interval T
CL_Rss	renal clearance (Aess/AUCss)
Cavss	average concentration at steady state (AUCss/T)
T	dosage interval
Vur	volume of urine
Cur	concentration in urine

Organ weights -
Males:
No statistically or biologically significant effects distinguishable at either wk52 or wk79 in the small number of animals examined (n=2-4).
Females:
Kidney: Relative wt increased in MD (1.2x) and HD (1.4x) at wk52, not at wk79. Effect not statistically significant.
Spleen: Absolute and relative wt increased (congestion) in 1/6 HDf at wk52 (3-4x control value), not at wk79. Unclear if effect was treatment related.

Gross pathology -

1. ANIMALS THAT DIED PREMATURELY

Males (0-0-0-2)

1 animal, sacrificed in wk38

Kidney: swelling/discoloration
Liver: discoloration
Lung: pneumonia
Stomach: bloody gastritis
Trachea: hemorrhage
Gallbladder: condensed bile
Blood: anemia
Bone: rib chondrocostal area, and proximal epiphyseal bone of extremities: nodular distension (chondrocostal = of costal (=rib) bone and costal cartilage)

1 animal, died in wk53

Kidney: capsular retraction
Testis: testicular atrophy
Lung: pulmonary emphysema
Urinary bladder: mucosa red
Stomach: overload
Bone: rib: nodular distension

Females (0-0-0-2, both sacrificed in extremis in wks 46,47)

Findings in 1 or 2 animals:
Kidney: discoloration and granular surface
Lung: discoloration and emphysema
Stomach: reddening
Small intestinal mucosa: reddening
Gallbladder: condensed bile
Feeding state: reduced
Bone: rib: nodular distension (1 animal)

2. ANIMALS SACRIFICED AS SCHEDULED

Males (4-4-4-3, scheduled sacrifice):

Kidney red spots 0-0-0-1
Thymus reduced size 0-0-0-1
Feeding state: lean 0-0-0-1
Skin: thin hair 0-0-0-1
Lymph node: Swelling 0-0-0-1
Bone: Nodular distension of chondrocostal rib region 1-1-0-1 (1-1-0-3??)

Females (4-4-3-2, scheduled sacrifice):

Lung: Discolored areas 0-0-2-1
Spleen: Congestion 0-0-0-1
Bone: Nodular distension of chondrocostal rib region 0-0-2-2

3. ANIMALS SACRIFICED AS SCHEDULED AFTER RECOVERY

Males (2-2-2-1, scheduled sacrifice after recovery):

Urinary bladder: reddening of mucosa 0-0-1-0
Skin: alopecia 0-0-0-1

Females (2-2-2-2-, scheduled sacrifice after recovery):

Kidney: granular surface 0-1-0-0
Heart: right ventricular dilatation 0-0-0-1
Bone: Nodular distension of chondrocostal rib region 0-0-1-2

Histopathology -

Males in 365-day treatment groups (+ 1 animal from recovery group that died in wk53)

		MALES				
		control killed	2 mkd killed	5mkd killed	10mkd killed	10mkd died
N		*4*	*4*	*4*	*3*	*2 (wk38, wk53*)*
Liver	Brown Pigmentation					0
	Vacuolated Cells					2
	Single Cell Necrosis					2
	Sinusoidal Dilation					2
	Focal Necrosis				1	
Kidney	Epithelial Hyperplasia			4	3	2
	Tubulonephrosis			1	1	2
	Tubular Dilation				2	1
	Interstitial Cell Infiltration				1	2
	Desquamation			1	1	1
	Glomerulopathy					1
	Interstitial Fibrosis				2	1
	Focal Basophilic Tubules			1	2	
	Epithelial Hypertrophy				2	
Spleen	Hemosiderosis			1		1
Lung	*Hemorrhage*					1
	Bronchopneumonia				0	2
	Emphysema	1	1	2	1	2
	Congestion				1	2
	Edema					2
	Focal Necrosis					2
Bronchus	Desquamation					1
	Leukocytic Infiltration					1
	Exsudation					2
Thymus	Brown Pigmentation					1
	Involution					2
Urinary Bladder	Congestion					1
Prostate	Dilation			1		
Testis	Atrophy					1
	Giant Cells					1
	Focal tubular atrophy			1	1	
Epididymis	*Reduced Sperm Content*					
Lymph Node	Brown Pigmenation				1	1
	Histiocytosis					2
Parathyroid Gland	Cyst				2	1
Parotid Gland	Leukocyte Infiltration					1
	Coagulated Secretion					1
Esophagus	Erosion					1
	Esophagitis					1
Skin	*Epithelial Hyperplasia*				1	
Brain	Congestion					1
	Edema					
Nerve	Wallerian Degeneration					1
Bone Marrow	Hemorrhage			1		1
	Fibrosis			2	1	2
	Edema			2	1	2
Bone	Focal Necrosis			1		
	Enlarged Endochondral Ossification		4	4	3	2
	Focal New Trabecular Bone Formation in marrow (young, woven bone)			2	1	2

*animal was "old", ie, 21-22 mo at start of treatment

Females in 365-day treatment groups

		FEMALES

		control	2 mkd	5mkd	10mkd	10mkd
		killed	killed	killed	killed	died
N		*4*	*4*	*3*	*2*	*2 (wk46, wk47*)*
Liver	Brown Pigmentation					2
	Vacuolated Cells					1
	Congestion					2
	Actrv of stellar cells					1
	Enlarged hepatocytes					1
	Sinusoidal Dilation					2
	Eosinophil cytoplasm					1
	Interstit cell infiltration				1	
Kidney	Epithelial Hyperplasia			3	2	2
	Tubulonephrosis				1	2
	Tubular Dilation				2	2
	Congestion				1	
	Edema					1
	Interstitial Cell Infiltration			1	1	1
	Desquamation				1	1
	Glomerulopathia					2
	Interstitial Fibrosis			1	1	2
	Epithelial Hypertrophy				1	1
Heart	Focal fibrosis			1		
Spleen	Congestion				2	2
	Bronchopneumonia					1
Lung	Desquamation			2	0	1
	Congestion					1
	Edema	1		2		2
	Focal Necrosis	1			0	1
	Interstitial cell infiltration	2	2	3	2	1
Bronchus	Desquamation					1
	Epithelial hyperplasia			2	0	2
	Exsudation					1
Thymus	Involution					2
	Crypt dilation					1
Unnary Bladder	Epithelial hyperplasia				1	
Ovary	Hyperpl sex cord strom					1
Lymph Node	Brown Pigmenation				1	2
	Edema					1
	Sinushistiocytosis				1	2
Thyroid	Ultimobranchial rest					1
	C-cell hyperplasia	1	1			2
Parathyroid Gland	Cyst	2	3		2	
Parotid Gland	Leukocyte Infiltration					1
	Resorptive inflammation			1		1
Esophagus	Erosion					1
	Mononuclear infiltration	1	1		1	2
Brain	Congestion					1
Nerve	Wallerian Degeneration			1		
Bone Marrow	Hemorrhage			3	1	2
	Cellular infiltration			2		
	Congestion					1
	Fibrosis			3	1	2
	Edema			3	1	2
Bone	Focal Necrosis	1		1		
	Enlarged Endochondral Ossification		3	3	2	2
	Focal New Trabecular Bone Formation			3	2	2
	Callus centr necr gap			2	2	

*animal was "old", ie, 21-22 mo at start of treatment

Males in 180-day recovery groups (1HD died in Week 53)

		MALES			
		control killed	2 mkd killed	5mkd killed	10mkd killed
N		*2*	*2*	*2*	*1*
Kidney	Calcification				1
	Interstitial Fibrosis				1
Lung	Emphysema	1	1	1	1
Unnary Bladder	Congestion			1	
	Desquamation				1
	Epithelial hyperplasia				1
Skin	Epithelial Hyperplasia				1
Nerve	Wallerian Degeneration			1	
Bone marrow	Fibrosis				1
	Edema				1
Bone	Enlarged Endochondral Ossification		2	2	1
	Focal New Trabecular Bone Formation			1	1
	Subepiphyseal zone with normal bone formation		2	1	1
	Callus central necr gap			1	1

Females in 180-day recovery groups

		FEMALES			
		control killed	2 mkd killed	5mkd killed	10mkd killed
N		*2*	*2*	*2*	*2*
Kidney	Calcification			1	
	Concrement(s)				1
	Tubular Dilation			1	1
	Interstitial Cell Infiltration				1
	Interstitial Fibrosis			1	2
Lung	Desquamation				1
	Fibrosis				1
	Edema				1
Brain	Congestion				1
Bone marrow	Fibrosis			2	
Bone	Enlarged Endochondral Ossification	1	2	2	2
	Focal New Trabecular Bone Formation			1	2
	Subepiphyseal zone normal bone formation		2	2	2
	Callus centr necr gap				2

SUMMARY

Doses 2, 5, 10 mg/kg/day (FAE), 12 months treatment with 6-mo recovery period

- Signs (GI) at MD and HD
- Mortality at HD. Animals that died had kidney pathology, lung pneumonia or emphysema, GI reddening or hemorrhage.
- BW reduced in HD
- No ECG changes

- Hematology (RBC, ESR reductions) changes at HD
- Renal and hepatic toxicity (clinical chemistry) at HD
- Serum Ca and P reduced at LD, MD, HD (pharmacologic drug effect)
- Glucosuria and ketonuria at HD
- Kidney weight increase in HD (f)
- Spleen weight increased in HDf
- Histopathological changes:
  - Renal tubular changes at MD and HD
  - Renal tubular nephrosis in MD and HD
  - Glomerulopathy in HD (dogs that died)
  - Hepatocyte vacuolation, necrosis at HD
  - Bronchopneumonia in HD (dogs that died)
  - Ulcerative esophagitis and gastritis at HD (dogs that died)
  - Testicular atrophy at HD (dogs that died)
  - New trabecular bone formation and endocondral ossification in almost all or all animals in all dose groups (not recovered)
- NOAEL 2 mg/kg/day (LD)

APPEARS THIS WAY
ON ORIGINAL

SUMMARY OF REPEAT DOSE ORAL STUDIES

RAT studies: LOAEL levels

Strain				SD	Wistar	Wistar	SD
Study Nr.				G1	H1	H3	H4
Duration				4 week	26 week +13 wk rec	26 week +13 wk rec	12 month +6 mo rec
N/s/g				10/s/g	30/s/g; 10/s/g/ rec	30/s/g; 10/s/g rec	40/s/g, 5/s/g rec
Route (oral)				gavage	gavage	gavage	gavage
Doses (mg/kg/day)				0.88, 2.65, 8 85	10.1, 30.3	1 0, 3 0, 10 1	3, 10, 20
Dose units				FAE	FAE	FAE	FAE
Feeding				Ad libitum,	Restricted feeding (O/N fast, food 2h-10h postdose)	Ad libitum	Ad libitum
TK				No	Yes	Yes	Yes
Mortality		Death			30 3 (30M, 22F) Deaths due to renal toxicity and GI hemorrhage		0 (2M,1F) 3 (4M, 2F) 10 (7M,13F) 20 (16M,14F) Deaths due to respiratory effects
Signs		Sedation, posture change, rough hair coat, emaciation, respiratory distress, fractured incisors			30.3		
		Wheezing					3
		Rough pelt, loss of teeth					10
Body weight		Decreased BWG			30.3	10.1	10 (M)
		Decreased FC			30.3	10 1	20 (M)
Hematology	RBC	Decrease in RBC, Hb, Hct			10.1	10 1	
		Increased RBC distribution width			10 1	3 0 (F)	
		Increase in MCV					3
	WBC	Decrease in WBC					20
		Increased segmented neutrophils		8 85	30 3 (F)	10 1	3
		Decrease in lymphocytes					3 (M)
	Platelets	Decrease in platelets					3
Clin Chem	Liver	Increase in AST			10.1		
		Decrease in ALP			10.1		
		Increase in ALT			30 3		
		Decreased serum protein/albumin			30.3		
	Bone/kidney	Decreased serum Ca		8.85	10.1		3 NR
		Decrease in serum P			10.1		3 NR
		Increase in serum P			30.3		
	Kidney	Decreased serum K		0 88 (M)	10.1		
		Increased Na and Cl			10.1	10 1	
		Increased creatinine			30.3		
Urine	Kidney	Specific gravity increase			10.1		
		PH decrease			10 1		
Organ weights	Kidney	Decrease in kidney weight				3 0 (M)	
		Increased kidney weight			10.1		20 (M)
	Spleen	Increased spleen weight			10.1	1.0 NR	3
	Liver	Decreased liver weight			10.1	1.0 (M) NR	
Pathology	Resp tract	Irritation of respiratory tract					3, 10, 20 (in animals that died)
	Kidney	Pale kidneys			30 3		
		Kidney medulla reddened			30.3		
		Renal medulla congestion			30.3		
		Renal tubulonephrosis (cloudy swelling)		8.85			
		Tubular dilation			30.3		
		Tubular vacuolation and necrosis			30 3		
		Medullary tubule swelling/basophilia			10.1 R		
		Renal tubulo-epithelial hypertrophy					10 R
	Stomach	Gastric irritation (surface hemorrhage,			30.3		

		irregularity)					
		Stomach muscle degeneration, inflammation			30.3		
	Liver	Liver focal necrosis		8 85			
	Brain	Brain basophilic bodies in cerebral vessels		≥			20 (M) NR
	Bone	Increase in trabecular bone mass		0 88	10.1	1 0	
		Increased endochondral ossification		0 88			3 NR
		Reduction in bone marrow space			10.1	1.0	
	Spleen	Increase in extramedullary hematopoiesis in spleen			10.1	1 0	3 NR
NOAEL							
General*	Mg/kg			2.65	<10.1	<1-3	<3
Kidney toxicity	Mg/kg			2.65	<10 1	≥10.1	3
Liver toxicity	Mg/kg			2.65	<10.1	≥10 1	≥20

* NOAEL excluding PD effect of drug on bone
(M) males, (F) females
NR not recovered, R recovered

RAT: NOAELs and multiples

STUDY				G1	H1	H3	H4
				fed	fasted	fed	fed
NOAEL for kidney toxicity	Dose	Mg/kg/day		2.65	<10.1	≥10.1	3
	AUC*	Ngxh/mL		11.1 (extrapolated from Study H4)	<111	≥12.55	12.6
	AUC multiple**	Multiple of human exposure at oral dose of 2.5 mg/day, based on PK data		3.3-6.2x	<34x-62x	≥4x-7x	4x-7x
	Mg/m2 multiple***	Multiple of human 2.5 mg/day dose, based on mg/m2 comparison		3.6x	<41x	≥14x	4.1x

*AUC at NOAEL were (extrapolated) values from pooled M and F data (Sponsor's TK Table 3)
**Based on human AUC values: 1.8-3.3 ngxh/mL(MF7159 and BP16304)
*** Human 2.5 mg dose: 0.04 mg/kg; Assumption: human BA = rat BA = 0 6% (fasted), and rat BA (non-fasted) = 0.2%

DOG studies: LOAEL levels

Strain			Beagle	Beagle	Beagle
Study Nr.			G3	H2	H5
Duration			4 week	26 weeks +13 wk rec	12 months + 6mo rec
N/s/g			4/s/g	5/s/g; 1/s/g rec	6M, 5-6F/s/g, 2/s/g rec
Formulation			Tablets (2x/day)	Gelatin capsule (1x/day)	Tablet (1x/day)
Dose (mg/kg/day)			0.9, 2.7, 9.0	2, 5, 13	2, 5, 10
Dose unit			FAE	FAE	FAE
Feeding			400g/day; unclear in relation to dosing	350 g/day + 300g meat/day Dosing in AM without food	300 g/day. Animals fed 2h after dosing
TK			No	Yes	Yes
Mortality		Death		13 (5M, 3F)	10 (1M, 2F)
Signs	General	Decreased activity; dehydration; pallidness/cyanosis; red-stained feces and/or urine		13	
	GI	Vomiting, apathy, alpecia, loss of appetite			5
Body weight		Decreased BWG		13	10
Food consumption		Decreased FC		13	10
Hematology	RBC	Anemia		13	10
	WBC	Increase in WBC	9.0		
	Other	ESR acceleration			10
Clin Chem	Liver	Increase in AST	9 0	13	10
		Decrease in ALP		13	
		Increased GGT		13	
		Serum glucose increase			10
		Bilirubin increase		13	
		Lipids, phospholipids, triglycerides, cholesterol increase		13	10
		Decrease in serum Fe		13	

		Decreased serum protein/albumin		13	
	Bone	Decreased serum Ca	2.7 (F), 9.0 (M)	13	2
		Decrease in serum P			2
		Increase in ALP	9 0		
	Kidney	Decrease in serum Na		13	
		Serum urea increase		13	10
		Creatinine increase		13	10
Urine	Kidney	Proteinurea		13	
		Glucosuria		13	10
		Ketonuria			10
Organ weights	Kidney	Increased kidney weight	0.9 (M)	13 (F)	10
	Spleen	Increased spleen weight	0.9 (M)		10
	Liver	Decreased liver weight			
Pathology	Kidney	Kidney discoloration			10
		Tubulonephrosis			5
		Renal tubular dilation		13	10
		Tubule basophilia			5
		Tubular cortex epithelium hyperplasia and/or hypertrophy			5 R
		Glomerulopathy			10
		Interstitial fibrosis			5
	Liver	Liver discoloration			10
		Liver fatty vacuolation		13	
		Hepatocyte vacuolation			10
	Thymus	Thymus involution			5
	Testes	Testicular atrophy			10
	Lung	Bronchopneumonia		13	10
	Esophagus	Acute ulcerative esophagitis		13	10
	Stomach	Gastric irritation			10
		Cachexia			10
	Bone	Increased spongiotic trabecular bone mass/density	0.9	13 R	5
		Enlarged zones of endochondral ossification	0.9		2
		Inflammationand necrosis at zone of endochondral ossification	9.0		
		Bone marrow fibrosis, hemorrhage, edema			10
		Deposition of mesenchymal tissue in medullary cavity		5 R	
		Costochondral junction, necrosis, hemorrhage, inflammation		13	
		Increased epiphyseal cartilage width		13	
	Spleen	Increase in extramedullary hematopoiesis in spleen			
NOAEL*					
General	Mg/kg		<0.9	5	2
Kidney	Mg/kg		<0.9	5	2
Liver	Mg/kg		2.7	5	5

(M) males, (F) females
NR not recovered, R recovered
* excluding PD effect of drug on bone

DOG: NOAELs and multiples

STUDY			G3	H2	H5
NOAEL for kidney toxicity	Dose	Mg/kg/day	<0.9	5	2
	AUC*	Ngxh/mL	<40 (extrapolated from Study H5)	350	90
	AUC multiple**	Multiple of human oral dose (2.5 mg/day) based on PK data	<12x-22x	106x-194x	27x-50x
	Mg/m2 multiple***	Multiple of human 2.5 mg/day dose, based on mg/m2 comparison	<18x	105x	42x

*AUC at NOAEL were (extrapolated) values from pooled M and F data (Sponsor's TK Table 3)
**Based on human AUC values: 1.8-3.3 ngxh/mL(MF7159 and BP16304)
*** Human 2.5 mg dose: 0.04 mg/kg; Assumption: human BA = 0.6%; dog BA =1%

In conclusion, in the repeat dose oral studies in rats and dogs, the main target organ was the kidney. Like other bisphosphonates, ibandronate is eliminated by renal excretion, and this may be related to the renal toxicity. Ibandronate also accumulates in renal tissue.
ECG changes were not observed in the dog.

In the rat, earliest signs of kidney toxicity were observed at 10 mkd in study H4 (non-fasted) consisted of reversible tubular hypertrophy. At 10.1 mkd in study H1 (fasted) kidney effects included renal tubular nephrosis, hypertrophy, tubule swelling and basophilia. Increased kidney weight was seen at 20 mkd (H4, fed) and 10.1 mkd (H1, fasted). At 30.3 mkd (H1), there were tubular dilation, vacuolation and necrosis, pale kidneys, medullary reddening and congestion, and increased serum creatinine. Other target organs affected were the liver at doses ≥10.1 mkd (increased enzymes, decreased protein) and stomach at 30.3 mkd (irritation, hemorrhage) in study H1.

In the dog, renal toxicity at 5 mkd (H5) consisted of tubulonephrosis, tubule basophilia, and interstitial fibrosis, and at 10 mkd there was tubular dilation, discoloration, glomerulopathy, increased serum BUN and creatinine, decreased serum Na, increased kidney weight, urine changes. GI tract toxicity (vomiting, loss of appetite, emaciation) was also seen at 5 mkd (H5). Other target organs affected in the dog at 10-13 mkd were liver, lung and testes (H2, H5). Stomach and esophagus histopathology and cachexia were also seen at 10-13 mkd (H2, H5).

In both rats and dogs, the changes in serum Ca and P and ALP and the histologic effects in bone were the results of the pharmacologic effect of ibandronate and were observed in all studies at almost all doses. The bone changes included trabecular bone mass increases, endochondral ossification and reduction in bone marrow space. The bone marrow changes secondarily resulted in hematologic changes (anemia) and extramedullary hematopoiesis in the spleen.