

dependent increase in the fraction of unbound drug. In all species zoledronic acid was more highly bound to plasma proteins than was ibandronate.

#### 2.6.4.5 Metabolism

**Title:** PCS(EU) R0400420: Analysis of ZOL446 in Plasma and Urine of Male Rats Following a Single Intravenous Dose of 0.6 mg/kg [<sup>14</sup>C]ZOL446

**Study number:** PCS(EU) R0400420

**Study design:** Three male Wistar (albino) rats received a single 0.6 mg/kg i.p. dose of [<sup>14</sup>C]-zoledronic acid. Plasma samples were collected at 0.25, 2, 4, 8 and 24 hr post dose, and urine samples were collected over two 24 hr periods (0-24 hr and 24-48 hr). The level of radioactivity in these samples was measured by LSC, and metabolite patterns were obtained by HPLC via fractionation into solid scintillator-coated microplates and off-line radioactivity counting using a \_\_\_\_\_ instrument.

#### Results:

**Table 4-1 Pharmacokinetic parameters in plasma**

Values refer to concentrations in plasma pools following a single intravenous dose of 0.6 mg/kg ZOL446

	Radioactivity <sup>b</sup>
$t_{max}$ (h) <sup>a</sup>	0.25
$C_{max}$ (μmol/L)	8.72
AUC <sub>0-24 h</sub> (μmol·h/L)	14.9
CL ((mL/min)/kg)	2.31
$V_d$ (L/kg)	0.18

a first time-point measured

b 95% of the radioactivity represents ZOL446

**Table 7-2 [<sup>14</sup>C]ZOL446 and related components in rat plasma**

Parent compound (ZOL446) and related components in rat plasma after a single intravenous dose of 0.6 mg/kg [<sup>14</sup>C]ZOL446 were determined by HPLC analysis with fraction collection and off-line radioactivity detection using a \_\_\_\_\_ instrument

Sample type	Male rats (0.6 mg/kg i.v. of [ <sup>14</sup> C]ZOL446)					AUC(0-24) [(μmol/L)·h] <sup>a</sup>	% of <sup>14</sup> C AUC
	Plasma pools of n=3						
Sample collection time [h]	0.25	2	4	8	24		
Compound	Concentration [μmol/L]						
P4.2	0.037	0.000	0.000	0.000	0.000	0.04	0.3
P5.1	0.039	0.009	0.001	0.001	0.000	0.07	0.5
P5.55	0.030	0.006	0.001	0.000	0.000	na	na
P6.0	0.014	0.000	0.000	0.000	0.000	na	na
ZOL446	8.227	1.400	0.263	0.084	0.032	13.89	94.6
P13.5	0.045	0.005	0.001	0.000	0.000	na	na
Sum traces	0.101	0.012	0.002	0.001	0.001	na	na
Total of recovered RA	8.493	1.432	0.270	0.087	0.033	14.31	97.4
Not recovered from HPLC	0.227	0.038	0.007	0.002	0.001	0.35	2.38
Not recovered from sample processing	0.000	0.000	0.000	0.000	0.000	0.00	0.00
Total RA concentration *)	8.720	1.470	0.277	0.089	0.034	14.69	100.00

<sup>a</sup> Concentration C(0) was calculated by extrapolation (linear-linear mode) of the concentrations obtained at t=0.25 h and at t=2 h to calculate AUC(0-24) [(μmol/L)·h]

**Table 7-3 Excretion of ZOL446 and related compounds with urine**

Excretion of ZOL446 and related compounds (expressed in % of administered dose) with urine after a single intravenous dose of 0.6 mg/kg [<sup>14</sup>C]ZOL446 to male rats (n=3). The proportions were semi-quantitatively determined from radiochromatograms of HPLC analyses with fraction collection and off-line radioactivity detection using a \_\_\_\_\_ instrument

Compound	Male rats 0.6 mg/kg i.v.		
	Excretion with urine		
	[% dose]		
	0-24 h	24-48 h	0-48 h
P2.6	0.01	0.03	0.04
P4.2	0.36	0.03	0.39
P4.8	0.20	0.03	0.23
P5.5	0.94	0.12	1.06
ZOL446	23.03	2.84	25.88
P12.3	0.11	0.03	0.14
P13.5	0.16	0.02	0.18
Sum of traces (not assigned)	0.05	0.01	0.06
Total of recovered RA	24.99	3.13	28.12
Not recovered from HPLC	0.00	0.00	0.00
Not recovered from sample processing	0.00	0.00	0.00
Total analysed (pool 0-48 h)	24.99	3.13	28.12

**Table 7-4 Excretion in urine**

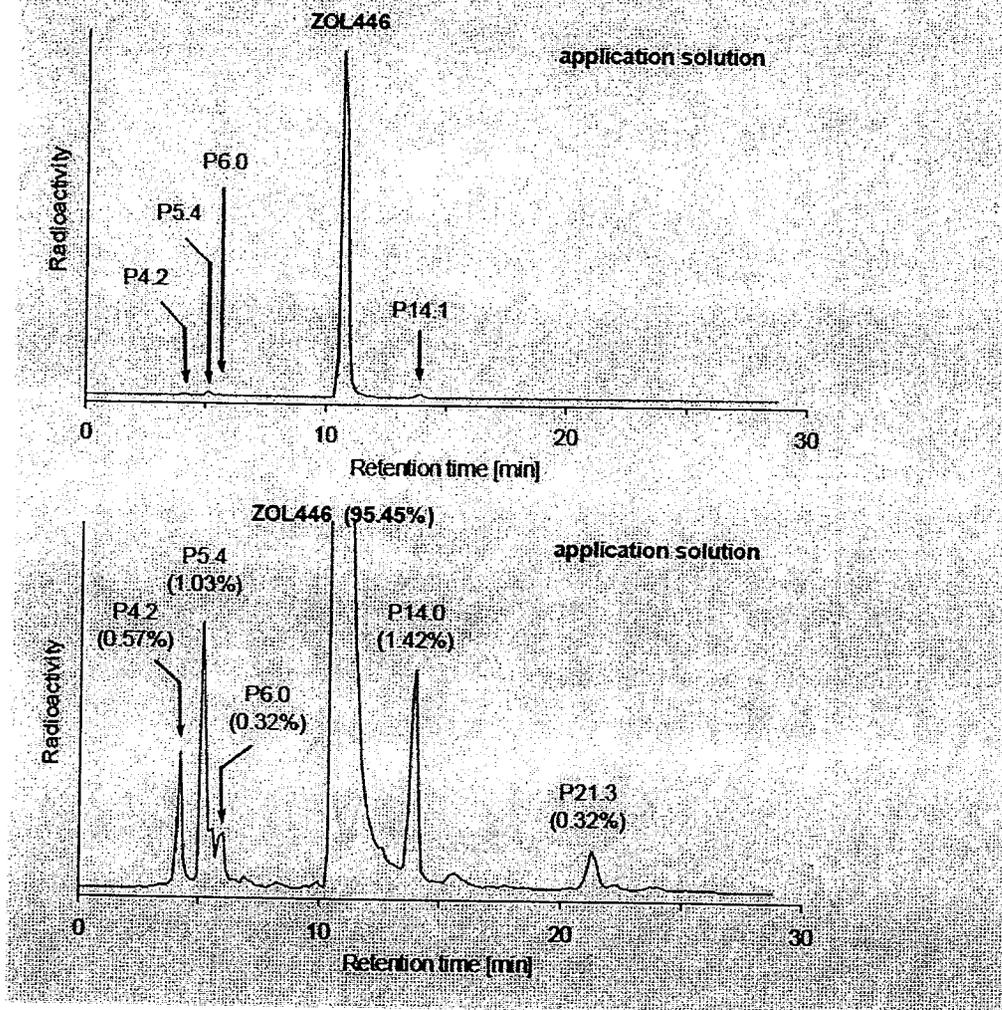
Recovery of radioactivity in urine of rats within 2 days after i.v. administration of 0.6 mg/kg [<sup>14</sup>C]ZOL446.

Excretion in % of administered radioactivity				
Time [h]	Rat 1	Rat 2	Rat 3	mean
0-24	28.7	22.3	24.0	25.0
24-48	4.16	3.31	1.92	3.13
0-48	32.9	25.5	25.9	28.1

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**Figure 8-1 Purity of application solution**

The purity of the application solution (containing 0.30 mg of [ $^{14}\text{C}$ ]ZOL446 (Batch Ch E-4007-101-20) per g of 0.9% saline) was investigated by HPLC with fraction collection and off-line radioactivity detection using a \_\_\_\_\_ instrument. An diluted sample of 10  $\mu\text{L}$  of the application solution was subjected to HPLC. Both pictures represent the same analysis (lower picture: enlarged y-scale).



**Conclusion:** Drug-related radioactivity was rapidly cleared from the plasma, with ~1% of the  $C_{\text{max}}$  level being present at 8 hr post dose. Zoledronic acid was the predominant species in the plasma ( $\geq 95\%$  of the signal) and urine ( $\geq 90\%$  of the signal) at all time points. Most of the other identified  $^{14}\text{C}$ -labeled peaks were present in the administered drug and likely represent trace impurities and/or degradation products. These data suggest that zoledronic acid is not metabolized in the rat.

#### 2.6.4.6 Excretion

No studies submitted.

**2.6.4.7 Pharmacokinetic drug interactions**

No studies submitted to the NDA. Data submitted to NDA 21-817 indicate no liability for inhibition of cytochrome P450 enzymes. The relatively modest plasma protein binding exhibited by zoledronate indicates that interactions with drugs that are highly bound to plasma proteins is also unlikely.

**2.6.4.8 Other Pharmacokinetic Studies**

No studies submitted.

**2.6.4.9 Discussion and Conclusions**

The pharmacokinetic properties of zoledronate are qualitatively similar to those reported for other bisphosphonates. There is high affinity for and slow elimination from bone tissue. Zoledronate exhibits rapid elimination from the circulation and soft tissues via renal excretion. There is no evidence that zoledronate undergoes biotransformation. As a consequence of renal excretion there is temporary accumulation in the kidneys as drug that is not bound to bone is eliminated. The degree of bone accumulation of zoledronate is proportional to the cumulative dose.

The additional information provided by the newly available studies does not alter the nonclinical pharmacokinetic profile of zoledronate that was determined from the previous studies.

**2.6.4.10 Tables and figures to include comparative TK summary**

**FROM PAGET'S SUBMISSION (NDA 21-817)**

Species	Dose (mg/kg)	Route	Day	Gender	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC (ng·hr/mL)	CL (L/hr/kg)	Assay	Ref.
Rat	0.15	iv	1	m	1500	0.08	900	0.17	LSC	DM 17/1993
Rat	0.1	sc	1	m	400	0.25	550	0.18	LSC	DMET (EU) 8/1996
Rat	0.6	iv	1	m	4800	0.08	3900	0.15	RIA	DMPK (CH) R99-1978
	0.6	sc	1	m	2100	0.25	3900	0.15		
Dog	0.1	iv	1	m			2100	0.05	EI	BPK(CH) 1996/067
			1	f			1800	0.06		
	87	m			2100	0.10				
Dog	0.1	iv	87	f			2000	0.10	EI	DMPK (CH) 1997/304
			1	m	670	0.08	1400	0.07		
			1	f	490	0.08	1400	0.07		
			190	m	710	0.08	1500	0.07		
			190	F	600	0.08	1300	0.08		
352	M	810	0.08	1900	0.05					

Species	Dose (mg/kg)	Route	N	Sex	Weight (kg)	C <sub>0</sub> (ng/mL)	C <sub>1</sub> (ng/mL)	C <sub>2</sub> (ng/mL)	AUC (ng·h/mL)	RIA	BAPK(F)
Dog	0.25	iv	1	M	1100	0.08	1600	0.16		RIA	BAPK(F) R0170102
			1	F	960	0.08	1300	0.19			
			85	M	940	0.08	1500	0.17			
			85	F	930	0.08	1300	0.19			
			1	M	4600	0.08	7000	0.14			
			1	F	3500	0.08	5800	0.17			
	1	iv	85	M	2400	0.08	6100	0.16			
			85	F	3700	0.08	7300	0.14			
			1	M	1000	0.08	1300	0.19			
			1	F	1200	0.08	1500	0.17			
			64	M	1100	0.08	1500	0.17			
			64	F	1200	0.08	1600	0.16			
Dog	0.25	iv	169	M	1300	0.08	1700	0.15			
			169	F	1200	0.08	1600	0.16			
			0.5	iv	1	M	2200	0.08	2800	0.18	
			1		F	2200	0.08	2900	0.17		
			64		M	2000	0.08	3000	0.17		
			64		F	2000	0.08	2900	0.17		
	169	M	2500		0.08	3700	0.14				
	169	F	2400		0.08	3400	0.15				
	1	iv	1	M	4600	0.08	6600	0.15			
			1	F	4700	0.08	6900	0.14			
			64	M	5400	0.08	8200	0.12			
			64	F	4300	0.08	7100	0.14			
169			M	5200	0.08	8100	0.12				
169			F	5300	0.08	9100	0.11				

Source: [Pharmacokinetics tabulated summary]

**2.6.7.3 Overview of toxicokinetics data**

Test article: Zoledronic acid

Steady-state AUC (ng·h/mL)

Daily dose (mg/kg)	Mice		Rats		Dogs		Rabbits	Humans	Study Number	Location in Module 4 or 5
	m	f	m	f	m	f	f			
0.0377	-	-	-	-	-	-	-	344 <sup>a</sup>	ZOL 3001	
0.0651	-	-	-	-	-	-	-	540 <sup>a</sup>	ZOL 3001	
0.1	-	-	-	-	1464 <sup>a</sup>	1305 <sup>a</sup>	-	-	93-6193	
0.139	-	-	-	-	-	-	-	1133 <sup>a</sup>	ZOL 3001	
0.2	-	-	-	-	2075 <sup>a</sup>	1982 <sup>a</sup>	-	-	92-6261	
0.25	-	-	-	-	1500 <sup>a</sup>	1300 <sup>a</sup>	-	-	0170102	
0.5	-	-	-	-	1664 <sup>a</sup>	1614 <sup>a</sup>	-	-	018037	
	-	-	-	-	3654 <sup>a</sup>	3447 <sup>a</sup>	-	-	018037	
1	-	-	-	-	6060 <sup>a</sup>	7270 <sup>a</sup>	-	-	0170102	
	-	-	-	-	8091 <sup>a</sup>	9144 <sup>a</sup>	-	-	018037	
3	-	-	-	-	35600 <sup>a</sup>	-	-	-	008189	

- = no data  
<sup>a</sup> Iv. bolus  
<sup>b</sup> Iv. infusion

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

**Nonclinical PK Data Collected Subsequent to Paget's Filing (NDA 21-817)**

**2.6.5.1 Pharmacokinetics overview**

Type of study	Test system or species / strain	Method of admin.	Duration of dosing	Concentrations or doses (mg/kg <sup>3</sup> )	GLP compliance	Testing facility	Study number
Absorption:							
Distribution:	Human	in vitro	-	50-5000 ng/mL	No	Novartis, CH	[PCS(EU) R0400374]
	Rat / Hanover Wistar	in vitro	-	2-2000 ng/mL	No	Novartis, CH	[DMPK R0500513]
	Dog / beagle						
	Human						
Metabolism:	Rat HAN.WIST(SPF)	I.V.	single dose	0.6	No	Novartis, CH	[PCS(EU) R0400420]
Excretion:							
Pharmacokinetic drug interactions:							
Other pharmacokinetic studies:							

**2.6.5.3 Pharmacokinetics: Absorption after a single dose**

Species:	Rat
Study number:	[PCS(EU) R0400420]
GLP compliance:	not required
Gender(MF) / Number of animals:	Male / 3
Feeding condition:	Free access to food and water
Vehicle / Formulation:	Saline / solution of [ <sup>14</sup> C]zoledronic acid, 7.027 MBq/mg, 95.5% radiochemical purity
Method of Administration:	I.V. bolus
Dose (mg/kg):	Single 0.6 mg/kg
Sample (Whole blood; plasma; serum etc.):	plasma, urine
Analyte:	zoledronic acid
Assay:	HPLC LSC and microplate scintillation counting for <sup>14</sup> C-labelled compounds

**PK parameters:**

Species:	Rat
T <sub>max</sub> :	0.25 h
C <sub>max</sub> :	8.72 µmol/L or 2.37 µg/mL
AUC:	14.9 µmol h/L or 4.05 µg h/mL
Cl:	2.31 mL/min/kg

**Additional information:**

After I.V. bolus dose, plasma radioactivity declined rapidly and multi-exponentially. By 8 hours, levels were only ~ 1% of the levels at 15 min.

Radiochromatography of plasma and urine samples revealed the presence of zoledronic acid and traces of radiolabeled impurities/degradation products, but no metabolites of zoledronic acid.

**2.6.5.6A Pharmacokinetics: Plasma protein binding**

Study title:	In vitro protein binding of [ <sup>14</sup> C]ZOL446 in human plasma and its dependence on the plasma calcium concentration	
Study number:	[FCS(EU) R0400374]	
GLP compliance:	not required	
Study system:	Human plasma (3 individuals pooled)	
Radionuclide:	<sup>14</sup> C	
Specific activity:	7.027 MBq/mg	
Method:	Ultrafiltration	
Analyte / Assay:	Total radioactivity / liquid scintillation counting	
Species:	Concentration tested	% Bound
Human (pooled N=3)	5000 ng/mL	25.5
	500 ng/mL	42.9
	50 ng/mL	52.8

**Additional information:**

Plasma protein binding of [<sup>14</sup>C]zoledronic acid in human was weak and concentration dependent.

Results from pooled plasma sample were consistent with the results from 5 individuals.

Protein binding was increased by addition of calcium and decreased by addition of a chelator (EDTA). However, the data indicate that in human plasma only major (non-physiological) changes in the calcium concentration result in changes in plasma protein binding. Calcium concentrations were in the range of 1.9-2.2 mM. Over this small range, no correlation to protein binding was observed.

**2.6.5.6B Pharmacokinetics: Plasma protein binding**

Study title:	Comparative analysis of the in vitro protein binding and its dependence on the concentration of calcium in rat, dog and human plasma for the two bisphosphonates [ <sup>14</sup> C]ZOL446 and [ <sup>14</sup> C]ibandronate		
Study number:	[DMPK R0500513]		
GLP compliance:	not required		
Study system:	Rat, dog and human plasma (pooled from 3 individuals for each species)		
Radionuclide:	<sup>14</sup> C		
Specific activity:	Zoledronic acid: 6.913 MBq/mg (monohydrate); ibandronate: 4.332 MBq/mg (trifluoroacetate)		
Method:	Ultrafiltration		
Analyte / Assay:	Total radioactivity / liquid scintillation counting		
Species:	Concentration tested	Zoledronic acid % Bound	Ibandronate % Bound
Rat	2000 ng/mL	83.6	51.5
	200 ng/mL	87.9	67.6
	20 ng/mL	86.9	75.8
	2 ng/mL	80.4	71.1
Dog	2000 ng/mL	36.0	24.9
	200 ng/mL	37.5	28.4
	20 ng/mL	45.3	35.3
	2 ng/mL	49.1	40.7
Human	2000 ng/mL	21.5	18.4
	200 ng/mL	27.7	22.0
	20 ng/mL	33.2	27.0
	2 ng/mL	44.1	34.3

Results from the human pooled sample were consistent with the results from 3 individual samples (not used for the pooled sample).

The bound fractions of zoledronic acid and of ibandronate decreased with increasing plasma concentration in dog and human plasma. This concentration dependency was not clearly evident for rat plasma.

For both zoledronic acid and ibandronate, the same species ranking was observed: plasma protein binding was highest for rat, medium for dog and lowest for human.

In all species plasma protein binding was increased in plasma supplemented with calcium chloride (2 mM, in human also 4 mM) and decreased in plasma supplemented with the chelator EDTA (2 mM, in human also 4 mM).

The data indicate that the protein binding of zoledronic acid in human plasma is similar to or slightly higher than that of ibandronate. In rat and dog plasma, the protein binding of zoledronic acid is higher than that of ibandronate.

2.6.5.13 Pharmacokinetics: Excretion			
Rat / albino HANWIST (SPF)			
Study number	[PCS(EU) R0400420]		
GLP compliance	not required		
Gender(M/F) / Number of animals	M / 3		
Feeding condition	Free access to food and water		
Vehicle / Formulation	Saline / solution of [ <sup>14</sup> C]zoledronic acid		
Method/ Route / Duration of administration	bolus / i.v. / single		
Dose (mg/kg)	0.6 mg/kg		
Specific activity	7.027 MBq/mg; 95.5% radiochemical purity		
Analyte / Radionuclide	Zoledronic acid / <sup>14</sup> C		
Assay	HPLC LSC and microplate scintillation counting for <sup>14</sup> C-labelled compounds		
Excretion route:	Urine	Faeces	Total
Time (h)	% of dose*	not studied	
0-24	25.0 ± 3.3		
24-48	3.13 ± 1.13		
0-48	28.1 ± 4.1		
* mean ± SD			
<b>Additional information:</b>			
Radioactivity excreted renally corresponded almost exclusively to unchanged zoledronic acid, except for trace impurities/degradation products contained in the administered [ <sup>14</sup> C]zoledronic acid batch. There were no metabolites found in urine (or plasma), consistent with the lack of metabolism of zoledronic acid, and of bisphosphonates in general.			

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

**General toxicology:** Toxicology studies submitted to NDA #21-223:

- Single dose toxicity studies carried out in rat, dog and mouse by various dosing routes.
- Repeated dose studies by the intravenous route in rats and dogs, by the subcutaneous route in rats, and by the oral route in mice, rats and dogs.

#### Single dose studies

Species	Test No.	Study Title	Doses (mg/kg)
Rat	88-6126	Acute i.v. findings study in rats	0.6, 6, 30, 60, 80
	997049	A comparative acute i.v. findings study in rats (w, w/o dimer)	0, 1.6, 8, 16, 32
	96-8002	Acute oral findings study in rats	300, 1000
	230142	Assessment of acute oral findings with zoledronic acid	200, 2000
Dog	93-6084	Acute i.v. findings study in dogs	2, 10
Mouse	93-6085	Acute s.c. findings study in mice	10, 20, 50

#### Repeated dose studies

##### Intravenous application

Species	Test No.	Study Title	Doses (mg/kg/day)
Rat	89-6036	10-day i.v. range finding study in rats	0, 0.06, 0.6, 6
	1486	A 2-week i.v. findings study in Sprague Dawley rats	0, 0.06, 0.6, 3.2
Dog	90-6157	10-day i.v. range-finding study in dogs	0.1, 1
	90-6180	4-week i.v. findings study in dogs	0, 0.02, 0.06, 0.2
	92-6261	3-month i.v. findings study in dogs	0, 0.01, 0.03, 0.1-0.2
	93-6193	26/52 week i.v. findings study in mature dogs	0, 0.005, 0.03, 0.1
	94-4045	Bone analysis: 26/52 week i.v. findings study in	0, 0.005, 0.03, 0.1

		mature dogs	
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## Subcutaneous application

Species	Test No.	Study Title	Doses (mg/kg/day)
Rat	90-6156	10-day s.c. range finding study in rats	0, 0.2, 0.6, 2.0
	90-6179	1-month s.c. findings study in rats	0, 0.02, 0.06, 0.2
	92-6259	3-month s.c. findings study in rats	0, 0.01, 0.03, 0.1
	93-6230	6/12-month s.c. findings study in rats	0, 0.001, 0.003, 0.01
	-98-00873	Effect on tibial cancellous bone in a 6/12-month s.c. findings study in rats. Bone histomorphometry	0, 0.001, 0.003, 0.01

## Oral application

Species	Test No.	Study Title	Doses (mg/kg/day)
Mouse	94-6024	Pilot 13-week oral toxicity study in mice	0, 0.3, 3, 10, 30-20
Rat	89-6306	10-day oral dose range-finding study in rats	0, 1, 10, 100
	90-6079	1-month oral findings study in rats	0, 6, 20, 60
	90-6191	6-month oral findings study in rats	0, 0.1, 1, 10
Dog	89-6307	10-day oral dose range-finding study in dogs	1-30, 10
	90-6080	1-month oral findings study in dogs	0, 3, 10, 30
	90-6190	6-month oral findings study in dogs	0, 0.01, 0.1, 1

These studies were reviewed for NDAs #21-223 and 21-817. Main target organs of toxicity identified in the animal studies were kidney, stomach, GI tract, liver, thymus, lung, and i.v. injection site. Zoledronate is concentrated and excreted by the kidney, and this is one of the most sensitive target organs for toxicity. Bone was the target organ for the compound's pharmacologic effect.

In the review of NDAs #21-223 and 21-817, it was concluded that for single dose treatment renal and GI tract are the most relevant toxicities, and calculated NOAEL and LOAEL multiples for the highest proposed 8 mg human dose. For renal effects LOAEL multiples were lower (2-10x) than for GI tract effects (10x-38x). Renal toxicity was concluded to be the main safety concerns. Pharmacology/Toxicology recommended approval (AP) of NDA#21-223 for the 4-8 mg i.v. dose (indication hypercalcemia of malignancy) and of the 5 mg i.v. dose for treatment of PMO (NDA 21-817).

No new toxicology studies were performed for the current NDA. What follows are summaries from the studies previously submitted to NDAs #21-223 & 21-817.

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TOXICITY STUDIES PREVIOUSLY REVIEWED UNDER NDA 21-223

In the single dose studies, moderate to marked toxicity was seen at doses  $\geq 6$  mg/kg. In an acute IV study in rats with 1.6, 8, 16, 32 mg/kg, 1.6 mg/kg was tolerated with local irritation only. In another acute rat study, 0.6 mg/kg was the NOAEL with renal changes at higher dose of 6 mg/kg and above. An acute IV study in the dog caused mortality at 10 mg/kg probably due to GI hemorrhage or cardiac arrest, and 2 mg/kg was the GI NOAEL. Renal findings were not reported but a histopathology evaluation was not done in this study.

With repeat dose i.v. administration there were effects on body weight, red blood cells, kidney, liver, GI tract, spleen, lung, and injection site (local irritation). Target organ toxicity was evidenced by changes in serum parameters (e.g. liver enzymes, renal toxicity markers), changes in organ weight, and inflammatory changes, necrosis and hemorrhage. In rat, there were decreases in serum Ca and P. Bone changes and secondary effects thereof were due to the pharmacologic activity of the compound and were seen in most studies at the lowest doses used. Findings were partially or totally recoverable. Kidney toxicity included renal tubular necrosis/regeneration, vacuolation, dilatation, inflammation, with BUN and serum creatinine elevations.

Note that GI lesions were observed in rat and dog studies with the i.v. dosing route. In one intermittent 18-day i.v. study in rat, gastric necrosis in females was seen at a dose (0.6 mg/kg) lower than the renally toxic dose (3.2 mg/kg). The GI irritation may partly be due to local effects in the tissue but may also be due to systemic effects such as mast cell effects or serum electrolyte changes. Effects on the GI system with both i.v. and p.o. dosing has also been observed with other bisphosphonates.

TOXICITY STUDIES PREVIOUSLY REVIEWED UNDER NDA 21-817

Single dose iv studies

In a single dose i.v. dog study (#0170119) with **1 mg/kg (5- or 15-min infusions)**, a 5-min dose had renal and GI effects, but a 15-minute infusion did not. Dose-related decreases in Ca and P were seen in both groups that persisted for 6 days. Kidney effects in the 5-min infusion group included renal cortical tubular vacuolation, tubule basophilia, necrosis, urothelial hyperplasia, and focal inflammation, hemorrhage and congestion. GI findings included inflammation, hemorrhage, congestion in stomach and intestine.

In a single dose i.v. study (#0270126) with **0, 1.25 mg/kg (15-min infusion)** with 1-wk, 13-wk or 26-wk recovery period (2/grp controls, 3/grp treated), and clinical pathology on Days 4, 8, 22, there was hypocalcemia and hypophosphatemia on Days 4, 8, and 22. BUN was decreased ( $<10$  mg/dL) in 3 dogs on Day 8 and 1/3 dogs with low BUN was isothermic (low urine specific gravity). The low serum P was thought to be due to drug-related renal tubule damage and dysfunction. At 1 week recovery, 1/3 dogs had microscopic tubule necrosis and basophilia. After 13- or 26-wk recovery kidney findings had resolved. The low BUN was thought to be related to transient medullary washout secondary to a diuretic-like effect and/or tubular damage. It is unclear whether the low Ca and P were related to the medullary washout or renal tubule damage or due to an other drug-related effect.

Based on single dose iv studies, the renal NOAEL was 1 mg/kg.

Intermittent dose iv studies

In a pilot 2-week repeat iv renal study in the male dog (008109), with doses of **0.2, 0.5, 0.75, 1, 3, 6 mg/kg (1 or 3 doses)**, and 5-, 15-, or 60-min infusions, 5-min and 60-min infusions appeared more toxic than a 15-min infusion. This was unexplained. There was no renal effect at 0.2 and 0.5 mg/kg (15-min), and a minimal microscopic effect at 0.5 mg/kg (60-min). Renal NOAEL was 0.5 mg/kg (15-min).

In the 13-week intermittent dog study (0170102) with **0.25 and 1 mg/kg (15-min infusions)**, once every 3 weeks, for 1, 3, or 5 doses, serum Ca and P were decreased in all treated, which recovered in females but not all males after 13 weeks. Urine specific gravity was decreased, probably due to tubular damage. Injection site irritation was present in all treated. Renal microscopic findings were seen at 1 mg/kg and included slight tubular cortical necrosis and debris mineralization, and slight tubular regeneration, inflammation and dilatation. PK data indicated dose proportional C<sub>max</sub> and no accumulation of compound. Renal NOAEL was 0.25 mg/kg (5 doses).

In the 26-week intermittent iv dose study in dogs (018037), with doses of **0.25, 5, 1 mg/kg, for 9 doses every 3 weeks**, 2/6 HD males died on D82 and D140 after clinical signs. These 2 dogs had increased BUN and creatinine, with kidney findings including degeneration/necrosis of tubule epithelial cells, mineralization in cortical tubules, inflammation, increased connective tissue, and papillary necrosis. 1 of the 2 dogs had GI lesions of congestion, erosion, hemorrhage and (sub)acute inflammation. Ca and P were decreased transiently at all doses. At interim sacrifice (Day 46, after 3 doses) there was minimal degeneration/ necrosis of kidney at 0.5 mg/kg. At terminal sacrifice (after 9 doses) there was kidney degeneration/necrosis of tubule epithelial cells, mineralization in cortical tubules, inflammation, increased connective tissue, and papillary necrosis, at 0.25, 0.5 and 1 mg/kg. At recovery (3 wks after 9 doses) there were kidney findings at 1 mg/kg, but no significant GI toxicity. PK: Exposure was dose-proportional and slight accumulation after 25 weeks. Renal NOAEL was 0.25 mg/kg after 3 doses, and <0.25 mg/kg after 9 doses. Mortality occurred at 3 or 6 doses of 1 mg/kg.

Note that hypocalcemia and hypophosphatemia occurred at doses lower than those causing microscopic renal damage. These events were likely to be related to the pharmacologic effect of the drug on bone resorption and possibly the ability of the compound to bind Ca. An effect of tubule function may also have contributed. The NOAEL for this effect was <1 mg/kg in single dose study 0170119 (<6.7x1.5=<10x human AUC), and <0.25 mg/kg (5 doses, 9 doses) in the 13-wk and 26-wk intermittent study, respectively (<10.8x human AUC)

In conclusion, in the iv studies (15-min infusions) with 2-3 week dosing intervals, renal NOAEL values were 0.5 mg/kg (3 doses), and 0.25 mg/kg (5 and 3 doses). At higher doses or more infusions, renal toxicity including tubule basophilia, necrosis, de- or regeneration, vacuolation, dilatation, inflammation, and papillary necrosis was observed. The data suggest a potential for accumulation of renal toxicity with multiple doses.

#### Impurities

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Qualification threshold for impurities in new drug substances is 0.15% (ICH Guidance Q3A). Specification limit in the drug product (for degradation products) is 0.2%.

The first —impurities were present in acute and repeat dose iv toxicity studies in rats and dogs, at percentages ranging from <1/10x – 1x the 0.2% values. However, the maximum tolerated dose (i.e. the dose that did not lead to mortality) in acute studies was 12x (rat) and 25x (dog), and in repeat dose studies of up to 26/52 week duration, it was 1x (dog)-5x (rat) the highest expected dose of 0.17 ng/kg in a 60 kg human, based on mg/kg dose comparison (NDA 21-817, Toxicology Written Summary, p.67). Based on mg/m<sup>2</sup> comparison these multiples are 2x (rat) and 12x (dog) for acute studies, and 0.5x (dog) and 1x (rat) for repeat dose studies. Therefore, these —impurities are adequately qualified for the proposed single dose treatment regimen.

Levels of \_\_\_\_\_ in the toxicology batches ranged from \_\_\_\_\_ Nevertheless, a separate 2-week repeat dose i.v. rat toxicity study was performed with 0.0005 and 0.005 mg/kg/day \_\_\_\_\_ (Study 0470043). The LOAEL for liver toxicity was 0.005 mg/kg. This is approximately 12x (based on mg/kg) and 2x (based on mg/m<sup>2</sup>) the expected human dose of 0.0004 mg/kg in a 5 mg clinical dose at a specification limit of \_\_\_\_ The NOAEL was 0.0005mg/kg (0.2x human dose, based on mg/m<sup>2</sup>; 14-day cumulative dose 2.8x human dose, based on mg/m<sup>2</sup>). Thus, \_\_\_\_\_ is adequately qualified.

**Safety margins (multiples)**

Renal NOAEL values from acute and repeat dose bolus iv studies and from intermittent iv infusion studies (rat and dog) were used by Sponsor to calculate multiples of the human single 5 mg dose. Multiples, or safety margins, were based on NOAEL-dose or exposure (AUC)-at-NOAEL in animals as compared to the dose (mg/m<sup>2</sup>) or exposure (AUC) in humans. For repeat dose data, the multiples are of cumulative dose or AUC. Cumulative dose (or AUC) was the dose (or AUC) multiplied by the number of doses (e.g. 90x in a 3-month study).

The calculations are acceptable for evaluation of safety for a single dose human treatment such as proposed for the current NDA.

**1. Based on mg/m<sup>2</sup> comparison**

**Table 5-3 Renal NOAELs in rat and dog (parenteral bolus administration)**

Bolus dose		Rat				Dog			
Zoledronate		Cumulative NOAEL				Cumulative NOAEL			
Dosing	No. doses	NOAEL (mg/kg)	(mg/kg)	(mg/m <sup>2</sup> )	x single clinical 5 mg dose <sup>2</sup>	NOAEL (mg/kg)	(mg/kg)	(mg/m <sup>2</sup> )	x single clinical 5 mg dose <sup>2</sup>
Single [88-6126]	1	0.6	0.6	3.6	1.2	2 <sup>1</sup> [93-6084]	2	40	13.4
Single [997049]	1	1.6	1.6	9.4	3.2	-	-	-	-
Daily [90-6156]	10	0.06	0.6	3.6	1.2	0.1 [90-6157]	1	20	6.7
Daily [906179]	28	0.2	5.6	33.6	11.3	0.02 [90-6180]	6	12	4
Daily [92-6259]	90	0.03	2.7	16	5.4	0.01 [92-6261]	0.9	18	6.0
Daily [93-6230]	365	0.01	3.7	22	7.4	-	-	-	-
Q2 d for 16 wk, then Q 3d to wk 52	141	-	-	-	-	0.005 [93-6193]	0.71	14.1	4.7

<sup>1</sup>Based on clinical signs and necropsy observations only, no clinical or histopathology  
<sup>2</sup>Comparison based on mg/m<sup>2</sup> BSA (60 kg human) Source: [Toxicology tabulated summary]

**Table 5-4 Renal NOAELs in rat and dog (iv infusion)**

Dosing	No. doses	NOAEL (mg/kg)	Cumulative NOAEL (mg/kg)	(mg/m <sup>2</sup> )	x single clinical 5 mg dose <sup>1</sup>
Q 3 d Rat for 18 d [998073]	6	0.6 (1-2min)	3.6	21.6	7.3
Single Dog [0170119]	1	1.0 (15 min)	1.0	20	6.7
Q 2 wk 28 days-Dog (pilot study) [008189]	3	0.5 (15 min)	1.5	30	10.1
Q 3 wk 13 wk Dog [0170102]	5	0.25 (15min)	1.3	25	8.7
Q 3 wk 26 wk Dog [018037]	3	0.25 (15 min)	0.8	16	5.4

<sup>1</sup> comparison based upon mg/m<sup>2</sup> BSA (60 kg human) Source: [Toxicology tabulated summary]

**2. Based on AUC comparison**

**Table 10-3 Comparative systemic exposure (AUC) at the animal renal NOAEL (parenteral bolus administration) versus 5 mg human equivalent**

Zoledronate Dosing	No. doses	NOAEL (mg/kg)	Rat			Dog			
			Cumulative NOAEL		X single clinical 5 mg dose <sup>4</sup>	Cumulative NOAEL		x single clinical 5 mg dose <sup>4</sup>	
			AUC <sup>2</sup> (ng·h/mL)	AUC (ng·h/mL)		NOAEL (mg/kg)	AUC <sup>3</sup> (ng·h/mL)		AUC (ng·h/mL)
Single [88-6126]	1	0.6	3917	3917	1.0	2 <sup>1</sup> [93-6084]	12820	12820	12.8
Single [997049]	1	1.6	10445	10445	2.8	-	-	-	-
Daily [90-6156]	10	0.06	392	3920	1.0	0.1 [90-6157]	641	6410	6.4
Daily [906179]	28	0.2	1306	36568	9.1	0.02 [90-6180]	128	3584	3.6
Daily [92-6259]	90	0.03	196	17640	4.4	0.01 [92-6261]	64	5760	5.8
Daily [93-6259]	365	0.01	65	23725	5.9	-	-	-	-
Q2 d for 16 wk; then Q 3d to wk 52	141	-	-	-	-	0.005 [93-6193]	32	4512	4.5

Source: [Toxicology tabulated summary]

<sup>1</sup>Based on clinical signs and necropsy observations only; no clinical or histopathology

<sup>2</sup>Rat AUC based on 3917 after 0.6 mg/kg sc [OMP K R99-1978]

<sup>3</sup>Dog AUC based on 6410 (day 1 average male/female) Day 1 after 1 mg/kg [BAPK R0170102]

<sup>4</sup>Human AUC based on AUC<sub>(0-∞)</sub> = 1601 after 8 mg [Clinical Study J001] ≈ 1001 for 5 mg

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**Table 10-4 Comparative systemic exposure (AUC) at the animal renal NOAEL (infusion studies) versus 5 mg human equivalent**

Dosing	No. doses	NOAEL (mg/kg)	AUC <sub>(0-24hrs)</sub> (ng·h/mL)	Cum AUC (ng·h/mL)	x single clinical 5 mg dose
Q 3 d Rat for 18 d [998073]	6	0.6 (1-2min)	3936	23616	5.9
Single Dog [0170119]	1	1.0 (15 min)	6410	6410	6.4
Q 2 wk 28 days Dog (pilot study) [008189]	3	0.5 (15 min)	2848	8544	8.5
Q 3 wk 13 wks Dog [0170102]	5	0.25 (15min)	1440	7200	7.2
Q 3 wk 26 wks Dog [018037]	3	0.25 (15 min)	1405	4215	4.2

Source: [Toxicology tabulated summary]

Rat AUC based on 3936 after 0.6 mg/kg iv [DMPK R99-1978]

Single dog AUC based on 6410 (day 1 average male/female) after 1 mg/kg [BAPK R0170102]

28d dog AUC based on 2848 (day 1 average male/female) after 0.5 mg/kg [BAPK R018037]

13w dog AUC based on 1440 (day 1 average male/female) after 0.25 mg/kg [BAPK R0170102]

26w dog AUC based on 1405 (day 1 average male/female) after 0.25 mg/kg [BAPK R018037]

Human AUC based on AUC<sub>(0-24)</sub> = 1601 after 8 mg [Clinical Study J001] = 1001 for 5 mg

For the AUC exposure multiple calculation, Sponsor used an AUC value of 1001ngxh/mL for humans dosed with 5 mg. This was extrapolated from the AUC value of 1601 ngxh/mL at 8 mg i.v. (Study J001). However, based on data from Study 1101, the AUC at a 5 mg single iv dose was calculated to be 650 ngxh/mL (simulation by Biopharmaceutics Reviewer NDA #21-817). Thus, this reviewer believes that sponsor's AUC multiples were underestimated by a factor 0.67x, and real values are 1.5x higher.

For AUC exposure multiples, the ratio of rat: human AUC was corrected by a factor 4x, since the unbound fraction in rat plasma (0.1) is about 4-fold lower than in human (0.4). The unbound fraction in dog and human plasma is approximately the same (0.4) and no correction was needed. This is acceptable.

The sponsor concluded that based on both single and repeat dose rat and dog toxicity studies, the safety margin for renal toxicity is adequate for the 5 mg single dose. The sponsor also stated that the fact that margins were based on multiple doses in several animal studies while intended human dose is single dose adds to the calculated safety margins. This reviewer does not agree since the use of cumulative dose or exposure values takes this into consideration. Therefore, reviewer concludes margins can be directly evaluated (after correction by a factor 1.5x for AUC exposure multiples).

#### Safety evaluation

Multiples calculated by Sponsor are appropriate to serve as basis for safety evaluation. The multiples are based on renal NOAEL dose levels for rat and dog derived from both single dose, intermittent dose, and daily dose IV studies. GI toxicity was the other main safety concern and occurred at similar or higher doses than renal effects. Toxicities in other organ systems were observed at higher doses with higher NOAEL's. In the review of NDA#21-223, it was concluded that based on acute studies in rats and dogs renal toxicity is the main safety concern for a clinical iv dose regimen.

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**Safety margins (AUC multiples, based on NOAEL) for renal toxicity**

Study type	IV dose	RAT		DOG	
		Study #	AUC multiple	Study #	AUC multiple
Single dose	Bolus	88-6126; 997049	1.5x-4x	0170119	9.6x
10-day repeat dose	Bolus	90-6156	1.5x	90-6157	9.6x
≥1-month repeat dose	Bolus	90-6179;92-6259;936259	6.6x-14x	90-6180; 92-6261	5.4x-8.7x
Intermittent doses	Infusion, 1-2 min	998073	9.1x	-	-
	Infusion, 15 min	-	-	008189;0170102;018037	6.3x-13x

AUC multiples, after a 1.5x adjustment (based on human AUC of 650 ngxh/mL), are 1.5-4x (rat, single dose, and 10-day repeat dose) and 9.6x (dog, single dose). Values are higher based on ≥1-month daily studies in both species. Based on intermittent dose studies, adjusted multiples are 9.1x (rat, every 3 days for 18 days) and 6.3x-13x (dog, bi- or triweekly intermittent dose). All multiples can be considered for safety evaluation. The lowest multiples from rat studies (1.5x) were from an acute study with no histopathology evaluation and with mortality at the next 10x-higher dose of 6 mg/kg (15x multiple), and from a 10-day study with kidney and liver effects at the next 10x higher dose of 0.6 mg/kg (15x).

Serum Ca and P decreases were seen at levels below the NOAEL and the safety margin for this was <9.6x and <10.8x based on AUC comparison (<1 mg/kg in single dose study 0170119, and <0.25 mg/kg, 5 doses, in the 13-wk intermittent study 0170102). It is unclear whether this finding is due to a direct drug Ca-binding effect or related to suppression of bone resorption or an effect on tubule function. Since the effect persisted for up to ca. 3 weeks and occurred in association with tubule dysfunction or damage (single dose study #0270126, 1.25 mg/kg) it may have a renal component.

The reviewer concludes that safety margins for the main toxicities (renal, GI) are adequate to support the proposed clinical treatment with a 5 mg iv dose, given as a 15-minute infusion. The reviewer also concludes that hypocalcemia and hypophosphatemia are potential adverse events that may be associated with the compounds pharmacologic activity, but their cause (bone and/or kidney effect, Ca-chelation) has not clearly been elucidated.

**Genetic toxicology:** There was no evidence of mutagenicity in three Ames tests, an in vitro Chinese Hamster cell V79 assay, an in vitro Chinese Hamster Ovary clastogenicity assay and an in vivo rat micronucleus assay.

**Carcinogenicity:** Carcinogenicity studies of 2-year duration were carried out using the oral gavage route in mice and rats, and were submitted and reviewed for NDA #21-223. Animals were fasted before dosing to facilitate oral absorption.

There was no evidence of carcinogenic in rats. In mice an increased incidence of Harderian gland adenomas/adenocarcinomas was observed in males at 0.1 and 1.0 mg/kg and in females at doses 0.3 and 1 mg/kg. Although there was no dose response, the incidence exceeded the historical control range in some groups, and it was concluded that the finding was biologically significant but its relevance is unclear (Executive CAC meeting minutes, May 23, 2000, NDA 21-223).

In the current label (Zometa), multiples of human dose are expressed as multiples based on mg/m<sup>2</sup> (dose/body surface area) comparison. The multiples are very low (<<1) since the doses in the long term carcinogenicity studies were given daily for lifetime duration, while the clinical dose is a relatively high single or infrequently repeated dose. Based on AUC, multiples in rats dosed by the s.c. route have been found to be larger than based on mg/m<sup>2</sup>. However, TK data for the oral carcinogenicity studies were not available. Therefore multiples are based on mg/m<sup>2</sup> comparison.

In the previous review of NDA #21-223, multiples were based on the assumption of 1% bioavailability, However, for the label sponsor and Division agreed that bioavailability in rats and mice can be assumed to be 2%.

**Reproductive toxicology:** Reproductive toxicity studies were carried out by the subcutaneous route in rats and rabbits, and were reviewed for NDA (#21-223). Due to the dystocia and periparturient mortality observed in the Segment I study probably resulting from drug-related hypocalcemia, a Segment III study was not performed. The dystocia was probably due to lowered serum calcium causing interference with the mobilization of calcium and contraction of uterine muscle.

Bisphosphonates cross the placental barrier and are taken up into the developing fetal skeleton. The teratogenicity observed in the rat Segment II study may be due to a decrease in serum calcium levels and binding to fetal bone.

For the rat Segment I and II studies, multiples were based on AUC comparison, based on data available from single dose s.c. studies at 0.1 and 0.6 mg/kg, and the finding from a 3-month toxicity study that C<sub>pl</sub> (plasma concentration) does not change upon repeated daily dosing with 0.1 mg/kg. For the rabbit study Segment II study, multiples were based on a mg/m<sup>2</sup> comparison.

**Special toxicology:** In an i.v. irritation study in rabbits, there were dose-dependent local inflammatory changes at the site of i.v. injection (ear) (Study 93-6282). A single sc injection of zoledronate in rats at doses of 0.2, 0.4, 2 and 4 mg/kg (concentrations 0.4, 0.8, 4 or 8 mg/ml) was not tolerated due to dose-dependent local irritation at the site of injection (edema, erythema, skin thickening, ulceration) in all dose groups (Study 007062). In a guinea pig sensitization study, zoledronate was considered a weak sensitizer in female guinea pigs. Zoledronate is irritating at the site of administration.

#### **2.6.6.2 Single-dose toxicity**

No studies submitted.

#### **2.6.6.3 Repeat-dose toxicity**

No studies submitted.

#### **2.6.6.4 Genetic toxicology**

Reviewed under NDAs 21-223, 21-386 & 21-817. See summary above.

#### **2.6.6.5 Carcinogenicity**

Reviewed under NDAs 21-223, 21-386 & 21-817. See summary above.

#### **2.6.6.6 Reproductive and developmental toxicology**

Reviewed under NDAs 21-223, 21-386 & 21-817. See summary above.

**2.6.6.7 Local tolerance**

No studies conducted.

**2.6.6.8 Special toxicology studies**

Reviewed under NDAs 21-223, 21-386 & 21-817. See summary above.

**2.6.6.9 Discussion and Conclusions**

See General Toxicity Summary and Executive Summary

**2.6.6.10 Tables and Figures**

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

**Table 2-1 Single dose toxicity studies**

Species	Route	Doses (mg/kg)	Findings
Mouse [93-6085]	Sc	10, 50	LD <sub>50</sub> = 10-50 mg/kg in males; > 10 mg/kg in females
Rat [88-6126]	iv	0.6, 6, 30, 60, 80	LD <sub>50</sub> = approximately 13 mg/kg
Rat [997049]	iv	1.6, 8, 16, 32	≥ 8 mg/kg: mortality, clinical signs, kidney, liver, GI tract ≥ 1.6 mg/kg: ↓ BW/F.C, injection site irritation maximum non lethal dose: 1.6 mg/kg minimum lethal dose: 8 mg/kg
Dog [0170119]	iv 5 & 15 min infusion	1.0	5 & 15 min infusion: clinical signs & moribund sacrifice, mainly due to infusion site leakage/irritation in 15 min grp. ↓ Ca & P in both groups considered pharmacol. side-effect in absence of other renal effects (e.g. histopath) 5 min infusion: kidney, esophagus, GI tract lesions in 5 min grps but not 15 min group
Dog [0270126]	iv 15 min infusion	1.25	↓ Ca & P, kidney lesions
Dog [936084]	iv inj	2, 10	2 mg/kg: no clinical signs 10 mg/kg: mortality

Source: [Toxicology tabulated summary]

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**Table 3-1 Repeat-dose parenteral toxicity studies in rats and dogs**

Study Type	Species	Route	Doses (mg/kg)	Findings
10-Day range-finding [89-6036]	Rat	iv inj	0.06, 0.6, 6	0.06 mg/kg: well tolerated 0.6 mg/kg: clinical signs; kidneys, liver 6 mg/kg: sacrifice due to severe clinical signs; bone, kidneys, stomach, liver, thymus, spleen, lymph nodes NOAEL: 0.06 mg/kg
2-Week [998073]	Rat	iv 1-2 min infusion	0.06, 0.6, 3.2 (every third day for 18 days)	> 0.06 mg/kg: local irritation, bone changes > 0.6 mg/kg: gastric lesions 3.2 mg/kg: mortality, clinical signs, ↓ BW/FC, clinical pathology, kidney, liver NOAEL: not established
10-Day range-finding [90-6156]	Rat	sc	0.2, 0.6, 2	≥ 0.2 mg/kg: injection site irritation ≥ 0.6 mg/kg: clinical signs 2 mg/kg: clinical signs; kidney, liver, spleen, thymus, lymph nodes, lung and adrenals NOAEL: not established
1-Month + 1 mo recovery [90-6179]	Rat	sc	0.02, 0.06, 0.2	≥ 0.06 mg/kg: clinical signs; inj. sites, spleen, muscle 0.2 mg/kg: liver, lymph nodes NOAEL: 0.02 mg/kg/day
3-Month + 1 mo recovery [92-6259]	Rat	sc	0.01, 0.03, 0.1	Tolerated without mortality at doses up to and including 0.1 mg/kg. Pharma bone changes at all doses. NOAEL 0.01 mg/kg/day
6/12-Month + 6 mo recovery [93-6230]	Rat	sc	0.001, 0.003, 0.01	≥ 0.001 mg/kg: clinical path, bone, bone marrow ≥ 0.003 mg/kg: kidney findings 0.01 mg/kg: equivocal changes in testes NOAEL: 0.001 mg/kg/day
6/12-Month bone analyses [93-6230]				Bone morphometry normal

**Table 3-1 Repeat-dose parenteral toxicity studies in rats and dogs**

Study Type	Species	Route	Doses (mg/kg)	Findings
10-Day range-finding [90-6157]	Dog	iv inj	0.1, 1	≥ 0.1 mg/kg: bone rb, inj. sites 1 mg/kg: clinical signs, GI, liver, lung, thymus NOAEL: 0.1 mg/kg
4-Week + 4wk recovery [90-6180]	Dog	iv inj	0.02, 0.06, 0.2	≥ 0.06 mg/kg: clinical signs 0.2 mg/kg: GI tract NOAEL: 0.02 mg/kg/day
3-Month + 4wk recovery [92-6261]	Dog	iv inj	0.01, 0.03, 0.1 → 0.2 (increased to 0.2 at 6 weeks)	≥ 0.01 mg/kg: genital tract (F), bone, spleen; lung, thymus ≥ 0.03 mg/kg: moribund sacrifice at 0.1 → 0.2 mg/kg due to inj. site irritation, ↓ BW/FC, clinical pathology, kidney, liver, genital tract (M), pancreas, urinary bladder, esophagus, stomach, inj. site irritation NOAEL: not established

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Renal Tox Pilot Study through 29 days [008189]	Dog	Intermittent inj or iv infusion, variable infusion times	0.2, 0.5, 0.75, 1.0, 3.0, 6.0	0.2 mg/kg: no effects 0.5 mg/kg: renal NOAEL when given at 2-wk intervals (3x) over 28 days as 5 or 15-minute infusion; kidney effects when infused over 60 min, same dosing regimen ≥ 0.75 mg/kg given 3x over 28 days: kidney effects regardless of infusion time ≥ 3 mg/kg: not tolerated
13-wk [0170102]	Dog	iv 15 min infusion once triweekly	0.25, 1	≥ 0.25 mg/kg: local irritation at infusion sites, ↓ Ca & P 1.0 mg/kg: kidney Renal NOAEL: 0.25 mg/kg
26-wk + 3 wk recovery [018037]	Dog	iv 15 min infusion once triweekly	0.25, 0.5, 1	0.25 mg/kg = Renal NOAEL after 1, 3 & 5 doses Minimal renal effects after 9 doses in 2/8 dogs 1.0 mg/kg: clinical signs, 2 dogs sacrificed moribund; kidney, GI tract
26/52-wk + 6 mo recovery [93-6193]	Dog	iv inj *	0.005, 0.03, 0.1	All doses: inj site irritation; bone ≥ 0.03 mg/kg: kidney, GI tract; clinical pathology NOAEL: 0.005 mg/kg
Bone analyses from 26/52-wk dog [93-6193]				All doses: ↑ mineralization at 12 mo No mineralization or structural defects at 6 or 12 mo; ↓ bone formation at 6 & 12 mo (reversible) ↑ connectivity parameters with ↑ dose (reversible) Biomechanical parameters assessing bone quality showed either no deleterious effect or ↑ quality

Source: [Toxicology tabulated summary]

\*Administered on alternating days for 16 weeks, then every third day through wk 52.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

See executive summary.

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**APPENDIX/ATTACHMENTS**

LABEL (with Reviewers corrections)

Sections relevant for Pharmacology/Toxicology are appended.

**13.1 CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY**

*Carcinogenicity:* Two-year oral carcinogenicity studies were conducted in mice and rats. Rats were given daily oral doses of zoledronic acid of 0.1, 0.5, or 2.0 mg/kg. No increased incidence of tumors was observed (at doses  $\leq 0.1$  times the human intravenous dose of 5 mg, based on a comparison of relative body surface areas). Mice were given daily oral doses of zoledronic acid of 0.1, 0.5, or 2.0 mg/kg/day. There was an increased incidence of Harderian gland adenomas in males and females in all treatment groups (at doses  $\geq 0.002$  times the human intravenous dose of 5 mg, based on mg/m<sup>2</sup> comparison).

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