CENTER FOR DRUG EVALUATION AND RESEARCH

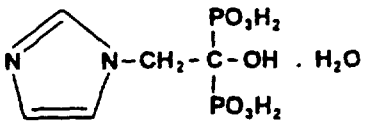
Application Number 21-223

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS: Bisphosphonate, zoledronate, tumor, hypercalcemia, bone, resorption, malignancy

NDA NUMBER:	**21,223**
DRUG:	**ZOLEDRONATE (ZOMETA™)**
Reviewers Names:	Gemma Kuijpers, Fred Alavi, John Gong
Division Name:	Division of Metabolic and Endocrine Drug Products
HFD # :	510 (DMEDP)
Review Completion Date:	August 1, 2000
Date of submission:	December 21, 1999
Information to Sponsor:	Yes (X) (Labeling Comments)
Sponsor:	Novartis Pharmaceuticals Corporation
Manufacturer for drug substance:	Novartis Pharma Basel and ' Switzerland
Drug substance:	Zoledronic acid for injection
Drug product:	4 mg zoledronic acid anhydrous, —— mannitol, USP and —— sodium citrate, USP
Dosage Form:	Sterile lyophilized powder for solution for injection (vial)
Code Name:	CGP 42446, ZOL446
Trade Name:	Zometa™
Chemical Name:	1-hydroxy-2-imidazol-1-yl-phosphonoethyl)phosphonic acid monohydrate
Molecular Formula:	$C_5H_{10}N_2O_7P_2.H_2O$
Molecular Weight:	290.1 g/mole
Drug Class:	Bisphosphonate
Structure:	



CLINICAL INFORMATION

Indication:	Treatment of tumor-induced hypercalcemia
Clinical formulation:	Lyophilized powder (4 mg) reconstituted in 50 ml 0.9% NaCl or 5% Dextrose Injection
Strength:	4 mg (anhydrous)
Dose:	4 mg (initial), or 8 mg (repeat) single dose
Route of administration:	Intravenous infusion
Disclaimer - use of sponsor's material:	Tables and Figures from the paper submission have been copied or scanned in for use in the review
Relevant INDs/NDAs/DMFs:	IND ———— (zoledronic acid) DMF ————

RECOMMENDATION CODE: **AP**

Cc:
NDA Arch
HFD-510
HFD-510/Kuijpers/El-Hage/Hedin

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/S/ 8/1/00

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/S/ 8/2/2000

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[1] Genetic Toxicology studies for this NDA were reviewed by Dr. Fred Alavi, Pharmacology Reviewer, HFD-510

[2] Reproductive toxicology studies for this NDA were reviewed by Dr. John Gong, Pharmacology Reviewer, HFD-510

INTRODUCTION

Bisphosphonates are analogues of inorganic pyrophosphate that can bind to divalent cations and to calcium hydroxyapatite in the skeleton. The pharmacological action of the bisphosphonates consists of an inhibition of osteoclastic bone resorption, partly through a chemical mechanism involving the binding of the compound to bone, and partly through a biological mechanism involving inhibition of cellular osteoclast activity.

Zoledronic acid is a nitrogen-containing hydroxy-bisphosphonate. Zoledronic acid has 2 nitrogen atoms in a heterocyclic imidazole ring attached to carbon atom 1, in contrast to other nitrogen-containing bisphosphonates that have a single nitrogen atom in an aliphatic side chain (e.g. pamidronate). Zoledronic acid is a potent inhibitor of osteoclastic bone resorption and skeletal calcium release. Bone resorption can be stimulated by a variety of stimuli ($VitD_3$, PTH, PTHrP, PGE_2). In tumor-induced hypercalcemia the increase in serum calcium level is thought to be due to the action of the calcemic hormone, parathyroid hormone related peptide (PTHrP), that is released from tumor cells. The compound has irritating properties at the site of application, especially when intravenous dosing is employed.

The current NDA is for zoledronic acid for the indication of tumor-induced hypercalcemia. The proposed treatment regimen is a single dose of 4 mg for intravenous infusion. Retreatment [redacted] mg for intravenous infusion is indicated in patients who do not respond to the initial 4 mg dose with normalization of serum calcium.

The NDA was submitted on December 21, 1999, and given priority review status. The UFGD was June 21, 2000. However, the Sponsor submitted an amendment to the NDA on June 9, 2000, which referred to new information obtained on the increased incidence of renal adverse events in patients treated with zoledronate (4 mg or 8 mg, monthly infusion) in clinical trials for the indication of preventing skeletal-related events in cancer patients with metastatic bone disease (IND [redacted] zoledronic acid for injection). This resulted in a 3-month extension of the review clock and a new UFGD (September 21, 2000) for the NDA. In the amendment the Sponsor stated that they plan to switch from the 8 mg to the 4 mg dose in the three ongoing bone metastases studies. In this NDA review, however, the doses at which preclinical findings were observed are in most instances compared to the maximum recommended human intravenous dose of 8 mg proposed in the NDA for tumor-induced hypercalcemia submitted on December 21, 1999.

Most preclinical studies submitted to this NDA were carried out with sodium salts and hydrates of zoledronic acid colectively known as zoledronate. However, their action is believed to be the same as those of zoledronic acid.

PHARMACOLOGY

Summary of Pharmacology

In vitro, zoledronate inhibits bone resorption at concentrations of 0.3-30 nM, and in vivo it inhibits bone resorption at doses of 0.3-30 µg/kg. In cultures of murine calvaria the IC_{50} value for inhibition of calcium release by zoledronate is approximately 1/100 (0.01x) times the value for pamidronate. In the calvarial cultures, zoledronate and other bisphosphonates also inhibit calcium incorporation. The ratio between the IC_{50} for calcium incorporation and the IC_{50} for calcium release varies largely, from approximately 3 for etidronate and 500 for pamidronate to 15000 for zoledronate. In the thyroparathyroidectomized rat zoledronate dose-dependently inhibits $1,25(OH)_2D_3$-induced acute hypercalcemia with an ED_{50} of ca. 0.07 µg/kg s.c., and is several orders of magnitude more potent than clodronate or etidronate. The inhibition of hypercalcemia in this model is presumably brought about by inhibition of osteoclastic bone resorption. In this animal model the action of zoledronate is thus analogous to its action in tumor-induced hypercalcemia, where the increase in serum calcium level is thought to be due to the stimulation of osteoclastic bone resorption by the calcemic hormone, parathyroid hormone related peptide, PTHrP, which is released by the tumor.

In short term studies in ovariectomized rats, zoledronate can completely prevent the OVX-induced bone loss at a dose of 0.3 µg/kg given 5x/week for 3 weeks. In long term studies in ovariectomized rats (12 months) and monkeys (16 months), zoledronate prevents OVX-induced changes in bone mineral density (BMD), bone biomechanics and bone metabolism at doses of 0.3-7.5 µg/kg/week s.c. and 0.5-12.5 µg/kg/week, respectively.
Histomorphometric studies in rats and monkeys have shown an increase in the amount and connectivity of cancellous bone in zoledronate treated animals. The studies have also shown that zoledronate can prevent the loss of bone volume and trabecular connectivity, and can prevent the increase in bone formation and activation frequency caused by ovariectomy. There was no indication of a deleterious effect on bone cell morphology, osteoid deposition or mineralization, and no woven bone was observed. In OVX rats, zoledronate did not interfere with the anabolic action of PTH on bone.

In intact dogs, treated for 52 weeks with i.v. doses up to 0.1 mg/kg (2-3x/week), zoledronate caused decreased bone turnover, but no mineralization defect. Trabecular bone density and biomechanical properties appeared increased after 6 and 12 months at 0.03 mg/kg in vertebrae but not in femoral head. An increase in tibial trabecular connectivity was also observed.
In intact rats, treated for 52 weeks with s.c. doses up to 0.01 mg/kg/day, zoledronate caused inhibition of cartilage and bone resorption in the growing long bone, resulting in an increased amount, connectivity and extension of the spongiosa located at the epiphyseal-metaphyseal junction. There were no mineralization effects such as osteomalacia.

[redacted]

The exact mechanism of action of zoledronate involved in the various cellular effects is unknown. In the osteoclast zoledronate appears to reduce the level of farnesylated and geranylgeranylated proteins, due to inhibition of isopentyl- and farnesyl-diphosphate synthases, possibly leading to inhibition of cell function and/or viability.

Zoledronate appears to have no significant CNS effects at doses up to 10 mg/kg i.v. in mice. It also has no significant effects on a variety of other safety pharmacology parameters, including gastrointestinal transit time, drug-induced convulsions, cardiac and smooth muscle contraction, respiration, hemodynamic and ECG parameters.

It is well known that renal toxicity can occur upon treatment with relatively high doses of bisphosphonates.

In a study in rats using a single 1-hr i.v. infusion (doses 1.5, 5, 15, 50 mg/kg), both zoledronate and pamidronate at doses of 1.5-50 mg/kg increased serum urea concentration over a 4-hr period in a time- and dose-dependent manner. At 1.5 and 5 mg/kg the effect was slight (<20%), at 15 and 50 mg the effect was marked (up to 100%). The potency of zoledronate to increase serum urea was a fraction of 1/2 to 1/3 of the potency of pamidronate. Taken together with the finding that zoledronate is ca. 100 times more potent than pamidronate in inhibiting bone resorption and hypercalcemia, these data suggest that in the clinical situation zoledronate may be less nephrotoxic than pamidronate. The NOAEL for the effect of zoledronate was <1.5 mg/kg (<1.9x the maximum human dose), the LOAEL was 1.5 mg/kg (1.9x the maximum human dose).

In a repeat-dose s.c. rat study with zoledronate and pamidronate 9 doses of 0.01, 0.1, and 1 mg/kg/day were given over a 10-day period and the urinary excretion of the cytosolic enzyme malate dehydrogenase (MDH) was measured as an early marker of renal damage. The cumulative excretion of MDH over the 10-day dosing period was increased dose-dependently at all doses of pamidronate up to about 2-fold at a dose of 1 mg/kg, but not by zoledronate. However, the daily excretion of MDH was increased in a dose-dependent manner at 0.1 and 1 mg/kg zoledronate, after 8 of the 9 injections given over a 10-day period. The NOAEL of 0.01 mg/kg dose of zoledronate represents a dose multiples of 0.013x the maximum recommended human 8-mg dose, and an exposure multiple of 0.06x the maximum human dose. The LOAEL levels is equivalent to 0.13x the human 8-mg dose or 0.6x the human exposure at the 8-mg human i.v. dose. Although the relevance of the NOAEL and LOAEL levels for a single dose clinical treatment regimen is unclear since the effect was not uncovered until after 8 or 9 daily doses, the low multiples suggest potential nephrotoxicity of zoledronate and pamidronate. The data also indicate differences in the time- and dose-response relationships of the renal toxicity induced by zoledronate and pamidronate.

GENERAL TOXICOLOGY

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

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
	NOTOX 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
	[redacted] 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 mature dogs	0, 0.005, 0.03, 0.1

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
	[redacted] 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 toxiicty 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

RAT AND DOG STUDIES

Summary Tables for rat and dog, listing the lowest dose (mg/kg) at which findings occurred:

RAT, Acute and subchronic i.v. toxicity studies.

Finding	Duration/Dose (mg/kg)			
	Acute i.v.	Acute i.v.	10-day i.v.	2.5-week i.v.
	0.6, 6, 30, 60, 80	1.6, 8, 16, 32	0.06, 0.6, 6	0.06, 0.6, 3.2 (every 3rd day)
Mortality	6	8	6	3.2
Clinical signs	6	8	0.6	3.2
Body weight reduction	NC	1.6	6	3.2 (m>f)
Food consumption reduction		1.6	6	3.2
Reduced RBC, increased WBC				3.2
AST increase			0.06	3.2
ALT increase			0.6	3.2
ALP increase				3.2
Ca, P decrease				3.2
Globulin increase			0.6	3.2
Creatinine, urea increase				3.2
Hematuria, proteinuria				3.2
Adrenal weight increase				3.2
Kidney weight increase	6			3.2
Liver weight increase				3.2
Local irritation at injection site		1.6	6	0.06
Pharmacological bone changes			6 (thickened epiphyseal cartilage)	0.06 (nonproliferative hyperostosis)
Renal findings	6 (enlargement, tubular degeneration)	8 (pale, red foci)	6 (tubular vacuolation, necrosis)	3.2 (enlarged, tubular nephropathy)
Liver findings		8 (pale)	6 (sinus dilatation, congestion)	3.2 (hepatocellular hypertrophy)
GI findings		8 (discoloration, dark contents)		
Adrenal findings		8 (discoloration)		3.2 (enlarged)
Lung findings		8 (discoloration)		
Stomach findings		8 (distension)	6 (mucosal degeneration)	0.6 (necrosis of glandular epithelium)
Thymus findings		8 (edema)	6 (lymphocytosis)	
Spleen findings		8 (pale)	6 (macrophages)	
Lymph nodes findings			6 (macrophages)	
Urinary bladder findings		8 (distension, bloody urine)		
Tail findings		8 (swelling, discoloration)		0.06 (vasculitis, cellulitis, fasciitis)
LD_{50}	13			
NOAEL	0.6 mg/kg	<1.6 mg/kg	0.06 mg/kg	0.06 mg/kg
LOAEL	6 mg/kg	1.6 mg/kg	0.6 mg/kg	0.6 mg/kg

NC = No Change

RAT, Subchronic and chronic s.c. toxicity studies.

Finding	Duration/Dose (mg/kg)			
	10-day s.c.	1-month s.c.	3-month s.c.	6/12-month s.c.
	0.2, 0.6, 2	0.02, 0.06, 0.2	0.01, 0.03, 0.1	0.001, 0.003, 0.01
Mortality	-	-	-	-
Clinical signs	0.6	0.06	0.03 (broken incisors in m)	
Body weight reduction	2		0.01 (m)	
Food consumption reduction	2		0.01 (m)	
Reduced RBC, increased WBC	0.6	0.2	0.01	0.003
Fibrinogen increase	0.6	0.2	0.03	0.003 (m)
AST increase	0.6	0.06	0.01	
ALT increase	0.6	0.2 (f)		
ALP increase	2 (bone ALP)			
ALP decrease		0.02 (liver ALP)		0.001 (bone ALP)
Ca, P decrease	2	0.2 (m)		
Albumin decrease	0.6	0.2		
Globulin increase	0.6	0.2	0.1	
Triglyceride decrease			0.01	
Creatinine, urea increase	2		0.1 (m)	
Protein decrease	2		0.1	
Adrenal weight increase	2			
Kidney weight increase	2			
Liver weight decrease	0.6	0.2		
Spleen weight increase	0.2	0.06		0.003 (m)
Thymus weight decrease	2		0.01	
Local irritation at injection site	0.2	0.2	0.1	- (>0.01)
Pharmacological bone changes		0.02 (extension of primary spongiosa)	0.01 (lengthened primary spongiosa)	0.001 (lengthened primary spongiosa)
Bone marrow hypercellularity		0.02		0.001
Renal findings	2 (pale, regeneration, focal necrosis)			0.003 (m) (tubular casts and focal basophilia)
Liver findings	0.6 (regeneration, extramedullary hematopoiesis)	0.06 (fatty change in f)		
Adrenal findings	2 (hypertrophy)			
Lung findings	2 (cellular infiltration)			
Stomach findings				
Thymus findings	2 (lymphocytolysis)		0.1 (atrophy)	
Spleen findings	2 (lympocytolysis) 0.2 (e.m. hematopoiesis)	0.06 (e.m. hematopoiesis)		0.003 (e.m. hematopoiesis, congestion)
Lymph nodes findings	2 (lymphocytolysis)	0.2 (enlargement)		
NOAEL	<0.2 mg/kg	0.02 mg/kg	<0.01 mg/kg	0.001 mg/kg
LOAEL	0.2 mg/kg	0.06 mg/kg	0.01 mg/kg	0.003 mg/kg

RAT, Acute, subchronic and chronic oral toxicity studies.

Finding	Duration/Dose (mg/kg)			
	Acute oral	10-day oral	1-month oral	6-month oral
	200, 2000	1, 10, 100	6, 20, 60	0.1, 1, 10
Mortality	2000	100	60	10
Clinical signs	200	100	20	1
Body weight reduction	200	100	20	10
Food consumption reduction	200	100	20	10
Hb, Hct, RBC, platelet increase, WBC increase		100	60 (f)	
RBC decrease				1
WBC increase			20	10
WBC decrease		100		
AST increase		100	20	10
ALT increase		100	20 (f)	
ALP decrease		100	60	10
Ca, P, albumin decrease		100	20	10 (Ca)
Protein decrease		100		
Creatinine, urea increase		100		
Adrenal, lung weight increase			60	
Spleen, thymus weight decrease		100	60	
Kidney weight increase		100		
Liver weight decrease		100		
Pharmacological bone changes			6 (extension of primary spongiosa)	0.1 (extension of primary spongiosa)
Bone marrow hypercellularity				1
Renal findings		100 (dilated tubules, with protein casts)	60 (inflammation)	
Liver findings		100 (hepatocyte dissociation)	20 (inflammation)	
GI findings		100 (distension, necrosis)	60 (distension, inflammation)	
Adrenal findings			60 (inflammation)	
Lung findings			20 (inflammation)	
Salivary gland findings			60 (inflammation)	
Stomach findings	200 (enlarged, red)	100 (gastritis)	60 (degeneration, inflammation)	
Thymus findings	200 (hemorrhage)	100 (lymphoid depletion)	60 (atrophy)	
Spleen findings		100 (lymphoid depletion)	20 (inflammation)	
Lymph nodes findings			20 (inflammation)	
NOAEL	<200 mg/kg	<100 mg/kg	6 mg/kg	0.1 mg/kg
LOAEL	200 mg/kg	100 mg/kg	20 mg/kg	1 mg/kg

NC = No Change

DOG, Acute, subchronic and chronic i.v. toxicity studies.

Finding	Duration/Dose (mg/kg)				
	Acute i.v.	10-day i.v. (f)	4-week i.v.	3-month i.v.	26/52-week i.v.
	2, 10	0.1, 1	0.02, 0.06, 0.2	0.01, 0.03, 0.1→0.2 (increase in wk.6)	0.005, 0.03, 0.1 (every 2nd day for 16 wks, then every 3rd day)
Mortality	10	-	-	0.1→0.2 (injection site irritation)	-
Clinical signs	10 (GI hemorrhage!)		0.06	0.1→0.2 (bradypnea)	
Body weight reduction	10		0.2	0.1→0.2	0.1
Food consumption reduction	10		0.06	0.1→0.2	
Reduced RBC, Hb, Hct			0.06	0.1→0.2	0.1
Leukocytosis				0.1→0.2	0.1
(A)PTT increase			0.2	0.1→0.2	
Fibrinogen increase		1		0.1→0.2	
AST increase			0.2	0.1→0.2	0.1
ALT increase		1	0.2	0.1→0.2	
ALP decrease			0.02 (bone ALP)	0.1→0.2 (bone ALP)	0.1 (m)
Cholesterol increase					0.1
CK increase			0.02		
Ca, P decrease			0.02		0.03
Globulin increase		1		0.1→0.2	0.03
Total protein increase			0.2	0.1→0.2	0.03
Albumin decrease			0.1	0.1→0.2	0.1
Triglyceride increase			0.2		
Urea, creatinine increase		1	0.2		
Urea, creatinine decrease				0.1→0.2	0.03
Urine proteinuria			0.06		
Kidney weight increase			0.2 (m)	0.03	0.1
Liver weight decrease			0.2 (m)	0.03	
Spleen weight increase				0.03	
Thymus weight decrease				0.1→0.2	
Genital organ weight decrease				0.01	
Local irritation at injection site		0.1 (hemorrhage, inflammation)	0.02 (cellulitis, phlebitis, hemorrhage)	0 (inflammation), 0.01 (edema, hemorrhage)	0.005 (inflammation, fibrosis, iv thrombosis)
Pharmacological bone changes		0.1 (extension of primary spongiosa in rib)	0.06 (increased bone deposition)	0.01(increased primary spongiosa, osteofibrosis)	0.005 (nonproliferative hyperostosis primary spongiosa)
Bone marrow hypercellularity				0.01	
Renal findings				0.1→0.2 (discoloration, enlargement, pyelitis, urothelial hyperplasia), 0.03 (tubular lesions) ®	0.005 (focal tubular necrosis with cell casts, basophilia, tubular dilatation) ®
Liver findings		1 (necrosis)			
GI findings	10 (hemorrhage)	1 (duodenal inflammation)		0.03 (pancreas acinar atrophy)	0.03 (smooth muscle degeneration, inflammation)
Lung findings		1 (mononucl cell infiltration)			
Stomach findings		1 (focal hemorrhage, microerosions)	0.2 (inflammation, ulceration, atrophy, edema)		0.03 (smooth muscle degeneration and inflammation)
Thymus findings		1 (lymphocytoclasia)		0.01 (atrophy)	
Spleen findings				0.1→0.2 (enlargement)	
Genital tract organ findings				0.01 (atrophy, degeneration)	0.03 (m) (atrophy, degeneration) (@26wks)
NOAEL	2 mg/kg	0.1 mg/kg	0.02	<0.01 mg/kg	<0.005 mg/kg
LOAEL	10 mg/kg	1 mg/kg	0.06 mg/kg	0.01 mg/kg	0.005 mg/kg

® reversible

DOG, Subchronic and chronic oral toxicity studies.

Finding	Duration/Dose (mg/kg)		
	10-day oral	1-month oral	6-month oral
	10, 30 mg/kg	3, 10, 30 mg/kg	0.01, 0.1, 1 mg/kg
Mortality	-	10	-
Clinical signs	-	30	-
Body weight reduction	30	30	
Food consumption reduction	30	30	
Reduced RBC, decreased WBC	30	10	
Thrombocyte decrease		30	
AST increase	30	30	
ALT increase			
ALP decrease		30	0.1
CK increase			0.1
Ca, P decrease	30		
Na, K decrease		30	
Protein decrease	30	30	
Urine: protein, blood, glucose, cells	30		
Kidney weight increase		3	
Liver weight decrease			
Spleen weight increase	30	3	
Thymus weight decrease	30		
Lung weight increase		3	
Local irritation		30 (gingival inflammation and hemorrhage)	
Pharmacological bone changes	10 (extension of primary spongiosa)	3 (extension of primary spongiosa, prominent osteoid seams)	0.01 (extension of primary spongiosa, metaphyseal widening)
Bone marrow changes		10 (necrosis, fibrosis, hypercellularity)	
Bone (rib)		10 (periosteal inflammation and/or hemorrhage)	
Renal findings	30 (congestion, tubular necrosis and dilatation, MN cell infiltrates)	3 (pale, red foci, tubular regeneration, casts, inflammation, congestion, hemorrhage, fibrosis)	
Liver findings	10 (congestion), 30 (inflammation)	10 (discoloration, necrosis, fatty change, congestion, hemorrhage, inflammation, cholestasis)	
Gallbladder findings		10 (edema, hemorrhage, inflammation)	
GI findings	30 (esophageal erosion)	3 (red foci, congestion, distension, erosion, inflammation, hemorrhage, edema, Ca deposits) 30 (pancreas inflammation)	
Adrenal findings	30 (congestion, cortical hemorrhage)	30 (hemorrhage, neutrophil infiltrate, cell necrosis)	
Lung findings		10 (red foci, inflammation, adenomatosis with hemorrhage), 30 trachea inflammation)	
Stomach findings			
Thymus findings	10 (lymphocytoclasia), 30 (decreased size, lymphoid depletion)	10 (lymphoid depletion, lymphocytoclasia, hemorrhage, inflammation)	
Spleen findings	10 (congestion)	3 (enlarged, soft, congestion, hemorrhage, em hematopoiesis, neutrophil infiltration)	
Lymph nodes findings	10 (lymphocytoclasia)	3 (reddened, clotted blood, hemorrhage, lymphocytoclasia, cell infiltration)	
NOAEL	<10 mg/kg	<3 mg/kg	0.01 mg/kg
LOAEL	10 mg/kg	3 mg/kg	0.1 mg/kg

SUMMARY OF TOXICITIES

Zoledronate caused multiple toxicities involving multiple target organs in i.v., s.c. and oral studies in rats and dogs. Local irritation at the injection site occurred in one acute and most (sub)chronic i.v. and s.c. studies, and pharmacological bone changes were seen in all (sub)chronic i.v., s.c. and oral studies. These drug effects occurred at the LOAEL or NOAEL levels, and were generally seen at lower dose as the study duration increased. The local irritation effect was most pronounced upon i.v. administration.

The NOAEL and LOAEL values from acute and up to 1-month i.v. and s.c. toxicity studies in rats and dogs are summarized in the following Table. In selecting these values, local irritation at the injection site and pharmacologic bone changes were not taken into consideration.

RAT	Acute i.v.	Acute i.v.	10-day i.v.	2.5-week i.v.	10-day s.c.	1-month s.c.	3-month s.c.	6/12-mo s.c.
NOAEL	0.6	<1.6	0.06	0.06	<0.2	0.02	<0.01	0.001
LOAEL	6	1.6	0.6	0.6	0.2	0.06	0.01	0.003
DOG	Acute i.v.	10-day i.v.	4-week i.v.	3-month i.v.	26/52-wk i.v.			
NOAEL	2	0.1	0.02	<0.01	<0.005			
LOAEL	10	1	6	0.01	0.005			

RAT	Acute oral	10-d oral	1-mo oral	6-mo oral
NOAEL	<200	10	6	0.1
LOAEL	200	100	20	1
DOG	10-day oral	1-month oral	6-month oral	
NOAEL	<10	<3	0.01	
LOAEL	10	3	0.1	

Toxicities at LOAEL levels

In the rat i.v. and s.c. studies, the toxicities at the LOAEL levels were:

clinical signs
reductions in body weight and food consumption
reductions in red blood cell parameters
fibrinogen increase
serum ALT and AST increase
serum Ca and P decrease
serum triglyceride decrease,
spleen weight increase
thymus weight decrease
kidney tubular de-and regeneration
stomach glandular necrosis (!)
liver fatty changes
spleen extramedullary hematopoiesis

In the rat oral studies, toxicities at LOAELs were:

clinical signs
reductions in body weight and food consumption
reductions in red blood cell parameters
increases in platelets and WBC
increases in serum AST and ALT
decrease in serum ALP
decreases in serum Ca, P, albumin, protein
increases in serum creatinine and urea
kidney weight increase
liver, spleen and thymus weight decrease
kidney tubular changes and casts
stomach enlargement and red foci, gastritis
GI tract distension, necrosis and inflammation
liver inflammation
lung inflammation
thymus lymphoid depletion and hemorrhage
spleen lymphoid depletion and inflammation
lymph node inflammation

In the dog i.v. studies, toxicities at LOAELs were:
reduction in food consumption
reductions in red blood cell parameter
fibrinogen increase
serum ALT increase
serum globulin increase
serum urea and creatinine increases
proteinuria
thymus weight decrease
liver necrosis
stomach erosion and hemorrhage (!)
duodenal inflammation (!)
kidney tubular necrosis and dilatation with cellular casts
lung inflammation
thymus atrophy and lymphocytoclasia
genital degeneration and atrophy

In the dog oral studies, toxicities at LOAELs were:
ALP decrease
CK increase
kidney, spleen and lung weight increase
GI tract red foci, congestion, distension, erosion, inflammation, hemorrhage and edema
liver congestion and inflammation
kidney red foci, tubular regeneration, casts, inflammation, congestion, hemorrhage and fibrosis thymus lymphocytoclasia
spleen enlargement, congestion, hemorrhage, inflammation and extramedullary hematopoiesis
lymph node red foci, hemorrhage, lymphocytoclasia and inflammation

Toxicities seen above the LOAEL levels were similar or related to thoses seen at the LOAELs. Additional findings at the higher doses were:
(A)PTT increases
serum Na and K decrease
serum ALP increase, urea decrease and creatinine decrease
hematuria
adrenal weight increase
genital organ weight decrease
bone marrow hypercellularity, necrosis and fibrosis associated with the pharmacological bone changes
bone periosteal inflammation
kidney tubular necrosis, pyelitis, urothelial hyperplasia and nephropathy
liver extramedullary hematopoiesis
liver cholestasis
GI tract smooth muscle degeneration and inflammation
gallbladder inflammation
esophageal erosion
tracheal inflammation
adrenal hypertrophy, inflammation and necrosis
adrenal congestion and cortical hemorrhage
pancreatic acinar atrophy and inflammation
broken incisors (rat)

DOSE AND EXPOSURE MULTIPLES

Calculation of dose multiples for i.v. and s.c. studies

Dose multiples for the NOAEL and LOAEL levels from the animal toxicity studies are calculated for i.v. and s.c. studies of up to 1 month duration. Dose multiples are based on dose comparison on the basis of body surface area (mg/m^2).

NOAEL AND LOAEL values (mg/kg/day) from animal i.v. and s.c. studies up to 1 month

RAT	Acute i.v.	Acute i.v.	10-day i.v.	2.5-week i.v.	10-day s.c.	1-month s.c.
NOAEL	0.6	<1.6	0.06	0.06	<0.2	0.02
LOAEL	6	1.6	0.6	0.6	0.2	0.06
DOG	Acute i.v.	10-day i.v.	4-week i.v.			
NOAEL	2	0.1	0.02			
LOAEL	10	1	0.06			

Dose multiples at animal NOAEL and LOAEL values of human 8 mg dose*

RAT	Acute i.v.	Acute i.v.	10-day i.v.	2.5-week i.v.	10-day s.c.	1-month s.c.
NOAEL	0.75x	<2x	0.075x	0.075x	<0.25x	0.025x
LOAEL	7.5x	2x	0.75x	0.75x	0.25x	0.075x
DOG	Acute i.v.	10-day i.v.	4-week i.v.			
NOAEL	7.5x	0.38x	0.08x			
LOAEL	38x	3.8x	0.23x			

*based on mg/m^2 (human dose: 8 mg/60 kg =0.133 mg/kg)

Calculation of exposure multiples for i.v. and s.c. studies

In a number of single dose and repeated dose studies in rats and dogs, AUC values were determined. On the basis of these AUC values we can determine the animal:human exposure multiples at the NOAEL and LOAEL values. For that purpose, we will use the exposure value (AUC_{0-24}) obtained for the 8 mg human dose of 1133 ngxh/ml (see ADME section of this NDA Review).

For rats, C_{max} and/or AUC values were obtained in a 0.6 mg/kg single i.v. or s.c. dose study (AUC_{iv} = AUC_{sc} = 3925 ngxh/ml), a 0.16 mg/kg single i.v. dose study, and a 0.1 mg/kg single s.c. dose study (AUC_{sc} = 600 ngxh/ml). Furthermore, C_{plasma} values (@1h post dose) were measured for the 0.1 mg/kg/day dose in a 3-month s.c.study. The C_{pl} values did not change between Day 1 and the end of the study. The data indicated that, for the rat:

(1) AUC is approximately proportional to dose within the range of 0.1-0.6 mg/kg
(2) AUC is the same after i.v. and s.c. dose administration
(3) AUC does not change between 1 day and 3 months of dosing.

Thus, for the rat, AUC's and exposure multiples for i.v. and s.c. studies are calculated for the 0.06-0.6 mg/kg/day dose range.

For dogs, C_{max} and AUC values were obtained in a 0.15 mg/kg single i.v. dose study (AUC = 210 ngxh/ml), and in a 3-month i.v. study at doses of 0.2 mg/kg/day (AUC_{Day1} = 1936 ngxh/ml), and a 26/52-week i.v. study at a dose of 0.1 mg/kg/2-3 days ($AUC_{Day\,0}$ = 1416 ngxh/ml).
For the dogs dose-proportionality of the AUC values is not obvious. Therefore, we will only use the AUC and calculate the exposure multiple for the 0.1 mg/kg i.v. dose (the NOAEL in the 10-day i.v. study)

NOAEL AND LOAEL values (mg/kg/day) from animal i.v. and s.c. studies up to 1 month

RAT	Acute i.v.	Acute i.v.	10-day i.v.	2.5-week i.v.	10-day s.c.	1-month s.c.
NOAEL	0.6	<1.6	0.06	0.06	<0.2	0.02
LOAEL	6	1.6	0.6	0.6	0.2	0.06
DOG	Acute i.v.	10-day i.v.	4-week i.v.			
NOAEL	2	0.1	0.02			
LOAEL	10	1	0.06			

AUC values (ngxh/ml) at NOAEL and LOAEL levels from animal i.v. and s.c. studies up to 1 month

RAT	Acute i.v.	Acute i.v.	10-day i.v.	2.5-week i.v.	10-day s.c.	1-month s.c.
NOAEL	3925	<10467	393	393	<1308	-
LOAEL	-	10467	3925	3925	1308	393
DOG	Acute i.v.	10-day i.v.	4-week i.v.			
NOAEL	-	1416	-			
LOAEL	-	-	-			

Exposure multiples at animal NOAEL and LOAEL values of human exposure at 8 mg dose*

RAT	Acute i.v.	Acute i.v.	10-day i.v.	2.5-week i.v.	10-day s.c.	1-month s.c.
NOAEL	3.5x	-	0.35x	0.35x	<1.2x	-
LOAEL	-	-	3.5x	3.5x	1.2x	0.35x
DOG	Acute i.v.	10-day i.v.	4-week i.v.			
NOAEL	-	1.25x	-			
LOAEL	-	-	-			

*human exposure at 8 mg maximum human i.v. dose = 1133 ngxh/ml

For the rat the multiples calculated on the basis of dose (mg/m^2) are approximately 1/5 times the ones based on exposure, and for the dog they are 1/3 times the ones based on exposure.

Multiples of NOAELs in acute i.v. rat and dog studies

The dose multiple for the NOAEL in the first acute rat study was 0.75x and the exposure multiple was 3.5x. The dose multiple in the second study was <2x. The dose multiple at the NOAEL for the acute dog study was 7.5x.

Multiples of LOAELs in acute i.v. rat and dog studies

For the first acute study in rats, the dose multiple of the LOAEL was 7.5x (exposure multiple not determined). The toxicity at this LOAEL value was mortality and renal toxicity. For the second acute study in rats the dose multiple was 2x, and the toxicity at this LOAEL was decreased body weight and food consumption, and irritation at the injection site. For the acute study in dogs, the dose multiple of 38x refers to the LOAEL value at which mortality and GI hemorrhage was observed. Since the real LOAEL values are somewhere between the NOAEL and the LOAEL values given in the tables above, the multiples for the LOAELs are possibly overestimated.

Multiples in oral rat studies

Dose or exposure multiples for oral rat studies are not calculated here. However, for the 2-year oral rat carcinogenicity study exposure and dose multipes are calculated in the ADME section of this review, assuming 1% bioavailability of an oral dose (p.83).

RENAL AND GI EFFECTS IN ACUTE SINGLE DOSE I.V. RAT AND DOG STUDIES

Since renal and GI toxicity constitute an important clinical safety concern upon short term infusion of bisphosphonates, the renal and GI findings in the single dose rat and dog i.v. studies and the associated NOAEL and LOAEL levels are discussed here in more detail.

In the first acute i.v. rat study (doses 0.6, 6, 30, 60, 80 mg/kg) all doses produced various clinical signs. One of the five 6 mg/kg animals died on Day 6 after dosing. The cause of the death in this

animal was unclear but seemed to be unrelated to kidney toxicity. Gross necropsy revealed one lesion namely enlarged and pale kidneys in one 6 mg/kg animal that did not die. Kidneys of the one 0.6 mg/kg and the five 6 mg/kg males used in the study were examined microscopically. The kidneys of the animal that died and of 3 other animals in the 6 mg/kg dose group showed renal changes of minimal to slight severity, consisting of tubular regeneration, dilation (in the cortex) and desquamation (in outer medulla), and of inflammation and/or fibrosis of the interstitium mainly in the inner stripe of the outer medulla. In the fifth male, in which the kidneys were enlarged, pale and soft, marked tubular and interstitial lesions that were similar to but much more severe than the lesions seen in the other animals in this group were observed. In this animal there were also granulocytic casts in tubules of the outer medulla and in collecting ducts. The kidney of the animal treated with 0.6 mg/kg had no pathological changes. The tubular lesions may be explained by the high renal clearance of the compound leading to the build up of large concentrations in the renal tubule. At necropsy, no GI lesions were observed at any dose.
In conclusion, the NOAEL for renal findings in this study was 0.6 mg/kg. This represents is a dose multiple of 0.75 x the maximum recommended 8-mg human dose, and an exposure multiple of 3.5x the maximum human dose. The LOAEL of 6 mg/kg is a dose multiple of 7.5 x the maximum human dose. Since at the LOAEL renal histopathology findings were marked in 1 out of the 5 animals, and since the separation between the LOAEL and the NOAEL doses was rather large (10-fold), the LOAEL and the associated dose multiple, or safety margin, of 7.5x may be overestimated.

In the second acute i.v. rat study (doses 1.6, 8, 16, 32 mg/kg) all animals but one treated with doses ≥8 mg/kg died starting at Day 4 of the study. At 1.6 mg/kg the only toxicity seen was a reduction in body weight and food consumption. At doses of 8 mg/kg and above a variety of clinical signs and macroscopic organ pathology was observed. Renal findings consisted of pale kidneys at 8 and 16 mg/kg, and red foci at 8 mg/kg. The other prominent gross findings were hemorrhagic GI lesions in stomach, small intestine and abdominal cavity (red foci or contents, distension, dark fluid in cavity) observed at 8, 16 and 32 mg/kg. Organ histopathology was not performed.
The NOAEL and LOAEL for renal findings were 1.6 and 8 mg/kg, respectively. These doses represent dose multiples of 2x and 10x the maximum recommended 8-mg human dose.
The NOAEL and LOAEL values for GI toxicity were also 1.6 and 8 mg/kg, respectively. These are dose multiples of 2x and 10x the maximum recommended 8-mg human dose.

In the dog acute i.v. toxicity study (doses 2 and 10 mg/kg, 1 male/dose group), there were no findings in the 2 mg/kg animal. In the animal treated with 10 mg/kg, there were adverse clinical signs starting 3 days post dosing, and after 6 days the animal died with hemorrhagic discharge from the anus. Macroscopic examination of this animal revealed red foci or reddened stomach mucosa, hemorrhage in the small and large intestine, thickened gall bladder contents, and spleen enlargement and dark discoloration. Renal findings were not reported.
The NOAEL and LOAEL values for GI toxicity in this acute i.v. dog study were 2 and 10 mg/kg, respectively. These doses represent dose multiples of 7.5x and 38x the maximum recommended 8-mg human dose.

MOUSE STUDIES

An acute s.c. and a 3-month oral toxicity study in the mouse revealed toxicities that were also seen in rat and/or dog. Since the mouse studies did not provide additional toxicology information they are not discussed here.

CARCINOGENICITY

RAT CARCINOGENICITY STUDY

GENERAL INFORMATION

Study Title: 104-Week Oral (Gavage) Carcinogenicity Study in Rats
Study Number: 951159
Volume Numbers: Vols. 1.47-1.52
Test Facility: Novartis Pharmaceuticals Corporation, Preclinical Safety Facilities, New Jersey, US
Study Period: June 1995 – June 1997
Date of Submission: December 21, 1999
QA Report: Yes
Dose-range-finding study: 1-month and 6-month toxicity studies

STUDY PROTOCOL AND METHODS

Species/strain: Rat (Crl:CD®(SD)BR strain)
Number of animals: 70/sex/dose group
Age at start of study: Approximately 6 weeks
Weight at start of study: 203-277g (males), 146-218g (females)
Animal housing: Individually
Drug Name: —— 42446 (zoledronic acid)
Drug Batch number(s): 800194
Drug Analysis: Aliquots of dosing solutions were taken in weeks 1,5,9,21,33,45,57,69,81,93, and 104 for concentration and pH analysis by ——
Drug Stability: Stable under conditions used.
Dosage form: NaOH was added to test article solutions to form the disodium salt of the test compound (zoledronate-Na).
Vehicle employed: Purified water (USP)
Test Article Administration: Test article was given daily, by oral gavage (syringe), after ca. 3.5h of fasting. Fasting was continued after dose administration for another 4h approximately.

Doses:

Group		Dose (mg/kg/day)	Dose Volume (ml/kg)	Main Study N/sex/group
1	Control	0	10	70
2	LD	0.1	10	70
3	MD	0.5	10	70
4	HD	2.0	10	70

Toxicokinetics: Urine samples collected but discarded due to lack of suitable analytic method.
Clinical Pathology: Hematology parameters were evaluated at study termination from the first 10 surviving animals/sex/group.
Anatomic Pathology: All animals were necropsied. Macroscopic observations were only recorded in the raw data. Tissue specimens were taken from the tissues in the list appended, and sections were examined from all tissues, from all animals. Histopathology incidence tables were provided for decedents and survivors.
Relation to Clinical Use: Recommended dose: 4 mg single dose by i.v. injection.

CAC Concurrence: Not available
Route of Administration: Oral (gavage)
Frequency of Administration: Daily
Control groups: One vehicle control group
Interim Sacrifices: None
Statistics: *Sponsor's evaluation*: Mortality data: test for equality (Mantel-Cox logrank test) and test for progressive trend.
Neoplastic lesions: Time-adjusted trend test (modified Peto test).
Non-neoplastic lesions: One-sided trend test.
CDER evaluation: See CDER Biometrics Review (APPENDIX II)

STUDY RESULTS

Clinical Observations
No treatment-related clinical signs.

Mortality
Mortality appeared to be slightly increased in the HD females.
However, according to Sponsors analysis, there was no treatment-related trends in mortality rates in either sex.

Survival

			MALES	FEMALES
Group		Dose (g/kg/day)	Number per 70 (%)	Number per 70 (%)
1	Control	0	19 (27%)	21 (30%)
2	LD	0.1	19 (27%)	17 (24%)
3	MD	0.5	19 (27%)	19 (27%)
4	HD	2.0	22 (31%)	15 (21%)

Body Weight
Small reduction in BW and BW gain through first 85 weeks of the study in MD and HD males. By week 105, however, BW of MD males was similar to controls.
Small reduction in BW and BW gain throughout study in MD and HD females.

Body weight (BW) in males

	Control	LD	MD	HD
BW wk 1 (g)	242	241	239	239
BW wk 85 (g)	908	932	848*	844*
BW wk 85 (% of control)	100%	103%	93%*	93%*
BW wk 105 (g)	809	866	824	760
BW wk 105 (% of control)	100%	107%	102%	94%
BW gain wk 105 (g)**	567	625	585	521
BW gain wk 105 (%)	100%	111%	103%	92%

*significantly different from control
**BW gain = avg BW wk105- avg BW wk1

Body weight (BW) in females

	Control	LD	MD	HD
BW wk 1 (g)	178	178	176	177
BW wk 85 (g)	605	592	549*	542*
BW wk 85 (% of control)	100	98%	91%*	90%*

BW wk 105 (g)	583	604	529	537
BW wk 105 (% of control)	100%	104%	91%	92%
BW gain wk 105 (g)**	405	426	353	360
BW gain wk 105 (%)	100%	105%	87%	89%

*significantly different from control
**BW gain = avg BW wk105- avg BW wk1

Food Consumption:
Minimal to slight reduction in food consumption through week 90, in MD and HD males and females. From week 90-104, slight reduction only in HD (m and f).

Food consumption (FC) in males

	Control	LD	MD	HD
FC (g/animal) wk 52	212	214	206	202*
FC (g/animal) wk 105	181	173	191	163

*p<0.05

Food consumption (FC) in females

	Control	LD	MD	HD
FC (g/animal) wk 52	159	163	153	144*
FC (g/animal) wk 105	165	156	157	149

*p<0.05

Ophthalmology:
No treatment-related changes in HD.

Palpable masses
No apparent dose-related increases in incidence of palpable masses.

Hematology
Small decreases in RBC count, Hb and Hct in LD, MD and HD males and females. Change was statistically significant only for RBC count in all female dose groups.
Small non-dose-related increases in MCV and MCHB in LD, MD and HD males and females, statistically significant only for MCV in MD and HD females
Moderate, non-dose-related and non-significant decrease in WBC and lymphocyte count in all female dose groups.
No apparent effect on other hematology parameters (WBC count, MCHC, differential count, red cell morphology)

Organ Weights:
No data.

Gross pathology:
Observations recorded only in raw data.

Histopathology
Neoplastic histopathology findings (incidence in all animals, ie, decedents and survivors)

Group #		Males				p-value trend test		Females				p-value trend test
Group		Ctrl	LD	MD	HD			Ctrl	LD	MD	HD	
Uterus	Endometrial stromal sarcoma							0	1	0	1	0.342
	Polyp							2	6	5	7	0.057
	Combined polyp							2	6	5	8*	0.032*

	or endometrial stromal sarcoma											

*statistically significant

Non-neoplastic histopathology findings (incidence in all animals, ie, decedents and survivors)

Group #		Males				p-value trend test		Females				p-value trend test
Group		Ctrl	LD	MD	HD			Ctrl	LD	MD	HD	
Bone	Nonproliferative hyperostosis	0	69*	70*	70*	0.000*		0	70*	70*	70*	0.000*
Liver	Hematopoiesis	1	2	2	6*	0.027*		5	10	9	13*	0.042*
Spleen	Hematopoiesis	8	10	3	6	0.897		7	13	9	13	0.166
Urinary bladder	Neutrophilic inflammation	1	2	6*	5*	0.033*		0	1	1	0	0.623
Kidney	Pyelonephritis	1	0	3	3	0.086		0	1	0	0	0.75

*statistically significant

Number of tumor-bearing animals

Group #		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
Number examined		70	70	70	70		70	70	70	70
Animals with benign neoplasms		59	60	54	54		64	67	65	64
Animals with malignant neoplasms		32	23	20	23		26	28	25	19
Total number of animals with neoplasms		62	66	56	59		67	68	68	67

STATISTICAL ANALYSIS OF TUMOR FINDINGS

Sponsor's statistical analysis

Neoplastic findings

Sponsor's analysis showed a statistically significant increase in the incidence of combined uterine polyp or endometrial stromal sarcoma. Although statistically significant, Sponsor considered the increased incidence of this combined tumor fortuitous and not treatment-related since the incidences for polyps in the control and the three dose groups (2/70-6/70-5/70-7/70) were within their (historical) control range of 3/60 to 9/60. The incidences of the individual neoplasms were not statistically significantly increased.

Sponsor concluded that the test compound, —— 42246 was not carcinogenic when administered for 104 weeks to rats.

Non-neoplastic findings

Test compound caused a significant increase in nonproliferative hyperostosis of femur and sternum, characterized by thickening and lengthening of the primary spongiosa. The phenomenon was seen in all animals but one LD male and tended to be more severe when dose was increased. It is caused by inhibition of bone resorption in the newly formed primary spongiosa (osseous tissue) located in the epiphyseal area of the long bones. Normally the primary spongiosa is remodeled to form the marrow space.

The statistically significant increase in liver hematopoiesis observed in males and females is thought to be a result of the decreased marrow space in drug-treated animals.

The increased incidence of urinary bladder inflammation observed in males was mostly due to an effect seen in decedent animals and was thought by Sponsor to be secondary to other bladder lesions (calculi, urethral plugs).
There was a slight increase in pyelonephritis incidence in males.

CDER reviewers statistical analysis (APPENDIX II)
The dose-mortality trend for the rats in either sex was not statistically significant ($p>0.05$), i.e., higher dose did not result in higher mortality.
The dose-tumor positive linear trends for uterine polyp ($p>0.005$) and combined uterine polyp and endometrial stromal sarcoma tumor ($p>0.005$) were not statistically significant.

SUMMARY AND EVALUATION

Dose selection

The high dose used in the rat carcinogenicity study was 2.0 mg/kg/day. The oral administration route was chosen because of the potential of the test compound to cause severe local irritation. Animals were fasted before and after dosing to ensure optimal absorption, which is assumed to be 1-2% in the fasted condition.
The doses selected for this study (0.1, 0.5, 2 mg/kg/day) were chosen in collaboration with the Reviewing Division (Dr. A. Jordan) prior to study start. However, there was no consultation with the Exec CAC. At all doses non-proliferative hyperostosis was seen in the bone, with incidence increased at higher doses. This effect probably caused a decrease in bone marrow space resulting in the observed compensatory increase in extramedullary liver hematopoiesis and the small perturbations in some hematological parameters (RBC, Hb and Hct in both sexes, and WBC in females; all dose groups). At the mid and high doses there were small decreases in body weight and body weight gain in both sexes. At the high dose there was a slight decrease in food consumption in both sexes.
In a previous 6-month oral gavage rat toxicity study at doses of 0.1, 1, and 10 mg/kg/day, mortality was seen at 10 mg/kg/day in 3/25 males and 3/25 females. The cause of death was either unclear (n=3) or related to inflammation of the lung or middle ear (n=2), or tracheal perforation (1). Clinical signs (spasms, sunken flanks, hunched posture, stiff gait, hypoactivity, emaciation, dehydration, tremor, rales, salivation) were seen in 1 out of 5 animals at 10 mg/kg (5 times the high dose in the carcinogenicity study). At 1 mg/kg (0.5 times the high dose in the carcinogenicity study), similar signs (dyspnea, rales, emaciation, salivation) were noted at a lesser degree in individual animals. The signs may have been related to hypocalcemia. Body weight was slightly reduced at 10 mg/kg/day in males (94% of control at 26 weeks) and at 1 and 10 mg/kg/day in females (92% and 88% of control at 26 weeks). Food consumption was slightly decreased at 10 mg/kg in both sexes. Other effects that occurred at 1 and 10 mg/kg (clinical chemistry and hematology) were relatively mild. Based on this information, the 10 mg/kg dose exceeds the MTD. From these results it can be concluded that the MTD is somewhere between 1 mg/kg and 10 mg/kg. The data from the actual carcinogenicity study, however, suggest that 2 mg/kg is below the MTD and a dose between 3 and 5 mg/kg would have been a more appropriate high dose.

Multiples of human dose

In the carcinogenicity studies the doses were given orally by gavage to fasting animals. The bioavailability of the compound under these conditions is approximately 1-2%. Since there are no toxicokinetic data from this study or from other oral rat toxicity studies carried out with this compound, comparison of the doses used in the carcinogenicity studies with the intended human dose is best done on the basis of mg/m^2 comparison. The intended human dose is 4 mg as a single dose intravenous infusion, for the treatment of hypercalcemia of malignancy. This dose is equivalent to 0.066 mg/kg, assuming a human body weight of 60 kg. A conservative calculation of the animal dose multiples can be done if we assume a bioavailability of e.g. 1%, and we compare the animal doses (in mg/kg/day) with the single 4-mg human dose expressed as mg/kg/day.

Rat and human dose comparison, based on mg/m²/day

Dose group	Dose (mg/kg/day)	Equivalent i.v. dose* (mg/kg/day)	Multiple of recommended human dose, basis m² body surface area**
LD	0.1	0.001	0.0025x
MD	0.5	0.005	0.013x
HD	2.0	0.02	0.05x

* Assumption: bioavailability = 1%

**Recommended human dose is 4 mg, or 0.066 mg/kg for a 60 kg person

These multiples apply to the intended 4 mg human dose by i.v. infusion. For the current indication (hypercalcemia of malignancy) retreatment with 8 mg i.v. infusion is recommended if the patient does not respond to the first 4 mg dose by a lowering of serum calcium. Thus, as compared to the 8 mg dose the multiples would be two-fold lower. When interpreting the very low values of the multiples we need to keep in mind that they are based on a single clinical dose, while animals were dosed daily for a lifetime duration.

Histopathology findings

Neoplastic findings

Neoplastic histopathology findings (incidence in all animals)

FEMALES						p-value trend test (Sponsor)
Group		Ctrl	LD	MD	HD	
Uterus	Endometrial stromal sarcoma	0	1	0	1	0.342
	Polyp	2	6	5	7	0.057
	Polyp or endometrial stromal sarcoma	2	6	5	8*	0.032*

*statistically significant (trend test)

There was an increase in the incidence of benign uterine polyps in all dose groups. Although the individual tumor findings were not statistically significant, the increased incidence in the combination of uterine polyp and endometrial stromal sarcoma was statistically significant, based on the Sponsor's trend test (p=0.032). The combined tumor incidence in the high dose group was significantly different from control.

The incidences of polyps, endometrial sarcoma and combined tumor in the current study were formally within the historical control range in all dose groups. For that reason Sponsor considered the increased incidence in the combined tumor incidence, even though statistically significantly according to their analysis, fortuitous and not due to treatment.

Historical control incidence of polyp and endometrial stromal sarcoma in female SD rats from studies conducted at Novartis Pharmaceuticals

Study	A	B	C	D
Polyp	3/60	9/60	3/60	3/59
Endometrial stromal sarcoma	1/60	1/60	0/60	1/59
Combination	4/60	10/60	3/60	4/59

The statistical analysis by CDER's Biometrics Reviewer showed that the increased incidence for the individual uterine polyp and the combined uterine polyp and endometrial stromal sarcoma was not statistically significant (trend test, p>0.005).

Non-neoplastic findings

Bone: Nonproliferative hyperostosis was seen in almost all animals of all dose groups, and was probably the result of the pharmacologic action of the test compound to inhibit bone resorption. In the growing animals this inhibition lead to the finding of hyperostosis, i.e., lengthening and

thickening of the newly formed primary spongiosa located in the epiphyseal area of the long bones.
Liver: Extramedullary hematopoiesis was seen in the liver, with statistically significantly increased incidence in male and female HD groups. This effect was probably the result of impaired bone marrow blood cell formation due to drug-induced hyperostosis.
Urinary bladder: Neutrophilic inflammation of the urinary bladder was increased in incidence in MD and HD males. The effect may have been related to the occurrence of urinary calculi. The cause of the effect is unclear.
Kidney: A slight non-significant increase in pyelonephritis incidence was seen in males.

CONCLUSIONS

CGP was tested in a rat bioassay for 104 weeks at doses up to 2 mg/kg/day. Mortality was not affected. Body weight and food consumption were slightly decreased in MD and HD males and females. Selected hematology parameters, i.e., RBC, Hb and Hct in both sexes, and WBC in females, were affected in all dose groups.
Tumor findings included an increased incidence of uterine polyps and an increased incidence of combined uterine polyp and endometrial stromal sarcoma. According to Sponsor's trend test the increased incidence in the combination of uterine polyp and endometrial stromal sarcoma was statistically significant. However, based on historical control values Sponsor considered the increased incidence fortuitous and not due to treatment. According to CDER's statistical analysis the dose-tumor positive linear trends for the individual and combined uterine tumors were not statistically significant ($p>0.005$ for common tumors).
Non-neoplastic findings included bone hyperostosis in all treated rats and a small increase in the incidence of extramedullary liver hematopoiesis in all treated male and female dose groups.
Based on the results from a 6-month oral rat toxicity study at 0.1, 1 and 10 mg/kg/day, the high dose of 2 mg/kg/day used in the current study was marginally adequate (approximately 0.5 times the MTD).
Doses applied in this study were very small multiples of the recommended human 4-mg dose (0.05x for the high dose, on the basis of mg/m^2). However, the human dose is intended to be a single dose given one time only with one possible re-administration, whereas animals were given daily doses for two years.

NDA 21-223
Zometa
Rat Carcinogenicity Study

Histopathology Inventory
(Tissues retained)

Sponsor Study #951159		
Species: Rat	Pathology	
Abnormalities	X	
Adrenals	X	
Aorta (thoracic)	X	
Blood smear	X	
Bone (cranial)	X	
Bone (sternum)	X	
Bone (femur)	X	
Bone marrow smear*	X	
Brain	X	
Cecum	X	
Colon	X	
Duodenum	X	
Epididymides	X	
Esophagus	X	
Eyes	X	
Gall bladder		
Harderian glands	X	
Heart	X	
Ileum	X	
Jejunum	X	
Kidneys	X	
Lacrimal glands	X	
Larynx		
Liver	X	
Lungs (all lobes)	X	
Lymph nodes, submaxillary	X	
Lymph nodes, mesenteric	X	
Mammary gland (inguinal)	X	
Optic nerves		
Ovaries	X	
Pancreas	X	
Pituitary	X	
Parathyroids	X	
Pharynx		
Prostate	X	
Rectum*	X	
Salivary gland	X	
Sciatic nerve	X	
Seminal vesicles	X	
Skeletal muscle (quadriceps)	X	
Skin (inguinal)	X	
Spinal cord (cervical)	X	
Spleen	X	
Stomach	X	
Teeth		

Testes	X	
Thymus	X	
Thyroid	X	
Tongue		
Tonsils		
Trachea	X	
Ureter		
Urinary bladder	X	
Uterus	X	
Vagina	X	
Zymbal glands		

*tissue retained but not evaluated

MOUSE CARCINOGENICITY STUDY

GENERAL INFORMATION

Study Title: 104-Week Oral (Gavage) Carcinogenicity Study in Mice
Study Number: 951021
Volume Numbers: Vols. 1.41-1.46
Test Facility: Novartis Pharmaceuticals Corporation, Preclinical Safety Facilities, New Jersey, US
Study Period: March 1995 – March 1997
Date of Submission: December 21, 1999
QA Report: Yes
Dose-range-finding study: 3-month oral gavage toxicity study

STUDY PROTOCOL AND METHODS

Species/strain: Mouse (Crl:CD1(ICR)Br) strain
Number of animals: 70/sex/dose group
Age at start of study: Approximately 6 weeks
Weight at start of study: 23.7-32.3g (males), 17.9-25.4g (females)
Animal housing: Individually
Drug Name: [redacted] 42446 (zoledronic acid)
Drug Batch number(s): 800194
Drug Analysis: Aliquots of dosing solutions were taken in weeks 1,5,9,21,33,45,57,69,81,93, and 104 for concentration and pH analysis [redacted]
Drug Stability: Stable under conditions used.
Dosage form: NaOH was added to test article solutions to form the disodium salt of the test compound (zoledronate-Na)
Vehicle employed: Purified water (USP)
Test Article Administration: Test article was given daily, by oral gavage (syringe), after ca. 3.5h of fasting. Fasting was continued after dose administration for another 4h approximately.

Doses:

Group		Dose (mg/kg/day)	Dose Volume (ml/kg)	Main Study N/sex/group
1	Control	0	10	70
2	LD	0.1	10	70
3	MD	0.3	10	70
4	HD	1.0	10	70

Toxicokinetics: Urine samples collected but discarded due to lack of suitable analytic method.
Clinical Pathology: Hematology parameters were evaluated at study termination from the first 10 surviving animals/sex/group.
Anatomic Pathology: All animals were necropsied. Macroscopic observations were recorded in the raw (individual animal) data. Tissue specimens were taken from the tissues in the list appended, and sections were examined from all tissues, from all animals, and from gross lesions. Histopathology incidence tables were provided for decedents and survivors.
Relation to Clinical Use: Recommended dose: 4 mg single dose by i.v. injection
CAC Concurrence: Not available

Route of Administration: Oral (gavage)
Frequency of Administration: Daily
Control groups: One vehicle control group
Interim Sacrifices: None
Statistics: *Sponsor's evaluation*: Mortality data: test for equality (Mantel-Cox logrank test) and test for progressive trend.
Neoplastic lesions: Time-adjusted trend test (modified Peto test).
Non-neoplastic lesions: One-sided trend test.
CDER evaluation: See CDER Biometrics Review (APPENDIX II)

STUDY RESULTS

Clinical Observations
No treatment-related clinical signs.

Mortality
Sponsors analysis: No dose-related trends in mortality rates in either sex.
However, mortality appeared less in high dose males.

Survival

			MALES	FEMALES
Group		Dose (g/kg/day)	Number per 70 (%)	Number per 70 (%)
1	Control	0	24 (35%)	27 (39%)
2	LD	0.1	18 (26%)	28 (41%)
3	MD	0.3	20 (29%)	30 (43%)
4	HD	1.0	37 (54%)	26 (37%)

Body Weight
Small to moderate dose-related reduction in BW and BW gain in LD, MD and HD males.
Small reduction in BW and BW gain in HD females.

Body weight (BW) in males

	Control	LD	MD	HD
BW wk 1 (g)	28.2	28.0	28.1	28.2
BW wk 105 (g)	38.2	36.6	35.7*	34.7*
BW change (%)	+34.4%	+32.4%	+28.5%	+24.3%*
BW gain wk 105 (g)**	10.0	8.6	7.6	6.5
BW gain wk 105 (%)	100%	86%	76%	65%*

*significantly different from control
**BW gain = avg BW wk105- avg BW wk1

Body weight (BW) in females

	Control	LD	MD	HD
BW wk 1 (g)	22.3	21.5*	21.8*	21.8*
BW wk 105 (g)	32.6	32.8	32.1	31.2
BW change (%)	+49.3%	+52%	+47.9%	+41.1%*
BW gain wk 105 (g)**	10.3	11.3	10.3	9.4
BW gain wk 105 (%)	100%	110%	100%	91%

*significantly different from control
**BW gain = avg BW wk105- avg BW wk1

Food Consumption
Minimal to small reduction in LD, MD and HD males throughout study.

Minimal to small reduction in LD, MD and HD females throughout study.

Food consumption (FC) in males

	Control	LD	MD	HD
FC (g/animal) wk 53	31.0	30.5	30.6	28.6*
FC (g/animal) wk 105	30.5	30.0	27.9*	27.8*

*p<0.05

Food consumption (FC) in females

	Control	LD	MD	HD
FC (g/animal) wk 53	30.8	30.3	30.3	28.8*
FC (g/animal) wk 105	30.7	28.7*	28.5*	27.2*

*p<0.05

Ophthalmology:
No treatment-related changes in HD

Palpable masses
No apparent dose-related increases in incidence of palpable masses.

Hematology
Small, statistically significant increase in MCHB in all male dose groups.
Moderate, non-dose-related and non-significant decrease in WBC and lymphocyte count in all male dose groups.
No apparent effect on other hematology parameters (RBC count, Hb, Hct, MCV, MCHC, differential count, red cell morphology)

Organ Weights:
No data

Gross pathology:
Observations recorded only in raw (individual animal) data

Histopathology

Neoplastic histopathology findings (incidence in all animals, ie, decedents and survivors)

Group #		Males				p-value trend test		Females				p-value trend test
Group		Ctrl	LD	MD	HD			Ctrl	LD	MD	HD	
Harderian gland	Adenocarcinoma	0	0	0	1	0.370		0	0	0	0	
	Adenoma	3	9	4	9	0.134		1	2	5	4	0.056
	Adenoma/Adeno carcinoma combined	3	9	4	10	0.089						
Uterus	Endometrial stromal carcinoma							1	1	2	2	0.233
	Polyp							11	12	7	8	0.864

Non-neoplastic histopathology findings (incidence in all animals, ie, decedents and survivors)

Group #		Males				p-value trend test		Females				p-value trend test
Group		Ctrl	LD	MD	HD			Ctrl	LD	MD	HD	
Bone	Nonproliferative hyperostosis	0	27**	64**	67**	0.000**		0	52**	68**	66**	0.000**

Large intestine	Dilation	1	4	2	8*	0.016*		0	0	0	6**	0.001**
Small intestine	Dilation	2	4	3	7	0.068		0	0	1	9**	0.000**
Lung	Hemorrhage	3	6	9	7	0.095		1	3	3	4	0.136
Uterus	Dilation							0	0	1	2	0.062
	Inflammation							0	2	2	3	0.086

*,** statistically significant (p<0.05, 0.01)

Number of tumor-bearing animals

There appeared to be a dose-related decrease in the number of female animals with benign or malignant neoplasms. Partly, this was due to a decrease in the number of females with malignant lymphoma (incidence control-LD-MD-HD: 20-18-15-10)

Number of tumor-bearing animals

Group #		Males					Females			
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
Number examined		70	70	70	70		70	70	70	70
Animals with benign neoplasms		18	24	18	20		31	30	25	23
Animals with malignant neoplasms		18	18	11	18		43	33	32	24
Total number of animals with neoplasms		32	36	25	33		54	50	47	35

STATISTICAL ANALYSIS OF TUMOR FINDINGS

Sponsor's statistical analysis

Neoplastic findings

Sponsor's analysis showed a statistically non-significant increase in the incidence of combined Harderian gland adenoma or adenocarcinoma in males and Harderian gland adenoma in females.

Sponsor considered the increased incidence of the combined tumor not biologically relevant because:

1. the incidence in the high dose groups (9/70 in males and 4/70 in females) was within the historical control range (3/70-9/60 in males, and 1/70-4/70 in females) (*Reviewers Note:* incidence in mid dose females, however, was outside historical control range)
2. the incidence in males of either tumor or the combination was not significantly increased over the controls and there was no dose-related trend
3. Harderian glands are not "part of human physiology"

There was no significant increase in uterine endometrial sarcoma or polyps (as was observed in the rat study).

Sponsor concluded that the test compound — 42246, was not carcinogenic to mice after administration for at least 104 weeks.

Non-neoplastic findings

Test compound caused a significant increase in nonproliferative hyperostosis of the femur and sternum in males and females. This non-neoplastic change was thought to result from the pharmacological effect of the test compound of inhibition of bone resorption.

There was an increase in the incidence of dilation of the large and the small intestine in males and females, which was statistically significant in males for the large intestine and in females for the large and the small intestine.

There appeared to be an increase in lung hemorrhage in males and females, and in uterine dilation and inflammation in females, but these changes were not statistically significant.

CDER reviewers statistical analysis (APPENDIX II)
The dose-mortality trend for the male rats was statistically significant ($p<0.05$), i.e., higher dose resulted in lower mortality. The dose-mortality trend for the female rats was not statistically significant ($p>0.05$), i.e., higher dose did not result in altered mortality.
The dose-tumor positive linear trend for Harderian gland adenoma in males and females ($p>0.005$) and for combined Harderian gland adenoma and adenocarcinoma in males and females ($p>0.005$) was not statistically significant.
A test using pairwise comparison showed that the incidence of harderian gland adenoma in male and female mice was not significantly increased in the high dose groups as compared to the controls.

SUMMARY AND EVALUATION

Dose selection

The high dose used in the mouse carcinogenicity study was 1.0 mg/kg/day. The oral administration route was chosen because of the potential of the test compound to cause severe local irritation. Animals were fasted before and after dosing to ensure optimal absorption, which is assumed to be 1-2% in the fasted condition.
The doses selected for this study (0.1, 0.3, 1 mg/kg/day) were chosen in collaboration with the Reviewing Division (Dr. A. Jordan) prior to study start. However, there was no consultation with the Exec CAC. At all doses non-proliferative hyperostosis was seen in the bone, with incidence increased at higher doses and larger in females than in males. Body weight and body weight gain were slightly reduced in the mid and high dose males. In females, these parameters were slightly reduced in the high dose group only. There were minimal to small reductions in food consumption in all dose groups in both sexes.
In a previous 13-week pilot oral gavage toxicity study in mice, at doses of 0.3, 3, 10, and 30→20 mg/kg/day, mortality was seen in males at all doses (1/15-2/15-8/15-14/15) and in females at the three higher dose levels ≥3 mg/kg/day (1/15-7/15-14/15). In the animals that died clinical signs included rales and labored breathing, and there was inflammation of various sites in the respiratory tract. Body weight gain and food consumption were reduced at doses ≥3 mg/kg/day in males and females. Based on this information, 1 mg/kg day was selected as the high dose for the carcinogenicity study. Since mortality and adverse signs were observed in males at 0.3 mg/kg in the 13-week study, the high dose selection in the carcinogenicity study of 1 mg/kg/day for the males was adequate. For the females, the 1 mg/kg/day dose was also adequate, since this was one third of the dose at which lethality was observed in the 13-week study.

Multiples of human dose

In the carcinogenicity study the doses were given orally by gavage to fasting animals. The bioavailability of the compound under these conditions is approximately 1-2%. Since there are no toxicokinetic data from this study or from other oral mouse toxicity studies, comparison of the doses used in the carcinogenicity study with the intended human dose is best done on the basis of mg/m^2 comparison. The intended human dose is 4 mg as a single dose intravenous infusion, for the treatment of hypercalcemia of malignancy. This dose is equivalent to 0.066 mg/kg, assuming a human body weight of 60 kg. A conservative calculation of the animal dose multiples can be done if we assume a bioavailability of e.g. 1%, and we compare the animal doses (in mg/kg/day) with the single human 4-mg dose expressed as mg/kg/day.

Mouse and human dose comparison, based on mg/m^2/day

Dose group	Dose (mg/kg/day)	Equivalent i.v. dose (mg/kg/day)*	Multiple of recommended human dose, basis m^2 body surface area**
LD	0.1	0.001	0.0013x
MD	0.3	0.003	0.0038x

HD	1.0	0.01	0.013x

* Assumption: bioavailability = 1%
**Recommended human dose is 4mg, or 0.066 mg/kg for a 60 kg person

These multiples apply to the intended 4 mg human dose by i.v. infusion. For the current indication (hypercalcemia of malignancy) [redacted] if the patient does not respond to the first 4 mg dose by a lowering of serum calcium. Thus, as compared to the 8 mg dose the multiples would be two-fold lower. When interpreting the very low values of the multiples we need to keep in mind that they are based on a single clinical dose, while animals were dosed daily for a lifetime duration.

Histopathology findings

Neoplastic findings

Neoplastic histopathology findings (incidence in all animals, ie, decedents and survivors)

		Males				p-value trend test (Sponsor)		Females				p-value trend test (Sponsor)
Group		Ctrl	LD	MD	HD			Ctrl	LD	MD	HD	
Harderian gland	Adenocarcinoma	0	0	0	1	0.370		0	0	0	0	N/A
	Adenoma	3	9	4	9	0.134		1	2	5	4	0.056
	Adenoma/Adeno carcinoma combined	3	9	4	10	0.089						

There was an increase in the incidence of Harderian gland adenoma in males and females. There was 1 Harderian gland adenocarcinoma in a high dose male, and the combined incidence of Harderian adenoma and adenocarcinoma in males was also increased. Although the individual and combined Harderian gland tumor findings were not statistically significant, the increased incidence was suggestive of a drug-related effect.

The historical control incidence of this tumor (and combination) is given in the following table:

Historical control incidence of Harderian gland adenoma and adenocarcinoma in CD-1 mice from studies conducted at Novartis Pharmaceuticals

Study	A	B	C
MALES			
Adenoma	4/60	3/70	9/70
Adenocarcinoma	5/60	0/70	0/70
Combination	9/60	3/70	9/70
FEMALES			
Adenoma	2/57	3/70	1/70
Adenocarcinoma	0/57	1/70	0/70
Combination	2/57	4/70	1/70

Studies conducted in 1994 and 1996 (2x)

The increased incidence of adenoma in males (9/70 in LD and HD males) observed in the current study is within the historical control range (3/70-9/70). The finding of adenocarcinoma (1/70) in the high dose males is within the historical control range. The combined tumor incidence of adenoma and adenocarcinoma in the high dose males (10/70) is within the control range for the tumor combination (3/70-9/60 = 3/70-10.5/70).
Sponsor concluded that the increased incidence in males of Harderian gland tumors was not relevant since it was statistically not significant and since the incidence was within the range of historical control values. Sponsor considered the increased incidence of the combined tumor in females not biologically relevant because it was statistically not significant and the incidence in the high dose group (4/70) was within the historical control range for the combined tumor incidence.

However, in females there were only adenomas and the incidence of this tumor in the mid and high dose was outside the control range for adenoma only. Although the increase in adenomas in males or females was indeed not statistically significant (according to both Sponsor's and CDER's analysis), this Reviewers feels that, since the tumors occurred in both sexes and the concurrent and historical control incidence was exceeded in both mid and high dose females, the effect is biologically significant. In a 92-week carcinogenicity study with another bisphosphonate, alendronate, at doses 0,0,1,2,5→10 mg/kg/day, an increased incidence of Harderian gland adenoma was also observed in female mice (incidence 0-1-1-2-6). In males the incidence was not increased (4-3-2-2-4).
Taken together, this Reviewer feels that the Harderian gland tumor finding in the current mouse carcinogenicity study is biologically significant.
Non-neoplastic findings
Bone: Nonproliferative hyperostosis was seen in almost all animals of mid and high dose groups, and was probably the result of the pharmacologic action of the test compound to inhibit bone resorption. In the growing animals this inhibition lead to the finding of hyperostosis, i.e., lengthening and thickening of the newly formed primary spongiosa located in the epiphyseal area of the long bones.
Intestine: The significant increase in the incidence of intestinal dilation in males and females is a drug-related effect, that is probably the result of the known gastrointestinal irritant properties of the bisphosphonates.
Lung: The cause of the increased incidence of hemorrhage in both sexes, although statistically not significant, is unclear. Possibly, during the oral gavage, some of the test compound enters the lungs and causes tissue irritation.
Uterus: Uterine dilation and/or inflammation were increased in all dose groups. The drug-relatedness of this effect is unclear. The finding is noted because of the increased incidence of uterine polyps observed with the test compound in the rat carcinogenicity study.

CONCLUSIONS

CGP was tested in a mouse bioassay for 104 weeks at doses up to 1 mg/kg/day. Mortality was not affected. Body weight was slightly decreased in MD and HD males and in HD females. Tumor findings included an increased incidence of Harderian gland adenoma and of combined adenoma and adenocarcinoma in treated males, and an increased incidence of Harderian gland adenoma in trreated females. The incidence of adenoma in mid and high dose females was outside the historical control range. Although the Harderian gland tumor finding was not statistically significant according to both Sponsor's and CDER's statistical analysis, the finding appears to be biologically significant because the increase in tumor incidence was seen in both sexes and the incidence was above historical control values in both mid and high dose females. In a 92-week mouse carcinogenicity study with alendronate, a related bisphosphonate, a significant increase in the incidence of Harderian gland adenoma was observed in high dose female mice (10 mg/kg/day).
Non-neoplastic drug-related findings included bone hyperostosis in all male and female dose groups, intestinal dilation in males and females, and uterine dilation and inflammation in females. Based on the results from a 13-week oral mouse toxicity study at 0.3, 3, 10, and 30→20 mg/kg/day, the MTD is ≤ 0.3 mg/kg in males and ≤ 3 mg/kg/day in females. Thus, the high dose of 1 mg/kg/day used in the current study appears to be an adequate high dose for both sexes. Doses applied in this study were very small multiples of the recommended 4-mg human dose (0.013x for the high dose, on the basis of mg/m^2/day). However, the human dose is intended to be a single dose given one time only with one possible re-administration, whereas animals were given daily doses for two years.

NDA 21-223
Zometa
Mouse Carcinogenicity Study

Histopathology Inventory
(Tissues retained)

Sponsor Study #951159		
Species: Rat	Pathology	
Abnormalities	X	
Adrenals	X	
Aorta (thoracic)	X	
Blood smear	X	
Bone (cranial)	X	
Bone (sternum)	X	
Bone (femur)	X	
Bone marrow smear*	X	
Brain	X	
Cecum	X	
Colon	X	
Duodenum	X	
Epididymides	X	
Esophagus	X	
Eyes	X	
Gall bladder	X	
Harderian glands	X	
Heart	X	
Ileum	X	
Jejunum	X	
Kidneys	X	
Lacrimal glands	X	
Larynx		
Liver	X	
Lungs (all lobes)	X	
Lymph nodes, submaxillary	X	
Lymph nodes, mesenteric	X	
Mammary gland (inguinall)	X	
Optic nerves		
Ovaries	X	
Pancreas	X	
Pituitary	X	
Parathyroids	X	
Pharynx		
Prostate	X	
Rectum	X	
Salivary gland	X	
Sciatic nerve	X	
Seminal vesicles	X	
Skeletal muscle (quadriceps)	X	
Skin (inguinal)	X	

APPEARS THIS WAY
ON ORIGINAL

Spinal cord (cervical)	X	
Spleen	X	
Stomach	X	
Teeth		
Testes	X	
Thymus	X	
Thyroid	X	
Tongue	X	
Tonsils		
Trachea	X	
Ureter		
Urinary bladder	X	
Uterus	X	
Vagina	X	
Zymbal glands		

*tissue retained but not evaluated

GENETIC TOXICOLOGY

The genotoxicity of zoledronic acid (CGP 42446) was tested in a battery of four assays, i.e., the Ames bacterial reverse mutation assay (three different tests), an in vitro CHO cell clastogenicity assay, an in vitro V79 Chinese Hamster cell mutagenicity assay, and an in vivo rat micronucleus assay.

Study Nr.	Study Title	Strain/Cell Type/Species	Doses	Treatment/ Recovery	Result
906159	CGP 42446B: Salmonella/mammalian-microsome mutagenicity test	S.Typhimurium (TA98, TA100, TA1535, TA1537)	Original experiment: 313-5000 ug/plate. Confirmatory experiment: 78-1250 ug/plate	-	Negative
926297	CGP 42446, monohydrate: Salmonella and Escherichia/liver microsome test	S.Typhimurium (TA98, TA100, TA1535, TA1537), E.coli (WP2 uvrA)	Original experiment: 78-1250 ug/plate. Confirmatory experiment: 78-1250 ug/plate	-	Negative
968001	162641*: Salmonella and Escherichia/mammalian-microsome mutagenicity test	S.Typhimurium (TA98, TA100, TA102, TA1535, TA1537), E.coli (WP2 uvrA)	Original experiment: 313-5000 ug/plate. Confirmatory experiment: 313-5000 ug/plate	-	Negative
926248	CGP 42446B: Cytogenetic test on Chinese Hamster cells in Vitro	CHO cell line CCL 61	Original study, Exp. 1 (-S9): 9.77-39.06 ug/ml Original study, Exp. 2 (+S9): 313-1250 ug/ml Confirmatory study, Exp.1 (-S9): 9.77-39.06 ug/ml Confirmatory study, Exp.2 (+S9): 313-1250 ug/ml Confirmatory study, Exp.3 (-S9): 1.22-4.88 ug/ml Confirmatory study, Exp.4 (+S9): 39-156 ug/ml	18h/0h 3h/15h 18h/0h 3h/15h 42h/0h 3h/39h	Positive Positive Negative Negative Negative Negative
926249	Gene Mutation test with Chinese Hamster Cells V79 (OECD Conform) in Vitro	Chinese Hamster V79 cells	Original study, Exp. 1 (-S9): 1-8 ug/ml Original study, Exp. 1 (+S9): 1.88-15 ug/ml Confirmatory study, Exp. 1 (-S9): 1-8 ug/ml Confirmatory study, Exp. 1 +S9): 0.5-4 ug/ml	21h 5h 21h 5h	Negative Negative Negative Negative
926247	Micronucleus Test, Rat (OECD Conform) in Vivo	Rat/Tif:Ralf, SPF, i.p.	2.6, 5.2, 10.4 mg/kg	48h	Negative

*PBS 162641 is an intermediate of CGP 42446

The three Ames tests and the cytogenicity test in Chinese Hamster Ovary cells in vitro were reviewed for this NDA by Dr. Fred Alavi, Pharmacology Reviewer (HFD-510). The in vitro mutagenicity test in Chinese Hamster V79 cells and the in vivo rat micronucleus assay were previously reviewed by Dr. Daniel T. Coleman, Pharmacology Reviewer in the Division (HFD-510). The reviews of the latter two studies are appended to the Genetic Toxicology section of this NDA review (p.61).

APPEARS THIS WAY
ON ORIGINAL

Study Title: [redacted] 446 B: Salmonella/Mammalian-microsome mutagenicity test
Study No: 906159 (NDA Volume #55)
Study Type: Ames test for reverse mutation
Conducting Laboratory: Toxicology II, CIBA-GEIGY limited, Basel, Switzerland
Date of Study Initiation/completion: May 7, 1990
GLP Compliance: Yes
QA- Report: Yes (X) No ( )
Drug Lot Number: 800189 (102.6% purity)
Study Endpoint: mutation (base-pair substitution and frameshift)

METHODOLOGY:
The preliminary toxicity test was carried out with Salmonella strain TA 100 without activation with the concentrations ranging from 20 to 5000 µg/ 0.1 ml/plate. This test also included the positive control, 4-nitroquinoline-N-oxide. In the mutagenicity tests the same protocol was used as above but in conjunction with and without microsomal activation and concentrations ranging from 313 to 5000 µg/0.1 ml/plate. In the confirmatory tests concentrations of the test substance ranged from 78 to 1250 µg/0.1 ml/plate.

Strains/Species/Cell line: *Salmonella typhimurium* (TA 98, TA100, TA1535, TA1537)
Dose Selection Criteria: Limit dose of 5000 µg/plate or toxicity
Basis of dose selection: Concentration of 5 mg/plate is generally considered to be limit dose for this assay. A dose greater than 5 mg/plate test substance may prevent bacterial growth or become crystallized. Sponsor used concentration in the range of 2 to 5000 µg/0.1 ml/plate.
Range finding studies: From the preliminary study, the doses selected for the initial study were 313, 625, 1250, 2500 and 5000 µg/plate. In the definitive study, concentrations of 78, 156, 313, 625 and 1250 µg/0.1 ml per plate were used.

Test Agent Stability: [redacted] 42 446 B was stable.
Metabolic Activation System: S9 liver fraction of rats treated with Aroclor 1254. One ml of S9 mixture was made up of 0.3 ml of S9 fraction and 0.7 ml of co-factor solution.
Exposure Conditions: Approximately 0.1 ml of the test [redacted]42446B solution, plus 0.1 ml of bacteria culture and 0.5 ml of S9 mix or 0.1 M sodium-potassium phosphate buffer (pH 7.4 in metabolically activated tests) were transferred to 20 ml of minimum agar and incubated for 48 hrs at 37 ± 1.5 °C.
Incubation and sampling times: 2 days
Doses used in definitive study: 78, 156, 313, 625 and 1250 µg/0.1 ml per plate.

CONTROLS:
Vehicle: bidistilled water
Positive Controls:
4–nitroquinoline-N-oxide,
2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide,
2-Aminoanthracene, sodium azide,
9-Aminoacridine hydrochloride monohydrate

STUDY DESIGN AND ANALYSIS:
No. slides/plates/replicates/animals analyzed: 3 plates/ dose
Counting method: manual
Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)
Genetic toxicity endpoints/results: revertant mutation
Statistical methods: No specific statistical methods were used.
Criteria for Positive Results:
The test substance is considered positive in this test system if one or both of the following conditions are met:

- at least a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration level for one or more of the following strains: TA 98, TA 1535 and TA 1537,
- a reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by at least a factor of 1.5 for strain TA 100.
- a generally concentration-related effect.

RESULTS:
Study Validity: Test validity was examined by use of several positive controls and a negative control (vehicle). The tester stain characteristics were checked for genotype (requirement of amino acid, ability of DNA repair and ampicillin resistance). A test is considered acceptable if the mean colony counts of the control values of all strains are within the acceptable ranges and if the results of the positive controls meet the criteria for a positive response.
Study Outcome:

- ~~42446B inhibited bacterial growth at doses equal to or higher than 1250 µg/plate in the direct method. In the metabolic activation method, bacterial growth inhibition occurred at lower concentrations.
- The highest concentration of ~~ 42446B used in the definitive assay was 1250 ug/plate (with or without S-9 mix).
- The revertant mutation test was found to be negative with all doses of ~~ 42446B in all the plates with or without S9 mix. Control colony counts were within appropriate range. Response to positive control indicated a valid assay system.

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 906159 Test substance: ⬭ 42446 B
Date of evaluation: March 23, 1990

STRAIN	TA 98	TA 100	TA 1535	TA 1537
Control	21	183	11	10
313 µg/0.1 ml	15	187	7	6
625 µg/0.1 ml	17	181	14	8
1250 µg/0.1 ml	6	178	6	7
2500 µg/0.1 ml	0	2	1	6
5000 µg/0.1 ml	0	0	0	2
Positive controls				
daunorubicin-HCl				
10 µg/0.1 ml	651			
4-nitroquinoline-N-oxide				
0.25 µg/0.1 ml		927		
sodium azide				
5.0 µg/0.1 ml			910	
9(5)aminoacridine-hydrochloride				
100 µg/0.1 ml				789

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITH MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 906159 Test substance: ~ 42446 B

Date of evaluation: March 23, 1990

STRAIN	TA 98	TA 100	TA 1535	TA 1537
Control	36	162	16	12
313 µg/0.1 ml	29	183	11	11
625 µg/0.1 ml	15	130	11	14
1250 µg/0.1 ml	7	43	7	12
2500 µg/0.1 ml	0	6	2	6
5000 µg/0.1 ml	0	0	0	2
Positive control of the microsomal activation				
cyclophosphamide				
250 µg/0.1 ml			565	
2-aminoanthracene				
5 µg/0.1 ml	1531	1067		145

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 906159 Test substance: ~ 42446 B

Date of evaluation: March 26, 1990

STRAIN	TA 98	TA 100	TA 1535	TA 1537
Control	20	166	9	8
78 µg/0.1 ml	20	174	10	8
156 µg/0.1 ml	15	157	11	7
313 µg/0.1 ml	22	175	13	7
625 µg/0.1 ml	18	208	11	7
1250 µg/0.1 ml	13	175	5	9
Positive controls				
daunorubicin-HCl				
10 µg/0.1 ml	764			
4-nitroquinoline-N-oxide				
0.25 µg/0.1 ml		1255		
sodium azide				
5.0 µg/0.1 ml			880	
9(5)aminoacridine-hydrochloride				
100 µg/0.1 ml				990

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITH MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN

No. of experiment: 906159 Test substance: 42446 B

Date of evaluation: March 26, 1990

STRAIN	TA 98	TA 100	TA 1535	TA 1537
Control	34	161	18	12
78 µg/0.1 ml	33	177	9	7
156 µg/0.1 ml	30	151	16	12
313 µg/0.1 ml	33	165	13	13
625 µg/0.1 ml	27	156	11	14
1250 µg/0.1 ml	24	120	7	8
Positive control of the microsomal activation				
cyclophosphamide				
250 µg/0.1 ml			372	
2-aminoanthracene				
5 µg/0.1 ml	1397	1157		187

TOXICITY TEST ON STRAIN TA 100 WITHOUT MICROSOMAL ACTIVATION
COLONIES PER PLATE AND ARITHMETIC MEAN (m)
No. of experiment: 906159 Test substance: 42446 B
Date of evaluation: March 19, 1990

Control	198		313 ug/0.1 ml	218	
	200			201	
	m	199		m	210
20 ug/0.1 ml	231		625 ug/0.1 ml	208	
	221			204	
	m	226		m	206
39 ug/0.1 ml	220		1250 ug/0.1 ml	124	
	243			174	
	m	232		m	149
78 ug/0.1 ml	245		2500 ug/0.1 ml	0	
	259			85	
	m	252		m	43
156 ug/0.1 ml	244		5000 ug/0.1 ml	0	
	220			0	
	m	232		m	0

Positive control (4-nitroquinoline-N-oxide)

0.25 ug/0.1 ml	1422	
	1315	
	m	1369

SUMMARY:
From the results of the cytotoxicity test, the highest concentration suitable for the mutagenicity test was selected to be 5000µg/0.1 ml/plate. An inhibiting effect of the test substance on the growth of the bacteria was observed in the experiments without microsomal activation in all strains at concentrations equal to or higher than 1250 µg/plate. In the experiments carried out with microsomal activation, this inhibitory effect was increased.
In the experiments performed without and with microsomal activation, treatment with — 42446B did not lead to an increase in the incidence of histidine-prototrophic mutants in comparison with the negative control in Salmonella test strains TA98, TA100, TA1535 and TA1537. Note that this assay did not include the TA specific strain TA102 or E.coli WP2uvrA as specified by the ICH standard battery.

Study Title: CGP 42446 (monohydrate): Salmonella and Escherichia liver microsome test
Study No: 926297 (NDA Volume #55)
Study Type: Ames test for reverse mutation
Conducting Laboratory: Toxicology, CIBA-GEIGY Limited, Basle, Switzerland
Date of Study Initiation/completion: Nov 4, 1992, May 12, 1993
GLP Compliance: Yes
QA- Report: Yes (X) No ( )
Drug Lot Number: 800392 (100.1% purity)
Study Endpoint: mutation (base-pair substitution and frameshift)

METHODOLOGY:
Strains/Species/Cell line:

Strain	Genotype	Type of mutation
Salmonella typhimurium		
TA98	HisD3052, rfa, uvrB, pKM101	Frame shift
TA100	HisG46, rfa, uvrB, pKM101	Base-pair substitution
TA1535	HisG46, rfa, uvrB	Base-pair substitution
TA1537	HisC3076, rfa, uvrB	Frame shift
Escherichia coli	trp, uvrA	Base-pair substitution
WP2 uvrA		

Dose Selection Criteria: Limit dose of 5000 µg/plate or toxicity
Basis of dose selection: A concentration of 5 mg/plate is generally considered to be limit dose for this assay. Sponsor used the maximum dose of 5000 µg/plate followed by 5 lower concentrations differing by a factor of three (5000, 1666.6, 555.5, 185.18, 61.72 and 20.57 µg/plate)
Range finding studies: Toxicity studies were carried out up to a maximal concentration of 5000 µg/plate. Concentrations greater than 1250 µg/plate were found to be toxic.
Test Agent Stability: CGP 42446 was stable in vehicle (bidistilled water).
Metabolic Activation System: S9 liver fraction from male RAI rats ([redacted]) was used as metabolic activator. To collect S9, rats were treated with Aroclor (500 mg/kg, i.p.) for 5 days prior to sacrifice. Rats were killed and liver was removed and the S9 fraction was stored at -85° C. The activation mixture was made by mixing the S9 fraction with buffer solution containing NADP, $MgCl_2$, KCl, Na phosphate buffer, glucose-6-phosphate.

CONTROLS:
Vehicle: Bidistilled water for test substance (pH 7.2) and DMSO for positive control vehicle
Negative Controls: bidistilled water
Positive Controls: 2-nitrofluorene (2NF), 4-nitroquinoline-N-oxide (4NQO), 2-Aminoanthracene (2AAT), sodium azide (NaN3), 9-Aminoacridine hydrochloride monohydrate (9AA), cyclophosphamide (CPA).

A) Experiment without metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	sodium azide	bidist. water	5.0 µg/ plate
TA 1535	sodium azide	bidist. water	5.0 µg/ plate
WP2 uvrA	4-nitroquinoline-N-oxide	DMSO	2.0 µg/ plate
TA 98	2-nitrofluorene	DMSO	20.0 µg/ plate
TA 1537	9(5)-aminoacridine	DMSO	150.0 µg/ plate

B) Experiment with metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/ plate
TA 1535	cyclophosphamide·H_2O	bidist. water	400.0 µg/ plate
WP2 uvrA	2-aminoanthracene	DMSO	50.0 µg/ plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/ plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/ plate

STUDY DESIGN AND ANALYSIS:

No. slides/plates/replicates/animals analyzed: 3 plate/ dose, with or without S9 mix

Counting method: Colonies were counted electronically with an

Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)

Genetic toxicity endpoints/results: revertant mutation

Statistical methods: No specific statistical methods were used.

Criteria for Positive Results: The test substance is considered to be mutagenic in this test system if one or both of the following conditions are met:

- At least a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration for one or more of the following strains: S. typhimurium TA 98, TA 1535, TA 1537 and E. coli WP2 uvrA.
- A reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by at least a factor of 1.5 for strain S. typhimurium TA 100.
- Generally, a concentration-related effect should be demonstrable.

RESULTS:

Study Validity: Test validity was examined by use of several positive controls and a negative control (vehicle). The characteristics of the strains were checked monthly. Histidine-auxotrophy of the Salmonella strains was demonstrated by the requirement for L-histidine. The presence of the rfa character was assayed by the sensitivity for crystal-violet. The deletion of the uvrB gene was demonstrated by the sensitivity for UV-light. The Salmonella strains containing the R-factor (TA 98 and TA 100) were additionally

checked for ampicillin resistance. The tryptophan-auxotrophy of E.coli WP2 uvrA was demonstrated by the requirement for tryptophan. The absence of the uvrA gene was demonstrated by the sensitivity of the strain for UV-light. Furthermore, all strains were checked for their characteristic reversion properties with known mutagens (positive controls).

Study Outcome:

- Toxicity test: Six concentrations of CGP 42 446 ranging from 20.57-5000 µg/plate were tested with strains S. typhimurium TA 100 and E.coli WP2 uvrA to determine the highest concentration to be used in the mutagenicity assay. The experiments were performed with and without metabolic activation. Normal background growth was observed with both strains. The numbers of revertant colonies were not reduced. From the results obtained, the highest concentration suitable for the mutagenicity test was selected to be 1250 µg/plate without and with metabolic activation.
- Original Mutagenicity test: In the experiments performed without and with metabolic activation, treatment of strains TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA with CGP 42 446 did not lead to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants in comparison with the negative control.
- Confirmatory Mutagenicity test: In the experiments performed without and with metabolic activation, again after treatment of strains TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA with CGP 42 446 no increase in the incidence of either histidine- or tryptophan-prototrophic mutants was observed in comparison with the negative control (Tables 7, 8 and 19-28). In the mutagenicity test, normal background growth was observed with all strains at all concentrations.
- Due to a toxic effect of the test chemical the numbers of revertant colonies were sometimes reduced at higher concentrations.
- The various mutagens, promutagens, sterility checks, sensitivity and resistance tests, etc., employed to ensure that the test system was acceptable, all produced results within sponsors established limits. There were no known circumstances or occurrences in this study that were considered to have affected the quality or integrity of data.

SUMMARY:
CGP 42446 was tested for mutagenic effects at five concentrations in the range of 78.13-1250 µg/plate without and with metabolic activation.
In the original experiment performed without and with metabolic activation, none of the tested concentrations of CGP 42446 led to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants when compared to the negative control.
In the confirmatory experiment performed without and with metabolic activation, again, the tested concentrations of CGP 42446 did not lead to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants by comparison with the negative control.
Based on the results of these experiments and CGP 42446 and its metabolites did not induce gene mutations in the strains of S. typhimurium and E.coli used.

Study Title: CGP 42446B — 162641): Salmonella and Escherichia /mammalian-microsome mutagenicity test

Study No: 968001 (NDA Volume #56)
Study Type: Ames test for reverse mutation
Conducting Laboratory: Toxicology, NOVARTIS Crop (CIBA-GEIGY Limited), Basle, Switzerland
Date of Study Initiation/completion: Sep 26, 1996/Feb 11, 1997
GLP Compliance: Yes
QA- Report: Yes (X) No ( )
Drug Lot Number: POP 3/95800392 (94.7% purity)
Study Endpoint: mutation (base-pair substitution and frameshift)

METHODOLOGY:
Strains/Species/Cell line:

Strain	Genotype	Type of mutation
Salmonella typhimurium		
TA98	HisD3052, rfa, uvrB, pKM101	Frame shift
TA100	HisG46, rfa, uvrB, pKM101	Base-pair substitution
TA 102		Base-pair substitution
TA1535	HisG46, rfa, uvrB	Base-pair substitution
TA1537	HisC3076, rfa, uvrB	Frame shift
Escherichia coli WP2 uvrA	trp, uvrA	Base-pair substitution

Dose Selection Criteria: Limit dose of 5000 µg/plate or toxicity
Basis of dose selection: Concentration of 5 mg/plate is generally considered to be limit dose for this assay. A dose greater than 5 mg/plate test substance generally is toxic or become crystallized. Sponsor used maximum dose of 5000 µg/plate followed by 5 lower concentrations with factor of three (5000, 1666.6, 555.5, 185.18, 61.72 and 20.57 µg/plate)
Range finding studies: Toxicity studies were carried out up to maximal concentration of 5000 µg/plate. Since even the maximal was not toxic, maximal plus and 5 lower concentrations (factor of two) were used in the mutagenicity test (5000, 2500, 12500, 625 and 312.5 µg/plate).
Test Agent Stability: CGP 42446B was stable in vehicle (bidistilled water).
Metabolic Activation System: S9 liver fraction of male RAI rats (Tif: RAIF[SPF]) was used as metabolic activator. To collect S9 fraction, rats were treated with Aroclor (500 mg/kg, i.p.) for 5 days prior to sacrifice. Rats were killed, and liver was removed and the S9 fraction was stored at -85° C. The activation mixture was made by mixing the S9 fraction with buffer solution containing NADP, MgCl2, KCl, Na phosphate buffer, glucose-6-phosphate.

CONTROLS:
Vehicle: Bidistilled water for test substance (pH 7.2) and DMSO for positive control vehicle
Negative Controls: bidistilled water

Positive Controls:

Experiment with metabolic activation

Strain	Mutagen	Solvent	Concentration
TA 100	2-Aminoanthracene	DMSO	1.5 µg/plate
TA 1535	Cyclophosphamide	Bidistilled water	200.0 µg/plate
WP2 uvrA	2-Aminoanthracene	DMSO	20.0 µg/plate
TA 102	2-Aminoanthracene	DMSO	5.0 µg/plate*
TA 98	2-Aminoanthracene	DMSO	1.5 µg/plate
TA 1537	2-Aminoanthracene	DMSO	1.5 µg/plate

* 4.0 µg/plate in the preincubation assay

Experiment without metabolic activation

Strain	Mutagen	Solvent	Concentration
TA 100	Sodium azide	Bidistilled water	2.0 µg/plate
TA 1535	Sodium azide	Bidistilled water	2.0 µg/plate
WP2 uvrA	4-Nitroquinoline (4-NQO)	DMSO	2.0 µg/plate
TA 102	Mitomycin-C	Bidistilled water	0.5 µg/plate
TA 98	2-Nitrofluorene	DMSO	5.0 µg/plate
TA 1537	9-Aminoacridine	DMSO	80.0 µg/plate

STUDY DESIGN AND ANALYSIS:
No. slides/plates/replicates/animals analyzed: 3 plate/ dose, with or without S9 mix
Counting method: Colonies were counted electronically with an
Cytotoxic endpoints: prevention of normal growth of bacteria (antibacterial toxic effect)
Genetic toxicity endpoints/results: revertant mutation
Statistical methods: No specific statistical methods were used.
Criteria for Positive Results: The test substance is considered to be mutagenic in this test system if one or both of the following conditions are met:

- At least a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration for one or more of the following strains: S. typhimurium TA 98, TA 1535, TA 1537 and E. coli WP2 uvrA.

- A reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by at least a factor of 1.5 for strain S. typhimurium TA 100 and TA102.
- Generally, a concentration-related effect.

RESULTS:

Study Validity: Test validity was examined by use of several positive controls and a negative control (vehicle). The characteristics of the strains were checked monthly. Histidine-auxotrophy of the Salmonella strains was demonstrated by the requirement for L-histidine. The presence of the rfa character was assayed by the sensitivity for crystal-violet. The deletion of the uvrB gene was demonstrated by the sensitivity for UV-light. The Salmonella strains containing the R-factor (TA 98 and TA 100) were additionally checked for ampicillin resistance. The tryptophan-auxotrophy of E.coli WP2 uvrA was demonstrated by the requirement for tryptophan. The absence of the uvrA gene was demonstrated by the sensitivity of the strain for UV-light. Furthermore, all strains were checked for their characteristic reversion properties with known mutagens (positive controls).

Study Outcome:

- Six concentrations of —— 162641 ranging from 20.6 to 5000.0 µg/plate were tested with strains of Salmonella and E. coli with and without metabolic activation. No toxicity on the bacterial strains used was observed so highest dose and 5 lower doses were used in the mutagenicity tests (5000, 2500, 1250, 625 and 312.5 µg/plate).
- In the original experiments performed with and without metabolic activation, —— 162641 did not lead to an increase in the incidence of histidine- or tryptophan-prototrophic mutants in comparison with the negative control.
- In the confirmatory experiments performed with and without metabolic activation, treatment with —— 162641, did not increase in the incidence of histidine- or tryptophan-prototrophic mutants in comparison with the negative control.
- In the mutagenicity test normal background growth was observed with all strains at all concentrations. The number of revertant colonies was not reduced. The test substance exerted no toxic effect on the growth of the bacteria.
- The concentrations of test substance in vehicle and culture were measured and found to be in agreement with intended concentrations in the original (91.7%) and confirmatory (99.4%) tests.

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2 pages

SUMMARY:
CGP 42446 was tested for mutagenic effects at five concentrations in the range of 312.5 to 5000 µg/plate without and with metabolic activation.
In the original experiment performed without and with metabolic activation, none of the tested concentrations of CGP 42 446 led to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants when compared to the negative control.
In the confirmatory experiment performed without and with metabolic activation, again, the tested concentrations of CGP 42 446 did not lead to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants by comparison with the negative control.
Based on the results of these experiments, CGP 42446B and its metabolites did not induce gene mutations in the strains of S. typhimurium and E.coli used.

Study Title: CGP 42446B: Cytogenetic test on Chinese Hamster cells in vitro.
Study No: 926248 (NDA Volume #55)
Study Type: in vitro clastogenicity test
Conducting Laboratory: [redacted], CIBA-GEIGY limited, Basle, Switzerland
Date of Study Initiation/completion: March 02, 1993
GLP Compliance: Yes
QA- Report: Yes (X) No ()
Drug Lot Number: 801290 (101% purity)
Study Endpoint: Examination of incidence of chromosomal abnormality including structural aberration and polyploidy

METHODOLOGY: Sponsor had carried out two main assays (18 hr incubation) with and without metabolic activation and four (18 and 42 hr incubation) confirmatory studies with and without metabolic activation (S9 mixture).
Strains/Species/Cell line: CHO cell line CCL 61 derived from Chinese Hamster ovary cells.
Dose Selection Criteria: Dose selection was based on cell growth inhibition test (IC_{50}) and the type, duration of test and solubility in the solvents.
Basis of dose selection: Cytotoxicity (greater than 50%).
Selection of concentrations for analysis: The highest concentration used or the concentration which suppresses mitotic activity by approximately 50 - 80% compared to the control group was selected as the highest for the analysis of chromosome aberrations together with the two lower concentrations.
For the determination of the mitotic index (M.I.) the preparations from the various cultures were examined first, uncoded. The percentages of mitotic suppression in comparison with the controls were evaluated by counting at least 2000 cells from one slide each of the treatment groups and the negative control group.
The determination of the mitotic coefficient was performed in the two experiments of the original study and in the third and fourth experiment of the confirmatory study with metabolic activation.
In the original study, CGP 42446B above 78 µg/ml in tests without metabolic activation and above 1250 µg/ml in tests with metabolic activation significantly inhibited cell growth. From these results, five "suitable" concentrations were determined for the first and second experiment of the confirmatory study. Data on M.I. for the latter two experiments were not given in the report.
Analysis of the original and confirmatory study was done on the three concentrations that had been selected for the first two experiments of the original study.
In the confirmatory study, in the third and fourth experiment, CGP 42446B above 4.88 µg/ml in the test without metabolic activation and above 156.25 µg/ml in the test with metabolic activation significantly inhibited cell growth. Analysis was done on three concentrations.
Test Agent Stability: Certified to be stable for the duration of the test (analyzed at the end of studies).
Metabolic Activation System:
Rat-liver post mitochondrial supernatant (S9 fraction) was prepared in advance from male RAI rats (Tif: RAIf[SPF]), reared at the Animal Farm of CIBA-GEIGY. Sisseln, Switzerland. The animals (150-250 g) were treated with Aroclor 1254 ([redacted] 500 mg/kg, i.p.) 5 days prior to sacrifice. The livers were homogenized with 3 volumes of 150 mM KC1. The homogenate was centrifuged at 9000x g for 15 minutes and the resulting supernatant (S9 fraction) was stored at approximately -80°C for no longer than one year (Lit. 6). S9 fraction was thawed immediately before use, mixed with NADP and isocitric acid and added to culture medium.

CONTROLS:
Vehicle: Ham's F12 culture medium
Negative Controls: Ham's F12 culture medium
Positive Controls: Without S9: Mitomycin C (0.2 µg/ml) and
with S9: cyclophosphamide (20 µg/ml)

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STUDY DESIGN AND ANALYSIS:

No. slides/plates/replicates/animals analyzed: quadruplicates

Counting method: About 200 cells per dose showing metaphase were examined. At least 50 metaphases were scored in the positive controls. Using the vernier scale on the microscope stage, the coordinates of all metaphases with specific aberrations were recorded.

Cytotoxic endpoints: Suppression of mitotic activity

Genetic toxicity endpoints/results: The slides were examined for the following aberrations:

- Specific aberrations; breaks, exchanges, deletions an fragments,
- Unspecific aberrations: gaps and chromosomes decay,
- Numerical alterations (metaphases with >21 centromeres) were registered but reported only in case of deviations

Statistical methods: The evaluated numbers of specific aberrations were subjected to statistical analysis. In the preliminary tests the data were assessed for flask effects (dependence of cells within each culture) using a chi-square test. The nonsignificant result of this test means there is no substantial evidence to conclude a flask effect (although a flask effect still might exist). Accordingly a chi-square test for trend was performed modeling all cells in a given experiment as independent (Lit. 8). That is, the individual cell is taken as the experimental unit. Consequently, the power of the test is substantially increased, resulting in a rather conservative judgement of the observed effects.

Criteria for Positive Results:

Under the standard conditions of our laboratories, the test substance is generally considered to be active in the Chinese Hamster cells if the following conditions are met:

- The percentage of metaphases containing specific aberrations in a treatment group is higher than 6.0 and the difference from the respective value of the negative control is statistically significant.
- A concentration-related response should be demonstrable.

Criteria for a negative response:

Under the standard conditions of our laboratories, the test substance is generally considered to be inactive in the Chinese Hamster cells if the following conditions are met:

- The percentage of metaphases containing specific aberrations in all treatment groups is less than or equal to 6.0 and the difference from the respective value of the negative control is not statistically significant.
- Exceptions: At the limits of the criteria for a positive or for a negative response or if the criteria for a positive response are only partially fulfilled or if effects are obtained at extremely high concentrations or in the toxic range of the test substance only, the Study Director will decide by experience about the interpretation of the results.

Assay acceptance criteria:

- The results of the experiments should not be influenced by a technical error, contamination or a recognized artifact.
- The quality of the slides should allow, at least to a large extent, the chromosomes to be easily identifiable.
- In the negative controls the percentage of metaphases showing specific chromosomal aberrations should be less than 6.0.
- The results of the positive control experiments should meet the criteria for a positive response.
- The highest concentration to which cells were exposed in the mutagenicity test should exert sufficient toxicity (suppression of mitotic activity by 50% or more), represent the limit of solubility of the test material, or be at least 5 mg/ml (or 10 mMol/l).

Flow cytometry: The DNA distribution of cell cultures was determined by flow cytometry. Cultures treated with the test substance or with the vehicle alone were fixed and stained with DAPI (4',6-diamino-2-phenylindole, Serva). Fluorescence of DAPI stained DNA was measured with a Partec PAS-II flow cytophotometer. A substantial shift in the DNA distribution pattern of cell cultures in comparison with the pattern of the vehicle control would indicate a disturbance of the cell cycle induced by the test substance.