subsequently caused inflammation of the trachea or lungs, and -via the auditory tube- of the middle ear." This hypothesis appears reasonable. However, the possibility that the inflammation is due to systemic effects of the drug can not be excluded.

The four spontaneous deaths are considered "compound related mortality" because they only occurred in the high dose group. A distinction must be made here between "drug mediated effects" and "treatment mediated effects". Although the inflammatory effects noted above are clearly treatment related, they do not appear to be systemic effects of the drug. Instead they are the result of giving this drug by gavage and, in part, the result of accidentally spilling it outside the stomach. It remains possible that all of the deaths and even the weight loss could have been avoided if the drug had faithfully been delivered only to the stomach.

CONCLUSIONS:

- The 4 spontaneous deaths in the HD (10 mg/kg) group and significant trend toward increased incidence of spasms, secretions from eyes, ears, nose and mouth, and poor general condition indicate that significant toxic effects are occurring at the 10 mg/kg HD group. The cause of these effects is not certain.

- Signs of inflammation in the stomach, in the HD group, indicate the capacity of this drug to irritate tissues. Evidence of drug induced damage to the esophagus, middle ear and lungs appear to be caused by accidental direct contact of these tissues with spilled drug.

- The decrease in plasma calcium in the 10 mg/kg group, although slight, may be physiologically important as the mechanism responsible for some of the toxicities seen in the HD group. This hypocalcemia is a known effect of bisphosphonates and could be responsible for spasms noted in the clinical signs.

- No important toxic effects were noted in the rats in the 1.0 and 0.1 mg/kg groups. This justifies consideration of 1.0 mg/kg group as NOAEL for 6 month oral exposure in rats.

- 0.1 mg/kg could be considered the NOEL for 6 month oral exposure in rats.

- Lack of PK data makes cross species comparisons and estimates of exposure difficult.

Daniel T. Coleman, Ph.D.

SPONSOR:	Ciba Geigy Corporation, Pharmaceutical Division, 556 Morris Ave., Summit, NJ 07901
DRUG:	CGP 42-446 (Zoledronate)
STUDY SUBMITTED:	May 2, 1995
STUDY RECEIVED:	May 3, 1995
CONTACT:	Ms. Lynn Mellor, (908) 277-7932
CATEGORY:	Bisphosphonate
INDICATIONS: (proposed)	Cancer patients with bone metastases Osteolytic lesions in patients with Multiple Myeloma Paget's Disease Postmenopausal Osteoporosis (no clinical studies yet)
Related INDs:	

Reviewer Recommendation Code: **Open**

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Daniel T. Coleman, Ph.D.

cc: IND Arch
HFD510
HFD510/Coleman,D./Hedin/Steigerwalt

PHARMACOLOGY AND TOXICOLOGY REVIEW
3-Month Oral Toxicity Study in Mice

PURPOSE:

To determine appropriate daily doses for subsequent the chronic/carcinogenicity study, and to obtain urine samples for metabolism studies.

EXPERIMENTAL DESIGN:

Testing Facility: [redacted]

Study Initiated: April 13, 1994
Study Report Written: Feb. 10, 1995

GLP statement, Q/A: **Not included.**

Dose & Formulation: 0, 0.3, 3, 10 and 30 mg/kg daily for 5 weeks. Then changed to 0, 0.3, 3, 10 and 20 mg/kg daily for 8 weeks.
Oral, by gavage.
Dosing volume was 10 ml/kg for all.

Diet: Ground rodent diet # 5001.
Fasted for 3.5 h. before dosing.
Fasted for 5 h after midpoint of dosing.
Food available ad lib. at night.
Tap Water was given ad libitum. (note: mice do not drink when fasted)

Term & # of animals: 3 months, 15/sex/group

Species, sex (strain): CD-1 Mice, M&F (Crl:CD1(ICR)Br)

Animal Supplier: [redacted]

Initial age of mice: approximately 6 weeks

Initial weight: M: 24.1 - 32.2 g, F: 18.0 - 24.1 g.

Housing: Individual cages

Dose Selection:

The only previous experience with this drug in mice was in an acute SC study #93-6085 in the initial application (Serial #000). Dr. Jordan's review of this study states "Albino mice were injected SC with 10-50 mg/kg. 1/1 died at 50 and 0/10 died at 10 mg/kg with no clinical signs."

Doses for this 3-month study were based on previous 1-month (Serial #000, #90-6079) and 6-month (Serial #31, #90-6191) oral toxicity studies in **rats**. In the 1-month rat study using 0, 6, 20 & 60 mg/kg doses; 8 HD rats died, the rest of the HD rats had to be killed in poor general condition, and 10% of the 10 mg/kg rats died. In the 6-month rat study using 0, 0.1, 1 & 10 mg/kg doses, there was minimal systemic toxicity but 6/50 of the 10 mg/kg rats died (apparently from aspirated drug). As a result slightly

higher doses were selected for this 3-month oral mouse study than the 6-month rat study.

Pharmacokinetic measurements were not made. 24-hour urine was collected using metabolism cages on week 2, 7 and 13. Urine was frozen for subsequent measurement of drug concentration.

Clinical Status when reviewed:
Studies begun:

1. Double-blind randomized dose ranging trial of IV Zoledronate vs. Aredia in cancer patients with osteolytic bone metastases, 0.4, 2, 4 & 8 mg infusion q. 4 weeks for 9 mo. 240 pat., Serial 18, 7/96-.
2. Open-Label, Fixed Ascending, Dose Ranging, Safety Trial of Intravenous Injection Bolus of Zoledronate in Non-Small Cell Lung and Prostate Cancer Patients with Bone Metastases.

1-16 mg, single dose, "at least" 40 patients. Serial 64.

3. Open-Label Extension Trial of Intravenous Injection Bolus of Zoledronate in Non-Small Cell Lung and Prostate Cancer Patients with Bone Metastases. Unspecified # of patients, 1-16 mg dose/q. 4 weeks.
4. Transdermal patch on Paget's patients

Studies reported:

1. Final Report, Protocol 01, Dose Ranging in Paget's Patients, Serial 22. 11/94.
2. Report, Protocol 02, A double blind placebo controlled trial using intravenous '42446 in patients with Paget's. Doses: 50, 100, 200, 400 ug IV infusion. Serial 45. 3/96
3. Phase I Irritation studies with patches (''

RESULTS:

CLINICAL SIGNS: (no grading scale used)

Clinical signs reflected dose dependent, treatment related effects:

1) Respiratory complications.
e.g. Rales, Labored breathing, Gasping.
2) Reduced food consumption and related dehydration.
e.g. Dehydration, Feces few, distended abdomen, perineal staining.
3) Overt clinical signs due to the animals poor physical condition prior to mortality due to 1 and 2. above.
e.g. Hunched posture, hypothermia, hypoactivity.
4) A number of additional clinical signs were sporadic and not believed to be treatment related due to lack of dose or time dependence, low incidence or occurrence in the control group.

The grades of these signs were not reported but the incidence is tabulated in the following table:

Incidence of Clinical Signs (n=15/group)										
Sex:	Male					Female				
Dose(mg/kg):	0	0.3	3	10	30→20	0	0.3	3	10	30→20
Dehydration:	0	1	0	11	12	0	1	2	9	9
Distended abdomen:	0	1	2	3	3	0	0	1	1	2
Feces few:	0	0	1	8	13	0	0	0	9	11
Gasping:	0	0	5	11	7	0	0	2	4	8
Hunched Posture:	0	0	0	1	6	0	0	0	2	8
Hypoactive:	0	0	0	2	5	0	0	0	3	3
Hypothermia:	0	1	0	3	5	0	0	0	3	2
Labored Breathing:	0	0	5	11	12	0	0	1	7	10
Perineal staining:	1	3	2	7	6	0	0	2	5	2
Rales:	0	1	5	11	4	0	0	1	5	4

MORTALITY:

Sex:	Male					Female				
Dose (mg/kg):	0	0.3	3	10	30→20	0	0.3	3	10	30→20
n:	15	15	15	15	15	15	15	15	15	15
Found Dead:	0	1	2	4	9	0	0	1	5	11
Killed moribund:	0	0	0	4	5	0	0	0	2	3
Total Dead:	0	1	2	8	14	0	0	1	7	14
Percent survival	100	93	87	47	7	100	100	93	53	7

HD Group: All but one male and one female died or were killed moribund. These deaths occurred "predominately" during the first five weeks of treatment. The remainder of the group died during the next 8 weeks of exposure to 20 mg/kg of drug.

10 mg/kg: Half (15/30) the animals died or were killed.

3 mg/kg: 2 males and 1 female died.

0.3 mg/kg: 1 male died.

Control: No deaths occurred.

BODY WEIGHT:

Body weight and growth rates were similar and normal in the control and 0.3 mg/kg groups. There was a decrease in the weight gain at ≥ 3 mg/kg and a corresponding decrease in body weight in males at ≥ 3 mg/kg, but a significant effect on female body weight was only seen at ≥ 10 mg/kg.

Body Weight (grams)										
Sex:	Male					Female				
Dose (mg/kg):	0	0.3	3	10	30→20	0	0.3	3	10	30→20
n:	15	14	13	7	1	15	15	14	8	1
Baseline weight:	28	29	28	27	28	21	21	22	21	21
Week 13 weight:	34	36	32*	26**	23**	27	26	25	21**	19**
Percent change:	21	21	15*	-5*	-19*	24	23	18*	9**	-3.6**

*=p<0.05, **=p<0.01, n=1-15, I don't know how they did statistics with an n=1 in the 30→20 groups.

FOOD CONSUMPTION:

There was a significant, dose dependent decrease in food consumption which reflected the changes in weight gain and body weight seen above. At 30→20 mg/kg there was a 50% reduction in food consumption throughout the study. At 10 mg/kg food consumption was reduced at least 30% throughout the study. At 3 mg/kg food consumption was slightly reduced in the females after 3 weeks, but was not significantly reduced in males.

EYE AND EAR EXAMINATION:

There were no significant or drug related findings of effects on hearing or vision.

HEMATOLOGY:

Due to low survival rates in the highest dose groups and in the male 10 mg/kg group, these groups were excluded from statistical analysis of hematology and serum chemistry data.

No biologically meaningful toxic effects were found at any dose examined. There was a slight statistically significant ($p<0.05$ vs. C) 50% increases in neutrophils in the 10 mg/kg female group..

COAGULATION:

No measurements of these parameters were made.

BLOOD CHEMISTRY:

Due to low survival rates in the highest dose groups and in the male 10 mg/kg group, these groups were excluded from statistical analysis of hematology and serum chemistry data.

No statistically significant, treatment related, changes were noted in any of the evaluated groups. Individual animals demonstrated elevated ALT (30 mg/kg male) or AP (3 mg/kg male) levels. These results were not corroborated by any histologic evidence of liver damage.

URINALYSIS:

No data reported. Urine collected will be used for drug metabolism studies and reported later.

ORGAN WEIGHTS:

Due to low survival rates in the highest dose groups and in the male 10 mg/kg group, these groups were excluded from statistical analysis of organ weight data. The organ weights of individual animals in these excluded groups were not unusual.

Slight decreases in absolute brain, heart, kidney, liver and spleen weights, and increases in adrenal (in males only) weights were seen at $\geq$ 3 mg/kg. The decreases in organ weights clearly correspond with decreases in the body weights. Relative to body weight, there were significant ($p < 0.01$), but slight (5-10%), decreases in the heart, kidney and liver of females only, exposed to $\geq$3 mg/kg.

GROSS PATHOLOGY:

No treatment related findings. GI distention (seen in the dead mice) was periodically noted throughout the study.

HISTOPATHOLOGY (No Grading scale used.):

The most common effects were in bone and respiratory system and are described below. Results are tabulated below for rates of abnormalities detected in bone and respiratory system as well as other

abnormalities in thymus, liver, spleen, adrenals and reproductive organs. Individual cases of numerous other minor abnormalities were reported but did not appear to be related to exposure to the drug.

BONE MORPHOLOGY: All drug exposed animals displayed treatment-related non-proliferative hyperostosis of the metaphysis within long bones. There was increased length density and number of trabeculae below the epiphyseal plate. Primary spongiosa at this site was not remodeled into secondary and tertiary spongiosa but persisted into the ends of the medullary cavity.

RESPIRATORY TRACT: There was treatment-related, dose-related local irritation of the respiratory tract including microscopic evidence of laryngeal, tracheal and/or bronchial inflammation. Although seen at all doses, these effects were much more frequent the 10 and 30 mg/kg animals and associated with breathing abnormalities noted above under clinical signs. Inflammation of the esophagus and non-glandular stomach was rare (1 HD male, 1 HD female).

Incidence of abnormal Histopathology findings: (n=15/group)

	Sex:	Male					Female				
	Dose (mg/kg):	0	0.3	3	10	30→20	0	0.3	3	10	30→20
Bone:	Hyperostosis	0	15	15	14	9	0	15	15	15	12
Resp. System:	Congestion	0	0	2	1	1	0	1	0	2	4
	Hemorrhage	1	0	0	0	1	0	1	0	3	3
	Bronchial inflammation	0	0	0	3	1	0	0	0	0	2
	Lung Inflammation	1	1	1	6	6	2	1	0	4	6
Liver:	Inflammation	0	0	0	0	4	0	0	0	0	0
	Necrosis	0	1	0	2	1	4	0	0	2	1
Adrenals:	Necrosis	0	0	0	0	0	0	0	0	0	1
	Pyknosis	0	0	1	3	4	0	0	0	0	0
Spleen:	Necrosis	0	1	0	0	2	0	0	0	0	3
	Lymphoid depletion	0	1	2	1	5	0	0	1	6	7
Thymus:	Atrophy	0	0	1	3	5	0	0	1	2	8
	Necrosis	0	1	1	0	1	0	0	0	0	3
Corp. lutea:	Reduction						0	0	0	7	5
Testes:	Atrophy	0	0	0	2	3					

Grades of effects were not reported.

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SUMMARY TABLE

(of all statistically significant findings in this study. Many of these effects are minimal or slight. Physiologically important findings are underlined. See main text for details, n=30 (M+F) per group)

Effect/dose	0	0.3	3	10	30→20
MORTALITY:	0	1	3	15	28
CLINICAL SIGNS:	0	0	2 dehydration 1 hypothermia	20 dehydration 18 breathing problems 6 hypoactive 6 hypothermia	21 dehydration 18 breathing problems 10 hypoactive 7 hypothermia
BODY WEIGHT:	0	0	12 % reduced growth	5% weight loss	20% weight loss
FOOD CONS.:	0	0	~10% reduced in females after 3 weeks	~30% reduced in M and F throughout	~50% reduced in M and F throughout
BIOCHEMISTRY:	0	0	0	n too small	n too small
EYE & EAR EXAM:	0	0	0	n too small	n too small
HEMATOLOGY:	0	0	0	n too small	n too small
BONE MORPHOLOGY:	0	All animals showed Non-proliferative hyperostosis of the metaphysis within long bones. There was increased length density and number of trabeculae below the epiphyseal plate. Primary spongiosa was not remodeled.			
BLOOD CHEMISTRY:	0	0	0	n too small	n too small
URINALYSIS:	Not conducted				
ORGAN WEIGHTS:	0	0	Females decreased (4-11%) relative to body weight: Brain, Heart, Kidney, Liver Males increased relative to body weight: Adrenals (+38-50%)		

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SUMMARY and DISCUSSION:

Dose selection:

The doses selected for this study were appropriate. The doses ranged from lethal (at 30 mg/kg) to slight effects (at 0.3 mg/kg). Significant toxic endpoints were reached in this study. Significant mortality, reduced growth or even weight loss, decreased food consumption and incidence of clinical signs were noted at doses ≥ 3 mg / kg. At the next lower dose (0.3 mg/kg) there was no indication of toxicity, although characteristic bisphosphonate effects on the bones were noted.

The only previous experience in mice was in an acute SC study #93-6085 in the initial application (Serial #000). Dr. Jordan's review of this study states "Albino mice were injected SC with 10-50 mg/kg. 1/1 died at 50 and 0/10 died at 10 mg/kg with no clinical signs."

Doses for this 3-month study were based on previous 1-month (Serial #000, #90-6079) and 6-month (Serial #31, #90-6191) oral toxicity studies in rats. In the 1-month rat study using 0, 6, 20 & 60 mg/kg doses; 8 HD rats died, the rest of the HD rats had to be killed in poor general condition, and 10% of the 10 mg/kg rats died. In the 6-month rat study using 0, 0.1, 1 & 10 mg/kg doses, there was minimal systemic toxicity but 4/50 of the 10 mg/kg rats died (apparently from aspirated drug). As a result slightly higher doses were selected for this 3-month oral mouse study.

Clinical trials are using 0.1 to 16 mg / dose, I.V. given either once or q. 4 weeks. This is equivalent to 0.07 to 12 mg / square meter. The maximum dose used in this rat study is 10 mg / kg, oral, given daily. This is equivalent to 90 mg / square meter (or 7.5 X the human dose). However, the bioavailability of the oral dose is unknown for this drug. PK data have not been submitted for oral exposure to this drug in any species. A similar drug, alendronate, is absorbed less than one percent when given orally, absorption may be further inhibited in the presence of food and absorption is slightly species dependent. Each animal exposure is therefore probably smaller (based on surface area and bioavailability) than the clinical dose. However, the animals receive a dose of drug every day in this study while the humans are exposed only once, or once per month, in the clinical trials. Note: No clinical trials have been proposed using oral dosing.

This 3-month oral mouse study was used by the sponsor to attempt to justify a maximum oral dose of 1 mg/kg for the mouse carcinogenicity trial. Dr. Alex Jordan approved the chosen doses of 1.0, 0.3 and 0.1 mg/kg for the mouse carcinogenicity trial.

Toxic Effects:

Significant mortality and many of the clinical signs (rales, gasping, labored breathing) appear to be secondary to inflammation caused by drug product spilled in the trachea and subsequently aspirated. This was evidenced by microscopic findings of ulcerative and fibrinous inflammation of the larynx, trachea, bronchi and occasionally the lungs. This reflects the capacity of this drug to irritate tissues and cause direct damage to these tissues. In contrast, Inflammation of the esophagus and non-glandular stomach was rare (1 HD male, 1 HD female). The sponsor suggests that "it seems likely that the stratified squamous epithelial lining is more protective than the thin respiratory mucosal lining. Inflammatory effects of Zoledronate in the digestive system have been observed previously, even when the drug was administered I.V. (1-year Dog I.V. study) .

The hypothesis that the respiratory effect (and the secondary clinical signs, deaths and weight loss) is secondary to inflammation caused by drug product spilled in the trachea, and subsequently aspirated, does not exclude other mechanisms which could cause the respiratory effects. Although respiratory irritation in response to Zoledronate has only been observed in oral gavage studies, there remains a slight possibility that this pulmonary effect is, at least in part, due to a systemic effect of the drug on the lungs.

Decreased food consumption is responsible for the dehydration and decreased body weights of the ≥3 mg/kg animals. The cause of the decreased food consumption is unknown. Perhaps it is secondary to the lung problems, or slight irritation of the upper digestive system, or because the animals just don't feel well as evidenced by the clinical signs. Individual animal data, over time, for body weight and occurrence of respiratory problems might help to resolve this question but the data were not provided.

Slight decreases in absolute brain, heart, kidney, liver and spleen weights, and increases in

adrenal (in males only) weights were seen at ≥ 3 mg/kg. The absolute increase in adrenal weight was attributed to pyknosis secondary to aging and stress of treatment and upper airway obstruction. The decreases in organ weights clearly correspond with decreases in the body weights. Relative to body weight, there were significant ($p < 0.01$), but slight (5-10%), decreases in the heart, kidney and liver of females only, exposed to ≥3 mg/kg. The sponsor states "There were no corroboration with microscopic findings in kidneys or adrenal glands" in the summary (page 20 and 118). The pathology report (on p 118) states that there was evidence of necrosis in the spleen and lymph nodes, atrophy of the thymus, as well as describing the pyknosis of the adrenals. These effects are attributed to stress of treatment, upper airway inflammation and weight loss.

A statistically significant ($p<0.05$ vs. C) 50% increase in neutrophils in the 10 mg/kg female group is believed to be secondary to the lung damage and weight loss.

A distinction must be made here between drug mediated effects and treatment mediated effects. Although the effects noted above are clearly treatment related, they do not appear to be systemic effects of the drug. Instead they are the result of giving this drug by gavage and, in part, the result of accidentally spilling it outside the stomach. It remains possible that all of the deaths and even the weight loss could have been avoided if the drug had faithfully been delivered only to the stomach.

The effects on bone clearly are direct systemic effects of the drug. This spectrum of effects on bone is an expected result of the pharmacological action of this drug to inhibit bone resorption.

Under the treatment conditions utilized in this study there is clearly dose limiting toxicity at ≥ 3 mg/kg. The sponsor concluded that the dose for the 2 year carcinogenicity study should be less than the 3 mg/kg dose. Dr. Alex Jordan approved the chosen doses of 1.0, 0.3 and 0.1 mg/kg for the mouse carcinogenicity trial.

CONCLUSIONS:

- Significant treatment related mortality and toxic effects were noted at doses ≥ 3 mg/kg in this study. 50% mortality was achieved at 10 mg/kg and almost all HD animals died.
- The dose limiting toxicity appears to be due to inflammation at the site of administration of the drug (in this case drug spilled from the gavage tube into the trachea) and not a systemic effect of the absorbed dose.
- No significant adverse effects were noted at the 0.3 mg/kg dose. This justifies consideration of the 0.3 mg/kg dose as the NOAEL.
- Lack of PK data make cross species comparisons and estimates of exposure difficult.

Daniel T. Coleman, Ph.D.

SPONSOR:	Ciba Geigy Corporation, Pharmaceutical Division, 556 Morris Ave., Summit, NJ 07901
DRUG:	CGP 42-446 (Zoledronate)
STUDIES SUBMITTED:	July 17, 1996
STUDIES RECEIVED:	July 22, 1996
CONTACT:	Ms. Lynn Mellor, (908) 277-7932
CATEGORY:	Bisphosphonate
INDICATIONS: (proposed)	Cancer patients with bone metastases Benign/malignant tumors w osteolytic lesions Paget's Disease Postmenopausal Osteoporosis (no clinical studies yet)
Related INDs:	

Reviewer Recommendation Code: **Open**

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Daniel T. Coleman, Ph.D.

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PHARMACOLOGY AND TOXICOLOGY REVIEW
3-Month Intravenous Toxicity Study in Dogs.

PURPOSE:

To assess the subchronic toxicity of Zoledronate in dogs when administered intravenously for 3 months, and after 1 month of recovery.

EXPERIMENTAL DESIGN:

Testing Facility: Ciba-Geigy Ltd., Basle, Switzerland.

Test #: 926261

Study Initiated: Mar. 31, 93
Study Written: Jan. 25, 95

Dose & Formulation: At the start of the study the test animals were divided as follows:
Control (5% mannitol), 0.01, 0.03 and 0.1 →.2 mg/kg daily IV.
3 dogs/sex/group (C,L,M,H): 3 months treatment.
3 dogs/sex/group (H): 3 mo. treatment, 1 mo. recovery.
after increasing the dose in the HD group:
4/sex/high dose and
2/sex/recovery group

Species, sex (strain): Dog, M&F (8-9 mo. old pedigree Beagles)

Supplier: Ciba Geigy

Batch of drug: 16/015/1

GLP statement: **Included.**

Dogs were allocated to four main groups: Control (5% mannitol), 0.01, 0.03 and 0.1 to 0.2 mg/kg, and a high dose recovery group, which were treated by daily IV injection. Injection sites not tabulated. Locations were noted when adverse reactions were noted at the sites. There seem to have been at least six injection sites: the four limbs and the ears. When the HD dose group dosage was increased from 0.1 to 0.2 mg/kg at week 6, one animal was moved from the recovery group to the treated group leaving 4 dogs in the high dose group and 2 dogs in the high dose recovery group.

Pharmacokinetic measurements were not made. Blood samples were taken for Pharmacokinetics. Results will be reported later.

RESULTS:

OBSERVED EFFECTS: (Grade Scale: 1-5)

Moderate swellings (average maximum grade/animal/week = 3) at the injection sites were seen in

all animals in the high dose group, most of the mid dose group (70%), the majority of the low dose group (50-60%). Many of the control animals also developed swelling, but they rarely reached grade 3 (10-30%, near the end of the study). In the HD group, the irritation and swelling became quite severe in individual animals by the end of the study. In the necropsy report the Grades for irritation at the injection sites in the 8 HD animals were 5,4,3,3,4,2,3,4. Hemorrhage at the injection site resulted in the premature sacrifice of 3 HD dogs (2 M, 1 F).

No other toxicologically relevant changes were demonstrated.

MORTALITY:

No treatment-related deaths occurred. Two male and one female HD dogs were killed before the end of the study for humane reasons (due to severe local irritation at the injection site).

BODY WEIGHT:

No treatment-related effect except weight loss in the one female HD dog sacrificed early.

FOOD CONSUMPTION:

No treatment-related effect.

VITAL SIGNS:

No treatment-related effects.

EYE AND EAR EXAMINATION:

No treatment-related effects.

HEMATOLOGY:

No statistically significant or biologically meaningful toxic effects were found. Slight increase in segmented neutrophils (~20% increase) noted in the HD dogs.

COAGULATION:

No statistically significant or biologically meaningful toxic effects were found. Slight effects noted in the HD dogs; fibrinogen (30-50% increase) and PTT (30% increase).

BLOOD CHEMISTRY:

No statistically and physiologically significant effects. The following minor effects and expected pharmacological effects were noted:

Liver AP was slightly (20-30%) increased in all drug treated groups. ALAT was increased (25-50%)and ASAT was increased 2-fold to 3-fold in HD animals by the end of the study. CK was elevated 4-fold in pretest HD males and declined throughout the study.

As expected for a drug in this (bisphosphonate) category Bone Alkaline Phosphatase levels were reduced (to 50-10% of control levels) in all treated animals regardless of dose.

Bone Alkaline Phosphatase (U/L at 13 weeks):				
Group	**C**	**0.01**	**0.03**	**0.1**
Males	**13.7**	**6.5**	**3.2**	**2.6**
Females	**16.4**	**3.0**	**2.7**	**5.0**

n = 4

URINALYSIS:

No statistically significant or toxicologically important effects.

ORGAN WEIGHTS: (No statistically significant differences)

Possible drug effects in the HD dogs are noted below:

1. Liver weight was 15-20% lower (as a % of brain wt.) in HD dogs than in control dogs.
2. Thymus weight(as a % of brain wt.) decreased 50% in HD males (as it did in the 3-month rat study) but increased 40% in HD females.(Note that results page 25 states that "thymus weights decreased in males and females". This statement is incorrect.)
3. Testes, prostate and ovaries were 30-40% lighter (as a % of brain wt.) in HD dogs than in controls.
4. Spleen weight (as % of brain weight) was increased 30-50% in HD dogs. This was not seen in the three-month rat study at 0.1 mg/kg but was seen in the 12 month rat study at 0.01 mg/kg.
5. Kidney weight (as % of brain weight) was increased 70-40% in female and male (respectively) HD dogs and was consistent with histological signs of renal toxicity in these dogs (see below).

GROSS PATHOLOGY and Histology: Grading scale: 1 - 5.

Incidence rate (but not average grade) of each effect was listed in a summary table (pages 316-322). Only effects on the injection sites, kidney, bones and thymus were Grade 3 (moderate) or higher. Microscopic findings are fully summarized in a table on pages 28-32.

Injection sites; Inflammation was present in 8/8 HD animals but also in 6/6 control animals. The grade of this response was dose dependent with control animals exhibiting grade 1 responses and HD animals exhibiting grade 4-5 responses with hemorrhage and necrosis.

Kidney; 7/8 HD dogs had various chronic tubular lesions (grade 1-3), 5/8 had suppurative pyelitis (grade 1-3) and 6/8 had urothelial hyperplasia (no grade). These effects were not seen at lower doses.

Bone morphology was characterized by lengthening of primary spongiosa in femur/tibia and sternum. This effect was seen in almost all animals in all drug treated groups (M,F;L,M,H). Increased metaphyseal osteoid and hypercellular marrow were dose dependent and reported in almost every HD animal. The grading for these symptoms were not tabulated, but these symptoms are part of the expected pharmacological effects of this drug.

Thymus; atrophy,(Grade 4) noted in 2/4 HD males and 3 of 4 HD females and 4/6 MD animals.

Liver; no effects were noted which might correlate with the slightly smaller size and altered biochemical parameters.

Testes; Tubular atrophy (grade 2) and oligospermia (no grade) was noted in 3/4 HD males.

Prostate; Atrophy (Grade 1) was noted in 4/4 HD males and 1/3 MD males. No microscopic findings were noted which might explain the slightly smaller size noted above.

Ovaries; no abnormalities noted. No microscopic findings were noted which explain the slightly smaller size noted above.

Spleen; Histiocytosis (Grade: 1), 7/8 HD animals and capsular thickening (Grade: 1), 4/8 HD animals.

NO OTHER TISSUES PRESENTED ABNORMAL FINDINGS.

SUMMARY TABLE

(Most of these effects are minimal or slight. Physiologically relevant effects of sufficient magnitude and dose dependence to be clearly drug related are underlined. See main text for details)

Effect/dose	0	0.01	0.03	0.1
MORTALITY:	0	0	0	0
BODY WEIGHT:	0	0	0	0
FOOD CONS.:	0	0	0	0
EYE & EAR EXAM:	0	0	0	0
HEMATOLOGY:	0	0	0	20 % increase in segmented neutrophil
COAGULATION:	0	0	30 % increase in Fibrinogen & PTT	30 % increase in Fibrinogen & PTT
BLOOD CHEMISTRY:	0	greatly reduced bone AP	greatly reduced bone AP	greatly reduced bone AP increased ASAT, ALAT and Liver AP
URINALYSIS:	0	0	0	0
ORGAN WEIGHTS:	0		30-40 % decreased thymus weight as % of body & brain weight	• 50% decreased thymus weight • 20% decreased liver weight • 50-70% increased spleen & kidney wt. • 30-40 % lighter gonads & prostate
OPATHOLOGY: njection Site	0	Slight inflammation	moderate inflammation	Severe inflammation with hemorrhage and necrosis
pleen	0	0	0	Histiocytosis & thickening
idney	0	0	0	signs of nephropathy
hymus/testes/ rostate		0	0	signs of atrophy
ONE MORPHOLOGY:	0	longer spongiosa	longer spongiosa	longer spongiosa Hypercellular marrow

APPEARS THIS WAY
ON ORIGINAL

SUMMARY & DISCUSSION:

This study describes the effects of 3-month daily I.V. exposure to 0, 0.01, 0.03 and 0.2 mg/kg Zoledronate in dogs. The most striking toxic reaction to this drug was hemorrhage at the injection site at the highest dose tested (0.2 mg/kg). This effect may determine the MTD for this route of exposure in dogs. The only other clear effects were the expected pharmacological actions of this drug class, namely decreased bone alkaline phosphatase, increased bone osteoid material, and longer primary spongiosa.

Other effects of possible toxicological relevance (but which were not statistically significant) were:

•Increased kidney weight (50-70%) and evidence of some moderate degree of nephropathy in the 7/8 HD dogs. These results corroborate a suggestion of nephrotoxicity in the 12-month rat study in the male HD (0.01 mg/kg) group.

•Atrophy of the thymus, gonads and prostate in most of the HD animals.

Perspective; Previous and planned studies:

Dose selection was based on a previous (Serial 000) 1-month I.V. dog study which was conducted at double the doses used in the present study. In the 1-month study, weak limbs, stiff gait, swelling at the injection site and bone changes were the only significant effects at the 0.2 mg/kg High Dose. These effects did not appear to be dose limiting. Nevertheless, the sponsor started this 3-month study at half the dose range used in the 1-month study and later doubled the high dose level (after six weeks), because of lack of toxic effects. The 3-month study demonstrated that the only major effect was hemorrhage at the injection site.

Toxicity studies are usually designed to determine the maximum tolerated doses and indicate the types of toxicity that develop at high exposure levels. Because of hemorrhaging at the site of injection this study cannot test the systemic exposure level necessary to see target organ toxicity.

CONCLUSIONS:

- Inflammation at the site of injection was the dose limiting toxicity of this drug using this route of exposure (IV).
- At the highest dose tested no other remarkable toxic effects were found.
- Many of the slight, yet statistically significant, toxic effects seen in the 3-month rat study were also noted in this dog experiment but were not statistically significant.

APPEARS THIS WAY
ON ORIGINAL

PHARMACOLOGY AND TOXICOLOGY REVIEW
3-Month Subcutaneous Toxicity Study in Rats.

PURPOSE:

To assess the subchronic toxicity of Zoledronate in rats when administered subcutaneously for 3 months, and after 1 month of recovery.

EXPERIMENTAL DESIGN:

Test #: 926259

Study Initiated: Apr. 23, 93
Study Written: Feb. 3, 95

Dose & Formulation: 0, 0.01, 0.03, 0.1 mg/kg
SC injection in 5% mannitol

Term & # of animals: 3 month, 10/sex/group
Satellite, 5/sex/group (C&H)
Recovery, 5/sex/group (C,M&H)

Species, sex (strain): albino Rats M & F, Tif:RAlf (SPF)

Supplier: ———

Batch of drug: 16/015/1

GLP statement: **Included.**

Rats were allocated to four groups: Control (5% mannitol), 0.01, 0.03 and 0.1 mg/kg which were treated by daily SC injection. Injection sites were not identified and schedule for use of these sites was not described. NOTE: In the 1-year study injections were made at seven distinct sequential sites (dorsal) following a weekly schedule.

Pharmacokinetic measurements were not made.

RESULTS:

OBSERVED EFFECTS: (Grade scale 1-5)

Slight swellings (average grade = 1) at the injection sites were seen in all HD animals.
In the recovery group 4/5 HD males and 3/5 MD male rats had broken incisors during the recovery period.
No other toxicologically relevant changes were demonstrated.

MORTALITY:

"No treatment related deaths were noted" (page 2 and 16).One MD male was killed before the end of the study for humane reasons (due to an eye injury).

BODY WEIGHT:

All male treatment groups were significantly (~20%) lighter than control groups at the end of the study, reflecting a decrease in body weight gain. This effect was not observed in females.

FOOD CONSUMPTION:

Food consumption was lower in all drug treated male groups. This difference increased in magnitude throughout the study.

VITAL SIGNS:

No treatment related effects.

EYE AND EAR EXAMINATION:

No treatment related effects.

HEMATOLOGY:

No biologically meaningful toxic effects were found. Several slight effects are noted here for future reference:

Small (< 10%), statistically significant, dose related reductions in red blood cell count, hematocrit and hemoglobin were seen. These effects were greater in the male rats than in females.

Minimal reticulocytosis and thrombocytosis was observed but is only mentioned because levels were significantly higher ($P<0.05$) than in controls at some higher concentrations and later time points. The magnitude of these differences however, was not of toxicological importance.

COAGULATION:

No physiologically significant effects despite some slight decreases (<10 %) in fibrinogen, PPT and TT which are statistically significant at H & M doses.

BLOOD CHEMISTRY:

No important treatment related toxicological effects. The following minor or pharmacological effects were noted:

Triglycerides were slightly reduced in the HD males and to a lesser extent HD females.

Bilirubin levels were slightly (~20% above control) elevated in HD male rats and to a lesser extent in HD female rats.

As expected for a drug in this (bisphosphonate) category Bone Alkaline Phosphatase levels were significantly reduced (50-60% of control levels) in all treated animals regardless of dose.

Bone Alkaline Phosphatase (U/L at 14 weeks):				
Group	**C**	**0.01**	**0.03**	**0.1**
Males	**33**	**16***	**11***	**11***
Females	**44**	**19***	**21***	**17***

n = 10, * = p<0.05%

Total Alkaline Phosphatase was slightly reduced as a result of the reduction in Bone AP.

Liver AP was slightly (20-30%) increased in MD and HD rats after 9 weeks and in all drug treated groups after 14 weeks.

Aspartate aminotransferase was slightly (20-30%) elevated in all drug treated groups. This effect was greater in males than females, but in no case was large enough to be toxicologically important.

Aspartate aminotransferase (U/L at 14 weeks):				
Group	**C**	**0.001**	**0.003**	**0.01**
Males	**90**	**124***	**122***	**125***
Females	**82**	**95**	**94***	**101***

n=10, * = p<0.05%

URINALYSIS:

No toxicologically important effects.

ORGAN WEIGHTS:

There were no differences in the mean data for any organ except:

1. A significant (p<0.05 vs. Control) 10% decrease in liver weight in all drug treated male rats. This was a significant decrease as percent of brain and body weight in only the HD males.
2. A significant (30-40%) decrease in the absolute thymus weight was concentration dependent in both male and female rats. A significant decrease was also seen when calculated as % of brain weight in HD and MD male and female rats(p. 292 & p. 296). The effect seems greater in males.

These decreases in weight did not reflect any treatment-related histologic abnormalities (see below).

GROSS PATHOLOGY: (Grade Scale 1-5)

3/5 MD and 5/5 HD recovery males had broken upper incisor teeth. No microscopic abnormalities were noted in the teeth.

Slight inflammation (Ave. Grade = 1) was noted at the injection sites. This was reversible by the end of the recovery period.

No morphological changes were noted in thymus, liver, kidney or bones.

TUMORS:

None found.

HISTOPATHOLOGY (By Tissue Type): Grading scale used: 1 - 5.

BONE MORPHOLOGY:

The most notable drug effect on bone morphology was lengthening of primary spongiosa in femur/tibia and sternum. This effect was seen in almost all animals in all drug treated groups (M,F;L,M,H) at all 3 time points. Osteoid Seams, Increased Metaphyseal Diameter and Hypercellular Marrow were also reported in almost every treated animal. The grading for these symptoms were not tabulated.

NO OTHER TISSUES PRESENTED ABNORMAL FINDINGS.

SUMMARY TABLE

(of all statistically significant findings in the 3 month study. Most of these effects are minimal or slight. Physiologically important findings are underlined. See main text for details)

Effect/dose	0	0.01	0.03	0.1
MORTALITY:	0	0	0	0
BODY WEIGHT:	0	males -(10-20)%	males -(10-20)%	males -(10-20)%
FOOD CONS.:	0	males -(10-20)%	males -(10-20)%	males -(10-20)%
EYE & EAR EXAM:	0	0	0	0
HEMATOLOGY:	0	0	Slight Dec.RBC, HB Reticulocytosis(M) Thrombocytosis(M)	Slight Dec.RBC, HB Reticulocytosis(M) Thrombocytosis(M)
COAGULATION:	0	0	Fibrinogen, PPT & TT decreased < 10%	Fibrinogen, PPT & TT decreased < 10%
BLOOD CHEMISTRY:	0	reduced bone AP increased Asp. Aminotransferase	reduced bone AP increased Asp. Aminotransferase	reduced bone AP increased Asp. Aminotransferase
URALYSIS:	0	0	0	0
ORGAN WEIGHTS:	0		**30-40 % decreased thymus weight & % of body & brain wt**	**decreased thymus.** Slightly (<10%) decreased liver wt.
PATHOLOGY: teeth:	0	0	1/5 broken in male recovery group	5/5 broken in male recovery group
BONE MORPHOLOGY:	0	longer spongiosa osteoid seams incr. met. Dia. Hypercellular marrow	longer spongiosa osteoid seams incr. met. Dia. Hypercellular marrow	longer spongiosa osteoid seams incr. met. Dia. Hypercellular marrow

APPEARS THIS WAY
ON ORIGINAL

SUMMARY & DISCUSSION:

This study describes the effects of 3-month daily S.C. exposure to 0, 0.01, 0.03 and 0.1 mg/kg Zoledronate in rats.

The only toxicological effect reported which was statistically significant and of great enough magnitude to be of possible toxicological importance at the doses given was a decrease in weight gain which resulted in A 10-20 % lower body weight in all drug treated male groups, than in controls, with a concomitant lower level of food consumption. This result may have been spurious. This effect was not dose dependent (occurring to a similar extent in all treated males regardless of dose), did not occur in females and did not occur at these doses in other longer or shorter studies.

The only other apparent toxic effect observed in this study was broken teeth seen in 7/10 males in the HD groups.

Some **statistically significant effects** of this drug were noted but were of **minimal magnitude**, including:

1. Minimally decreased (less than 5%) HB, HCT and RBC in mid and high dose groups.
2. Minimal reticulocytosis and thrombocytosis in mid and high dose males.
3. Elevated (max.: 30%) aspartate aminotransferase activity in all drug treated groups.
4. Slight decreases (<10 %) in fibrinogen, PPT and TT in mid and high dose animals.
5. A 30-40 % lower thymus weight in mid and high dose animals than in controls (no morphological correlates were seen).

Other **statistically significant effects** of this drug were of greater magnitude but are of **uncertain toxicological importance** or relate to the desired effects of the drug, including:

1. Decreased (max.: 50%) bone alkaline phosphatase activity in all drug treated groups.
2. Lengthening of the primary spongiosa, osteoid seams, increased metaphyseal diameter and hypercellular marrow.

Perspective; Previous and planned studies:

Dose selection was based on a previous (Serial 000) 1—month study (SC rat) conducted at double the doses used in this study. In the 1 month study, no major toxicological findings were reported and the low dose (0.02 mg/kg) was considered the no adverse effect level by the sponsor. Nevertheless, the sponsor conducted this 3-month study at a 2-fold lower dose range than the 1-month study. The current 3-month study demonstrated that the only potential toxicity seen when —— 42-446 was administered for 3 months was: possibly decreased weight gain (without weight loss) in male rats at all doses and broken teeth in the HD male and mid and high dose recovery groups (n=5/group).

Dose Levels (mg/kg) used in S.C. Rat Studies				
Study	Low	Mid	High	#/group
1-month	0.02	0.06	0.2	10
3-month	0.01	0.03	0.1	10
6/12-mo.	0.001	0.003	0.01	10+20

Toxicity studies are usually designed to determine the maximum tolerated doses and indicate the target organs of toxicity. The 1-, 3- and 6/12-month studies fail to address these issues because the doses tested are too low.

Occasionally, toxicity studies may attempt to determine a NOAEL. The expected human exposure level should then provide an additional margin of safety. This appears to be the sponsor's approach in these two studies.

To compare the doses used in this study to the proposed clinical levels:

- The rat NOAEL suggested by this study is:
  0.01 mg/kg SC daily * 6 = 0.06 mg/m^2.
- The highest dose used in the clinical trial (serial #51) is:
  4 mg /50 kg IV monthly * 36 = 2.88 mg/m^2.
- The human dose is 48 times the NOAEL in rats, but the doses are not comparable due to different schedules and routes of administration.

CONCLUSIONS:

- The non dose-related diminution of weight gain in all male rats receiving drug, and the tooth loss in the high dose recovery males indicates that some toxicological effects are occurring at the HD level used in this study.

- The minimal degree of statistically and physiologically significant adverse reactions to the selected doses of drug indicates that this study was conducted below the optimal range for determination of potential toxic effects and the maximum tolerated dose of this drug.

- A lower magnitude of toxic effects were noted in female rats at all doses tested. This justifies consideration of 0.01 mg/kg the NOAEL for 3-month exposure in female rats as suggested by the sponsor.

Daniel T. Coleman, Ph.D.

APPEARS THIS WAY
ON ORIGINAL

SPONSOR:	Ciba Geigy Corporation, Pharmaceutical Division, 556 Morris Ave., Summit, NJ 07901
DRUG:	CGP 42-446 (Zoledronate)
STUDY SUBMITTED:	May 22, 1996
STUDY RECEIVED:	May 28, 1996
CONTACT:	Ms. Lynn Mellor, (908) 277-7932
CATEGORY:	Bisphosphonate
INDICATIONS: (proposed)	Cancer patients with bone metastases Benign/malignant tumors w osteolytic lesions Paget's Disease Postmenopausal Osteoporosis (no clinical studies yet)
Related INDs:	

REVIEWER RECOMMENDATION CODE: **Open**

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Daniel T. Coleman, Ph.D.

cc: IND Arch
HFD510
HFD510/Coleman,D./Hedin/Steigerwalt

PHARMACOLOGY AND TOXICOLOGY REVIEW
6 / 12 Month Subcutaneous Toxicity Study in Rats

PURPOSE:

To assess the chronic toxicity of Zoledronate in rats when administered subcutaneously for 26 and 52 weeks, and after 4 weeks recovery.

EXPERIMENTAL DESIGN:

Testing Facility: [redacted]

Study Initiated: 6/10/94
Study Report Written: 1/12/96

GLP Statement: Included.

Dose & Formulation: 0, 0.001, 0.003, 0.01 mg/kg daily.
SC injection in 5% mannitol. Injections were made at seven distinct sequential sites (dorsal) following a weekly schedule.

Batches of drug: 16/719/1 and 16/718/1

Diet: Food and water ad lib.

Term & # of animals:

6 month,	10/sex/group
12 month,	20/sex/group
1 month recovery	5/sex/group

Species, sex (strain): Albino Rats, M&F (Tif:RAlf SPF)

Animal Supplier: [redacted]

Pharmacokinetic measurements were not made.

Clinical Status when reviewed: Phase II Studies begun: Double Blind randomized dose ranging trial of IV Zoledronate vs. Aredia in cancer patients with osteolytic bone metastases (Serials 40, 42, 49, 51) 240 patients. 0.4. 2. 4 mg infusion q. 4 weeks for 9 mo. 7/96-. [redacted]

Previous Human Experience:
Phase I Dose Ranging in patients w osteolytic metastases, IV infusion 0.1-8 mg q. month for 3 months 7/95-7/96, Serial 55.
Phase I and II Comparisons with Aredia in [redacted]

Outstanding requests from sponsor: Informal phone request of ref. for historical controls and lists of clinical studies(1/15/97).
Reply received 1/30/97.

RESULTS:

OBSERVED EFFECTS:

"No toxicologically relevant changes were found" (Page 2).
"No obvious treatment-related signs were found" (Page 15). Some individuals in each group (including controls) showed adverse effects (induration, lesions) at various sites of injection. These symptoms were generally slight (maximum weekly grade 1-3).
Summary tables were not provided.

MORTALITY:

"No treatment related deaths were seen in this study" (page 2 and 15). 10 of 280 animals (in the entire study) were killed before the end of the study for humane reasons:
3 Males (C,L,H) were moribund following blood sampling.
1 Female (M) had urolithiasis (common in these rats).
6 Females (1C, 2L, 2M, 1H) killed with mammary tumors.

Note: Mammary tumors were stated to be common in these rats and the distribution indicates lack of drug effect. (See also **Mammary Tumors:** below.)

BODY WEIGHT:

"No toxicologically relevant changes were noted" (pages 2 & 15). Growth rates were similar and normal in all groups.

FOOD CONSUMPTION:

"No toxicologically relevant changes were noted" (pages 2 & 15). Food consumption was similar and normal in all groups.

VITAL SIGNS:

No treatment related effects.

EYE AND EAR EXAMINATION:

"No findings were evident." (pages 2 & 15)

HEMATOLOGY:

No biologically meaningful toxic effects were found. Several slight effects are noted here for future reference;
Small (< 5%), statistically significant, dose related reductions in red blood cell count, hematocrit and hemoglobin were seen at some time points.

Minimal "Reticulocytosis in males at all doses from week 13 on" was observed with significant (P<0.05) 10-20% increases in the number of reticulocytes vs. controls. There was a concomitant increase in males in the percentage of immature reticulocytes from 15% of total in controls to 20% of total in all treated groups (page 322). Conversely, mature reticulocytes in males were lower in all drug exposed groups (46% of total) than in controls (50% of total). These results were very consistent in all male drug treated groups after week 13.

In females, (pages 433-4) there was an even less pronounced shift to a population of less mature reticulocytes. There was a significant (p<0.05) increase in the percentage of immature reticulocytes (23-26% in treated groups vs. 17-21% in controls) together with a significant decrease in the percentage of mature reticulocytes (42-44% in treated groups vs. 47-49% in controls). Overall there was no significant difference in the total reticulocytes but they did tend to be more elevated in the treated groups than in controls.

Reticulocytes (at 53 weeks)				
Effect (units)	**C**	**0.001**	**0.003**	**0.01**
Reticulocytes (#) Males	**0.027**	**0.030***	**0.033***	**0.031***
Mature Retics. (%)	**46.6**	**42.2***	**41.5***	**42.5***
Immature Retics (%)	**21.7**	**27.2***	**27.7***	**26.6***
Reticulocytes(#) Females	**0.022**	**0.025**	**0.025**	**0.026**
Mature Retics. (%)	**47.1**	**42.9***	**43.7***	**43.7***
Immature Retics (%)	**21.4**	**26.6***	**27.4***	**26.4***

n=19-20, * = p<0.05%

There were significant (p<0.05 vs. C) 10-50% increases in segmented neutrophils in all male drug-treated groups in weeks 6 & 13 and M or H groups in weeks 28, 38 and 53 (page 323). No effects were seen in female's segmented neutrophils. The toxicological importance of this finding is unknown.

Segmented Neutrophils (# at 6 weeks)				
Group (n=9-10/group)	**C**	**0.001**	**0.003**	**0.01**
Males	**0.08**	**0.12***	**0.12***	**0.12***
Females	**0.08**	**0.07**	**0.09**	**0.08**

* = p<0.05%

COAGULATION:

No physiologically significant effects despite some slight decreases (<10 %) in fibrinogen, PPT and TT which are statistically significant at some doses and time points.

BLOOD CHEMISTRY:

No treatment related effects except:

As expected for a bisphosphonate, Bone Alkaline Phosphatase levels dropped steadily throughout the study (from 81 to 12 U/L in control males and from 68 to 12 U/L in control females). In males bone AP levels were significantly lower in almost all treated groups vs. Controls (p<0.05), at each dose, at almost every time point. In females the results were qualitatively similar but the differences were not statistically significant. Inhibition of bone AP is consistent with the effect of bisphosphonates to inhibit bone formation.

Bone Alkaline Phosphatase (U/L at 6 weeks):				
Group	**C**	**0.001**	**0.003**	**0.01**
Males	**41**	**28***	**26***	**25***
Females	**30**	**28**	**19**	**18**

n = 19 - 20, * = p<0.05%

Total Alkaline Phosphatase was not affected by treatment (pages 129-30 & 224-5) as reported on page 16. The Total AP levels rise and then fall in all groups with age. This change seems to reflect the simultaneous changes in liver, intestinal and bone AP as the animals age. Like total AP, liver and intestinal AP were not affected by exposure to drug.

AP activity in male control rats:						
Parameter	WK 1	WK 6	WK 13	WK 28	WK 38	WK 53
Bone AP	81	41	22	12	16	8
Liver AP	25	30	38	59	98	80
Intestinal AP	60	67	79	75	58	38
Total AP	162	138	137	138	169	148

n=19-20.

"Cholinesterase activity was slightly elevated in high dose males" (677 U/L in H vs. 483 U/L in C, $p<0.05$) and recovered by the end of the recovery period. PAGE 131. The physiological relevance of this apparent effect is unknown.

Cholinesterase (U/L at 53 weeks):				
Group	**C**	**0.001**	**0.003**	**0.01**
Males	**483**	**556**	**551**	**677***
Females	**1149**	**1282**	**1267**	**1273**

n=19-20, * = p<0.05%

URINALYSIS:

"No treatment related changes were recorded." (PAGE 16).

ORGAN WEIGHTS:

I observed no differences in the mean data for any organ except a significant ($p<0.05$ vs. Control) 16% increase in spleen weight in high dose males (page 595) and low dose females (page 598) at 12 months. Corresponding differences were also seen in the relative (to body and brain) weights.

Spleen Weight (grams at 53 weeks):				
Group	**C**	**0.001**	**0.003**	**0.01**
Males (n=19-20)	**0.886**	**0.936**	**0.944**	**1.03***
Females (n=19-20)	**0.562**	**0.672****	**0.609**	**0.605**

* = p<0.05%, ** = p<0.01%

GROSS PATHOLOGY:

"Induration of bones in individual animals of both sexes at all dose levels." No summary table is given, however there are individual necropsy reports (e.g., page 771, 785) containing the statement "Bone: indurated." The microscopic analysis of the bones of these two individuals (page 771,785) was reported to be normal. The magnitude, frequency or significance of this finding was not reported.

TUMORS:

The following table outlines the distribution of various tumor types in study animals including seven animals that were killed before the end of the study:

ammary Tumors	6 month study				12 month study			
Dose (mg/kg)	0	0.001	0.003	0.01	0	0.001	0.003	0.01
# of animals:	10	11	11	10	20	19	20	20
Adenoma	1				1			
Fibroadenoma		1				5	1	1
Adenocarcinoma				1			2	
Totals:	1	1		1	1	5	3	1

Tumor rates: 2/30 (6.7 %) in control group
11/91 (12.1 %) in treated group
13/121 (10.7 %) overall.

These tumor rates seem higher than the historical control rates (<2%) obtained from the sponsor. The tumor rates are higher than historical control rates in the control animals as well as in the treated animals. In the treated animals the tumor rate is not dose dependent. These results are inconclusive but emphasizes the importance of more thorough carcinogenicity studies.

HISTOPATHOLOGY (By Tissue Type): Grading scale used: 1 - 5.

BONE MORPHOLOGY:

Dose dependent increase in density of spongiosa was seen only in males after the 12 month study: Marrow spaces in the tibia were noted in 14/7/1 of 20/20/20 L/M/H rats. The Mean grade was 1.1.

The most notable drug effect on bone morphology was lengthening of primary spongiosa in femur/tibia. This effect was seen in almost all animals in all drug treated groups (M,F;L,M,H) at all 3 time points. Average grading 3.0 in femur/tibia and 1.0 in sternum.

Lengthening of Primary Spongiosa in Females at 12 Months: Incidence percent (Average magnitude)				
Dose:	C	0.001	0.003	0.01
Femur/Tibia	0	100 (3.0)	100 (3.0)	100 (3.0)
Sternum	0	100 (1.0)	100 (1.0)	100 (1.1)

n=19-20, similar results were obtained for males.

BONE MARROW: (Grading scale used: 1 -5.)

All drug treated groups showed only "slight" (Average Grading 1.0 -1.7) hypercellularity of the marrow. (See tables in appendix)

SPLEEN:

Most groups, including controls, showed only "minimal-slight" (Average grading 1-1.6); increased extramedullary hematopoiesis, hemosiderosis and congestion of the spleen, at all doses in both sexes throughout the study.(See tables in appendix)

KIDNEYS:

Males, after 12 months of exposure, developed "minimal-slight" (Average grading 1-1.6) hyaline casts and focal basophilia at about twice the rate of the control animals. These effects were not evident in the recovery groups (n=5/s/g).

Increased incidence of progressive nephropathy was found in the high dose males but not the recovery group exposed to high dose Zoledronate. The degree of this symptom however was minimal-

slight (average grading 1.6).

Renal Pathology in Males at 12 Months: Number (Average magnitude)				
Dose:	**C**	**0.001**	**0.003**	**0.01**
Hyaline casts	**9 (1.1)**	**12 (1.1)**	**18 (1.1)**	**18 (1.4)**
Basophilia	**8 (1.0)**	**8 (1.0)**	**15 (1.0)**	**11 (1.1)**
Nephropathy	**1 (1.0)**	**2 (1.5)**	**2 (1.0)**	**5 (1.6)**

n=19-20

TESTES:

Slightly increased incidence of tubular atrophy may have occurred in the 12 month study group and may be evident in the recovery group:

Testicular Tubular Atrophy: Incidence (Average magnitude):				
Group (#/group)	**C**	**0.001**	**0.003**	**0.01**
12 Mo. (19-20)	**2 (3.0)**	**3 (1.0)**	**2 (3.5)**	**5 (2.4)**
13 Mo. (5)	**1 (1.0)**	**0**	**2 (2.5)**	**1 (5)**

SUMMARY TABLE

(of all statistically significant findings in either the 6 or 12 month study. Most of these effects are minimal or slight. Physiologically important findings are underlined. See main text for details)

Vdose	**0**	**0.001**	**0.003**	**0.01**
MORTALITY:	0	0	0	0
BODY WEIGHT:	0	0	0	0
FOOD CONS.:	0	0	0	0
BIOCHEMISTRY:	0	reduced bone AP	reduced bone AP	reduced bone AP
EYE & EAR EXAM:	0	0	0	0
HEMATOLOGY:	0	Decrease RBC, HB Reticulocytosis(M)	Decreased RBC, HB Reticulocytosis(M) Inc. fibrinogen(M)	Decreased RBC, HB Reticulocytosis(M) Inc. fibrinogen (M)
COAGULATION:	0	0	0	0
BONE MORPHOLOGY:	0	longer spongiosa	longer spongiosa higher density	longer spongiosa higher density
BONE MARROW	0	slight hypercellularity in all treated groups		
BLOOD CHEMISTRY:	0	0	0	0
URINALYSIS:	0	0	0	0
ORGAN WEIGHTS:	0	Increased spleen weight in females		Increased spleen weight in males
KIDNEY:	0	0	Hyaline casts (M) Basophilia (M) (all slight)	Hyaline casts (M) Basophilia (M) Nephropathy (M) (all slight)
TESTES:	0	0	0	Tubular atrophy not drug related
ORS:(mammary)	2	6	3	2

SUMMARY and DISCUSSION:

No toxicological effects were reported which were statistically significant and of great enough magnitude to be of toxicological importance at the doses given.

Numerous **statistically significant effects** of this drug were noted but were of **minimal magnitude**, including:

1. Minimally decreased (less than 5%) HB, HCT and RBC in mid and high dose groups after 13 weeks.
2. Minimal reticulocytosis (10-20 %) in all drug exposed males after 13 weeks.
3. Slight decreases (<10 %) in fibrinogen, PPT and TT at a few doses and time points.

Other **statistically significant effects** of this drug were of greater magnitude but are of **uncertain toxicological importance** or relate to the desired effects of the drug, including:

1. Elevated (max. 41%) cholinesterase activity in H dose males.
2. Elevated (max.: 50%) segmented neutrophils at several time points in H and M dose males and occasional female groups.
3. Decreased (max.: 50%) bone alkaline phosphatase activity primarily in males.
4. Lengthening of the primary spongiosa in virtually all drug treated animals was moderate (3) in femur/tibia and minimal (1) in sternum.

Several microscopic findings demonstrated **possibly increased incidence** in drug treated animals but were **of "minimal" average grading** including:

1. Increased metaphysis diameter (most noticeable after 53 wks).
2. Marrow spaces (most noticeable after 53 weeks).
3. Hypercellularity of the marrow (most noticeable after 53 wks).
4. Increased hematopoiesis, hemosiderosis and congestion in the spleen (most noticeable after 53 weeks).
5. Increased hyaline casts, basophilic tubuli and progressive nephropathy in the kidneys of male rats (only seen after 53 weeks, and not after the recovery period).

Finally, the testicular diffuse tubular atrophy seen in the high dose males after 53 weeks (5/20) was only marginally more common than in controls (3/20). "In most cases this finding was seen in one testis only and its severity was not increased." In addition this irreversible condition was not observed in the smaller recovery group one month later.

Perspective; Previous and planned studies:

The previous 3 month SC rat toxicology study (serial 50) demonstrated that 0.01 mg/kg was a NOAEL for females but not males (who had some slight effects at this "low" dose). No major toxicological findings were reported in the 3 month study even at the "high" dose 0.1 mg/kg. Nevertheless the sponsor conducted the 6/12 month study at a 10-fold lower dose range than the 3 month study. The current 6/12 month study demonstrated that no additional major effects were seen when 0.01 mg/kg [redacted] 42-446 was administered for a full year. Many of the "slight" effects seen in the three month study were not seen in the 12 month study. Novel effects in this study (not seen in the 3 month study) were increased spleen weights, reticulocytosis, hyaline casts, basophilia nephropathy and tubular atrophy. None of these effects were of great magnitude even at the 0.01 mg/kg level.

Toxicity studies are usually designed to determine the maximum tolerated doses and indicate the types of toxicity that develop at high exposure levels. The 3 and 6/12 month studies described here fail to address these issues because the doses tested are too low.

Occasionally, toxicity studies may attempt to determine a NOAEL when this level is expected to be (at least) an order of magnitude above the highest expected human exposure level. This appears to be the sponsor's approach in these two studies.

To compare the doses used in this study to the proposed clinical levels:

- The rat NOAEL suggested by this study of 0.001 mg/kg SC daily * 6 = 0.006 mg/m^2.
- The highest dose proposed for the clinical trial (serial # 51 is 4 mg /50 kg IV monthly * 36 = 2.88 mg/m^2.
- The human dose is 480 times the NOAEL in rats. The doses are not comparable due to different schedules of administration. (Daily in rats, monthly in humans) However, the monthly human dose is still 10X the total accumulated doses in a rat over a month.

CONCLUSIONS:

- The apparent lack of statistically and physiologically significant adverse reactions to the selected doses of drug invalidates the study in terms of determining potential toxic effects and the maximum tolerated dose of this drug.

- No important toxic effects were noted in female rats at any dose tested. This justifies consideration of 0.01 mg/kg the NOAEL for 12 month exposure in female rats as suggested by the sponsor.

- Indications of minimal and slight adverse effects on kidneys (hyaline casts, basophilic tubuli and nephropathy) at the mid and high doses in males, and possible effects on testes (tubular atrophy) the highest doses tested suggest that the low dose (0.001 mg/kg) is an appropriate NOAEL for 12 month exposure in male rats.

Daniel T. Coleman, Ph.D.

SPONSOR: Novartis Pharmaceuticals Corporation,
556 Morris Ave.,
Summit, NJ 07901

STUDY SUBMITTED: Feb. 24, 1997
STUDY RECEIVED: Feb 28, 1997

DRUG: CGP 42-446 (Zoledronate)

CATEGORY: Bisphosphonate

INDICATIONS:
(proposed)

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Daniel T. Coleman, Ph.D.

cc: IND —
HFD510
HFD510/Coleman,D./Hedin/Steigerwalt

PHARMACOLOGY AND TOXICOLOGY REVIEW
26/52-Week Intravenous Toxicity Study in Dogs.

Amendment to IND

Sponsor: Novartis Pharmaceuticals Corporation

Testing Facilities: Ciba Geigy, Preclinical Safety, Summit NJ

Drug: CGP 42-446 (Zoledronate)

Jacket #s: C22.1 & 22.2

Test #: 94-4045

Study Initiated: Aug. 18, 1994. **Study Written: Nov. 20, 1996.**

Dose & Formulation: 0, 0.005, 0.03, 0.1 mg / kg q2 days for 112 days (16 weeks).
0, 0.005, 0.03, 0.1 mg / kg q 3 days for remainder of study.
IV injection in 5% mannitol

Term & # of animals: 2/sex/group, 26 weeks
4/sex/group, 52 weeks
2/sex/group, 52 weeks + 26 weeks recovery

Species, sex (strain): Dog, M&F (8-13 kg, 15-17 mo. old Beagles)
Supplier:

Batches of drug: 800492, 117123, 16/720/1, 117124, 16/723/1.
All were reanalyzed.

PURPOSE:

To assess the subchronic toxicity of Zoledronate in dogs when administered intravenously for 6 and 12 months, and after 1 month of recovery.

EXPERIMENTAL DESIGN:

At the start of the study the test animals were divided as follows:
2 dogs/sex/group (C,L,M,H): 6 months treatment.
4 dogs/sex/group (C,L,M,H): 12 months treatment.
2 dogs/sex/group (H): 12 mo. treatment, 6 mo. recovery.

Dogs were allocated to four groups: Control (5% mannitol), 0.005, 0.03 and 0.1 mg/kg, which were treated by IV injection once every two days. After 16 weeks,

injection frequency was reduced to one injection every three days. Injection sites were not tabulated. Locations were noted when adverse reactions were noted at the sites. Injections were made into the cephalic and occasionally the saphenous veins. When a mid-dose female was sacrificed for humane reasons on the day before dosing initiation, a replacement was shifted over from the recovery group, leaving the recovery group one short.

Pharmacokinetic measurements and bone analyses are not included in the report and will be issued separately as report addendums.

GLP statement included.

RESULTS and DISCUSSION:

OBSERVED EFFECTS:

Swellings at the injection sites were seen in all drug treated animals. The severity of this swelling was moderate to severe and the severity and incidence were dependent on dose and duration of exposure. The severity was not tabulated and was not reported in the data on individual animals. MD and HD males and all treated females had non-weight bearing appendages. This was generally associated with moderate to severe swelling. No signs of swelling and non-weight bearing were evident after 8 weeks of recovery.

No other toxicologically relevant changes were demonstrated.

MORTALITY:

No treatment-related deaths occurred. One female MD dog was killed just before the start of the study for humane reasons.

BODY WEIGHT:

No statistically significant treatment-related effect on weight or weight gain was noted, however it is evident that the high dose males and females were 10 % lighter than the respective control groups at the 52 week mark. Although the differences in the percent weight gains in each group were not statistically significant, the percent weight gain in the HD male and female groups were 43 and 74 % smaller respectively than the percent weight gains in the control groups. Percent weight gain relative to control = (weight gain of group - weight gain of control) / weight gain of control * 100 (See table 10.4 on page 58 and figures on p 43 and 44.)

FOOD CONSUMPTION:

The sponsor states that "There were no compound-related changes in food consumption at any dose level in the study". The data were not reported.

VITAL SIGNS:

No treatment-related effects. EKGs (described in Appendix 11.2) revealed no treatment related abnormalities.

EYE AND EAR EXAMINATION:

No treatment-related effects reported.

HEMATOLOGY:

No biologically meaningful toxic effects were noted. Statistically significant toxic effects were found almost exclusively in the high dose group and were similar in both sexes (except as noted). RBC, HGB and HCT were all decreased (10-20%) in all HD animals throughout the drug exposure period. WBC, PMN and Monocytes were elevated (10-20%) in the HD groups at most time points. These effects were not evident at the end of the recovery period.

Hematology Parameters, affected by treatment, at 12-months					
Dose (mg/kg):		0	0.005	0.03	0.1
Males:	RBC	7.4	7.1	7.9	6.8*
	Hg B	17	16	18	15*
	HCT	52	50	54	46*
Females:	RBC	7.2	7.1	7.0	6.3*
	Hg B	16	16	16	14*
	HCT	50	50	47	42*

COAGULATION:

No statistically significant or physiologically significant effects.

BLOOD CHEMISTRY:

Several statistically significant effects were noted. Aside from the hypocalcemia,

these changes were all slight indications of hepatotoxicity in the HD group. These results suggest the possibility that the HD may be starting to have some adverse effect on the liver. None of these effects were evident at the end of the recovery period.

AP was not separated into liver and bone components. Total AP was significantly (~100%) increased in the HD male group during the first 6 months before returning to normal range.

ALAT was not significantly elevated but ASAT was increased 2-fold to 3-fold throughout the exposure period in HD animals.

Albumin levels were decreased (10-20%) and globin levels were increased (10-20%) in all HD groups throughout the exposure period.

Moderate decreases in BUN in some groups may be statistically significant but are not likely to be physiologically relevant unless there is more direct evidence of severe liver toxicity.

Plasma Calcium levels decreased significantly (8-10%) in the HD male group after 1 year and continuing in the recovery period. This may result from drug induced inhibition of bone resorption which may persist long after exposure to the drug.

Blood Chemistry Parameters, affected by treatment, at 12-months

		0	0.005	0.03	0.1
Males:	AP	44	32	26	71
	ASAT	50	46	40	61
	ALAT	35	39	37	95**
	Albumin	3.8	3.7	3.9	3.4*
	Globin	2.7	2.6	2.7	3.3
	BUN	19	19	16	12*
	Calcium	10.4	10.2	10.4	9.8**
Females:	AP	50	42	82	93
	ASAT	40	34	42	35
	ALAT	34	35	46	67**
	Albumin	3.8	3.6	3.7	3.45*
	Globin	2.7	2.4	3.1	3.3*
	BUN	14.5	13	13.4	11.0
	Calcium	10.2	9.9	9.8	9.9

* = $p<0.05$, ** = $p<0.01$

URINALYSIS:

No physiologically or toxicologically important effects. Sponsor states that: "Reductions in mean urine specific gravity were noted in the HD animals." These effects were not statistically significant. No factor in the urinalysis reflects any renal toxicity.

Urinalysis Parameters, affected by treatment, at 12-months

Dose (mg/kg):		0	0.005	0.03	0.1
Males:	mean urine	1.034	1.040	1.033	1.028
Females:	specific gravity	1.031	1.033	1.026	1.020

ORGAN WEIGHTS:

Only individual data are reported. no summary or statistics were reported. I will review this when I have summary tables.

Possible drug effects noted in the HD dogs from the 3-month study are noted below:

1. Liver weight was 15-20% lower (as a % of brain wt.) in HD dogs than in control dogs.
2. The data shows that thymus weight(as a % of brain wt.) decreased 50% in HD males (as it did in the 3-month rat study) but increased 40% in HD females. (please note that results page 25 states that "thymus weights .. .decreased in males and females". This statement is incorrect.
3. Testes, prostate and ovaries were 30-40% lighter (as a % of brain wt.) in HD dogs than in controls.
4. Spleen weight (as % of brain weight) was increased 30-50% in HD dogs. This was not seen in the three-month rat study at 0.1 mg/kg but was seen in the 12 month rat study at 0.01 mg/kg.
5. Kidney weight (as % of brain weight) was increased 70-40% in female and male (respectively) HD dogs and was consistent with histological signs of renal toxicity in these dogs (see below). This effect was also consistent with a suggestion of nephrotoxicity in the 12-month rat study in the male HD (0.01 mg/kg) group.

In the 3-month rat study (92-6259) liver and thymus weights decreased to a similar magnitude but were statistically significant due to the larger sample size:

GROSS PATHOLOGY and Histology: Grading scale: not graded.

Incidence rate (but not average grade) of each effect was listed in a summary table (pages 349-383). Average, median or maximum grade of effects were not reported. I will finish reviewing this section when the severity grades are tabulated. Compound related findings occurred in the bone, kidneys, GI tract and at injection sites.
Injection sites; Inflammation was present at the injection site in 1/8, 5/8, 6/8 & 8/8 of C, L, M & HD animals respectively.
Kidney; 2/4 HDM and 4/4 HDF dogs had cellular casts at 12-months. 4/4 HDM but only 1/4 HDF had tubular dilatation. 1/8, 2/8, 5/8 8/8; C, L,M,HD dogs had tubular necrosis.
Bone morphology 4/4 MD and HD males had hypercellular marrows and 2/4 and 3/4 MD and HD females showed some degree of hypercellularity after 12 months. Only 2/4 HD males showed such symptoms after 6-months, and 1/2 HD females after the recovery period. Half of the animals exposed to any level of Zoledronate had nonproliferative primary spongiosa. Primary spongiosa was lengthened in most
Thymus; atrophy was noted in almost all the animals in the study, including control dogs.
Testes: Several treated animals showed testicular atrophy, tubular casts and mineralization. These effects were more prominent in the interim sacrifice.
Liver; no effects were noted which might correlate with the slightly smaller size and altered biochemical parameters noted above in Blood Chemistry.
GI Tract: Watch out for novel effects Inflammatory cell infiltration was noted in Stomach, intestine and colon. Novel, minimal to moderate, multifocal smooth muscle fiber degeneration of the muscularis accompanied by mononuclear cell infiltration in males at doses > 0.03 mg/kg and in one female at 0.1 mg/kg. This was seen at the 6-month time point and not at the 1-year mark.

TUMORS:

None found.

SUMMARY TABLE

(Most of these effects are minimal or slight.
Physiologically relevant effects of sufficient magnitude and dose dependence to be clearly drug related are **underlined**. See main text for details)

Effect/dose	0	0.005	0.03	0.1
MORTALITY:	0	0	0	0
BODY WEIGHT:	0	0	0	10% LIGHTER
FOOD CONS.:	?	?	?	? report states only "no significant difference"
EYE & EAR EXAM:	0	0	0	0
HEMATOLOGY:	0	0	0	**20 % less RBC, HGB, HCT** **20% more WBC, PMN, Monocyte**
COAGULATION:	0	0	0	0
BLOOD CHEMISTRY:	0	0	0	**increased ASAT, ALAT and AP** **Increased globin and decreased albumin, BUN & Ca.**
URINALYSIS:	0	0	0	0
ORGAN WEIGHTS:	0	?	?	? Not averaged or summarized.
HISTOPATH: Injection Site	1/8	**2/8 cases of inflammation**	**6/8 cases of inflammation**	**8/8 cases of swelling**
GI Tract, stomach, intestine, colon	0	0	0	inflammatory cell infiltration degen. stomach musculari
Testes	0	0	0	Tubular atrophy, casts, mineralization
Kidney	0	**2/8 tubular necrosis**	**5/8 tubular necrosis**	**6/8 cellular casts** **5/8 tubular dilatation** **8/8 tubular necrosis**
Thymus	atrophy	signs of atrophy	signs of atrophy	signs of atrophy
BONE MORPHOLOGY:	0	**nonproliferative spongiosa**	**nonproliferative spongiosa** **6/7 hypercellular marrow**	**nonproliferative spongiosa** **7/8 Hypercellular marrow**

SUMMARY:

This study describes the effects of 6/12-month I.V. exposure to 0, 0.005, 0.03 and 0.1 mg/kg Zoledronate. Dogs were injected every other day for 16 weeks. Due to inflammation and swelling at the injection site, injection frequency was reduced to every third day.

The most striking toxic reaction to this drug was inflammation at the injection site. This effect was dose and time dependent, occurring in a few animals to a small extent at the low dose and increasing to 100 % severe reactions at the high dose (0.1 mg/kg). This effect may determine the MTD for this route of exposure in dogs.

Expected pharmacological actions of the drug to inhibit bone resorption resulted in expected changes in bone morphology. This effect may have been strong enough to severely curtail bone turnover. Evidence for this is suggested by the "nonproliferative spongiosa" and the observed inability to maintain calcium homeostasis (hypocalcemia) in the HD males. Expected effects on bone morphology were not fully reported but will be submitted in the future.

Other compound related effects of possible toxicological relevance occurred in the liver, kidneys, blood, testes and GI tract:

•Liver: There were statistically significant, yet physiologically moderate to slight, increases in ASAT, ALAT and liver AP in HD dogs. There were also slightly increased levels of globin and decreased serum albumin and BUN indicating possible adverse effect on liver at HD. These results however are not reflected in major morphological changes in the liver.

•Kidney: There was dose dependent; tubular necrosis, tubular dilatation, cellular casts and evidence of nephropathy. The effects on the kidney however are not reflected in changes in the blood chemistry or urinalysis.

•Decreased RBC, HGB, HCT and hypercellular marrow's combined with increased WBC, PMNs and Monocytes in the HD animals indicating interference with red blood cells and a stimulation of WBCs.

•Testes: Minimal atrophy, mineralization and degeneration was observed.

•GI Tract: Inflammatory cell infiltration into the mucosal layer of the stomach, intestines and colon was observed along with (at six months) moderate multifocal smooth muscle degeneration in the stomach of HD males.

Perspective; Previous and planned studies:

Dose selection was based on a previous (Serial 050) 3-month I.V. dog study conducted at double the doses used in this study. In the 3-month study swelling at the injection site, lengthening of primary spongiosa and hypercellular marrow were the only significant effects at the 0.2 mg/kg High Dose. These effects did appear to be dose limiting. The sponsor started the current 12-month study at a 2-fold lower dose range than the 3-month study, and dosed the dogs every other day instead of every day. After 16 weeks they had to reduce the injection frequency to every third day due to inflammation at the injection sites. The 3-month study demonstrated that the only major

effect was hemorrhage at the injection site. In this longer term study additional toxic reactions were noted in the liver, kidney, GI tract, testes, and bone as described above..

CONCLUSIONS:

- Inflammation at the site of injection was the dose limiting toxicity of this drug using his route of exposure (IV).
- Longer term studies can be carried out at the same dose level as shorter studies by reducing frequency of dosing to every other day or every third day.
- Even at the highest dose tested no severe toxic effects were found. Several moderate reactions were clear however. Many of the slight, not statistically significant, toxic effects seen in the 3-month study were noted in this experiment and achieved statistically significance. Among these are:
    - Moderate to slight, increases in ASAT, ALAT and liver AP in HD dogs.
    - Slightly increased levels of globin and decreased serum albumin and BUN indicating possible adverse effect on liver at HD.
    - Dose dependent; tubular necrosis, tubular dilatation, cellular casts and evidence of nephropathy.
    - Decreased RBC, HGB, HCT and hypercellular marrow's combined with increased WBC, PMNs and Monocytes in the HD animals.
    - Hypocalcemia in HD males.

Additional moderate toxic effects noted for the first time in dogs were:

- Testicular atrophy in HD males, accompanied by casts and mineralization.
- Inflammatory cell infiltration of the mucosal layer of the stomach, intestines and colon and degeneration of the muscularis (at six months) in HD males.

The testicular atrophy and the renal nephropathy (which were both also seen in the 1 year rat study) and the hypocalcemia were the most potentially serious toxicities found.

Daniel T. Coleman, Ph.D.

APPENDIX II

(Statistical Review of carcinogenicity studies)
(Reviewer T. Guo, Ph.D.)

APPEARS THIS WAY
ON ORIGINAL

STATISTICAL REVIEW AND EVALUATION
CARCINOGENICITY

Date	May 23, 2000
NDA No.	21-223
IND No.	
Applicant	Novartis
Name of Drug	Zometa™ (zoledronic acid)
Document Reviewed	• Rat Study: Vol. 1.47 • Mouse Study: Vol. 1.41
Statistical Reviewer	Ted J. Guo, Div II/OEB, HFD-715
Pharmacologist	Gemma Kuijpers, DMEDP, HFD-510

Analysis based on Combined Tumor Types

Upon request from Dr. Kuijpers, this reviewer analyzed the female-rat data by combining uterine endometrial stromal sarcoma and uterine polyps (Table 1). Conclusion: The dose-tumor positive linear trend for the combined tumor was not statistically significant. This determination was based on the decision rules used by CDER Office of Biostatistics (Table 7).

Table 1. Trend Test for Uterine Endometrial Stromal Sarcoma and Uterine Polyps for Female Rats

```
Analysis of Carcinogenic Potential in Female Rat
                    Test of Dose-Response (Tumor) Positive Linear Trend
                              Study No. 951159
                    Run Date & Time:  May 22, 2000 (15:11)

  Note:      Dose Levels Included:  CTRL LOW MED HIGH (0 0.1 0.5 2)
             Missing value in Tumor-Caused Death is treated as tumor not causing death
             Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME          (ORG#)   TUMOR TIME     ROW    2xC CONTINGENCY     EXACT ASYMP ASYMP
PROB
  AND TUMOR NAME           (TMR#)   TYPES STRATA   NO.   -------TABLES-------  PROB  PROB  /CONT
CORR
                                                                               =P(STAT .GE.
OBSERVED)

UTERUS                     (UTERUS  ) IN 0-52     1      0  0  1  0          0.048 0.042 0.056
POLYP+ENDOMETRIALSTROMAL   (ENDOPOLY) IN 0-52     2      5  3  1  5
                                      IN 53-78    1      0  1  0  2
                                      IN 53-78    2     18 23 24 19
                                      IN 79-91    1      0  1  2  1
                                      IN 79-91    2     18 12 11 15
                                      IN 92-103   1      0  1  1  0
                                      IN 92-103   2      7 11  9 12
                                      IN 104-105  1      2  2  1  4
                                      IN 104-105  2     20 15 20 11
                                      FA 78       1      0  0  0  1
                                      FA 78       2     50 44 46 43
                                      FA 104      1      0  1  0  0
                                      FA 104      2     22 17 21 15
Spontaneous tumor pct: 3%     in ctrl. - Total    -      2  6  5  8
```

In addition, this reviewer analyzed the male- and female-mouse data by combining harderian gland adenoma and adenocarcinoma (Table 2 and Table 3 for male and female mice, respectively). Conclusion: The dose-tumor positive linear trend for the combined tumor was not statistically significant in either males or females. This determination was based on the decision rules used by CDER Office of Biostatistics (Table 7).

Table 2. Trend Test for Harderian Gland Adenoma and Adenocarcinoma for Male Mice

Analysis of Carcinogenic Potential in Male Mouse

Test of Dose-Response (Tumor) Positive Linear Trend
Study No. 951021
Run Date & Time: May 22, 2000 (15:26)
Source

Note: Dose Levels Included: CTRL LOW MED HIGH (0 0.1 0.3 1)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME	(ORG#)	TUMOR TIME	ROW	2xC CONTINGENCY	EXACT PROB	ASYMP PROB	ASYMP /CONT
AND TUMOR NAME	(TMR#)	TYPES STRATA	NO.	-------TABLES-------			
OBSERVED)					=P(STAT .GE.		
HARDERIANGLAND	(HARDERIA)	IN 53-78	1	0 3 0 1	0.052	0.045	0.078
ADENOMA+ADENOCARCINOMA[M	(ADENCARC)	IN 53-78	2	16 17 11 9			
		IN 79-91	1	0 2 1 1			
		IN 79-91	2	10 11 11 6			
		IN 92-103	1	1 2 2 2			
		IN 92-103	2	12 12 11 5			
		IN 104-105	1	2 2 1 6			
		IN 104-105	2	22 17 19 31			
Spontaneous tumor pct: 4%	in ctrl. - Total		-	3 9 4 10			

Table 3. Trend Test for Harderian Gland Adenoma and Adenocarcinoma for Female Mice

Analysis of Carcinogenic Potential in Female Mouse
Test of Dose-Response (Tumor) Positive Linear Trend
Study No. 951021
Run Date & Time: May 22, 2000 (15:33)
Source

Note: Dose Levels Included: CTRL LOW MED HIGH (0 0.1 0.3 1)
Missing value in Tumor-Caused Death is treated as tumor not causing death
Tumor Type: IN: Incidental (nonfatal) tumor, FA: Fatal tumor.

ORGAN/TISSUE NAME	(ORG#)	TUMOR TIME	ROW	2xC CONTINGENCY	EXACT PROB	ASYMP PROB	ASYMP /CONT
AND TUMOR NAME	(TMR#)	TYPES STRATA	NO.	-------TABLES-------			
OBSERVED)					=P(STAT .GE.		
HARDERIANGLAND	(HARDERIA)	IN 79-91	1	0 0 2 0	0.128	0.115	0.207
ADENOCARCINOMA[M]	(ADENCARC)	IN 79-91	2	16 14 13 12			
		IN 92-103	1	0 1 1 1			
		IN 92-103	2	9 9 10 11			
		IN 104-105	1	1 1 2 3			
		IN 104-105	2	30 27 28 24			
Spontaneous tumor pct: 1%	in ctrl. - Total		-	1 2 5 4			

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Analysis based on All Tumor Types

This reviewer analyzed all tumors reported. Conclusion: The dose-tumor positive linear trend was not statistically significant for all individual tumors reported. The statistical decision was based on the decision rules used by CDER Office of Biostatistics (Table 7).

Signoff Page

Statistical Reviewer: Ted J. Guo Signature: _____ Date: _____

Concur: Karl K. Lin, Ph.D. Signature: _____ Date: _____

CC:
Archival NDA 21-223
HFD-510/Division file
HFD-510/GKuijpers

HFD-715/Division file
HFD-715/KLin
HFD-715/Tguo

HFD-700/CAnello

Signoff Page

Statistical Reviewer: Ted J. Guo Signature: _____ 5/23/00

Concur: Karl K. Lin, Ph.D. Signature: Date: 5/25/2000

CC:
Archival NDA 21-223
HFD-510/Division file
HFD-510/GKuijpers

HFD-715/Division file
HFD-715/KLin
HFD-715/Tguo

HFD-700/CAnello

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ON ORIGINAL

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Appendix

Analysis of Male Rats

Table 4. Number of Male Rats Died by Treatment by Time

Number of Animals
Species: Rat
Sex: Male

	Treatment Group				Total
	CTRL	LOW	MED	HIGH	
	N	N	N	N	N
Week					
0-52	1	3	4	5	13
53-78	16	18	15	16	65
79-81	17	17	20	15	69
82-103	16	13	9	11	49
104-105	20	18	22	23	84
Total	70	70	70	70	280

Source:

Table 5. Analysis of Mortality for Male Rats by Treatment by Time

Analysis of Mortality
Species: Rat
Sex: Male

	Dose											
	CTRL			LOW			MED			HIGH		
	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died	Num. of Dead	Num. at Risk	Cumu Pct. Died
Week												
0-52	1	70	1.4	3	70	4.3	4	70	5.7	5	70	7.1
53-78	16	69	24.3	18	67	30.0	15	66	27.1	16	65	30.0
79-81	17	53	48.6	17	49	54.3	20	51	55.7	15	49	51.4
82-103	16	36	71.4	13	32	72.9	9	31	68.6	11	34	67.1
104-105	20	70	28.6	18	70	27.1	22	70	31.4	23	70	32.9

Table 6. Analysis of Dose-Mortality Trend for Male Rats

Dose-Mortality Trend Tests

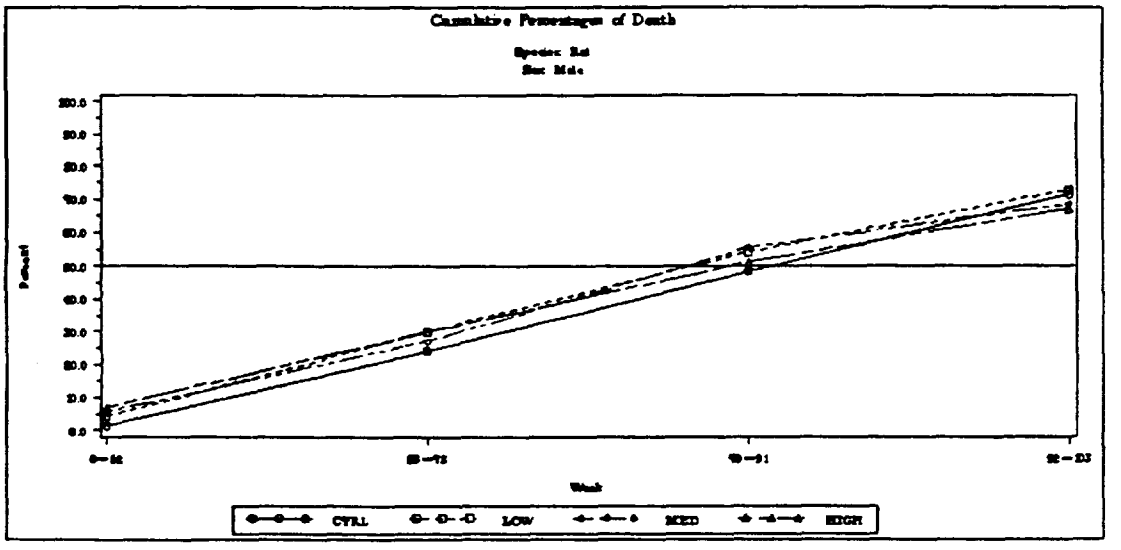
This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Rat
Sex: Male

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	0.09	0.7654
	Depart from Trend	0.23	0.8936
	Homogeneity	0.31	0.8574
Kruskal-Wallis	Dose-Mortality Trend	0.00	0.9929
	Depart from Trend	0.42	0.8110
	Homogeneity	0.42	0.9363

Source:

Figure 1. Cumulative Percentages of Death in Male Rats



APPEARS THIS WAY
ON ORIGINAL