RESULTS:

Study Validity:

- Negative control, vehicle had no effect on chromosomal aberration.
- Positive controls caused a significant increase in chromosomal aberration in the 14 and 42 hr studies.

Study Outcome:

- The clastogenic activity was assessed at concentrations between 9.77 and 1250 µg/ml.
- The highest concentration of 39.06 µg/ml selected for analysis in the first experiment of the original study caused 65.71% suppression of mitotic activity. The next higher concentration (78.1 µg/ml) revealed a suppression of mitotic activity of 90.7%.
- The highest concentration of 1250.0 µg/ml selected for analysis in the second experiment of the original study caused a 66.8% suppression of mitotic activity. The next higher concentration (2500 ug/ml) revealed an inhibition of 73.3% and the next higher concentration (5000 ug/ml) of 76.4%.
  Reviewers note: It can be argued that the highest concentration used should have been 5000 ug/ml since the inhibition of mitotic activity at this concentration was still <80%.
- For the first and second experiment of the confirmatory study the highest concentrations used for analysis were 39.06 ug/ml and 1250 ug/ml, respectively, based on the results on mitotic activity from the original study
- The highest concentration of 4.88 µg/ml selected for analysis in the third experiment of the confirmatory study caused a 45.9% suppression of mitotic activity. The next higher concentration of 9.77 ug/ml revealed a suppression of mitotic activity of 87.7%.
- The highest concentration of 156.25 µg/ml selected for analysis in the third experiment of the confirmatory study caused a 2.4% suppression of mitotic activity. The next higher concentration of 312.5 ug/ml revealed a suppression of mitotic activity of 81.5%.

Original Clastogenicity Study

- In the first experiment without metabolic activation 2.0% of metaphases with specific chromosomal aberrations were detected in the negative control. At the concentrations of 9.77 µg/ml, 19.53 µg/ml and 39.06 µg/ml 2.0%, 3.0% and 4.5% of cells showed specific chromosomal aberrations. At the concentration of 39.06 µg/ml the difference as compared to the control value was statistically significant.
- In the second experiment with metabolic activation 1.0% of metaphases with specific chromosomal aberrations were seen in the negative control. At the concentrations of 312.5 µg/ml, 625.0 µg/ml and 1250.0 µg/ml the respective values were 1.0%, 3.0% and 4.5%. Again, at the highest concentration of 1250 ug/ml the value found showed a statistically significant difference in comparison to the negative control.

Confirmatory clastogenicity study

- In the first experiment without metabolic activation, 1.5% of metaphases showed specific chromosomal aberrations in the negative control. At the concentrations of 9.77 µg/ml, 19.53 µg/ml and 39.06 µg/ml the respective values were 2.0%, 3.0% and 2.5%.
- In the second experiment with metabolic activation,1.5% of metaphases showed specific chromosomal aberrations in the negative control. At the concentrations of 312.5 µg/ml, 625.0 µg/ml and 1250.0 µg/ml the respective values were 3.0%, 2.0% and 4.0%.
- In the third experiment performed without metabolic activation 1.0% of metaphases with specific chromosomal aberrations were detected in the negative control cultures. At the concentrations of 1.22 µg/ml, 2.44 µg/ml and 4.88 µg/ml the corresponding values were 3.0%, 1.5% and 1.5%.
- In the fourth experiment performed with metabolic activation 3.0% of metaphases with specific chromosomal aberrations were registered in the negative control cultures. At the concentrations of 39.06 µg/ml, 78.13 µg/ml and 156.25 µg/ml the corresponding values were 1.5%, 1.5% and 3.0%.

THIS SECTION WAS DETERMINED **NOT** TO BE RELEASABLE

3 Pages

RAW DATA

SUMMARY:

42 446 was investigated for clastogenic effects in Chinese Hamster Ovary cells in vitro with and without metabolic activation (S9). The clastogenic activity was assessed at concentrations between , with an incubation time of 18 h, and between with an incubation time of 42 hours, in the absence of metabolic activation. Concentrations were between with an incubation time of 3 hours followed by recovery periods of 15 or 39 hours, respectively, in the presence of a metabolic activation system. Final concentrations greater than 36.06 ug/ml without metabolic activation and greater than 1250 µg/ml with metabolic activation could not be achieved due to toxic effects of the test chemical.

Summary Table: Clastogenic study 42446B in Chinese hamster ovary cells

Study	Time	Concentration range (ug/ml)	Concentrations used for analysis	Results	Comments
Original (–S9)	18 hr		9.77, 19.53, 39.06	Positive	Chromosomal aberrations seen at 9.77, 19.53 and 39.06 µg/ml were 2%, 3% and 4.5% respectively. At 39.06 µg/ml the value was significantly different ($p<0.05$) from the negative control (1%). All these values were below the limit historical control value of 6% which the Sponsor defined for a positive response.
Original (+S9)	3 hr + 15 hr		312.5, 625, 1250	Positive	Chromosomal aberrations seen at 312.5, 625 and 1250 µg/ml were 1%, 3% and 4.5%. At 1250 µg/ml, the value was significantly different ($p<0.05$) from the negative control (1%). All these values were below the limit value of 6% which the Sponsor defined for a positive response.
Confirmatory (–S9)	18 h		9.77, 19.53, 39.06	Negative	Findings (2%, 3%, 2.5%) were not different from negative control (1.5%)
Confirmatory (+S9)	3 hr + 15 hr		312.5, 625, 1250	Negative	Findings (3%, 2%, 4%) were not different from negative control (1.5%)
Confirmatory (–S9)	42 hr		1.22, 2.44, 4.88	Negative	Findings (3%, 1.5%, 1.5%) were not different from negative control (1%)
Confirmatory (+S9)	3 hr + 39 hr		39.06, 78.13, 156.25	Negative	Findings (1.5%, 1.5%, 3%) were not different from negative control (3%)

The positive result obtained in the original study performed in the absence of metabolic activation was not reproduced in the confirmatory study with either incubation time. The number of chromosome aberrations was within the historical control range at all doses in both studies. Under these study conditions a weak depression of cell cycle activity was observed.

The highest concentration used in the first experiment of the original study was appropriate based on the toxicity results. The highest concentration used in the second experiment of the original study could have been higher (5000 ug/ml). Nevertheless, based on the results of the concentrations used the findings were positive in both experiments.

The concentration range in the first two experiments of the confirmatory study was chosen based on the toxicity results from the original study. However, data on toxicity were not presented for these two experiments and there is a possibility that the highest concentration used was too low, particularly in the second experiment with metabolic activation where the choice of the highest concentration was disputable. However, it should be noted that within the concentration range used for analysis there was no indication of a dose-related effect in either of these two experiments of the confirmatory assay.

The positive result obtained in the original study performed in the presence of metabolic activation was not reproduced in the confirmatory study with either incubation time. The number of chromosome aberrations was within the historical control range at all doses in both studies. Again, under these conditions a weak depression of cell cycle activity could be verified.

The Sponsor regarded the positive outcome in the original experiment with and without metabolic activation as fortuitous and not related to the treatment with the test chemical, since the results were not reproduced in the confirmatory assays and the highest values were below the limit chosen for a positive response.

GENETIC TOXICOLOGY SUMMARY

The mutagenicity of [redacted] 42446B was examined in three different Ames tests. In the initial study, Salmonella strains TA 98, TA100, TA1535 and TA1537 were used. In the second study both Salmonella (TA989, TA100, TA1535 and TA1537) and E.coli (WP2uvrA) were used. In the third Ames test Salmonella (TA98, TA100, TA102, TA1535 and TA1537) and E.coli WP2uvrA were used. None of the tested concentrations of [redacted] 42446 led to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants when compared to the negative control, either in the absence or in the presence of metabolic activation. Thus, all Ames tests were negative.

The clastogenicity of [redacted] 42446 was tested using Chinese Hamster Ovary (CHO) cells in vitro, with and without metabolic activation (S9). In the original study, there were statistically significant increases in the % of cells with structural aberrations, in the absence and in the presence of metabolic activation, and with an 18-hr treatment period. Although the values were within historical control range, this Reviewer considers the results positive based on dose-relatedness and extent of the response. The positive findings were, however, not reproduced in a confirmatory study with 18-hr or 42-hr treatment periods. Since the original clastogenicity study was positive and the confirmatory study was negative, this Reviewer considers the results of the CHO cell clastogenicity assay equivocal, both in the absence and the presence of metabolic activation. The conclusion of this Reviewer for the condition with metabolic activation is strengthened by the fact that the highest concentration in the experiments with metabolic activation with an 18-hr treatment period was suboptimal.

The in vivo rat micronucleus assay and the in vitro mutagenicity test in Chinese Hamster V79 cells were previously reviewed by Daniel T. Coleman, Pharmacology Reviewer in the Division (HFD-510). The reviews of these two studies are appended to this NDA review section. This reviewer concurs with the conclusions.

In the micronucleus test, the effect of [redacted] 42446B on the appearance of micronuclei in polychromatic erythrocytes was examined in rat bone marrow samples. Symptoms of toxicity in all animals were piloerection and ataxia. [redacted] 42446 was not clastogenic *in vivo* in the rat micronucleus assay under the conditions described. Appropriate exposure was demonstrated by testing at >60% of the LD_{50}.

In the Chinese Hamster V79 cell mutagenicity test, no significant increase in the frequency of mutations was observed at any concentration of [redacted] 42446 in comparison to the negative control, in the absence and presence of metabolic activation. The slight increase in mutation frequency in the confirmatory experiment with activation did not show concentration dependence and was of insufficient magnitude to be considered a positive response. Thus, [redacted] 42446 was not mutagenic at the hgprt locus in V79 Chinese Hamster cells in vitro in the absence or presence of metabolic activation.

Reviewers Conclusion: The results of the three in vitro bacterial reverse mutation (Ames) tests, the in vitro mutagenicity test in Chinese Hamster cell V79 cells, and the in vivo rat micronucleus assay were negative, with and without metabolic activation. The results of the in vitro clastogenicity test using Chinese Hamster Ovary cells were equivocal, with and without metabolic activation.

Gene Mutation Test with Chinese Hamster Cells V79 *In Vitro.*
(from: [redacted]

Purpose:

To assess the potential for [redacted]-446 to induce mutations (base pair substitutions, frame shift or deletions) resulting in 6-thioguanine resistance in V79 Chinese Hamster cells in the presence or absence of Aroclor-induced rat liver post mitochondrial supernatant (S9 fraction).

Experimental design

Testing Facility:	[redacted] iba-Geigy, Basle, Switzerland.	
Study #:	926249	
Study Initiated:	Sept 29, 92	
Study Completed:	July 7, 93.	
GLP statement	Included.	
Dose & Formulation:	Cytotoxicity: 4.88 - 5000 ug/ml (+/- act.)	
	Mutagenicity: 1.88 - 15 ug/ml (+/- act.)	
Batch of drug:	Lot 801290	
Conditions:	Growth:	Hams F-10 + 10% FCS
	Experimental:	Hams F-10 + 3% FCS - antibiotics
		5 h with metabolic activation
		21 h w/o metabolic activation
	Selection:	Hams F-10 + 10% FCS
		+ 8 ug/ml 6-thioguanine

Preliminary cytotoxicity tests were performed to determine the maximum concentration of [redacted] 42-446 (with S9 for five hours or without S9 for 21 hours) at which 10-50% of cells remained viable. Viability was determined by measuring cloning efficiency (number of colonies to grow in a flask after 100 cells were plated) after exposure to a wide range of concentrations of drug (11 concentrations; 4 to 5000 ug/ml).

Mutagenicity was determined for:
negative control, HF-10
positive control (-S9), Ethyl Methane Sulphonate
positive control (+S9), Nitroso-dimethylamine
[redacted] 42 446 -S9, [1, 2, 4 & 8 ug/ml] in 2 experiments.
42 446 +S9, [1.88, 3.75, 7.5, & 15 ug/ml] in one experiment.
42 446 +S9, [0.50, 1.00, 2.0, & 4 ug/ml] in a confirmatory experiment.

Cells were plated and allowed to grow overnight.They were then exposed to test compounds (with S9 for five hours or without S9 for 21 hours), washed, subcultured, allowed to grow for 2-3 days and subcultured again. After this 7-8 day "expression period", aliquots were seeded into high density cultures in the presence of 8 ug/ml 6-thioguanine. After 7-8 days the number of colonies reflects the number cells mutated at the hgprt locus.
Cytotoxicity was determined in parallel with mutagenicity by measuring cloning efficiency in an aliquot of cells immediately after treatment with test compound.

Criteria for significance:

- Viability at the concentration tested must exceed 15%.
- The mutation frequency must exceed the frequency in the solvent control by 2.5-fold.
- The mutation frequency must be concentration dependent, or
- The mutation frequency in any treated group exceeds three times the control number, or the control number plus 20.

Results:

- Without activation 95% toxicity was seen at 10 ug/ml. Mutagenicity was therefore assayed between 1 and 8 ug/ml.
- With metabolic activation 99% toxicity was seen at 19 ug/ml. Mutagenicity was therefore assayed at 2 to 15 ug/ml with metabolic activation.
- In the initial mutagenicity experiment there was excessive cytotoxicity at the highest doses tested with activation. The confirmatory experiment with metabolic activation was therefore conducted at 0.5 to 4 ug/ml.
- In the presence and absence of metabolic activation. no significant increase in frequency of mutations was observed at any concentration of —— 42-446 in comparison to the negative control. The slight increase in mutation frequency in the confirmatory experiment with S-9 did not show concentration dependence and was of insufficient magnitude to be considered a positive response. In contrast, the positive controls gave a clear positive response.

Conclusions:

—— 42-446 is not mutagenic at the hgprt locus in V79 Chinese hamster cells in vitro in the presence or absence of S-9.

Micronucleus Test, Rat (OECD Conform) *In Vivo.*

Purpose: To assess the potential for —— 42-446 to induce chromosomal damage *in vivo* as indicated by the appearance of micronuclei in polychromatic erythrocytes in rats exposed to the drug. This assay detects clastogens that break chromosomes and can detect aneuploidy.

Experimental design:

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

Report #: 926247

Study Initiated: May 7, 1993
Study Completed: Nov. 17, 1993

Dose & Formulation: 2.6 - 10.4 mg/kg IV in 0.9% NaCl.

Batch of drug: lot 16/015/1

GLP statement: **Included.**

Based on the known LD_{50} of 13 mg/kg b.w. i.v. in rats, the doses selected were; 2.6, 5.2, and 10.4 mg/kg. Animals were killed 16, 24 and 48 hours after a single i.v. bolus. The positive control substance was Cyclophosphamide, 40 mg/kg i.v. The negative control was 0.9% saline.

The number of animals assigned to each group were as follows:

Time	HD	ID	LD	Pos.Ctrl.	Neg.Ctrl.
16 h	5M + 5F				5M + 5F
24 h	5M + 5F	5M + 5F	5M + 5F	5M + 5F	5M + 5F
48 h	5M + 5F				5M + 5F

Marrow was harvested from femurs in calf serum. Erythrocytes were separated, resuspended in fetal calf serum, smeared on slides, and stained with May-Grunwald/Giemsa solution.

Results:

Symptoms of toxicity in all animals were piloerection and ataxia. No animals died. None of the groups treated with — 42-446 had significantly ($P < 0.05$, Chi-Square-Test) more polychromatic erythrocytes (PCEs). There was a highly significant increase in the number of PCEs in the positive control samples.

Conclusions:

—— 42-446 was not clastogenic *in vivo* in the rat micronucleus assay under the conditions described. Appropriate exposure was demonstrated by testing at >60% of LD_{50}.

REPRODUCTIVE TOXICOLOGY

Three reproductive toxicity studies were carried out, a Segment I study in rats, and two Segment II studies, one in rats and one in rabbits. In the Segment I study females were dosed throughout gestation, delivery and lactation. In this study part of the pregnant females were sacrificed on Gestation Day 13, and part of the females were scheduled for sacrifice on Lactation Day 21.

A Subcutaneous Fertility and Reproductive Toxicity (Segment 1) Study in Rats

Study No: 971010
Site and testing facility: Novartis pharmaceuticals Corporation, Preclinical Safety, Toxicology/Pathology, Safety Evaluation Facility (SEF), Summit, New Jersey
GLP compliance: Yes
QA- Reports: Yes
Lot and batch numbers: 800492
Protocol reviewed by Division: No

Methods:

- Species/strain: Sprague-Dawley rats [Crl:COBSCD(SD)BR]
- Doses employed: 0.01, 0.03, and 0.1 mg/kg/day. These doses were selected based on the range finding and definitive Segment II studies and the 3 month subcutaneous toxicity study.
- Route of Administration: Subcutaneous injection
- Study Design:
  Parental males were dosed for 71 days prior to mating, during the two-week mating period and until study day 97. Males were sacrificed on day 98. Parental females were dosed for 15 days prior to mating and during the mating period. Females scheduled for gestation day 13 necropsy were dosed through day 12 of gestation, and those originally scheduled for lactation day 21 necropsy were dosed throughout gestation and through lactation day 6 due to early termination of the study.
- Number of animals/sex/dosing group: 12 males and 24 females
- Parameters and endpoints evaluated:
  In-life examinations: parental (F_0) mortality, clinical signs, food consumption, body weight. F_1 clinical signs, sex and number, body weight, developmental parameters (righting reflex and pinna detached). Litters were culled to 8 pups (4/sex) on post partum Day 4.
  Necropsy examinations: testes in F_0 males. Numbers of corpora lutea, implantation sites, and resorption in F_0 females. External body in F_1 pups.
- Statistical evaluations:
  SAS program was used to evaluate parental body weight and food consumption, organ weights, reproductive parameters, pregnancy rates, pup sex ratios and F_1 developmental parameters.

Results:

- Clinical signs and mortality:
  Parental F_0 males: 1 death in HD due to treatment. 1 death in LD, cause could not be determined. No significant toxicological finding was observed at necropsy.

Observations	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
Alopecia	0/12	2/12	1/12	0/12
Chromodacryorrhea	1/12	3/12	4/12	5/12
Death	0/12	1/12	0/12	1/12[a]
Sore/scab	1/12	1/12	0/12	1/12
Stained fur	0/12	0/12	0/12	1/12[a]
Unthrifty	0/12	0/12	0/12	1/12[a]

[a]Same animal (male no. 39).

Parental F_0 females:
No mortality or significant clinical signs were observed in rats scheduled for gestation day 13 necropsy.
Mortality and moribund sacrifices occurred at parturition in rats scheduled for lactation day 21 necropsy in all dose groups. Out of the 8-12-6 (LD-MD-HD) animals 4-9-4 (LD-MD-HD) animals died around parturition time, leaving 4-3-2 (LD-MD-HD) animals and litters for study during lactation. This was presumably due to disruption of the delivery process caused by the calcium depleting effect of the drug. Due to these deaths F1 data on development, behavior and fertility were not obtained, and the study was terminated on lactation day 7.
Clinical signs of hypoactivity were seen in low and mid dose animals before death.
No treatment related necropsy findings were noted.

Observations	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
Alopecia	6/24	2/24	5/24	5/24
Chromodacryorrhea	0/24	1/24	0/24	0/24
Death	0/24	3/24	7/24	3/24
Hypoactivity	0/24	2/24	2/24	0/24
Moribund sacrifice	0/24	1/24	2/24	1/24

- Body weight:
  Parental F_0 males: Dose-related decreases (up to 18% at HD) in body weight and body weight gain throughout most of the treatment period in LD, MD and HD. The decreases were statistically significant in MD and HD.

Summary of male body weight (grams)
(Mean ± Standard deviation)

Days	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
0	375.4 ± 13.4 (12)[a]	373.4 ± 14.3 (12)	352.5 ± 13.2 (12)	359.1 ± 17.0 (12)
7	424.6 ± 18.2 (12)	419.9 ± 16.1 (12)	396.0 ± 15.5** (12)	405.9 ± 23.1** (12)
14	464.9 ± 22.8 (12)	450.2 ± 17.5 (11)	428.9 ± 19.8** (12)	436.1 ± 26.5** (12)
21	499.0 ± 26.8 (12)	486.9 ± 19.3 (11)	461.6 ± 22.8** (12)	468.8 ± 33.6** (12)
28	527.3 ± 30.9 (12)	510.6 ± 24.5 (11)	486.3 ± 27.2** (12)	491.6 ± 37.4** (12)
35	557.1 ± 29.0 (12)	543.3 ± 26.2 (11)	515.5 ± 30.5** (12)	519.6 ± 38.4** (12)
42	580.1 ± 33.9 (12)	562.9 ± 31.6 (11)	537.0 ± 31.4** (12)	540.3 ± 41.0** (12)
49	599.3 ± 35.1 (12)	583.4 ± 34.8 (11)	552.3 ± 33.8** (12)	554.0 ± 40.3** (12)
56	618.8 ± 39.4 (12)	597.2 ± 42.3 (11)	569.4 ± 35.9** (12)	565.5 ± 37.2** (12)
63	632.8 ± 44.1 (12)	614.6 ± 43.7 (11)	580.1 ± 34.3** (12)	568.4 ± 39.3** (12)
70	644.9 ± 42.5 (12)[a]	625.5 ± 45.3 (11)	582.2 ± 35.4** (12)	560.1 ± 37.4** (12)
77	646.3 ± 40.4 (12)	624.6 ± 44.1 (11)	580.8 ± 32.8** (12)	556.8 ± 36.8** (12)
84	662.3 ± 42.6 (12)	637.1 ± 50.8 (11)	594.3 ± 34.7** (12)	565.4 ± 40.0** (12)
91	676.7 ± 43.8 (12)	654.2 ± 53.1 (11)	603.1 ± 38.8** (12)	562.8 ± 49.0** (12)
98	695.5 ± 47.0 (12)	671.5 ± 53.0 (11)	603.1 ± 41.5** (12)	570.2 ± 40.1** (11)

[a]Numbers in parenthesis ( ) equal number of animals used in mean.

**Statistically different from the control group at $p \leq 0.01$.

Parental F_0 females: No effect on premating body weight. Gestational body weight was significantly decreased in MD and HD on gestation day 13. Decrease was also seen in MD and HD on gestation day 20 but was not statistically significant on this day, probably due to lower number of animals examined.
In animals dosed through lactation day 6, body weight and body weight gain were decreased in HD.

Summary of female gestation body weight (grams)
(Mean ± Standard deviation)

Days	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
0	273.3 ± 22.6 (21)[a]	267.3 ± 15.4 (19)	262.3 ± 16.2 (23)	264.3 ± 16.9 (14)
6	304.4 ± 20.1 (21)	298.1 ± 15.7 (19)	293.9 ± 14.4 (23)	294.9 ± 20.9 (14)
13	336.7 ± 20.3 (21)	334.4 ± 21.7 (19)	325.9 ± 16.6* (23)	322.9 ± 24.5* (14)
20	412.6 ± 29.9 (10)	408.5 ± 17.8 (8)	409.6 ± 21.9 (12)	395.7 ± 37.5 (6)

[a]Numbers in parenthesis ( ) equal number of animals used in mean.
*Statistically different from the control group at $p \leq 0.05$.

- Food consumption:

Parental F_0 males: Dose-related decreases (up to 30% at HD) were noted throughout the treatment period in LD, MD and HD.

Summary of male food consumption (grams/day)
(Mean ± Standard deviation)

Days	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
0-7	32.7 ± 2.9 (12)[a]	32.2 ± 2.1 (12)	30.4 ± 2.1* (12)	30.6 ± 2.5* (12)
7-14	32.5 ± 3.1 (12)	30.7 ± 2.0 (11)	30.0 ± 2.5* (12)	29.6 ± 2.9* (12)
14-21	32.5 ± 3.6 (12)	31.2 ± 1.6 (11)	29.7 ± 2.5** (12)	29.2 ± 2.7** (12)
21-28	33.1 ± 3.5 (12)	30.6 ± 2.7* (11)	29.9 ± 2.5** (12)	29.2 ± 2.7** (12)
28-35	31.9 ± 3.1 (12)	30.4 ± 2.4 (11)	29.6 ± 2.3* (12)	28.9 ± 2.5** (12)
35-42	32.8 ± 2.7 (12)	30.4 ± 2.8* (11)	29.6 ± 2.4** (12)	28.5 ± 2.1** (12)
42-49	33.1 ± 3.1 (12)	30.5 ± 2.9** (11)	28.9 ± 1.7** (12)	28.1 ± 2.2** (12)
49-56	32.7 ± 2.7 (12)	29.9 ± 3.7** (11)	28.7 ± 1.8** (12)	26.4 ± 2.0** (12)
56-63	32.3 ± 3.0 (12)	30.0 ± 3.2* (11)	27.5 ± 2.2** (12)	24.1 ± 3.0** (12)
63-70	33.0 ± 2.3 (12)	30.7 ± 2.9* (11)	27.0 ± 2.9** (12)	23.3 ± 2.5** (12)

[a]Numbers in parenthesis ( ) equal number of animals used in mean.
*Statistically different from the control group at $p \leq 0.05$.
**Statistically different from the control group at $p \leq 0.01$.

Parental F_0 females: No marked treatment related changes in food consumption during premating and gestation periods in females scheduled for sacrifice on gestation day 13. A decrease in food consumption during lactation period (day 0-7) was noted in HD females.

- Fertility in Males

- Terminal and Necroscopic evaluations: Absolute testes weight was not affected by the drug. Relative testes weight (to body weight) was increased in MD and HD due to the treatment-related decreases in terminal body weight.

Summary of male organ weight data
(Mean ± Standard deviation)

Organ	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
Testes (grams)	3.883 ± 0.31 (12)[a]	3.916 ± 0.21 (11)	3.904 ± 0.28 (12)	3.856 ± 0.54 (11)
Terminal body weight (grams)	695.5 ± 47.0 (12)	671.5 ± 53.0 (11)	603.1 ± 41.5** (12)	570.2 ± 40.1** (11)
Testes/body weight (ratio)	0.559 ± 0.04 (12)	0.586 ± 0.05 (11)	0.650 ± 0.07** (12)	0.675 ± 0.06** (11)

[a]Numbers in parenthesis ( ) equal number of animals used in mean.
**Statistically different from the control group at $p \leq 0.01$.

- Fertility and Early Embryonic Development in Females
  - In-life observations:
    - Precoital interval was not affected by treatment.
    - Pregnancy rate was decreased in HD. Calculated fertility index (# animals pregnant/# animals that mated) (21/23, 22/24, 23/24, 14/20) was also decreased in HD.

Summary of (F_0) parental fertility

Observations	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
Number of males cohoused	12	11	12	12
Number of females cohoused	24	24	24	24
Percent inseminated (number females inseminated/ number cohoused) x 100	95.8	91.6	100	83.3
Number pregnant	21	20	23	14
Percent pregnant (number females pregnant/ number cohoused) x 100	87.5	83.3	95.8	58.3*

*Statistically different from the control group at $p \leq 0.05$.

  - Terminal and Necroscopic evaluations (Females sacrificed on gestation day 13):
  - Reduction in the number of corpora lutea (not significant, n.s.) in HD, reduction in number of implants (n.s.) in MD and HD, reduction in number of live fetuses (n.s.) in MD and HD, and significant increase in preimplantation loss in MD and HD.

Summary of the reproductive parameters derived from parental (F_0) females sacrificed on gestation day 13 (Mean ± Standard deviation)

Parameters	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
No. of pregnant females	11	11	11	8
No. corpora lutea	16.30 ± 1.77	17.36 ± 2.69	15.64 ± 3.83	14.88 ± 2.64
No. of implants	15.73 ± 1.19	15.73 ± 2.37	12.27 ± 6.42	11.88 ± 5.74
No. of resorptions	0.64 ± 0.50	0.73 ± 1.19	0.64 ± 0.81	0.50 ± 1.07
No of live fetuses	15.09 ± 1.51	15.00 ± 2.14	11.64 ± 6.14	11.38 ± 5.48*
Preimplantation loss	0.70 ± 1.34	1.64 ± 1.50	3.36 ± 3.64*	3.00 ± 3.55*
% preimplantation loss	3.80 ± 6.94	9.11 ± 8.24	27.53 ± 34.84*	24.42 ± 33.31*
Postimplantation loss	0.64 ± 0.50	0.73 ± 1.19	0.64 ± 0.81	0.50 ± 1.07
% postimplantation loss	4.18 ± 3.33	4.22 ± 6.83	4.19 ± 5.33	3.24 ± 6.75

*Statistically different from the control group at $p \leq 0.05$.

- Prenatal and postnatal development, including maternal function
  - In-life observations:
  - Dams: Gestation duration was not affected by treatment.
  - Offspring: The number of viable litters and viable litter size was slightly decreased in MD and markedly decreased in HD. Survival was slightly decreased in MD and HD during lactation day 0-4. This was entirely due to an effect in male pups. Pup weight was decreased by 20% in HD on lactation day 7. However, the latter effect was not statistically significant due to small sample size. No treatment related findings were noted on clinical signs, sex ratio, and developmental parameters (righting reflex and pinna detachment).

	control	LD	MD	HD
Number of viable litters	10	4	3	2
Viable litter size (postpartum day 0)	14.1	13.3	11.3	7.5
Mean % pups surviving Day 0-4 (precull)	98	98	91.9	92.9
Mean % pups surviving Day 4-7 (postcull)	98.8	100	100	100
Pup weight PP Day 0 (g)	6.7	6.7	6.9	6.7
Pup weight PP Day 7 (g)	16.5	16.2	16.4	13.8

  - Terminal and Necroscopic Evaluations (Females sacrificed on lactation day 7):
    - Dams: Decrease in number of implantations in MD and HD, decrease in number of viable newborns in LD, MD and HD, increase in the number of stillbirths in LD and MD, and increase in postimplantation loss in LD. However, small number of full term females particularly in HD group (n=2) limited conclusive interpretation of the data.

Summary of the reproductive parameters derived from full-term parental (F_0) females
(Mean ± Standard deviation)

Parameters	Dose level (mg/kg/day)			
	Control (0)	0.01	0.03	0.1
No. of females with viable litters	10	4	3	2
No. of implants	15.30 ± 1.95	16.75 ± 1.26	12.33 ± 4.51	7.50 ± 0.71
No. of viable newborns	14.10 ± 2.51	13.25 ± 1.50	11.33 ± 5.13	7.50 ± 0.71
No. of stillbirths	0.10 ± 0.32	0.25 ± 0.50	0.67 ± 1.15	0.00 ± 0.00
% stillbirths	0.83 ± 2.64	1.92 ± 3.85	6.56 ± 9.62	0.00 ± 0.00
Postimplantation loss	1.20 ± 1.32	2.50 ± 1.00	1.00 ± 1.00	0.00 ± 0.00
% postimplantation loss	8.08 ± 8.70	15.89 ± 8.17	9.72 ± 8.67	0.00 ± 0.00

- Offspring: No treatment related findings at necropsy.

Conclusions:

42446 was administered to rats subcutaneously at doses of 0.01, 0.03 and 0.1 mg/kg/day during premating and mating in males, and during premating, mating, gestation and lactation in females.

F_0 males

- Death was observed in one animal at 0.1 mg/kg/day. Body weight and food consumption were decreased in all dose groups (0.01, 0.03, 0.1 mg/kg/day).

F_0 females

- Pregnancy rate was decreased at 0.1 mg/kg/day. Gestational body weight was decreased at doses ≥0.03 mg/kg/day. Lactational body weight and food consumption were decreased at 0.1mg/kg/day.
- In females sacrificed on Gestation Day 13, number of corpora lutea, number of implantations and number of live fetuses were reduced at doses ≥0.03 mg/kg/day. Preimplantation loss was increased at doses ≥0.03 mg/kg/day.
- Death or moribund sacrifices occurred in rats scheduled for necropsy on lactation day 21, concomitant with disruption of the delivery process (dystocia). Presumably, this was caused by the inhibition of calcium mobilization from the skeleton by the test compound and the resulting hypocalcemia.
- In females that delivered and were sacrificed on Lactation Day 7, number of implantations and number of viable newborns were decreased at doses ≥0.03 mg/kg/day, number of stillbirths was increased at 0.01 and 0.03 mg/kg/day, and postimplantation loss was increased at 0.01 mg/kg/day. However, for stillbirths and postimplantation loss, the data for the 0.03 and 0.1 mg/kg groups were not reliable due to small sample size.

F_1 offspring

- Survival was slightly decreased at doses ≥0.03 mg/kg/day, and viable litter size was decreased at doses ≥0.03 mg/kg/day. Body weight was decreased in the 0.1 mg/kg/day group on lactation day 7.

Based on this study 42446 caused impairment of fertility at doses ≥0.03 mg/kg/day, and periparturient mortality at doses ≥0.01 mg/kg/day. Maternal toxicity during gestation was evidenced by a decrease in body weight at doses ≥0.03 mg/kg/day, and during lactation by a decrease in food consumption and body weight at 0.1 mg/kg/day. Fetal toxicity was observed at doses ≥0.03 mg/kg/day.

Segment II (Teratology) Study in Rats
Study No: 936059
Site and testing facility: [redacted]
GLP compliance: Yes
QA- Reports: Yes
Lot and batch numbers: 16/015/1
Protocol reviewed by Division: No

Methods:

- Species/strain: Sprague-Dawley rats/Hsd/Ola
- Doses employed: 0.1, 0.2, and 0.4 mg/kg/day. These doses were selected based on the range finding study in pregnant rats at doses of 0.2, 0.6, and 2 mg/kg.
- Route of Administration: Subcutaneous injection.
- Study Design:
  Mated female rats were dosed once daily at 1 ml/kg from day 6 to day 15 of gestation and sacrificed on day 20 of gestation.
- Number of animals/sex/dosing group: 24 mated females
- Parameters and endpoints evaluated:
  In-life examinations: Dam (F_0) mortality, clinical signs, food consumption, body weight.
  Necropsy examinations: numbers of corpora lutea, implantation sites, and resorption.
  F_1 weight, sex, external observation, visceral and skeletal evaluation.
- Statistical evaluations:
  DART program was used to evaluate body weight and food consumption, organ weights, and reproductive parameters. Only animals with a status of pregnant to term with live young were included.

Results:

- Clinical signs:
  Moderate to marked skin thickening at the injection sites was noted at doses ≥ 0.2 mg/kg.
- Mortality: 1 control animal littered on gestation day 15 and was sacrificed. All other animals were sacrificed on day 20 of gestation as scheduled.
- Pregnancy status:

Group : Dosage (mg/kg) :	1 Control	2 0.1	3 0.2	4 0.4
Number of dams:	24	24	24	24
Number pregnant to term with viable young	17	19	22	14
Number pregnant to term with resorptions only	0	0	0	9
Number killed, littered	1	0	0	0
Number not pregnant	6	5	2	1

- Body weight:
  Body weight and body weight gain were decreased at doses ≥ 0.2 mg/kg from gestation day 10-16. The reduction persisted after cessation of drug treatment on gestation Day 16. The effect on both body weight and weight gain was statistically significant from gestation Day 10 at 0.4 mg/kg/day, and from gestation day 16 at doses ≥ 0.2 mg/kg/day.

Group Mean Body Weight Change (g) during Gestation

Day of Gestation	Group	1	2	3		4	
	Dosage (mg/kg)	0	0.1	0.2		0.4	
0–6	Mean	–25	–24	–23		–23	
	SD	4	5	5		5	
	N	17	19	22		14	
6–10	Mean	13	13	14		16	*
	SD	4	4	4		3	
	N	17	19	22		14	
6–16	Mean	49	49	45		29	***
	SD	8	9	8		8	
	N	17	19	22		14	
6–20	Mean	109	103	93	**	46	***
	SD	15	18	15		15	
	N	17	19	22		14	

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

- Food consumption:

Food consumption was decreased at doses ≥ 0.2 mg/kg from gestation day 10, and persisted after cessation of treatment. The effect was statistically significant from gestation day 10 at 0.4 mg/kg/day, and from gestation day 16 at doses ≥ 0.2 mg/kg/day.

Group Mean Food Consumption during Gestation (g/animal/period)

Day of Gestation	Group	1	2	3		4	
	Dosage (mg/kg)	0	0.1	0.2		0.4	
0–6	Mean	137	133	131		132	
	SD	10	10	8		9	
	N	17	19	22		14	
6–10	Mean	97	95	93		92	
	SD	8	6	7		8	
	N	17	19	22		14	
10–16	Mean	156	151	147	*	132	***
	SD	11	10	14		9	
	N	17	19	22		14	
16–20	Mean	120	113	104	***	92	***
	SD	11	14	11		9	
	N	17	19	22		14	

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

- Embryo-fetal Development
  - Dams: Increase in pre-implantation loss at 0.4 mg/kg (drug-related?). Increase in postimplantation loss and in number of late resorptions at 0.4 mg/kg. Decrease in number of implantations and decrease in viable fetuses at 0.4 mg/kg.

Dosage (mg/kg)		0	0.1	0.2		0.4	
Number of Corpora Lutea	Mean	17.06	16.95	15.45	*	16.29	
	SD	2.59	2.44	2.67		3.56	
	N	17.00	19.00	22.00		14.00	
Number of Implantations	Mean	13.82	13.79	13.05		11.57	*
	SD	2.30	2.74	2.44		4.24	
	N	17.00	19.00	22.00		14.00	
Early Resorptions	Mean	0.59	1.00	0.91		0.71	
	SD	1.28	1.05	1.15		0.83	
	N	17.00	19.00	22.00		14.00	
Late Resorptions	Mean	0.06	0.16	0.09		7.14	***
	SD	0.24	0.37	0.29		4.97	
	N	17.00	19.00	22.00		14.00	
Total Resorptions	Mean	0.65	1.16	1.00		7.86	***
	SD	1.27	1.01	1.11		5.16	
	N	17.00	19.00	22.00		14.00	
Number of Viable Fetuses	Mean	13.18	12.63	12.05		3.71	***
	SD	2.48	3.50	2.94		3.56	
	N	17.00	19.00	22.00		14.00	
Pre-implantation Loss (%)	Mean	18.49	18.08	15.06		28.73	
	SD	11.04	15.92	12.10		24.15	
	N	17.00	19.00	22.00		14.00	
Post-implantation Loss (%)	Mean	4.53	10.04	8.40		64.92	***
	SD	8.56	10.18	10.49		30.21	
	N	17.00	19.00	22.00		14.00	

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

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- Offspring: Decrease in fetal body weight at doses ≥ 2 mg/kg.

Dosage (mg/kg)		0	0.1	0.2		0.4	
Litter Mean Fetal Weight (g)	Mean	3.51	3.48	3.33	**	2.20	***
	SD	0.19	0.26	0.33		0.56	
	N	17.00	19.00	22.00		14.00	
Mean Fetal Weight of Male Fetuses (g)	Mean	3.59	3.59	3.45	*	2.14	***
	SD	0.24	0.27	0.38		0.32	
	N	17.00	19.00	22.00		12.00	
Mean Fetal Weight of Female Fetuses (g)	Mean	3.43	3.38	3.20	**	2.29	***
	SD	0.22	0.30	0.30		0.72	
	N	17.00	19.00	21.00		7.00	
Proportion of Male Fetuses (%)	Mean	49.03	46.74	53.75		65.81	*1
	SD	14.97	15.40	16.53		38.59	
	N	17.00	19.00	22.00		14.00	

1 The high male proportion at 0.4 mg/kg is a consequence of the number of litters with only one male fetus.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Malformations

A treatment-related increase in external, visceral and skeletal malformations, and in visceral and skeletal variations was noted at doses of 0.2 and 0.4 mg/kg/day.
Malformations are summarized in the next table (% by litter, i.e., % of dams affected, or, maternal incidence). Most malformations occurred only in the 0.4 mg/kg group, but some were also seen at 0.2 mg/kg.

Malformations (%) by litter

External –

Shortened lower jaw	0	0	0	64.3
Cleft palate	0	0	0	28.6
Oedematous	0	0	0	35.7

Visceral –

Lens reduced	0	0	0	50.0
Cerebellum rudimentary	0	0	4.6	12.5
Caudate lobe of liver reduced by 50%	0	0	9.1	12.5
Caudate lobe of liver absent	0	0	0	50.0
Left and median lobes of liver reduced	0	0	0	12.5
Kidney reduced	0	0	0	12.5
Adrenals enlarged	0	0	0	12.5
All lobes of lung reduced	0	0	4.6	75.0
Aorta dilated	0	0	0	25.0
Right subclavian artery and pulmonary trunk dilated	0	0	0	12.5

Skeletal –

Interparietal – not ossified	0	0	0	7.1
Occipital – not ossified	0	0	0	7.1
Rib – wavy	0	0	27.3	71.4
Rib thickened	0	0	9.1	14.3
Scapula curved	0	0	18.2	14.3
Scapula shortened	0	0	0	7.1
Clavicle curved	0	0	0	7.1
Humerus shortened	0	0	0	21.4
Humerus thickened	0	0	0	7.1
Humerus curved	0	0	0	7.1
Radius curved	0	0	4.6	7.1
Radius shortened	0	0	0	14.3
Ulna thickened	0	0	0	7.1
Ulna curved	0	0	4.6	7.1
Ulna shortened	0	0	0	14.3
Femur curved	0	0	0	7.1
Femur shortened	0	0	0	21.4
Tibia shortened	0	0	0	14.3
Fibula shortened	0	0	0	14.3
Number of litters with a malformation	0	0	8	14

Variations

External variations are summarized in the next table (% by litter, i.e., maternal incidence). One variation appeared to occur in all dose groups.

External variations (%) by litter

Dose group	control	0.1	0.2	0.4
hemorrhagic placenta (hp)	0	5.3	22.7	7.1

Visceral variations are summarized in the next table (% by litter, i.e., maternal incidence). Most variations occurred only in the 0.4 mg/kg group, but one appeared to occur with increased incidence in the 0.2 and 0.4 mg/kg groups (ventricular walls reduced in thickness). The numerical incidence of the one abnormality in the 0.1 mg/kg group (atria enlarged) was only 1 fetus in 1 litter and was therefore deemed not biologically significant.

Visceral variations (%) by litter

Dose group	control	0.1	0.2	0.4
posterior lobe of lung reduced by 30% (pl)	0	0	4.6	0
dilated lateral ventricle (dl)	12	0	4.6	50
reduced thickness of ventricular walls (tw)	0	0	4.6	37.5*
submaxillary glands reduced by 50% (sr)	0	0	0	12.5
atria enlarged (ae)	0	5.3	0	37.5*
left ventricle displaced posteriorly (vd)	0	0	0	12.5

*statistically significant effect

Skeletal variations were increased in the 0.4 mg/kg group, and consisted of non-ossified bones and incomplete ossification of a variety of bones, thickened and shortened bones, and reduced numbers of bones (e.g. sacrals). The maternal incidence of skeletal variations in the 0.4 mg/kg group ranged from ~~~~ Some of the variations (incomplete ossification of several bones) were also increased in the 0.2 mg/kg group, usually to a lesser degree, with an incidence ranging from ~~~~ A few variations were increased in the 0.1 mg/kg group, i.e., incompletely ossification of occipital and pubic bones, with relatively low incidence. The skeletal malformations and variations indicated retarded development and were in agreement with the reduced fetal weights.

Summary Of All Malformations And Variations With Increased Incidence

Dose (mg/kg)	Gestation Day	Effect (as compared to control):			External abnormalities	Visceral abnormalities	Skeletal abnormalities
		Food consumption	Body weight	Body weight gain			
0.1	GD 10	-2%	-1%	-	MALF: None treatment-related VAR: hp	MALF: None treatment-related VAR: None treatment-related	MALF: None treatment-related VAR: o, nc, ts, pb, nm
	GD 16	-3%	-1%	-			
0.2	GD 10	-4%	-2%	+8%	MALF: None treatment-related VAR: hp	MALF: cr, cl, ll VAR: tw	MALF: wr, br, sb, rb, uc VAR: o, f, p, l, wf, sq, pv, nc, rc, oc, ts, pb, nm
	GD 16	-6%	-3%	-8%			
0.4	GD 10	-15%	0.5%	+23%	MALF: sj, cp, od VAR: hp	MALF: lr, cr, cl, ca, lm, kr, ae, ll, ad, ab VAR: dl, tw, sr, ea, vd	MALF: ni, no, wr, br, sb, ss, cc, hs, ht, hc, rb, ri, ut, uc, us, fc, fs, tl, fl VAR: n, f, p, l, o, wf, sq, oi, tb, po, pv, nv, pc, nc, rs, rc, oc, ts, sc, cv, pb, nb, is, hi, ui, mo, mt, nm
	GD 16	-23%	-8%	-40%			

Abbreviations:
External:
MALF: sj shortened jaw, cp cleft palate, od oedematous
VAR: hp placenta hemorrhagic
Visceral:
MALF: lr lens reduced, cr cerebellum rudimentary, cl caudate liver lobe reduced by 50%, ll all lobes of lung reduced, ca caudate liver lobe absent, lm left+median liver lobes reduced, kr kidney reduced by 25%, ae adrenals enlarged, ad aorta dilated, ab artery dilated
VAR: dl dilated lateral ventricle, tw reduced thickness of ventricle walls, sr submax glands reduced by 50%, ea atria enlarged, vd left ventricle displaced posteriorly
Skeletal:
MALF: ni interparietal not ossified, no occipital not ossified, wr wavy rib, br rib thickened, sb scapula curved, ss scapula shortened, cc clavicle curved, hs humerus shortened, ht humerus thickened, hc humerus curved, rb radius curved, ri radius shortened, ut ulna thickened, uc ulna curved, us ulna shortened, tc femur curved, fs femur shortened, tl tibia shortened, fl fibula shortened
VAR: incomplete ossification of: n nasal bone, f frontal bone, p parietal, i interparietal, o occipital, sq squamosal, oi basisoccipital, tb tympanic bulla, po cranial, pv vertebral arch, pc, centra, sc scapula, cv clavicle, pb pubic bone, is ischium bone, hi humerus, ui ulna, mt metatarsal ; wf widened anterior fontanelle; no ossification of: nv, vertebral arch, nc centra, oc caudals, ts sternebra (≥3), nb pubic bone, mo metacarpal, nm metatarsal; rs sacrals reduced in number, rc caudals reduced in number.

Discussion:

Maternal toxicity and teratogenic effects

Maternal effects on food consumption and body weight occurred concomitant with fetal abnormalities, mainly at 0.4 mg/kg. The Sponsor hypothesized that the test compound might reduce plasma calcium levels, which would lead to the observed maternal toxicity and fetal abnormalities. Sponsor concluded that the teratogenicity was drug-related and not a consequence of maternal toxicity.
In this Reviewer's opinion the hypothesis about hypocalcemia is not supported by data. Results from a 10-day and a 1-month s.c. rat toxicity study (doses 0, 0.2, 0.6, 2 mg/kg/day, and doses 0, 0.02, 0.06, 0.2 mg/kg/day, respectively) did not show reductions in total plasma calcium levels in females up to dose levels of 0.6 and 0.2 mg/kg/day, respectively. However, these data do not give any information on ionized calcium levels.

As shown in the Summary table above, one external and a few skeletal variations were seen at 0.1 mg/kg, one external, a few visceral and several skeletal malformations and variations were seen at 0.2 mg/kg, and a few external, several visceral and several skeletal malformations and variations were seen at 0.4 mg/kg. The number of different abnormalities, and their litter and fetal incidence was dose-related. Most abnormalities were skeletal malformations and variations (no ossification or incomplete ossification of various bones, and thickened, curved, or shortened bones). In the opinion of this Reviewer, the malformations and variations at 0.1 and 0.2 mg/kg were not related to maternal toxicity, since body weight and food consumption parameters were reduced by less than 10% during the dosing, i.e., the organogenesis period. The malformations and variations at 0.4 mg/kg, however, were possibly also related to maternal toxicity evidenced by reduced body weight and food consumption during the dosing period.

The skeletal abnormalities may be caused by the pharmacological action of the test compound, i.e., inhibition of bone resorption. The compound is likely to cross the placental barrier and bind to fetal bone where it can inhibit osteoclastic bone resorption and interfere with bone (re)modeling. The cause of the external and visceral abnormalities is unclear. Distribution studies in the rat have shown accumulation of the test compound not only in bone but also in soft tissues, and the presence of the compound in fetal tissues and its affinity for calcium may be related to the observed teratogenicity. Again, at the high dose of 0.4 mg/kg, maternal toxicity may also play a role in the occurrence of these abnormalities.

Conclusions:

- [redacted] 42446 was administered to rats subcutaneously at doses of 0.1, 0.2 and 0.4 mg/kg during gestation day 6 to day 20.

F_0 females

Maternal toxicity was indicated by a decreased food consumption and body weight gain at doses ≥ 0.2 mg/kg. At 0.2 mg/kg these effects were minimal and/or not statistically significant until after Gestation Day 16. At 0.4 mg/kg these effects were slight to moderate and statistically significant from Gestation Day 10. Increases in pre- and post-implantation loss and in late resorptions, and decreases in number of implantations and viable fetuses were noted at 0.4 mg/kg.

F_1 offspring

Body weight was decreased at doses ≥ 0.2 mg/kg. Some variations were noted at external and skeletal examinations at 0.1 mg/kg. Several malformations and variations were noted at external, visceral and skeletal examinations at 0.2 and 0.4 mg/kg. At 0.1 and 0.2 mg/kg, these abnormalities, including poor skeletal ossification, were most likely due to a fetal effect of the drug, while at 0.4 mg/kg they were possibly also due to maternal toxicity.

Based on this study, [redacted]42 446 was teratogenic in the rat at doses ≥ 0.2 mg/kg.

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Segment II (Teratology) Study in Rabbits by Subcutaneous Administration.
(from: IND review of 7-31-1997 by Dan Coleman, Ph.D.)

Purpose:
To evaluate the teratogenicity of zoledronate in rabbits dosed subcutaneously daily from day 7 to 20 (inclusive) of gestation.

Experimental design:

Testing Facility:	[redacted]
Report #:	936063
Study Initiated:	Nov. 18, 93.
Study Completed:	Sept. 23, 94.
Dose & Formulation:	0, 0.01, 0.03 & 0.1 mg/kg, SC, in 5% Water on days 7-20 after mating (inclusive).
Batch of drug:	Lot 16/015/1
GLP statement:	Included

Mated female New Zealand White rabbits were dosed daily by S.C. injection from day 7 to 20 after mating with the following doses of zoledronate:

Group:		Dose (mg/kg):		# of Animals:	
	1		0		20
	2		0.01		20
	3		0.03		20
	4		0.1		20

Data were recorded for mating, mortality, clinical signs, body weight, food consumption. Blood samples were taken for calcium measurements in two control animals (terminal kill), one 0.03 mg/kg animal (humane kill) and six 0.1 mg/kg animals (one terminal kill Day 29, three humane kill Days 24,25, and two aborted kill Days 25,28). Females were killed on Day 29 and necropsy was carried out of dams and fetuses. Pharmacokinetic measurements were not made.

Dose Selection:
Doses selected were based on range finding studies in non-pregnant rabbits (#936060) and in pregnant rabbits (#936061) described in Serial #28 (IND [redacted], and summarized here:
The non-pregnant rabbits were exposed to 0, 0.2, 0.6 and 2 mg/kg zoledronate. Weight loss (>10%) and injection site reactions (up to grade 4) were the major toxicities seen which made the high and mid doses unsuitable for use with pregnant rabbits.
In the dose range finding study with pregnant rabbits 0, 0.1, 0.2, and 0.4 mg/kg zoledronate were given S.C. daily from day 6 to 18, and animals were scheduled to be killed on day 29. High and mid doses were terminated early because of poor general condition, skin irritation at injection site, hypoactivity, decreased food and water consumption and weight loss. In the low dose group all fetuses appeared normal, but were light as expected due to maternal weight loss. The conclusion of these studies was that the low dose (0.1 mg/kg) should be the highest dose tested in the Segment II Study.

Results and discussion:
Clinical observations:
At the injection sites there was some moderate-slight reddening in all animals including controls. Only in treated animals was there some moderate-slight skin thickening.
Mortality:
Several (14/80) animals were killed early because of poor health or abortion (L,M,H;1,4,9). One was found dead in each the mid and high group. All animals killed early had a number of the following signs: lowered food consumption, weight loss, lethargy, body tremors, decreased respiration, loss of limb function, ataxia and marked hypocalcemia.

Observation:	Control	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg
initial #	20	20	20	20
not pregnant	1	1	3	2*
abort then killed	0	0	1	4
humane kill	0	1	2	4*
found dead	0	0	1	1
# Pregnant to term	19	18	13	10

* One animal killed early for humane reasons was found to be not pregnant.

Body Weight:
In all animals that survived to term, there was no effect on body weight.

Food Consumption:
In all animals that survived to term, there was no effect on food consumption.

Blood Chemistry:
Blood samples were taken from seven treated animals and two control animals, on the day of their death. All drug-treated animals were markedly hypocalcemic.

Gross Pathology:
No drug related changes were noted except increased skin thickening and bruising in all drug treated groups regardless of dose.

Litter observations:
Average number, weight and sex of fetuses was unaffected except at HD where there was a slight decrease in the number of viable fetuses (from 10.1 to 9.5) and an apparent increase in the number of total resorptions (from 4.7 to 9.5).

Observation:	Control	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg
# pregnant to term	19	18	13	10
# total resorptions	4.69	4.97	4.88	9.47
# viable fetuses	10.11	10.06	9.46	9.50
% postimplantation loss	3.79	5.66	5.14	7.64
mean fetal weight (g):	39	41	41	39

Fetal Alterations:
External:
One low dose fetus had an umbilical hernia, but this is probably not related to treatment since this effect was not seen at higher doses. Aberrations seen more than once:

	# of occurrences:			
Abnormality: Group (n)	C (183)	L (172)	M (117)	H (86)
carpal flexure	0	5	1	1
placenta hemorrhagic	0	0	2	0

Visceral:
No treatment related findings. Aberrations seen more than once:

	# of occurrences:			
Abnormality: Group (n)	C (183)	L (172)	M (117)	H (86)
extra blood vessel	1	1	2	3
blood in stomach	0	0	0	2
stomach distended	0	0	1	2
kidney displaced	1	2	0	0
bladder hemorrhagic	3	0	1	0

gall bladder hemorrhagic	2	3	1	1
gall bladder enlarged	2	2	1	3
gall bladder reduced	0	4	0	0

Head:
No treatment related findings. Aberrations seen more than once:

	# of occurrences:			
Abnormality: Group (n)	C (183)	L (172)	M (117)	H (86)
nasal cavities dilated	5	4	5	3
lateral ventricles dilated	1	0	0	1
hemorrhage in olfactory lobes	1	2	2	0
folded retina	2	1	0	1

Skeletal:
No treatment related findings. Aberrations seen more in a drug treated group than in control:

	# of occurrences:			
Abnormality: Group (n)	C (183)	L (172)	M (117)	H (86)
interparietal bone incompletely ossified	1	0	3	0
median phalange not ossified	0	0	0	1

Individual incidence of > 60 types of malformations were reported. The distribution and incidence did not suggest a relationship to treatment. There was also no effect on the total incidence of all malformations.

Conclusions:
Mortality or humane kill of pregnant dams were noted in all dose groups (doses ≥ 0.01 mg/kg). Signs associated with mortality were weight loss, lethargy, tremors, decreased respiration, loss of limb function and ataxia. Signs were indicative of hypocalcemia. Abortions occurred at doses ≥ 0.03 mg/kg. Marked hypocalcemia was noted in animals that died after abortion or humane kill at 0.03 and 0.1 mg/kg. Thus, maternal toxicity including mortality was noted at all doses tested.
There were no increases in the incidence of fetal malformations at any dose tested ≥ 0.01 mg/kg, even at the highest dose of 0.1 mg/kg at which several animals aborted or were killed moribund. The findings indicate that zoledronate is not teratogenic in rabbits.
Effects of drug treatment on parturition were not evaluated.

Reproductive Toxicology Summary

Segment I

Rat

A fertility and reproductive toxicity study was carried out in rats. The study was conducted with subcutaneous doses of 0.01, 0.03, and 0.1 mg/kg/day. In this study, many females died or were sacrificed in moribund condition at or around the time of parturition due to difficulty in delivery (dystocia). This effect occurred at doses as low as 0.01 mg/kg/day. It was most likely due to a zoledronate-induced inhibition of Ca mobilization from the skeleton, i.e., inhibition of osteoclastic bone resorption, and hypocalcemia. This results in insufficient supply of calcium needed at the time of parturition for uterine contractions. The deaths lead to early termination of this study on lactation day 7. The effect on parturition has also been observed in reproductive toxicity studies with other bisphosphonates (alendronate, risedronate).
Other reprotoxic effects observed in this study were a decrease in pregnancy rate (fertility index) at 0.1 mg/kg, a decrease in number of corpora lutea, implants and live fetuses and an increase in % preimplantation loss at doses ≥ 0.03 mg/kg, and an increase in % stillbirths and decrease in the number of viable newborns at doses ≥ 0.01 mg/kg.

Segment II

Rat

A rat study conducted with subcutaneous doses of 0.1, 0.2 and 0.4 mg/kg/day showed dose-related teratogenicity at doses ≥ 0.1 mg/kg/day. One external and a few skeletal variations were seen at 0.1 mg/kg, one external, a few visceral and several skeletal malformations and variations were seen at 0.2 and a few external, several visceral and several skeletal malformations and variations were seen 0.4 mg/kg. The litter and fetal incidences of the abnormalities were dose-related. Most abnormalities were skeletal malformations and variations (no ossification or incomplete ossification of various bones and thickened, curved, or shortened bones). The malformations and variations at 0.1 and 0.2 mg/kg were probably not related to maternal toxicity, since body weight and food consumption parameters were reduced by less than 10% during the dosing, i.e., the organogenesis period. The malformations and variations at 0.4 mg/kg, however, were possibly also related to maternal toxicity evidenced by reduced body weight and food consumption during the dosing period. Fetal body weight was also decreased in the 0.2 and 0.4 mg/kg groups.
The skeletal malformations may be due to binding of zoledronate to fetal bone and interference with bone modeling and remodeling. Like pamidronate, another bisphosphonate, zoledronate is expected to readily cross the placental barrier and to be taken up into the developing fetal skeleton.

Rabbit

In a rabbit study, carried out with subcutaneous doses of 0.01, 0.03 and 0.1 mg/kg/day, no teratological or embryo/fetal effects were observed at doses up to 0.1 mg/kg. However, maternal toxicity, i.e., mortality and hypocalcemia were observed at doses ≥ 0.01 mg/kg.

Calculation of dose and exposure multiples

Plasma exposures in rats treated with single or 3-month repeated i.v. or s.c. doses have been calculated for the dose range of 0.1-0.6 mg/kg (see ADME section of this NDA review). Exposure (AUC_{0-24h}) multiples in animals of the expected clinical exposure were shown to be approximately 5 times larger than multiples based on dose (mg/m^2). Since exposure multiples more accurately reflect relative drug exposure, this Reviewer recommends that, in the label, the rat doses at which reproductive toxicity was observed, although ranging from a dose smaller than 0.1 mg/kg (0.03 - 0.4 mg/kg) are given as human exposure multiples. For rabbits, exposures and exposure multiples have not been determined. Thus, for the rabbit findings to be mentioned in the label doses can be expressed as multiples based on dose (mg/m^2).

Exposure multiples in the reproductive rat toxicity studies as compared to human exposure after a single 8 mg i.v. dose

Species	Toxicity study	Dose (mg/kg)	Exposure ratio [AUC(0-24h, animal/ AUC (0-24h, human, 8 mg)*]
Rat	Segment I	0.01, 0.03, 0.1	0.06x, 0.18x, 0.6x
	Segment II	0.1, 0.2, 0.4	0.6x, 1.2x, 2.4x

* Human AUC(0-24h) after 8 mg i.v. dose = 1133 ngxh/ml

Dose multiples in the reproductive rabbit toxicity studies as compared to the human 8-mg dose (basis mg/m^2)

Species	Toxicity study	Dose (mg/kg)	Dose multiple
Rabbit	Segment II	0.01, 0.03, 0.1	0.03x, 0.08x, 0.25x

Labeling comments
See Labeling Review

ADME

Pharmacokinetics Summary

Upon i.v. administration, zoledronate is rapidly eliminated from the circulation, mainly by renal excretion. Results from an i.v. disposition study in the rat showed that after a single radioactive i.v. dose (0.15 mg/kg) the plasma level of radioactivity after 48h was about 0.001 times the level after 5 minutes. A major part of the radioactivity was taken up by the skeleton where it was retained for a long time. After 12 months, about 40% of the dose was still in the skeleton. Radioactivity was also detected in the following soft tissues, in order of magnitude: bone marrow >> kidney >> spleen, liver, thyroid > stomach, small intestine, adrenal, skin > aorta, heart, thymus, lung, heart > brain, fat, muscle. Tissue distribution was similar in the dog.

Elimination from soft tissues was fast but slower than from plasma: 96h after a single i.v. dose in the rat the concentration in soft tissues was on average 1/60 times the concentration after 5 minutes. However, in many tissues, except white fat, aorta, brain and nerve tissue which did not retain the compound, tissue levels after 96h were still 30%-100% of the levels after 24h (e.g., in thymus, thyroid, kidney, lung, heart, liver, pancreas, spleen, adrenal, lymph nodes, testis, muscle, bone marrow, stomach, intestine, skin). Soft tissue levels were two orders of magnitude lower than those in bone.

When i.v. doses (0.15 mg/kg) were given to rats, daily for 16 days, the uptake in bone was approximately proportional to cumulative dose. Although soft tissue levels were much lower than those in bone, accumulation in soft tissues occurred and was marked, i.e., 9-fold after 16 days as compared to 1 day. After termination of repeated dosing, bone levels did not decline until after ca. 240 days, while soft tissue levels declined very slowly with apparent terminal half-lives of 150-200 days.

In a kinetics and metabolism study in rats, radioactivity and unchanged drug in plasma and urine were assayed by LSC and RIA, respectively. HPLC analysis of the urine was also carried. The dose given in this study was a single i.v. or s.c. dose of 0.6 mg/kg, or a single i.v. dose of 0.16 mg/kg. It was shown that plasma AUC levels of unchanged drug after s.c. administration (0.6 mg/kg) were about the same as those after i.v. dosing (0.6 mg/kg). T_{max} after s.c. was larger than after i.v. administration (ca. 15 minutes vs. ≤ 5 minutes). There were virtually no metabolites present in plasma and urine after an i.v. dose of 0.16 mg/kg. From this study with 0.6 mg/kg and 0.16 mg/kg, and from another single dose s.c. study with 0.1 mg/kg, and from the 16-day i.v study with 0.15 mg/kg it was concluded that systemic exposure is proportional to dose within the i.v. dose range of 0.1 to 0.6 mg/kg.

PK parameters of zoledronate after a single i.v. or s.c. dose of 0.6 mg/kg

Dose	Route	Dosing	Treatment Day	N	Cmax (ng/ml)	AUC (ngxh/ml)
0.16 mg/kg	i.v.	Single	1	3	1449 (@5min)	(1192)* (0-24h)
0.6 mg/kg	i.v.	Single	1	3	4784 (@5 min)	3936 (0-24h)
0.6 mg/kg	s.c.	Single	1	3	2105 (@15-30min)	3917 (0-24h)
0.15 mg/kg	i.v.	16 days	1	3m	850-1600 (@5min)	600-1000 (5min-96h)
0.1 mg/kg	s.c.	Single	1	3m	200-410 (@15min)	350-610 (5min-120h)

*extrapolated value

Data from a single s.c. dose study in male rats confirmed that s.c. doses are taken up rapidly and completely from the application site into the system. Once taken up systemically, disposition and elimination of the s.c. dose is similar as of an i.v. dose. The major part of the dose (50-65%) was taken up in bone, 25-28% was rapidly excreted mainly in the urine, and some (2.4-6.5%) was excreted in the feces. Soft tissues retained 1.8-2.1% of the dose.
Disposition of zoledronate in the dog was comparable to that observed in the rat.

In all species, the fraction of zoledronate taken up by red blood cells was small (<10%) and independent of concentration within the tested range. In the rat, at 37°C, zoledronate is highly bound to protein (96.4%). In dog and human plasma binding is much lower (8.7% and 22%). The species dependence of protein binding has also been observed with alendronate.

There are no data on distribution of zoledronate in pregnant animals. Sponsor assumes that like pamidronate, zoledronate can cross the placental barrier and be taken up into fetal bone. It is also assumed that zoledronate can be excreted in milk, although it would be bound to calcium in the milk to a large extent.

Zoledronate did not appear to act as a reversible or irreversible inhibitor of the P450 enzymes, CYP1A2, CYP 2A6, CYP 2B6, CYP 2C9, CYP 2C19, CYP 2D6, CYP 2E1, CYP 3A4/5, or CYP 4A9/11.

Toxicokinetics Summary

Plasma concentrations of zoledronate were determined in a 3-month s.c. toxicity study in rats, at 0 and 0.1 mg/kg/day. Zoledronate was not detected in the controls. Levels at 1 hr post dose are given in the following Table.

Plasma levels at 1hr post dose (0.1 mg/kg/day) in a 3-month s.c. rat toxicity study

Week	Plasma level (ng/ml)	Plasma level (ng/ml)
	Males	Females
1	247	276
6	396	303
13	332	322

Exposure multiples in rat i.v. and s.c. studies

Data from the single dose s.c. and i.v. study using 0.6 mg/kg (C_{max} and AUC) were used to calculate plasma exposure (AUC) in rats after chronic s.c. dose administration. The AUC values in the 0.01-0.6 mg/kg range were extrapolated assuming dose proportionality, and assuming no difference in AUC between single and multiple dosing regimens. The former was supported by the results from PK studies at doses between 0.1 and 0.6 mg/kg discussed above, and the latter was supported by the similarity in C_{pl} values at 1, 6 and 13 weeks at 0.1 mg/kg/day in the 3-month s.c. toxicity study. The ratio of the AUC(0-24h) in the rats and the AUC(0-24h) in humans after an 8 mg single i.v. dose was then calculated. The results are shown in the following Tables.

Exposure multiples in rat and dog in repeated dose toxicity studies as compared to humans after a single 8 mg i.v. dose

Species	Study	Dose (mg/kg)	AUC (ngxh/ml)	Exposure ratio [AUC(0-24h, animal/ AUC (0-24h, human, 8 mg)*]
Rat	Single dose, s.c.	0.1	350-610	0.3-0.5x
Rat	16 days, i.v.	0.15	600-1000	0.5-0.9x
Rat	Single dose, i.v.	0.6	3936	3.5x
Rat	Single dose, s.c.	0.6	3917	3.5x
Rat	3 months, s.c.	0.1*	-	0.6x [b]
Rat	26/52-weeks, s.c.	0.01*	-	0.06x [b]
Dog	3-month, i.v.	0.2*	1760-2112	1.6-1.8x
Dog	26/52-week, i.v.	0.1**	1304-1852	1.2-1.6x

* dosing once daily
** dosing every second day for 112 days, and every third day thereafter
*** dosing every third day
[a] Human AUC(0-24h) after 8 mg i.v. dose = 1133 ngxh/ml
[b] AUC (0-24) in the rat was extrapolated from the AUC measured after a single 0.6 mg/kg s.c. dose assuming dose proportionality

In humans, values for C_{plasma} (5 min) and AUC_{plasma} (0-24h) were determined after doses of 2, 4, and 8 mg (5 minute i.v. infusions):

PK parameters of zoledronate after a single i.v. dose in humans

Dose	Route	Treatment Day	N	Cpl (5 min) (ng/ml)	AUC (0-24h) (ngxh/ml)
2 mg	i.v.	1	3	453	344
4 mg	i.v.	1	3	668	540
8 mg	i.v.	1	3	1142	1133

This Reviewer recommends that in the label exposure multiples are used for the description of the rat reproductive toxicity findings in the 0.03-0.6 mg/kg dose range, assuming dose-AUC proportionality in this dose range.
At an s.c. dose of 0.1 mg/kg/day in the rat, which was a dose used in the rat reprotoxicity study, the exposure multiple in rats vs. humans is approximately 0.6x for the human 8 mg dose. On the basis of dose per body surface area (mg/m^2) the animal dose multiple at the 0.1 mg/kg s.c. rat dose of the 8 mg i.v. human dose (8mg/60 kg= 0.133mg/kg) is 0.125x (0.02:6:0.133). Thus, the exposure multiple (0.6x) is ca. 5 times the dose multiple based on mg/m^2 dose comparison (0.125x). Similar results are obtained for the 0.2 mg/kg and 0.4 mg/kg rat doses.

Exposure multiples in the 2-year rat oral carcinogenicity study
At an oral dose of 2 mg/kg/day in the rat, which was the high dose used in the rat carcinogenicity study, assuming 1% bioavailability and dose-AUC proportionality, the exposure multiple in rats vs. humans is approximately 0.12x for the human 8 mg dose. On the basis of dose per body surface area (mg/m^2) the animal dose multiple at the 2 mg/kg oral rat dose of the 8-mg human dose (8mg/60 kg= 0.133mg/kg) is 0.025x (0.02:6:0.133). Thus, the exposure multiple (0.12x) is ca. 5 times the dose multiple based on mg/m^2 dose comparison (0.025x).

Exposure multiples in the dog
From the results of PK and TK studies in the dog, it can be concluded that the AUC in dogs is not obviously proportional to dose. Therefore, for the (short-term) dog studies that were carried out at relatively high doses >0.2 mg/kg exposure multiples can not be established.

SUMMARY AND EVALUATION

The current application (NDA 21,223) is for the use of the Zometa™ (zoledronic acid for injection) for the treatment of tumor-induced hypercalcemia. The compound is a bisphosphonate which inhibits bone resorption and skeletal calcium mobilization. Zoledronic acid is to be administered as a single dose intravenous infusion over 5 minutes. The recommended dose is 4 mg. For retreatment a dose of 8 mg is recommended, given as single dose infusion over 5 minutes. Pharmacology and toxicology studies submitted and reviewed for this application include *in vitro* and *in vivo* non-clinical pharmacology studies, toxicity studies in rats and dogs, oral carcinogenicity studies in rats and mice, genotoxicity studies, reproductive toxicity studies in rats and rabbits, and studies on distribution and excretion in rats and dogs.

Pharmacology

Zoledronate inhibits bone resorption *in vitro* and *in vivo*, at concentrations that do not affect bone mineralization. Zoledronate appears to be a much more potent resorption inhibitor than etidronate, clodronate and pamidronate. The inhibition of bone resorption is evidenced *in vitro* and *in vivo* as a reduction of calcium release from bone stimulated by calcemic hormones such as $VitD_3$ and PTHrP. *In vivo* zoledronate inhibits the prevention of bone loss, and the increased bone turnover caused by ovariectomy in animal models of postmenopausal osteoporosis. The bone resorption effect of zoledronate appears at least partly to be caused by an inhibition of osteoclastogenesis and induction of osteoclast apoptosis. Additional pharmacological effects of zoledronate described in the NDA include induction of apoptosis of myeloma cells and *in vitro* inhibition of their proliferation, *in vitro* inhibition of tumor cell invasion into extracellular matrix, and *in vitro* and *in vivo* inhibition of angiogenesis. The exact mechanism of action of zoledronate is unknown. Apart from the renal toxicity discussed below, no adverse pharmacological effects of zoledronate were reported.

Renal effects

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 in a time- and dose-dependent manner. Pamidronate was 2-3 fold more potent than zoledronate. The NOAEL for the effect of zoledronate was <1.5 mg/kg (equivalent to <2x the maximum recommended human 8-mg i.v dose, on the basis of body surface area, mg/m^2), and the LOAEL was 1.5 mg/kg (equivalent to 2x the maximum recommended human 8-mg dose, on the basis of body surface area, mg/m^2). For the 4-mg human dose the dose multiples are twice as large. These low dose multiples indicate a small safety margin for zoledronate for clinical renal adverse events in the intended 4-mg or 8-mg single dose treatment regimen (see Table in General Toxicity Section below).

In rats given 9 s.c. doses of 0.01, 0.1 or 1 mg/kg zoledronate or pamidronate over a 10-day period (daily equivalents of 0.6-6 times the maximum human exposure), zoledronate caused a dose-dependent increase in the daily urinary excretion of malate dehydrogenase (MDH), an early marker of renal damage, starting after 8 doses. The cumulative excretion of MDH over the 10-day dosing period was, however, twice as large for pamidronate as for zoledronate.
The NOAEL for the effect of zoledronate was 0.01 mg/kg, which represents a dose multiple of 0.013x the maximum recommended human 8-mg dose (basis mg/m^2), and an exposure multiple of 0.06x the maximum human dose. The LOAEL level of 0.1 mg/kg represents a dose multiple of 0.13x the maximum recommended human 8-mg dose (basis mg/m^2), and an exposure multiple of 0.6x the maximum 8-mg human dose. For the 4-mg human dose the dose multiples are twice as large. Although interpretation of NOAEL and LOAEL levels from a 10-day repeat-dose study is difficult for a single dose clinical treatment the very low multiples again suggest potential nephrotoxicity of zoledronate at the intended human i.v. dose for the current indication. The data, however, do not permit conclusions as to the comparative effects of pamidronate and zoledronate.

General Toxicology

A vast number of toxicology studies were carried out in rats and dogs by the intravenous (i.v.), subcutaneous (s.c.) or oral route, varying in duration from single dose to 6 or 12 months. In i.v. and s.c. studies a common finding was local irritation at the injection site, which occurred in studies of daily dosing lasting 10 days or more. Also, in all studies, regardless of dosing route, pharmacological bone changes of nonproliferative hyperostosis, extension of the primary spongiosa and metaphyseal widening were observed after repeated dosing for 10 days or more.

In general, most toxicities were seen in both rats and dogs and occurred regardless of dosing route. Also, in most cases, toxicities such as renal and GI tract lesions that occurred at relatively high doses in the short term studies were reproduced in the longer term repeat-dose studies at lower dose levels.

The main findings observed in part or all of the toxicity studies included reductions in body weight and food consumption, reductions in red cell parameters, leukocytosis, increases in fibrinogen and (A)PTT, increases in serum AST, ALT, and CK, increases or decreases in serum ALP, decreases in serum Ca and P, decrease in serum albumin and increase in globulin, organ weight changes (kidney, spleen and thymus and adrenal weight increase, liver weight decrease), and a variety of histopathological changes. These included renal lesions, GI lesions, liver changes, adrenal gland lesions, thymus and spleen lesions, lymph node changes, and lung and tracheal inflammation in both rats and dogs, and genital atrophy and degeneration in dogs. Liver lesions including congestion, inflammation, necrosis and hemorrhage were predominantly seen in oral toxicity studies in dogs.

The organ toxicities that occurred in both rats and dogs as early as after one single i.v. dose were renal, stomach, GI tract, liver, lung and thymus lesions. Most other organ lesions mentioned above occurred only in one species or after more than 10 days of repeated dosing. Thus, the main target organs were kidney, GI tract, liver, thymus and lung. Renal toxicity, reflected in serum urea and creatinine levels and microscopic tubular changes of de- and regeneration, vacuolation, dilatation, necrosis and tubular casts was observed in single and/or repeated dose studies, in both rats and dogs, using all dose administration routes. Gastrointestinal irritation evident as distension, inflammation, hemorrhage, erosion, necrosis and edema was seen in oral repeat-dose studies of 10 days or more, in both rats and dogs. However, GI toxicity (hemorrhage, discoloration, inflammation, ulceration, edema) was also observed in rats and dogs dosed for 1 or 10 days via the i.v. route.

Macroscopic and/or microscopic kidney lesions were observed in both single dose i.v. studies in rats, and GI tract lesions were seen in one of the two single dose i.v. rat studies and the single dose i.v. study in dogs. Since the renal and GI tract pathology findings were obtained in single dose i.v. studies in both animal species they are most relevant for the intended single i.v. dose clinical treatment regimen. For that reason, and also because renal and sometimes GI events are a clinical safety concern upon short term infusion of bisphosphonates, the renal and GI toxicities in the single dose rat and dog i.v. studies and the associated NOAEL and LOAEL levels are discussed below in more detail. Renal toxicity and its adverse effect levels have also been discussed in the pharmacology summary above. The meaning of the low dose multiples of the NOAEL and LOAEL levels of toxicities observed in the repeated dose studies is unclear, since the multiples are based on comparison of the doses used in these repeat-dose studies with the intended single, and therefore relatively large, human dose.

Renal and GI effects in acute single dose i.v. rat and dog studies

In the first acute i.v. rat study (doses 0.6, 6, 30, 60, 80 mg/kg) the NOAEL for renal findings was 0.6 mg/kg. This represents 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 represents a dose multiple of 7.5 x the maximum human dose. Since at this LOAEL the severity of the renal pathology findings (enlargement and paleness, tubular regeneration, dilation (in the cortex) and desquamation (in the outer medulla), and inflammation and/or fibrosis of the outer

medullary interstitium) was 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. GI toxicity was not reported in this study.

In the second acute i.v. rat study (doses 1.6, 8, 16, 32 mg/kg) the NOAEL and LOAEL for renal gross pathology findings (paleness, red foci) 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 (hemorrhage in stomach, small intestine and abdominal cavity) were also 1.6 and 8 mg/kg, respectively, i.e., dose multiples of 2x and 10x the maximum recommended 8-mg human dose. Thus, on the basis of the data from this study the safety margin for both renal and GI toxicity is 10-fold.

In the dog acute i.v. toxicity study (doses 2 and 10 mg/kg, 1 male/dose group), the NOAEL and LOAEL values for GI toxicity (reddened stomach mucosa, intestinal hemorrhage) were 2 and 10 mg/kg, respectively. These doses represent dose multiples of 7.5x and 38x the maximum recommended 8-mg human dose. Thus, on the basis of the data from this study the safety margin for GI toxicity is 38-fold. Renal findings were not reported in this study.

STUDY		Renal effects		GI effects	
		NOAEL	LOAEL	NOAEL	LOAEL
Single i.v. dose rat pharmacology study	Dose	<1.5 mg/kg	1.5 mg/kg	-	-
	Multiple of 8-mg human dose	<2x	2x		
Acute i.v. rat toxicity study (1)	Dose	0.6 mg/kg	6 mg/kg	-	-
	Multiple of 8-mg human dose	0.75x	7.5x		
Acute i.v rat toxicity study (2)	Dose	1.6 mg/kg	8 mg/kg	1.6 mg/kg	8 mg/kg
	Multiple of 8-mg human dose	2x	10x	2x	10x
Acute i.v. dog toxicity study	Dose	-	-	2 mg/kg	10 mg/kg
	Multiple of 8-mg human dose			7.5x	38x

In conclusion, the NOAEL levels and the LOAEL levels and associated safety margins for renal lesions in the acute, single dose rat i.v. toxicity studies suggest that renal events may constitute an important clinical safety concern. The GI tract lesions in rat and dog i.v. toxicity studies and the associated NOAEL and LOAEL levels and safety margins suggest that GI events are of lesser clinical concern.

Carcinogenicity

Carcinogenicity studies of 104-week duration were carried out in rats and mice. The oral route was chosen because the compound causes marked injection site irritation. The studies were discussed with the Executive CAC on May 23, 2000, and the Meeting Minutes including the Committee's recommendations are appended to this NDA Review (APPENDIX III).

RAT

For the rat study dose selection was based on the results from a 6-month oral toxicity study. The Executive CAC concluded that the high dose selection (2 mg/kg) was not adequate and the 2 mg/kg dose appeared to be below the MTD. At this dose, mortality was not affected and little toxicity was observed. Body weight and food consumption were only 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 slightly affected in all dose groups.

Neoplastic findings

Significant neoplastic histopathology findings (incidence in all animals)

FEMALES		Dose (mg/kg/day)				p-value trend test (Sponsor)
		0	0.1	0.5	2.0	
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 (according to both Sponsor's and CDER's Statistical Reviewer's analysis), the increased incidence in the combination of uterine polyp and endometrial stromal sarcoma was statistically significant according to the Sponsor's trend test.

In the opinion of this Reviewer, comparison of the incidence of the uterine neoplasms with historical control values, and the dose-relatedness of the combined tumor effect, suggests that the increased incidence in uterine neoplasms was treatment-related. However, since the high dose appeared to be below the MTD, the Executive CAC judged the study to be inconclusive and recommended that the results should not be mentioned in the label, at least not for the single dose indication of the current NDA. If, however, future evidence becomes available that the MTD was reached at 2 mg/kg, mention of the uterine finding in the label shall be reconsidered.

MOUSE

For the mouse study dose selection was based on a 3-month pilot oral toxicity study, and the high dose selected was 1 mg/kg. Since mortality and adverse signs were observed in males at 0.3 mg/kg and in females at 3 mg/kg in the 3-month study, the Executive CAC concluded that the high dose selection of 1 mg/kg for the carcinogenicity study was adequate for males and females. In the carcinogenicity study mortality appeared to be decreased in high dose males and was otherwise not affected. Body weight was slightly reduced and body weight gain was slightly to moderately 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.

Neoplastic findings

Neoplastic histopathology findings (incidence in all animals)

		MALES					FEMALES				
		Dose (mg/kg/day)				p-value trend test (Sponsor)	Dose (mg/kg/day)				p-value trend test (Sponsor)
		0	0.1	0.3	1.0		0	0.1	0.3	1.0	
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/ Adenocarcinoma comb	3	9	4	10	0.089					

There was an increase in the incidence of Harderian gland adenoma in males and females. The combined incidence of Harderian adenoma and adenocarcinoma in males was also increased. The individual and combined Harderian gland tumor findings were not statistically significant according to either the Sponsor's or CDER's Statistical Reviewer's analysis.

The executive CAC concluded, however, that the Harderian gland tumor finding was drug-related and biologically significant, since it occurred in both sexes, was at the limit of the historical control range in high dose males and outside the historical control range in mid and high dose females,

respectively, and was increased above concurrent control values in low and high dose males and in mid and high dose females. Therefore mention of the finding in the label was recommended. In a 92-week oral mouse carcinogenicity study with alendronate, a related bisphosphonate, a significant increase in the incidence of Harderian gland adenoma was also observed in the high dose females (10 mg/kg/day).

Calculation of exposure and dose multiples

Plasma exposures in rats treated with single i.v. or s.c. doses or 3-month repeated s.c. doses were measured and/or calculated (see ADME section of this NDA review). For rats, multiples of the clinical exposure at the intended single i.v. human dose of 4-8 mg (AUC_{0-24h}) were approximately five times larger than multiples based on dose (mg/m^2) comparison. For mice, exposures and exposure multiples were not determined. Thus, mouse doses in the label can be translated into multiples based on dose (mg/m^2) comparison. For calculation of the exposure and dose multiples for the oral carcinogenicity studies it will be assumed that the oral bioavailability of the drug is 1%.

Exposure and dose multiples in the oral rat carcinogenicity study as compared to human exposure after a single 8 mg i.v. dose

Species	Oral dose (mg/kg)	Equivalent i.v. or s.c. dose (mg/kg)	Exposure multiple [AUC(0-24h, animal/ AUC (0-24h, human, 8 mg)[a]]	Dose multiple
Rat	0.1, 0.5, 2.0	0.001, 0.005, 0.02	0.006x, 0.03x, 0.125x	0.001x, 0.006x, 0.025x

[a] Human AUC(0-24h) after 8 mg i.v. dose = 1133 ngxh/ml

Dose multiples in the oral mouse carcinogenicity study as compared to the human 8-mg dose (basis mg/m^2)

Species	Dose (mg/kg)	Equivalent i.v. or s.c. dose (mg/kg)	Dose multiple
Mouse	0.1, 0.3, 1.0	0.0001, 0.003, 0.01	0.0006x, 0.002x, 0.006x

The very low dose and exposure multiples in the tables shown above are related to the fact that they are based on doses given daily in 2-year bioassays translated into multiples of a realtively large human dose that is to be given as a single intravenous infusion. The meaning of these low multiples is therefore unclear.

Genetic Toxicology

The mutagenicity of — 42446B was examined in three different Ames tests with Salmonella strains TA 98, TA100, TA 102, TA1535 and TA1537, and E.coli (WP2uvrA). None of the tested concentrations of — 42446 led to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants when compared to the negative control, either in the absence or in the presence of metabolic activation. Thus, all three Ames tests were negative.

The clastogenicity of —42446 was tested using Chinese Hamster Ovary (CHO) cells *in vitro*, with and without metabolic activation (S9). In the original study, there were statistically significant increases in the % of cells with structural aberrations, in the absence and in the presence of metabolic activation, with an 18-hr treatment period. Although the values were within historical control range, this Reviewer considered the results positive based on dose-relatedness and extent of the response. The positive findings were not reproduced in a confirmatory study with 18-hr or 42-hr treatment periods. Since the original clastogenicity study was positive and the confirmatory study was negative, this Reviewer concludes that the results of the CHO cell clastogenicity assay were equivocal, both in the absence and the presence of metabolic activation.

The mutagenicity of — 42446 was also tested in the *in vitro* mammalian Chinese Hamster V79 cell mutagenicity assay. In this test, no significant increase in the frequency of mutations was observed at any concentration of — 42446 in comparison to the negative control, in the absence and presence of metabolic activation. The slight increase in mutation frequency in the confirmatory experiment with activation did not show concentration dependence and was of insufficient magnitude to be considered a positive response. Thus, 42446 was not

mutagenic at the hgprt locus in V79 Chinese Hamster cells in the absence or presence of metabolic activation.

The clastogenicity of [redacted] 42446 was also examined in an *in vivo* rat micronucleus assay. [redacted] 42446 was not clastogenic in this assay under the conditions described. Appropriate exposure was demonstrated by testing at >60% of the LD_{50}.

In conclusion, the results of the three *in vitro* bacterial reverse mutation (Ames) tests, the *in vitro* mutagenicity test in Chinese Hamster cell V79 cells, and the *in vivo* rat micronucleus assay were negative, with and without metabolic activation. The results of the *in vitro* clastogenicity test using Chinese Hamster Ovary cells were equivocal, with and without metabolic activation.

Reproductive Toxicology

Segment I

Rat

A fertility and reproductive rat toxicity study was conducted with subcutaneous doses of 0.01, 0.03, and 0.1 mg/kg/day. Part of the females were sacrificed on gestation day 13, and part of them were dosed throughout gestation, parturition and lactation. In this study, many females died or were sacrificed in moribund condition at or around the time of parturition due to difficulty in delivery (dystocia). This effect occurred at doses as low as 0.01 mg/kg/day. It was most likely due to a zoledronate-induced inhibition of calcium mobilization from the skeleton, through inhibition of osteoclastic bone resorption resulting in hypocalcemia. The insufficient supply of calcium, which is needed at the time of parturition for uterine contractions, presumably lead to the observed dystocia. Periparturient moribundity and mortality have also been observed in reproductive toxicity studies with other bisphosphonates (alendronate, risedronate).
Other reprotoxic effects were a decrease in pregnancy rate (fertility index) at a dose of 0.1mg/kg, a decrease in number of corpora lutea, implants and live fetuses and an increase in % preimplantation loss at doses ≥ 0.03 mg/kg in females sacrificed on gestation day 13, and an increase in % stillbirths and decrease in the number of viable newborns at doses ≥ 0.01 mg/kg in females allowed to deliver.

Segment II

Rat

A rat study conducted with subcutaneous doses of 0.1, 0.2 and 0.4 mg/kg/day showed dose-related teratogenicity at doses ≥ 0.1 mg/kg/day. One external and a few skeletal variations were seen at 0.1 mg/kg, one external, a few visceral and several skeletal malformations and variations were seen at 0.2 and a few external, several visceral and several skeletal malformations and variations were seen 0.4 mg/kg. The litter and fetal incidences of the abnormalities were dose-related. Most abnormalities were skeletal malformations and variations (thickened, curved, or shortened bones, or no ossification or incomplete ossification of various bones). The malformations and variations at 0.1 and 0.2 mg/kg were probably not related to maternal toxicity, since body weight and food consumption parameters were reduced by less than 10% during the dosing, i.e., the organogenesis period. The malformations and variations at 0.4 mg/kg, however, were possibly also related to maternal toxicity evidenced by reduced body weight and food consumption during the dosing period. Fetal body weight was also decreased in the 0.2 and 0.4 mg/kg groups.
The skeletal malformations may be due to binding of zoledronate to fetal bone and interference with bone modeling and remodeling. Like pamidronate, another bisphosphonate, zoledronate is expected to readily cross the placental barrier and to be taken up into the developing fetal skeleton.

Rabbit

In a rabbit study, carried out with subcutaneous doses of 0.01, 0.03 and 0.1 mg/kg/day, no teratological or embryo/fetal effects were observed at doses up to 0.1 mg/kg. However, maternal toxicity, i.e., mortality and hypocalcemia were observed at doses ≥ 0.01 mg/kg.

Calculation of dose and exposure multiples

Plasma exposures in rats treated with single i.v. or s.c doses or 3-month repeated s.c. doses were measured and/or calculated for the 0.1-0.6 mg/kg dose range. For rats, multiples of the clinical exposure at the intended single i.v. human dose of 4-8 mg (AUC_{0-24h}) were approximately fives times larger than multiples based on dose (mg/m^2) comparison. Since exposure multiples more accurately reflect relative drug exposure, this Reviewer recommends that in the label rat doses in the 0.03-0.6 mg/kg range, at which toxicity was observed, are expressed as animal:human exposure multiples, based on the maximum recommended human dose of 8 mg. For rabbits exposures and exposure multiples have not been determined. Thus, rabbit doses in the label can be translated into multiples on the basis of dose (mg/m^2) comparison.

Exposure multiples in the reproductive rat toxicity studies as compared to human exposure after a single 8 mg i.v. dose

Species	Toxicity study	Dose (mg/kg)	Exposure multiple [AUC(0-24h, animal/ AUC (0-24h, human, 8 mg)*]
Rat	Segment I	0.01, 0.03, 0.1	0.06x, 0.18x, 0.6x
	Segment II	0.1, 0.2, 0.4	0.6x, 1.2x, 2.4x

* Human AUC(0-24h) after 8 mg i.v. dose = 1133 ngxh/ml

Dose multiples in the reproductive rabbit toxicity studies as compared to the human 8-mg dose

Species	Toxicity study	Dose (mg/kg)	Dose multiple (basis body surface area, mg/m^2)
Rabbit	Segment II	0.01, 0.03, 0.1	0.03x, 0.08x, 0.25x

ADME

Preclinical distribution and elimination studies showed that, similar to other bisphosphonates, zoledronate is rapidly eliminated from the circulation mainly by renal excretion, and that a major part of the compound in the systemic circulation is bound to bone in a cumulative fashion. However, drug is also taken up and retained in many soft tissues, including thymus, kidney, lung, heart, liver, stomach, small intestine, pancreas, spleen, adrenal, lymph nodes, thyroid, testis and bone marrow. Upon single i.v. or s.c. dose administration T_{max} values are <5 minutes and approximately 15 minutes, respectively. Exposure, i.e., plasma AUC, is the same upon i.v. and s.c. dosing. C_{plasma} and AUC values appear to be dose-proportional in both single and repeated-dose studies.

Toxicokinetic data were obtained from a 3-month s.c. study in rat, and from 3-month and 6/12-month i.v. studies in dogs. In the 3-month s.c. rat study only plasma levels were measured in weeks 1,6, and 13 of the study at 1hr post dose. No AUC's were determined. Comparison with the data from single dose studies suggests that upon repeated dosing plasma levels and exposure values are the same as upon single dosing. In the dog toxicity studies both C_{plasma} and AUC values were determined.

Exposure or dose multiples of animal vs. human exposure or dose (mg/m^2) achieved in the toxicology, carcinogenicity and reproductive toxicology studies were used to evaluate and interpret the clinical relevance of the toxicity findings. Exposure multiples achieved in rat studies were used in the label in the section on reproductive toxicology findings. For mouse and rabbit findings mentioned in the label the doses were expressed as multiples of the intended clinical dose (mg/m^2). Calculation of dose multiples in orally dosed animals was based on the assumption that the bioavailability of an oral dose is 1%.

RECOMMENDATIONS

Pharmacology/Toxicology recommends approval of NDA #21,223 for Zometa™ for the indication of treatment of tumor-induced hypercalcemia with a single intravenous dose of 4 mg.

Approval is pending agreement with the Sponsor on the relevant sections of the package insert (see Labeling Review (B), p.97).

The low safety margin with respect to renal toxicity in single dose i.v. rat pharmacology and toxicity studies supports the clinical finding of an increased incidence of renal adverse events in patients treated with 4-mg infusions of zoledronate in bone metastasis trials, carried out under the related . These preclinical findings warrant continued clinical renal function monitoring and careful review of the clinical renal safety data submitted to this NDA.

The validity of the rat carcinogenicity study should be re-evaluated if chronic use of the drug is proposed in future applications.

Reviewer signatures

/S/ 8/1/00

Gemma Kuijpers, Ph.D.

/S/ 8/2/2000

Fred Alavi, Ph.D.

/S/ 8/1/00

John Gong, Ph.D.

Team Leader signature [Concurrence/Non-concurrence]

/S/ 8/1/00

Jeri El-Hage, Ph.D.

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

LABELING REVIEW

PHARMACOLOGY/TOXICOLOGY SECTIONS OF THE PACKAGE INSERT:
REVIEWERS PROPOSAL

(1)
CLINICAL PHARMACOLOGY

General
The principal pharmacologic action of ZOMETA (zoledronic acid) is inhibition of bone resorption. Although the antiresorptive mechanism is not completely understood, several factors are thought to contribute to this action. In vitro, zoledronic acid inhibits osteoclast activity and induces osteoclast apoptosis. Zoledronic acid also blocks the osteoclastic resorption of mineralized bone and cartilage through its binding to bone. Zoledronic acid inhibits the increased osteoclast activity and skeletal calcium release induced by various stimulatory factors released by tumors. In long-term animal studies, zoledronic acid inhibits bone resorption without adversely affecting bone mineralization or bone biomechanical properties.

(2)
Carcinogenesis, Mutagenesis , Impairment of Fertility

Carcinogenesis: Zoledronic acid was administered orally by gavage to mice at daily doses up to 1.0 mg/kg for 104 weeks. The incidence of Harderian gland adenoma was increased in males and females at doses ≥0.1 mg/kg/day (equivalent to approximately 0.001 times the maximum recommended human intravenous dose of 8 mg, on the basis of body surface area, mg/m^2). The clinical significance of this finding is unknown. In a 104-week oral carcinogenicity study in rats, zoledronic acid at daily doses up to 2 mg/kg (equivalent to approximately 0.05 times the maximum recommended human intravenous dose of 8 mg, on the basis of body surface area, mg/m^2) had no tumorigenic effects.

Mutagenesis: Zoledronic acid did not exhibit genetic toxicity *in vitro* in the bacterial mutagenesis assay using *S.typhimurium* and/or *E.coli* (Ames test) or the Chinese Hamster V79 hgprt gene mutation assay in the absence or presence of metabolic activation. However, the results of an *in vitro* mammalian clastogenicity test with Chinese Hamster Ovary cells were equivocal in the absence and presence of metabolic activation. *In vivo*, there was no evidence of clastogenicity in the rat micronucleus assay.

Impairment of Fertility:
In female rats ovulation was inhibited and, when mated with treated males, a decrease in the number of pregnant animals occurred at a subcutaneous dose of 0.1 mg/kg (equivalent to approximately 0.6 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison). An increase in preimplantation loss and a decrease in the number of implantations and live fetuses was seen at subcutaneous doses ≥ 0.03 mg/kg (equivalent to approximately 0.2 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison). In rats treated during gestation a reduction in the number of implantations, an increase in late resorptions and a decrease in viable fetuses was observed at a subcutaneous dose of 0.4 mg/kg (equivalent to approximately 2 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison).

Pregnancy.
Pregnancy Category C. Survival of neonates was decreased, and the number of stillbirths was increased in rats treated throughout (pre)mating and gestation with subcutaneous doses ≥0.03

mg/kg/day (equivalent to approximately 0.2 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison).

At a subcutaneous dose of 0.4 mg/kg/day (equivalent to approximately 2 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison) fetal skeletal, visceral and external malformations and variations were observed including unossified or incompletely ossified bones, thickened, curved or shortened bones and wavy ribs, reduced lens, rudimentary cerebellum, reduction or absence of liver lobes, reduction of lung lobes, vessel dilation, cleft palate, shortened jaw and edema. Skeletal and visceral malformations and variations were also observed at 0.2 mg/kg (approximately 1.2 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison), and skeletal variations were also seen at 0.1 mg/kg (equivalent to approximately 0.6 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison). At 0.4 mg/kg, reductions in maternal body weight and food consumption occurred and may have contributed to the observed effects.

In rabbits treated during gestation with subcutaneous doses up to 0.1 mg/kg/day (equivalent to approximately 0.3 times the maximum recommended human intravenous dose of 8 mg, on the basis of body surface area, mg/m^2) no fetal teratogenic effects were seen. However, hypocalcemia-induced mortality and abortions occurred in rabbits at doses ≥0.01mg/kg/day and ≥0.03 mg/kg/day, respectively (equivalent to approximately 0.03 and 0.08 times the maximum recommended human intravenous dose of 8 mg, on the basis of body surface area, mg/m^2). As with other bisphosphonates, treatment of rats during mating and gestation with doses as low as 0.01 mg/kg (equivalent to approximately 0.06 times the maximum recommended human intravenous dose of 8 mg, based on AUC comparison) caused dystocia and periparturient mortality in pregnant rats allowed to deliver. This was probably due to the drug-induced inhibition of skeletal calcium mobilization resulting in periparturient hypocalcemia. There are no studies in pregnant women. ZOMETA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

SPONSORS PROPOSAL (Submissions: December 21, 1999 and May 5, 2000)

(1)
CLINICAL PHARMACOLOGY

General
The principal pharmacologic action of ZOMETA (zoledronic acid) is inhibition of bone resorption. Although the antiresorptive mechanism is not completely understood, several factors are thought to contribute to this action. Zoledronic acid accumulates in bone, where it blocks the resorption of mineralized bone and cartilage. In vitro, zoledronic acid inhibits osteoclastic activity and induces apoptosis in osteoclasts, as well as reducing the formation and recruitment of osteoclasts into bone. In vitro, zoledronic acid has a very large ratio between the desired inhibition of bone rersorption and the adverse effects on bone mineralization. Zoledronic acid inhibits the osteoclastic hyperactivity and accelerated bone resorption induced by various stimulatory factors released by tumors. In long-term animal studies, doses of zoledronic acid similar to those recommended for the treatment of hypercalcemia inhibit bone resorption without adversely affecting the formation, mineralization, or mechanical properties of bone.

In addition to inhibiting osteoclastic bone resorption, zoledronic acid exerts direct anti-tumor effects on cultured human myeloma and breast cancer cells, inhibiting their proliferation and inducing apoptosis. Zoledronic acid also inhibits the proliferation of human endothelial cells in vitro and is anti-angiogenic in animal tumor models. In vitro zoledronic acid reduces the invasion of breast cancer cells into the extracellular matrix.

(2)
Carcinogenesis, Mutagenesis , Impairment of Fertility

In carcinogenicity studies, zoledronic acid was administered orally by gavage to rats and mice at daily doses of 0.1, 0.5 and 2.0 mg/kg and 0.1, 0.3 and 1.0 mg/kg, respectively, for at least 104 weeks without evidence of carcinogenic potential. Chronic parenteral administration was not feasible given the potential of the compound to cause severe local irritation. The pharmacologic bone changes (nonproliferative hyperostosis) typically observed following long term bisphosphonate administration to young animals with growing skeletons gave clear evidence of systemic exposure to zoledronic acid in both species at all doses.

Six mutagenicity studies were conducted with zoledronic acid. Three bacterial reverse mutation assays (Ames test) using *E.coli* and/or *S.typhimurium*, and one mammalian cell gene mutation assay using V79 Chinese hamster cells, did not show evidence of mutagenic potential with or without metabolic activation. The results of a cytogenetics test with Chinese Hamster Ovary cells with and without metabolic activation were equivocal. An *in vivo* micronucleus assay in rats was negative.

Pregnancy Category C
There are no adequate and well-controlled studies in pregnant women. Consequently, ZOMETA should not be used during pregnancy unless the benefits to the mother outweigh the risks to the fetus.
In animal reproduction studies zoledronic acid was administered subcutaneously to rats and rabbits. Teratogenicity manifested by external, visceral and skeletal malformations was observed in the rat at doses ≥0.2 mg/kg (2.5x the maximum recommended human doses on a mg/m2 body surface area basis). There was also evidence of maternal toxicity at ≥0.2 mg/kg as well as fetal toxicity at 0.4 mg/kg (5x the maximum recommended human doses on a mg/m^2 body surface area basis). No teratological or embryo/fetal effects were observed in the rabbit. However, maternal toxicity was marked at ≥0.1 mg/kg (1.4x the maximum recommended human doses on a mg/m^2 body surface area basis) due to decreased serum calcium. Bisphosphonates readily cross

the placental barrier and are taken up into the developing fetal skeleton; which may have contributed to the teratogenicity observed in the rat.

THIS SECTION WAS DETERMINED **NOT** TO BE RELEASABLE

3 pages Draft Label

APPENDIX I

(Reviews of repeated dose toxicity studies)
(Reviewer D. Coleman, Ph.D.)

SPONSOR: Ciba Geigy Corporation,
Pharmaceutical Division,
556 Morris Ave.,
Summit, NJ 07901

DRUG: CGP 42-446 (Zoledronate)

STUDY SUBMITTED: March 30, 1995
STUDY RECEIVED: April 3, 1995

CONTACT: Ms. Lynn Mellor, (908) 277-7932

CATEGORY: Bisphosphonate

INDICATIONS: Cancer patients with bone metastases
(proposed) Osteolytic lesions in patients with Multiple Myeloma
Paget's Disease
Postmenopausal Osteoporosis (no clinical studies yet)

Related INDs:

TABLE OF CONTENTS

Daniel T. Coleman, Ph.D.

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

PHARMACOLOGY AND TOXICOLOGY REVIEW
6-Month Oral Toxicity Study in Rats

PURPOSE:

To assess the toxicity profile of Zoledronate in rats when given for 6-months, orally, by gavage, and after 6-month exposure followed by 4 weeks of recovery.

EXPERIMENTAL DESIGN:

Testing Facility:	Ciba Geigy Pharmaceuticals Division, Basel, Switzerland.
Study Initiated:	May 3, 1990
Study Report Written:	Feb. 25, 1995
GLP statement:	Included.
Dose & Formulation:	0, 0.1, 1, 10 mg/kg. Dissolved in dH_2O. Given orally by gavage between 12-4PM. (see questions in "Discussion" and "To Be Communicated" sections regarding food/drug interaction).
Batches of drug:	800190 (white powder with agglomerates).
Diet:	Animals were fed ad libitum at night (beginning approximately 4 p.m.). Water was distilled and given ad libitum.
Term & # of animals:	6 month, 20/sex/group 6-Mo + 1 month recovery 5/sex/group
Species, sex (strain):	Albino Rats, M&F (Tif:RAlf SPF)
Supplier:	Ciba Geigy, Animal Production, Switzerland.
Initial Age of rats:	6-8 weeks
Initial Weight:	M:252-335g, F:125-234g.
Housing:	5 rats/cage

Dose Selection: Was based on previous 1-month oral toxicity study in rats #90-6079.
Serial #000, 0, 6, 20 & 60 mg/kg, fasted. In the 1-month study, 8 HD rats died and the rest had to be killed for humane reasons (in poor general condition). 10% of the MD rats also died.

Pharmacokinetic measurements were not made.

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: (Grade 1-5)

In the 10 mg/kg dose group, from the third week onward, typically 3/20 animals would show some adverse clinical signs, including; poor general condition, rolling spasms, sunken flanks, hunched posture, stiff gait, decreased activity, dehydration, rough coat, tremor, rales, salivation, rhinorrhea, chromodacryorrhea (colored discharge from the eye), discharge from the ear, and abnormal head posture. These symptoms were all transient (occurring in each animal for a period of a few days and then remitting) and reversible (not occurring in the recovery period) and were of slight to moderate grade (grade 1-3). One exception to this was the abnormal head posture of animal #464 that was due to otitis media. This condition was not reversible and the animal was killed before the scheduled date.

At the 1 mg/kg dose transient dyspnea, deep respiration, emaciated appearance and salivation were noted in individual animals.

In the 0.1 mg/kg and control groups there were no treatment related signs.

MORTALITY:

There were a total of eight deaths. Three were due to misintubation and five were possibly drug related. One control female (#338), one 1 mg/kg male (#420) and one 10 mg/kg high dose female (#489) were killed in extremis due to intubation errors. The drug related deaths all occurred in the 10 mg/kg high dose group:

- One male (#464) was killed due to abnormal posture that was determined to be due to otitis media (week 25), possibly drug related.
- Of four spontaneous deaths (animals numbered; 469, 473, 492 and 497, on weeks; 9, 20, 24 and 21), one male had pneumonia (#469), the other male and 2 females had some degree of pulmonary edema, possibly drug related. (It is not clear if this effect is systemic or contact mediated).

BODY WEIGHT:

Body weight and growth rates were slightly reduced in the 10 mg/kg groups. This difference was not significant in the male rats. However, the female 10 mg/kg group was significantly (10-12%) lighter than the control females at almost all time points after the first 3 weeks of the study. This was due to

slower weight gain in the 10 mg/kg animals than in the controls. The 10 mg/kg females did not lose weight, rather, they had slightly slower weight gain, which resulted in a slightly lower weight.

Decreased body weight was not reversed at the end of the 1-month recovery period. Note, the statistically significant finding in the 1 mg/kg females could be due to a lower initial weight in this group.

Body Weights (grams): (percent difference from Control)								
Group:	n	Control:	0.1 mg/kg		1 mg/kg		10 mg/kg	
Males at 26 wks.	22-25	508	539	(+ 6%)	532	(+5%)	478	(-6%)
Females at 26 wks.	22-25	314	300	(-5%)	290	(-8%)	**276****	**(-12%)**
Recovery Females at + 1 wk	5	354	312	(-12%)	**279****	**(-21%)**	**288***	**(-19%)**
Recovery Females at +5 wks.	5	360	324	(-10%)	**284****	**(-22%)**	**300***	**(-17%)**

* = $p < 0.05$; ** = $p < 0.01$, Dunnett's Test

FOOD CONSUMPTION:

Food consumption paralleled the weight gain results. There was a slight (not significant) decrease in food consumption in the 10 mg/kg animals compared to the controls. This difference was even smaller at the end of the 1-month recovery period.

EYE AND EAR EXAMINATION:

There were no significant or drug related findings in the hearing or vision tests. There was one ear related incident; a case of otitis media in a high dose male rat that was killed because of abnormal head posture. The middle ear effect was attributed to accidental direct contact with drug (see Mortality above for further discussion).

HEMATOLOGY:

No biologically meaningful toxic effects were found at any dose. Several slight effects in the HD animals 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 and after the recovery period.

There were significant ($p<0.05$ versus C) 10-50% increases in segmented neutrophils in all male HD groups in weeks 13-27 (page 347). Smaller effects were seen in female's segmented neutrophils. These effects were not noted in the recovery period.

Differential WBC Count, Segmented Neutrophils (#/1 at 6-months)				
Group	**C**	**0.1**	**1**	**10**
Males	**0.11**	**0.12**	**0.16***	**0.15***
Females	**0.10**	**0.14***	**0.11**	**0.12**

n = 22, * = $p<0.05$

COAGULATION:

No physiologically significant effects were noted.

BONE MARROW: (Grade Scale, 1-5)

All animals in the study showed some degree of "cellularity" in the various bone marrows examined. The range of grades reported in the control animals (grades 1-3) seemed slightly lower than in the exposed animals (grades 1-4) and seemed to support the sponsors statement that "Most high- and mid-dose rats showed a slightly increased cellularity of the vertebral marrow." This was not supported

statistically by the "Trend analysis" .

BLOOD CHEMISTRY:

Findings related to expected physiologic actions of Zoledronate included:

1. Bone AP activity was significantly reduced in almost all groups. Total Alkaline Phosphatase was decreased in the 10 mg/kg groups reflecting the changes in the bone AP. Liver AP was not affected by treatment to any physiologically important extent.

Bone Alkaline Phosphatase (U/L at 6-months):				
Group	**C**	**0.1**	**1.0**	**10**
Males	**40**	**20***	**21***	**18***
Females	**42**	**35**	**29***	**26***

n = 22, * = p<0.05%

2. There was a slight hypocalcemia in the 10 mg/kg animals. The plasma calcium levels in HD males and females were statistically significantly reduced. This reduction was less than 5%.

Plasma Calcium (mMols/L at 6-months):				
Group	**C**	**0.1**	**1.0**	**10**
Males	**2.60**	**2.60**	**2.58**	**2.52***
Females	**2.52**	**2.49**	**2.51**	**2.45***

n = 22, * = p<0.05

In addition, ASAT, and CK activities were statistically significantly elevated in a dose and time dependent manner possibly related to drug exposure. However, the magnitude of the increase was physiologically slight, the increase was not noted after the recovery period and there was no histopathological correlation when individual animal reports were checked for liver, heart or muscle damage and enzyme levels.

URINALYSIS:

No treatment related changes were noted.

ORGAN WEIGHTS:

Small, yet statistically significant, differences were noted in several of the mean absolute organ weights from the HD groups. Similar results were obtained for male and female rats, and as % of brain, or % of body weight. Only the male absolute data will be presented here, as an example. Kidney weight was increased 12%, liver weight was decreased 14%, and spleen weight was increased by 7% in the 10 mg/kg group compared to controls.

Mean Absolute Organ Weight (grams)				
Group:	Control	0.1 mg/kg	1 mg/kg	10 mg/kg
Kidney	2.77	3.04*	2.88	3.10**
Liver	16.53	16.76	15.17	14.21**
Spleen	0.715	0.749	0.713	0.761

n = 17-20, * = $p < 0.05$; ** = $p < 0.01$

GROSS PATHOLOGY:

No treatment related findings at terminal necropsy.

HISTOPATHOLOGY (Grade scale 1-5):

BONE MORPHOLOGY:

Dose dependent increase in length and density of the primary spongiosa and prominence of osteoid seams were seen in treated animals after the 6 month study. There was no recovery from this effect in the recovery groups. This effect was more pronounced in the femur/tibia (Grade 4-5) and less in the ribs and vertebra and slight in the sternum (Grade 1-3). Prominent osteoid seams were noted in all bones examined from drug treated animals.

STOMACH:

Inflammatory cell infiltration into the gastric submucosa was slightly increased (grade 1-3) in half of the HD animals compared with controls (none detected) resulting in a highly significant trend. This effect was reversed in the recovery period.

TRACHEA:

Damage to the tracheal mucosa was noted as "tracheitis" in all groups (including controls). Tracheitis, though mild (grade 1-2), was relatively more severe (grade 3) in a few (474, 484, 481) 10 mg/kg animals than in controls. This effect was reversible in the recovery period. This effect may be due to direct exposure to drug spilled from the gavage tube.

LUNGS:

5/20 HD (10 mg/kg) females and 1/20 HD males, had moderate to severe edema in the lungs. One of these was definitely associated with pneumonia but the others are of unknown cause. This effect may be due to direct exposure to drug spilled from the gavage tube.

TESTES:

Slight ($p < 0.08$) trend toward increased tubular mineralization was observed. No other remarkable effects observed.

HEART:

3/20 HD and 1/1 MD male rats demonstrated an inflammatory focus (grade 1-2) in their hearts. Only one control female demonstrated this symptom. This effect did demonstrate a significant ($p< 0.04$) trend, but the toxicological importance is unclear at this time.

LYMPH NODES:

The sponsor points out (p. 38) that there were small aggregates of macrophages in the mesenteric lymph nodes in the 10 mg/kg animals, and that this effect was partially reversed in the recovery period. The sponsor also states that this is a normal reaction after oral resorption of a xenobiotic. There was no statistically significant trend toward any lymph node abnormality reported.

SPLEEN, LIVER, KIDNEYS:

No remarkable effects observed.

SUMMARY TABLE

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

Effect: / Dose:	0	0.1 mg/kg	1 mg/kg	10 mg/kg
CLINICAL SIGNS:	0	0	individual incidence of the same indications as in the high dose groups	**15% reporting moderate-severe spasms, stiff gait, poor general condition, rales, salivation ...**
MORTALITY:	1 k	0	1 killed	**4 spontaneous 2 killed**
BODY WEIGHT:	0	0	0	reduced ~10% vs. Control
FOOD CONSUMP.:	0	0	0	reduced ~10% vs. Control
EYE & EAR EXAM:	0	0	0	0, **1 case of otitis media in an animal killed early**
HEMATOLOGY:	0	0	0	5% Decreased RBC, HB 50% increased segmented neutrophils
COAGULATION:	0	0	0	0
BONE MARROW	Slight hypercellularity in controls. Slightly increased hypercellularity of the marrow in all treated groups.			
BLOOD CHEMISTRY:	0	**reduced bone AP**	**reduced bone AP**	**reduced bone AP Hypocalcemia**
URINALYSIS:	0	0	0	0
ORGAN WEIGHTS:	0	0	0	kidney + 12%, liver - 14%, spleen +7%
GROSS PATHOLOGY:	0	0	0	0
HISTOPATHOLOGY: BONE MORPHOLOGY:	0	**osteoid seams longer spongiosa**	**osteoid seams longer spongiosa** increased density	**osteoid seams longer spongiosa** increased density
STOMACH:	0	not examined	0	**23/40 inflammatory cell infiltration (grade 1-3).**
TRACHEA:	Tracheitis, which was seen in all groups, was slightly increased in severity in HD. Erosion was increased in incidence and severity with dose.			
LUNGS:	Inflammatory foci, seen in almost all animals, were increased in severity in HD. The 10 mg/kg HD group also had 5/40 cases of **moderate-severe edema in lungs**.			

Summary of significant target tissues identified in this study:

Target Tissue:	Effect:	Dose: (mg/kg)	Grade (1-5)	Affected:
Stem derived cells	-RBC, -HB, +granulocytes, +marrow cellularity	10	2	mean value
Bone Cells	Decreased AP activity	0.1-10	4-5	mean value
Bone cells	Hypocalcemia	10	2	mean value
Bone	Lengthened Spongiosa	0.1-10	4-5	mean value
Bone	Higher density	1-10	3-4	mean value
Stomach	Inflammatory cell infiltration	10	1-2	50%
Trachea	Erosion - possibly local not systemic exposure	10	1	50%
Lungs	Edema - possibly local not systemic exposure	10	5	20%
dle ear	inflammation- possibly local not systemic exposure	10	3-5	5%

SUMMARY & DISCUSSION:

Dose selection:

Dose selection in this study was based on the previous rat, 1-month, oral toxicology study (serial 000, Doses: 0, 6, 20 and 60 mg/kg). 8/20 rats died at the high dose and the rest had to be killed in poor general condition. 10 % of the 20 mg/kg animals also died in the 1-month study. As a result 10 mg/kg was selected as the highest dose for this 6-month 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 60 mg / square meter (or 5X 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.

The 1-month oral and this 6-month oral study were used by the sponsor to attempt to justify a maximum oral dose of 0.5 mg/kg for the rat carcinogenicity trial. Due to the lack of deaths and lack of severity of clinical signs at the 1 mg/kg dose in this 6-month study, the CAC suggested 0, 0.1, 0.5 and 2 mg/kg for the rat carcinogenicity trial.

In the earlier 1-month oral study, rats were fasted before and after gavage. Further clarification of the feeding and dosing schedule will be requested from the sponsor regarding the 6-month study described here (see "To Be Communicated" section). The absorption of this class of drugs is generally known to be poor and to be inhibited by food in the stomach. Concern was expressed by the sponsor in the Methods section regarding mixing of drug and food. The sponsor states on page 11, "In order to avoid complexing of the compound with the diet (i.e. Ca^{++}) diet was available only during the night time (as of approximately 4:00 p.m.)". As it is described, this protocol would not prevent the possibility of mixing food and drug in the animal's stomachs. The protocol seems to permit animals to eat immediately following dosing if they were gavaged at 4:00 p.m.. This would influence the exposure to the drug in an uncontrolled fashion.

Toxic Effects:

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

1. High dose rats weighed (~10%) less than control rats at the end of the study.
2. Minimally decreased (less than 5%) HB, HCT and RBC in high dose groups after 13 weeks (as seen in the 12-month study).
3. Slightly elevated (~40%) segmented neutrophils at several time points in 10 mg/kg males and some female groups.
4. Slightly elevated CK (<4X) and ASAT (<40%) in HD animals.
5. Moderate bone marrow hypercellularity in all drug exposed animals.
6. Slight changes in kidney, liver and spleen weights in HD animals.
7. Slight degree of inflammatory cell infiltration into the gastric mucosa of 23/40 HD animals.
8. Mild hypocalcemia in the HD groups.

These effects are not physiologically meaningful when taken alone. They also have no morphological correlates (except the marginal increase in hemopoietic cell turnover, seen in the hematology section, which might relate to the spleen enlargement). However, several effects have been consistent across several studies and taken together indicate possibly physiologically significant toxic effects. The toxicological importance of the hematology findings are unknown. They suggest a marginal

increase in hemopoietic cell turnover and possible response to infection or inflammation.

Effects on spleen liver and kidney weight are consistent with effects in these organs seen in other studies and so are confirmatory: Increased spleen and kidney weight and decreased liver weight was observed in the 3-month dog IV study. Increased spleen weight was also observed in the 1-year rat SC study where it was also probably due to extramendullary hematopoiesis.

Inflammatory cell infiltration into the gastric mucosa is consistent with local irritation caused by the drug at other sites and in other studies. Although this effect was slight and was reversed in the recovery period it reflects the capacity of this drug to irritate the stomach.

The slight effect to elevate liver enzymes indicates the potential to cause liver damage at higher doses. At the doses used in this study the effects on stomach and liver are slight and reversible.

Although the hypocalcemia was mild in absolute terms, even a slight change in calcium could be physiologically important. This hypocalcemia may have caused the spasms noted in the clinical signs. Hypocalcemia is a known clinical "side effect" of overly aggressive bisphosphonate therapy.

Other **statistically significant effects** of this drug were of greater magnitude but relate to the **known pharmacological effects** of the drug, including:

1. Lengthening of the primary spongiosa in virtually all drug treated animals.
2. Prominent osteoid seams from all drug treated animals.
3. Increased bone density and thickness.
4. Decreased bone alkaline phosphatase activity in all drug exposed animals.

Inhibition of bone AP is consistent with the effect of bisphosphonates to inhibit bone formation. This "side effect" occurs at concentrations of drug greater than those required for the drugs intended effect (to decrease bone resorption). This decrease in AP is considered a negative effect because it reflects a decrease in bone formation

The above effects are expected pharmacological and therapeutic actions of this type of drug, as is the increase of the metaphyseal and rib diameter. However, at the high dose, the effect of this drug to inhibit resorption may be reaching levels that could be undesirable. By very strongly slowing bone resorption, the drug may be interfering with the bone remodeling process to a point which may have negative effects on bone structure as well as calcium homeostasis. The increased density of the primary spongiosa and the hypocalcemia seen in the 10 mg/kg animals suggests that this level of effect may have been reached. This effect may be exacerbated if it continues for longer periods of time. The fact that these effects were not reversed to any extent in the recovery period indicates that the 6-month exposure period has effects which are much longer term.

Several **statistically significant and/or toxicologically important** effects noted in the 10 mg/kg group were probably due to accidental release of drug from the gavage tube in the esophagus instead of the stomach of the rats.

1. Slightly increased erosion and inflammation in the trachea of HD animals.
2. Edema (5 cases ranging from slight to severe) in lungs.
3. One case of severe otitis media.
4. Four spontaneous deaths.

The spilled drug may have also caused the rales and discharge from eyes, ears, nose or mouth seen in Clinical Signs.

Clearly the high dose animals (10 mg/kg) exhibited toxic effects not seen at lower doses or in control animals. Four animals in the 10 mg/kg group died spontaneously (not from intubation errors). The most significant findings in the pathology report on these animals were lesions in the lungs. 15% of this HD group consistently showed signs of poor general condition, rales, spasms, stiff gait and discharge from eyes, ears, nose or mouth. This 10 mg/kg group also gained ~10% less weight and ate ~10% less. Nevertheless, no toxic effects were reported which clearly caused these toxic endpoints.

The sponsor hypothesizes (page 39) that " Inflammation in trachea, lungs and middle ear of individual animals in the high dose group may be explained by the fact, that in the course of application, small amounts of the compound had accidentally local contact with the oropharynx. The local irritative effects