CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-386
21-223/S-003

PHARMACOLOGY REVIEW(S)

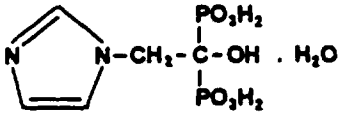
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-386
Review number: 1
Sequence number/date/type of submission: 000/8-22-01/Type 6
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Novartis Pharmaceuticals Corporation
Manufacturer for drug substance : Novartis

Reviewer name: John K. Leighton
Division name: Oncologic Drug Products
HFD #: 150
Review completion date: 1/3/02

Drug:

Trade name: Zometa
Generic name (list alphabetically): Zoledronic acid
Code name: CGP 42446, ZOL446
Chemical name: (1-hydroxy-2-imidazol-1-yl-phosphonoethyl)phosphonic acid monohydrate
CAS registry number: 118072-93-8
Mole file number: none
Molecular formula/molecular weight:$C_5H_{10}N_2O_7P_2.H_2O/290.1$
Structure:



Relevant INDs/NDAs/DMFs: NDA 21-223
Drug class: bisphosphonate
Indication: "Zometa is indicated for the treatment of osteolytic, osteoblastic, and mixed bone metastases of solid tumors and osteolytic lesions of multiple myeloma, in conjunction with standard antineoplastic therapy."
Clinical formulation: 4 mg zoledronic acid anhydrous, 220 mg mannitol, USP and 24 mg sodium citrate, USP. Lyophilized powder (4 mg) reconstituted in 5 mL sterile water; further diluted in 0.9% NaCl or 5% Dextrose Injection
Route of administration: intravenous
Proposed use: treatment of patients with bone metastases

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability: approvable

B. Recommendation for Nonclinical Studies: no additional studies recommended

C. Recommendations on Labeling: suggest deleting label wording on mechanism of action added to the current draft version of the proposed label.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

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

No toxicology studies were reviewed in this application. Pharmacology studies in this application related to the primary mechanism of action support the current labeling and thus were not reviewed in detail. Pharmacology studies conducted to support additional mechanism of action claims used doses and schedules that are not used clinically (e.g., 4 mg q 3 weeks human dose vs. up to 100 µg/kg daily for 6 days in the murine model of growth factor induced angiogenesis). These studies are reviewed in the pharmacology section. In general, the studies suggest that zolendronic acid may have multiple pharmacologic activities, but that these other activities have not been demonstrated to be relevant to the clinical use of zoledronic acid.

B. Pharmacologic Activity: no additional comments

C. Nonclinical Safety Issues Relevant to Clinical Use: none

III. Administrative

A. Reviewer signature: _____

B. Secondary reviewer signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:
BensonKi
WilliamsG

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: no studies were reviewed

Mechanism of action:

IN VITRO STUDIES

Antiproliferative activity of zoledronic acid against human breast cancer cells in vitro.

Results: Zoledronic acid inhibited growth of MCF-7, MCF-7/ADR, and MDA-MB-231 cells in culture with IC_{50} values of 13.7, 24.5, and 8.9 μM, respectively. EDTA had no effect on cell growth at eqimolar concentrations. The highest drug concentration tested was 100 μM.

Antiproliferative activity of zoledronic acid against human prostate cancer cells in vitro.

Results: Zoledronic acid inhibited growth of human Du 145 and PC-3M (metastatic prostate carcinoma) cells in vitro. IC_{50} values were 13.1 and 28.8 μM, respectively. EDTA at equimolar concentrations was not effective. The highest drug concentration tested was 100 μM. In an in vivo study ("Effect of zoledronic acid on the bone destruction induced by human prostate cancer, PC-3MM2, cells injected into bone"), PC-3MM2 cells injected directly into the tibia of nude mice, zoledronic acid at twice a day sc for 5 weeks at doses up to 125 μg/kg had no effect on tumor load or incidence of metastasis to the lymph nodes. Doses of 25 μg/kg or higher completely protected against the osteolytic effects of the tumor in this model.

Antiproliferative activity of zoledronic acid against human prostate cancer cells in vitro.

Results: Zoledronic acid inhibited growth of T24 transitional bladder carcinoma cells in culture with an IC_{50} of 8.9 μM. EDTA had no effect on cell growth at eqimolar concentrations. The highest drug concentration tested was 100 μM.

Antiproliferative activity of zoledronic acid against human lung cancer cells in vitro.

Results: Zoledronic acid inhibited growth of human A549 (non-small cell lung adenocarcinoma) and NCI-H460 (large cell lung carcinoma). The IC_{50} values were 19.4 and 31.2 μM, respectively. EDTA had no effect on cell growth at eqimolar concentrations. The highest drug concentration tested was 100 μM.

The bisphosphonate, zoledronic acid, induces apoptosis of breast cancer cells: evidence of synergy with paclitaxel. Br J Cancer (2001) 84: 1126-1134.

Results: Zoledronic acid caused concentration and time dependent decreases in number of MCF-7 and MDA-MB-231 cells. A concentration of 100 μM effectively inhibited growth of both cell types; 10 μM was not effective in inhibiting growth of MDA-MB-231 cells. The decreased cell number could be prevented by incubation with geranyl geraniol. The combination of 10 μM zoledronic acid and 2 μM paclitaxel resulted in a 5-fold increase in apoptosis of MCF-7 cells compared to zoledronic acid alone.

Bisphosphonates directly regulate cell proliferation, differentiation, and gene expression in human osteoblasts. Cancer Res (2000) 60: 6001-6007.

Most of the experiments in this report used pamidronate as the test compound. In a comparative study, parmidronate and zoledronate were approximately equpotent in inhibiting growth of immortalized human fetal osteoblasts (hFOB). ED_{50}s were 42 and 40 μM, respectively. Adding free calcium or magnesium did not affect the growth curves for pamidronate or zoledronate. Pamidronate also induced the differentiation of the hFOB cells, as indicated by increased total cellular protein, alkaline phosphatase activity, and secretion of type I collagen.

IN VIVO STUDIES

The bisphosphonate zoledronic acid inhibits tumour growth in vivo by reducing angiogenesis.

Key study findings: Zoledronate inhibited growth of MDA-MB-231 and CHO tumor cells in xenograft models. Daily zoledronic administration prevented formation of radiographically detectable osteolytic bone metastases in MDA-MB-231 injected mice.

Document no: RD-2001-00293
Volume #, and page #: 5; 5-36
Formulation/vehicle:

Methods:

Dosing:

Species/strain:	Balb/c nu/nu mice
#/sex/group:	6 (tumor xenograft) or 10 (bone metastasis study) ♀/group
Age:	4 weeks
Weight:	not stated
Doses:	150 μg/kg

Route:	sc
Frequeney:	daily for 21 d (CHO cells) or 27 days (MDA-MB-231)
Volume:	100 µL

Anti-angiogenic effects of the bisphosphonate compound zoledronic acid.

Key study findings: zolendronic acid inhibited bFGF but not VEGF induced angiogenesis.

Document no: RD-2001-00171
Volume #, and page #: 5; 5-258
Formulation/vehicle:

Methods:

Dosing (in vivo studies):

Species/strain:	mice
#/sex/group:	6 ♀/group
Age:	not stated
Weight:	17-20 g
Doses:	1, 10, or 100 µg/kg/d
Route:	sc
Frequency:	daily
Duration:	6 days

Results: Zoledronic acid inhibited serum- (FCS), VEGF- and bFGF-induced proliferation of HUVEC with IC_{50} values of 4.1, 6.9 and 4.2 µM, respectively. EDTA at 30 µM inhibited HUVAC proliferation induced by serum, VEGF and bFGF 23.7% (not statistically significant versus control), 55.6% and 49.5%, respectively.

Using the —— assay, a 4-fold increase in DNA fragmentation was observed at 100 μM zoledronic acid. The finding was confirmed by flow cytometry and morphology.

In the aortic ring assay, zoledronic acid completely inhibited sprouting of capillaries; EDTA was without effect. In the —— assay, a slight reduction in angiogenesis was observed at 0.1 mM zoledronic acid 24 hours after application. No capillaries were observed at 1 mM zoledronic acid. EDTA did not affect angiogenesis in the test system.

In mice implanted with chambers containing growth factors, zoledronic acid inhibited the angiogenic response induced by bFGF, measured by blood content and tissue weigh, reaching statistical significance at 10 and 100 μg/kg/d. Zoledronic acid inhibition of bFGF stimulated growth was > 80% for both parameters at the 2 doses (dose-dependent compared to bFGF alone). With VEGF stimulation, zoledronic acid at 100μg/kg/d inhibited the increase in the weight of vascularized tissue by 57% (statistically significant) compared to VEGF treated animals alone. With VEGF and zoledronic acid treatments, blood content at 1-100 μg/kg/d and tissue weight at 1 and 10 μg/kg/d were not affected.

Conclusion: The sponsor states that it is unlikely that the low doses used in the in vivo experiments could produce the sustained micromolar concentrations needed to inhibit endothelial cell proliferation and adhesion in vitro. Alternatively, the sponsor suggests that local effects of zoledronic acid on endothelial progenitor cells originating in bone marrow were affected by the high concentrations on zoledronate in bone.

Drug activity related to proposed indication: no additional studies reviewed

Secondary pharmacodynamics: no studies were reviewed

Pharmacology summary:

In vitro and in vivo studies support the hypothesis that zoledronic acid may inhibit cell and tumor growth, possibly through inhibition of the mevalonate biosynthetic pathway. The inhibition does not appear to involve chelating the divalent cations calcium and magnesium. The potential anti-angiogenic effect of zoledronic acid may preferentially target the bFGF pathway rather than the VEGF pathway.

Pharmacology conclusions:

A dose of 4 mg/patient per month translates to approximately 2.1 μg/kg/day or 15 μg/week. The in vivo studies submitted to support potential anti-tumor activity of zoledronic acid generally require doses 1-2 orders of magnitude higher than the intended clinical dose and different schedules to observe the anti-tumor effect. Thus, while the studies described are interesting, they are not sufficient to demonstrate that zoledronic acid inhibits tumor growth at a clinically relevant dose.

II. SAFETY PHARMACOLOGY:

No studies were submitted.

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters: no studies were reviewed

Absorption: no studies were reviewed

Distribution: no studies were reviewed

Metabolism: no studies were reviewed

Excretion: no studies were reviewed

Other studies: no studies were reviewed

PK/TK summary: no changes from NDA 21-223

PK/TK conclusions: no changes from NDA 21-223

IV. GENERAL TOXICOLOGY: no studies were reviewed

V. GENETIC TOXICOLOGY: no studies were reviewed

VI. CARCINOGENICITY: no studies were submitted

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY: no studies were submitted.

VIII. SPECIAL TOXICOLOGY STUDIES: no studies were submitted.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: see discussion below under Labeling

General Toxicology Issues: no issues were identified for this supplemental review

Recommendations: see discussion under labeling

Labeling with basis for findings:

The sponsor proposes the addition of the following language to the current label.

This section should be deleted for the following reasons:

- Fetal osteoblastic differentiation studies were conducted with a related bisphosphonate, pamidronate, and not zoledronate.
- In vivo studies generally used different schedules and doses 1-2 orders of magnitude higher than the clinical dose of 4 mg/patient every 3-4 weeks. The impact of dose and schelule were not assessed in the in vivo studies.
- Synergism with other anti-cancer agents was explored in vitro. No in vivo or clinical experience assessing synergism of zolendronic acid with other anti-cancer agents was provided.

X. APPENDIX/ATTACHMENTS:

Addendum to review: none

Other relevant materials (Studies not reviewed, appended consults, etc.): none

Any compliance issues: none identified

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

John Leighton
1/31/02 12:13:48 PM
PHARMACOLOGIST

Kimberly Benson
1/31/02 12:17:23 PM
PHARMACOLOGIST

APPEARS THIS WAY
ON ORIGINAL

Pharm/Tox Amendment from C to D to be finalized on 2/22/02

(Pink copy is a draft.)

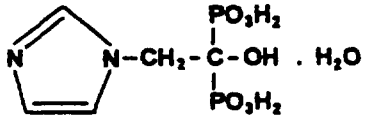
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-386
Review number: 2
Sequence number/date/type of submission: 000/8-22-01/Type 6
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Novartis Pharmaceuticals Corporation
Manufacturer for drug substance : Novartis

Reviewer name: John K. Leighton
Division name: Oncologic Drug Products
HFD #: 150
Review completion date: 2/22/02

Drug:

Trade name: Zometa
Generic name (list alphabetically): Zoledronic acid
Code name: CGP 42446, ZOL446
Chemical name: (1-hydroxy-2-imidazol-1-yl-phosphonoethyl)phosphonic acid monohydrate
CAS registry number: 118072-93-8
Mole file number: none
Molecular formula/molecular weight: $C_5H_{10}N_2O_7P_2.H_2O/290.1$
Structure:



Relevant INDs/NDAs/DMFs: NDA 21-223
Drug class: bisphosphonate
Indication: "Zometa is indicated for the treatment of osteolytic, osteoblastic, and mixed bone metastases of solid tumors and osteolytic lesions of multiple myeloma, in conjunction with standard antineoplastic therapy."
Clinical formulation: 4 mg zoledronic acid anhydrous, 220 mg mannitol, USP and 24 mg sodium citrate, USP. Lyophilized powder (4 mg) reconstituted in 5 mL sterile water; further diluted in 0.9% NaCl or 5% Dextrose Injection
Route of administration: intravenous
Proposed use: treatment of patients with bone metastases

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability: approvable

B. Recommendation for Nonclinical Studies: no additional studies recommended

C. Recommendations on Labeling: suggested label wording on pregnancy category.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

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

No toxicology studies were reviewed in this application. Pharmacology studies for this NDA were summarized in Review 1. This review details the rationale for changing Zometa from Pregnancy category C to D. The sponsor has agreed with this change.

B. Pharmacologic Activity: no additional comments

C. Nonclinical Safety Issues Relevant to Clinical Use: none

III. Administrative

A. Reviewer signature: _____

B. Secondary reviewer signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:
BensonKi
WilliamsG

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY: no additional comments

II. SAFETY PHARMACOLOGY: No studies were submitted.

III. PHARMACOKINETICS/TOXICOKINETICS: no studies were reviewed

IV. GENERAL TOXICOLOGY: no studies were reviewed

V. GENETIC TOXICOLOGY: no studies were reviewed

VI. CARCINOGENICITY: no studies were submitted

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

After review of the reproductive toxicity of zolendronic acid, assessment by the Pharmacology/Toxicology Reproductive Toxicity Committee (see Appendixes 1 and 2), and discussions within the Division of Oncology Drug Products Zometa review team, it was decided by DODP that the most appropriate pregnancy category for Zometa was "D". The DODP recognizes that every bisphosphonate currently available in the US is labeled "C". However, the DODP believes that the class effects of bisphosphonates on reproductive toxicity that are considered related to the mechanism of action of these products, as well as potential conditions of use, warrant the "D". The chronicity of dosing for the proposed indication as well as the similarity of the therapeutic mode of action and site of toxicity in the reproductive toxicity study are cause for concern. For these reasons, the DODP recommended changes to the sponsor's proposed label.

After discussions with the sponsor, the following label changes were made.

In the WARNINGS section:

PREGNANCY: ZOMETA SHOULD NOT BE USED DURING PREGNANCY. Zometa may cause fetal harm when administered to a pregnant woman. In reproductive studies in the pregnant rat, subcutaneous doses equivalent to 2.4 or 4.8 times the human systemic exposure (an i.v. dose of 4 mg based on an AUC comparison) resulted in pre- and post-implantation losses, decreases in viable fetuses and fetal skeletal, visceral and external malformations (See PRECAUTIONS, Pregnancy Category D).

There are no studies in pregnant women using Zometa. I f the patient becomes pregnant while taking this drug, the patient should be apprised of the potential harm to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

In the PRECAUTIONS section; change Pregnancy Category C to D: **See WARNINGS.**

VIII. SPECIAL TOXICOLOGY STUDIES: no studies were submitted.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: see discussion below under Labeling

General Toxicology Issues: no issues were identified for this supplemental review

Recommendations: see discussion under labeling

Labeling with basis for findings: Change from pregnancy Category C to D: see Section VII. Reproductive and Developmental Toxicology, and appendix/addendum below.

X. APPENDIX/ATTACHMENTS:

Addendum to review: none

Other relevant materials (Studies not reviewed, appended consults, etc.):
Review for Reproductive Toxicology Committee
OPDRA Review (author Jenny Craig, under NDA 17-831)

Any compliance issues: none identified

Use of Bisphosphonates in Pregnancy

Prepared for the Reproductive Toxicity Assessment Committee
Reviewer: John K. Leighton, Ph.D., DABT
Division of Oncology Drug Products, FD-150

Final 2/22/02

1. Overview

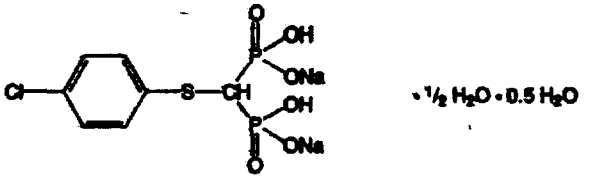
The bisphosphonates are a class of drugs currently indicated for the treatment of osteoporosis, Paget's disease, osteolytic bone metastases and hypercalcemia of malignancy. Bisphosphonate structures are shown in Figure 1; Table 1 lists the bisphosphonates and foscarnet (a monophosphate) currently approved or studied in the US and abroad. The bisphosphonates are characterized by a core phosphate-carbon-phosphate structure; second and third generation bisphosphonates also contain one or more nitrogens that may also be important in biological activity. These agents are presently classified as Pregnancy Category C. The following review contains both toxicology and pharmacology data regarding the use of these agents and their potential risk to the fetus. This information was compiled in order to assess whether a similar risk profile for reproductive toxicity may exist for these compounds. If a similar risk profile is observed, then the possibility of a common mechanism of action responsible for reproductive toxicity should be assessed.

Table 1. Summary Information Regarding Bisphosphonates and Foscarnet.

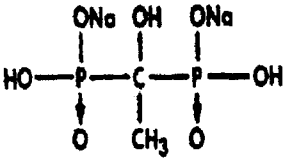
Drug Name	**Trade/Code Name**	**Dose**	**Company**	**Status**
Alendronate	Fosamax	40 mg/d po to 6 mo	Merck	Approved in US
Etidronate	Didronel	11-20 mkd to 3 mo po	Procter & Gamble	Approved in US
Incadronate or cimadronate	YM175		Yamanouchi	Not Approved in US
Minodronate	YM529		Yamanouchi	Not Approved in US
Neridronate				Not Approved in US
Olpadronate			Gador	Not Approved in US
Pamidronate	Aredia	60-90 mg iv single dose	Novartis	Approved in US
Risedronate	Actonel	30 mg/d to 2 mo po	Procter & Gamble	Approved in US
Tiludronate	Skelid	400 mg/d po to 3 mo	Sanofi-Synthelabo	Approved in US
Zoledronate	Zometa	4 mg iv	Novartis	Approved in US
Foscarnet	Foscavir	90 mg/kg q12 h for 2-3 wks iv	AstraZeneca	Approved in US

[1] Dosing cited from the PDR unless noted. The dosing and schedule shown is the highest dose approved. [2] Dosing information from Prescribing Information (6/4/98).

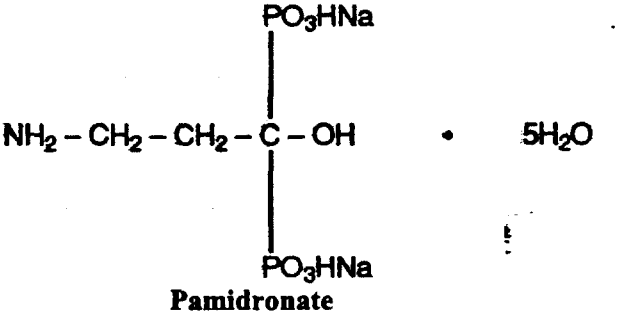
Figure 1. Bisphosphonate Structures



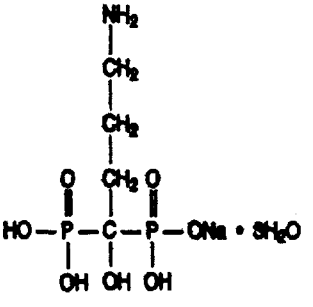
Tiludronic Acid



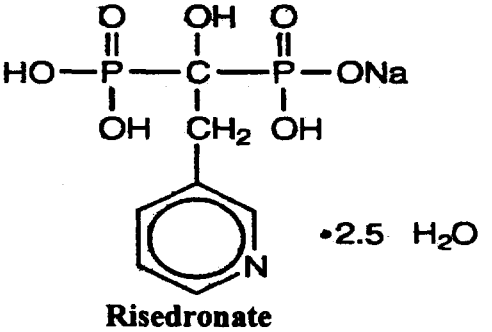
Etidronate



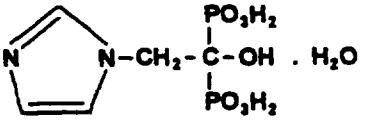
Pamidronate



Alendronate



Risedronate



Zoledronic Acid

2. Retention and Plasma Half-life of Bisphosphonates

Initially, bisphosphonates are widely distributed throughout the body. For example, 5 min after a single iv dose, 63% of administered alendronate is distributed in noncalcified tissue. This amount drops to 5% at 1 hour post dose (Porras et al., 1999). Results from a disposition study for zoledronate in the rat showed that 12 months after a single radioactive iv dose, 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 (Pharmacology/Toxicology Review, NDA 21-223).

The plasma half-life and bone retention of the bisphosphonates is shown in Table 2. The long retention time of bisphosphonates in bone may be a concern for use prior to and during pregnancy.

Table 2. Plasma half-life (terminal phase), bone distribution and retention of selected bisphosphonates.

Drug	Plasma $t_{½}$	Bone Distribution and Retention	Reference
Alendronate	Estimated single dose $t_{½}$ of 300 days in rats, 1000 days dog, 10.5 yrs human	Uptake in bone proportional to dose in rats from 0.2-5 mg/kg iv or 1-25 mg/kg po; uptake not saturated with repeat doses; 60-70% of dose sequestered in bone in rats; 40-60% of human dose still resident in body after 72 hr, with little subsequent urinary excretion	NDA 20-560; Porras et al., 1999
Etidronate	1-6 h	50% excreted in urine in 24 h in human, remainder distributed to bone	NDA 20-082
Pamidronate	28 h in humans	50-60% of iv dose of labeled pamidronate absorbed by bone in rats, with a terminal $t_{½}$ estimated at 300 d	NDA 20-927
Risedronate	200-480 h in humans	60% of dose distributed to bone in rats and dogs; 87% recovered in urine at 28 days, the remainder reflecting bone incorporation	NDA 20-835; Williams et al., 2001
Tiludronate	150 h after single dose in pagetic patients	Slow release from bone with a $t_{½}$ in rats of 30 d or longer depending upon the status of bone turnover.	NDA 20-707
Zoledronate	167 h after single dose in humans	After 12 months, about 40% of the iv dose in rats was still in the skeleton.	NDA 21-223

3. Labeling and Literature Information Summary for Specific Bisphosphonates

3.1. Alendronate

3.1.1. Current Labeling-US

Reproduction studies in rats showed decreased postimplantation survival at 2 mg/kg/day and decreased body weight gain in normal pups at 1 mg/kg/day. Sites of incomplete fetal ossification were statistically significantly increased in rats beginning at 10 mg/kg/day in vertebral (cervical, thoracic, and lumbar), skull, and sternebral bones. The above doses ranged from 0.26 times (1 mg/kg) to 2.6 times (10 mg/kg) a maximum recommended daily dose of 40 mg (Paget's disease) based on surface area, mg/m^2. No similar fetal effects were seen when pregnant rabbits were treated at doses up to 35 mg/kg/day (10.3 times a 40 mg human daily dose based on surface area, mg/m^2).

Both total and ionized calcium decreased in pregnant rats at 15 mg/kg/day (3.9 times a 40 mg human daily dose based on surface area, mg/m^2) resulting in delays and failures of delivery. Protracted parturition due to maternal hypocalcemia occurred in rats at doses as low as 0.5 mg/kg/day (0.13 times a 40 mg human daily dose based on surface area, mg/m^2) when rats were treated from before mating through gestation. Maternotoxicity (late pregnancy deaths) occurred in the female rats treated with 15 mg/kg/day for varying periods of time ranging from treatment only during pre-mating to treatment only during early, middle, or late gestation; these deaths were lessened but not eliminated by cessation of treatment. Calcium supplementation either in the drinking water or by minipump could not ameliorate the hypocalcemia or prevent maternal and neonatal deaths due to delays in delivery; calcium supplementation IV prevented maternal, but not fetal deaths.

There are no studies in pregnant women. FOSAMAX should be used during pregnancy only if the potential benefit justifies the potential risk to the mother and fetus.

3.1.2. NDA review

Developmental toxicity studies were conducted in rats and rabbits. Rats were dosed by gavage at 5, 10 or 25 mg/kg/d from GD 6-17 and sacrificed on day GD 20. An increase in the number of litters with incomplete ossification was seen in all dose groups. Rabbits were dosed by gavage at 3.5, 10 or 35 mg/kg/d from GD 6 to 18 and sacrificed on day 28. External examination revealed 1/15 mid dose litters with malformations and 1/15 low dose litters with variations. An increase in skeletal malformations/variations was observed in high dose animals. Additional study details can be found in Appendix V.

The studies described below were reviewed in the NDA and also published by the sponsor (Minsker et al., 1993).

To assess female fertility, alendronate was administered to female rats at doses of 5, 10, and 15 mg/kg/d orally by gavage for 14 days prior to mating through day 20 of gestation. A second experiment dosed animals at 15 mg/kg/d for 6-7 days during the following time periods: day 6-1 of premating; GD 0-6; GD 7-13; GD 14-20; day 14 premating through GD 20. The purpose of the second experiment was to examine the critical time period of reproductive toxicity. A third experiment dosed animals at 15 mg/kg/d for GD 1-21 in order to examine the effect of maternal hypocalcemia induced by alendronate on reproductive toxicity. A fourth experiment examined

the effect on calcium supplementation when administered on GD 21 to animals dosed with alendronate starting 4 days prior to mating and continuing through GD 20.

The results of this study are as follows. In the female fertility study, tremors, dystocia, labored breathing, lethargy and death and associated neonatal deaths due to protracted deliveries were seen at 10 (4/19 dams) and 15 mg/kg/d (5/18 dams). One dam dosed at 5 mg/kg without physical signs of distress failed to deliver; sacrificed on D 24, this dam had 4 dead intrauterine pups. No treatment-related effects on fertility were observed. No gross malformations were seen in any treatment group upon external evaluation.

In the second study, no critical period of treatment associated with physical signs and deaths in late gestation was identified. Maternal deaths occurred on GD 20-22 in all treatment groups except control and animals dosed from premating days 6 through 1. In the third experiment, serum Ca was decreased by 23% relative to control animals. No difference in fetal plasma Ca levels was seen. In the fourth experiment, iv Ca supplementation prevented the adverse physical signs in dams as previously reported. No treatment-related effects on pups were observed. Skeletal events were outside the scope of the study.

3.1.3. Literature Review of Animal Studies

Decreased locomotion, hypothermia and dyspnea were observed in female pregnant rats treated with doses as low as 0.5 mg/kg/d (dosing period not stated). The findings were attributable to hypocalcemia. This dose was about 3 times the human dose on a weight basis. There was no increase in adverse pregnancy outcome, congenital anomalies, or subsequent behavioral abnormalities of offspring after maternal doses up to 2.5 mg/kg/d. At the top dose, however, incisor eruption was delayed and incisors came in at abnormal angles. Other studies showed similar incisor deformation in offspring after maternal doses of 1 mg/kg/d and in the subset of male offspring, after maternal doses of 0.5 mg/kg/d. Rats did not show fertility impairment at doses of alendronate up to 0.5 mg/kg/d, although the number of corpora lutea and implants was slightly decreased in pregnant animals at the top dose. Teratogenicity testing in rabbits was negative (cited from REPROTOX, 2000; original studies published in Japanese).

In rats treated with either saline or alendronate at 0.1 mg/kg/d sc on days 11-20 of pregnancy (approximately equivalent to a human dose of 10 mg/kg, based on body weight), diaphyseal length was significantly ($p<0.05$) reduced in the alendronate group (2.97 mm vs 3.48 mm). There was a 2-3 fold increase in the volume of diaphyseal bone with a concomitant decrease in the volume of bone marrow in the fetuses of dams treated with alendronate in comparison to control. No effect was seen on the distal or proximal epiphyses (Patlas et al., 1999).

3.1.4. Transplacental Transfer

The transplacental transfer of alendronate has been demonstrated in 2 rats dosed sc on day 20 of gestation with a single injection of 0.03 mg/kg ^{14}C-alendronate (Patlas et al., 1999). Maternal serum contained 0.0675% of the administered dose 2 hr after dosing. The placenta (N=3) contained 0.202%, and the fetuses (n=8) 0.020% at the 2 hour timepoint. Twenty-four hours after the injection, maternal serum ^{14}C-alendronate dropped to 0.0056%, and the amount of ^{14}C-alendronate decreased in the placenta (N=3) to 0.096% but increased in the fetuses (N=10) to 0.051% of the dose.

This is consistent with accumulation of the radionucleotide ^{90m}Tc-MDP (metheylene diphosponate) in the placenta and fetal skeleton when the radionucleotide was given to two

women receiving 740 MBq and 540 MBq ^{99m}Tc-MDP at weeks 32 and 30 weeks gestation, respectively (McKenzie et al., 1994).

3.1. Cimadronate

3.2.1 Literature Review of Animal Studies

Cimadronate was administered iv to female rats at 0.16, 0.31 or 0.62 mg/kg/d from 2 weeks prior to mating through GD 7 with sacrifice on day 20; from GD 7 through 17 with sacrifice of some animals on GD 20; or GD 17-21. Dosing was based on maternal toxicity observed at 1.25 mg/kg/d. A separate male fertility study was negative. A similar result was obtained in the Segment I study (dosing to GD 7). In the Segment II study, one female in the 0.62 mg/kg/d dose group died on lactation day 2 with one dead fetus in utero. No external abnormalities were seen in pups in any dose group; visceral findings were considered minor and/or incidental in nature. Skeletal examination indicated delayed ossification in the high dose group resolved by day 22 of lactation. In the Segment III study, 6, 4, and 5 females (n = 22/group) in the low to high dose groups, respectively, were found dead or sacrificed moribund close to or during parturition. A dose dependent increase in malocclusion and uneven growth of the lower incisors was recorded around the time of weaning, and tooth eruption was delayed in the offspring of the high dose group. A teratology study in rabbits was negative (Okazaki et al., 1995).

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3.4. Etidronate

3.4.1. Current Labeling-US

In teratology and developmental toxicity studies conducted in rats and rabbits treated with dosages of up to 100 mg/kg (5 to 20 times the clinical dose), no adverse or teratogenic effects have been observed in the offspring. Etidronate disodium has been shown to cause skeletal

abnormalities in rats when given at oral dose levels of 300 mg/kg (15 to 60 times the human dose)[1]. Other effects on the offspring (including decreased live births) are at dosages that cause significant toxicity in the parent generation and are 25 to 200 times the human dose. The skeletal effects are thought to be the result of the pharmacological effects of the drug on bone.

There are no adequate and well-controlled studies in pregnant women. Didronel (etidronate disodium) should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

3.4.2. NDA review

Etidronate was added to the diet of rats at 0.1 or 0.5% either continuously for 3 generations or days 6-15 of gestation. Pregnant rats were sacrificed on days 13 or 21 for teratology. Pups were examined for gross malformations and skeletal defects. Rabbits were dosed with etidronate at 25, 50 or 100 mg/kg/d via diet on pregnancy days 2-16 and sacrificed on day 29. A previous study in rabbits indicated that at a dose of 500 mg/kg/d via gavage, dams died after 4-5 days of dosing. Examination was similar as for rats. In these studies the frequency of malformations was not increased among the offspring of rats or rabbits. The results of the study were published (Nolen and Buehler, 1971). According to the authors, the dietary dose in rats was one-twelfth and one-third the LD50 dose (1.34 g/kg).

The results of this dietary study should be considered in light of the recommendation that bisphosphonates not be given with food (Physicians' Desk Reference). The study appears to for the basis for the label recommendations. No maternal toxicity was observed in rats in the study, and no pharmacokinetic data were provided. The label for alendronate includes the following information on the effect of diet.

"A study examining the effect of timing of a meal on the bioavailability of alendronate was performed in 49 postmenopausal women. Bioavailability was decreased (by approximately 40%) when 10 mg alendronate was administered either 0.5 or 1 hour before a standardized breakfast, when compared to dosing 2 hours before eating. In studies of treatment and prevention of osteoporosis, alendronate was effective when administered at least 30 minutes before breakfast.

Bioavailability was negligible whether alendronate was administered with or up to two hours after a standardized breakfast. Concomitant administration of alendronate with coffee or orange juice reduced bioavailability by approximately 60%."

3.4.3. Literature Review of Animal Studies

Mice were injected once ip at 200 mg etidronate/kg during days 7-11 of pregnancy and sacrificed on day 18. Fetal weight was significantly decreased in all treated groups when compared to control mice. No effect was observed on implantation, number of live fetuses, or maternal body weight (Sakiyama et al., 1985).

In another investigation, mice were treated as above and examined primarily for external anomalies. In this report, anomalies were classified as cranial or facial and further classified with regard to hemorrhage. The total incidence of fetal malformation and internal hemorrhage is

[1] The exact source of these data are unclear. The data may be taken from Hirohashi et al., Reproduction studies of SM-5600 in rats. The Clinical Report 23: (4): 91-89, 1989. Data tables in English; text in Japanese.

presented in the table below. Sites of internal hemorrhage included the cranial suture; tip of the nose; eyelid; ear; mandibular; forehead; angle of mouth; nasal ala; tongue; and buccal region. Hemorrhage appeared at 1, 2, or 3 sites per fetus. Single cases of hemorrhage always appeared on the nose or eyelids. Types of malformation included exencephalia; cleft palate; and cleft lip. Cleft palate appeared in every treatment group. Cleft lips were seen in combination with cleft palate in one fetus on treatment days 7-9. Litter incidences were generally not reported, except the observation that malformations were concentrated in a single mother. In one example, a dam treated on day 7 had 7 of 13 fetuses with exencephalia (53.8%). In another example, 3 of 12 fetuses showed this finding. In a third dam treated on day 10, 3 of 16 fetuses (18.8%) showed this finding (Sakiyama et al., 1986).

Day	No. of implants	Normal	Internal hemorrhage	Malformation	Internal hemorrhage + Malformation
Control	218	211 (96.8)	6 (2.8)	1 (0.5)	0
7 th	241	99 (41.1)	100 (41.5)	25 (10.4)	17 (7.1)
8 th	249	147 (59.0)	79 (31.7)	20 (8.0)	3 (1.2)
9 th	238	101 (42.2)	105 (44.1)	21 (8.8)	11 (4.6)
10 th	245	107 (43.7)	84 (34.3)	34 (13.9)	20 (8.2)
11 th	242	39 (16.1)	143 (59.1)	23 (9.5)	37 (15.3)

( ): %

In a review of the available literature, alterations in ossification were reported among the offspring of rats treated with 1.5-5 times the human dose of etidronate during pregnancy in one study. In another study reversible skeletal anomalies were seen at higher doses in offspring of pregnant rats treated with <1-75 times the human therapeutic dose (TERRIS, 1999).

3.5. Foscarnet

3.5.1. Current Labeling-US

FOSCAVIR did not adversely affect fertility and general reproductive performance in rats. The results of peri- and post-natal studies in rats were also negative. However, these studies used exposures that are inadequate to define the potential for impairment of fertility at human drug exposure levels.

Daily subcutaneous doses up to 75 mg/kg administered to female rats prior to and during mating, during gestation, and 21 days post-partum caused a slight increase (< 5%) in the number of skeletal anomalies compared with the control group. Daily subcutaneous doses up to 75 mg/kg administered to rabbits and 150 mg/kg administered to rats during gestation caused an increase in the frequency of skeletal anomalies/variations. On the basis of estimated drug exposure (as measured by AUC), the 150 mg/kg dose in rats and 75 mg/kg dose in rabbits were approximately one-eighth (rat) and one-third (rabbit) the estimated maximal daily human exposure. These studies are inadequate to define the potential teratogenicity at levels to which women will be exposed.

There are no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, FOSCAVIR should be used during pregnancy only if clearly needed.

3.5.2. Literature Review of Animal Studies

Administration of foscarnet (phosphonoformic acid) to newborn rats (n = 5) at 10 mg/kg sc at the age of 3, 4, 5, 6, 7, 10 or 15 days. The pups were sacrificed 24 hr after injection in order to examine effects on developing enamel of rat molars. Molars of rats injected at days 10 and 15 showed no changes. Foscarnet induced subameloblastic cysts after injection to 4-7 day old rats. The authors conclude that injected monophosphates can induce pathologic changes in the developing enamel organ and hypoplasias in the enamel (Caracatsanis, 1989).

3.5.3. Literature Review of Human Case Reports

A 21-year-old pregnant female at 18 weeks' gestation with a history of AIDS for 3 years and recurrent genital HSV infection was unresponsive to high dose oral acyclovir. The patient received foscarnet 40 mg/kg (2,920 mg) IV through a central venous catheter infused over 2 hours every 12 hours for 8 days (15 doses). The baby was born HIV negative and developed normally for the first year of follow-up. No adverse effects to the skeleton related to in utero exposure to foscarnet were seen (Alvarez-McLeod et al. 1999). In this report, 2 other cases involving foscarnet use in pregnancy are cited from Astra Pharmaceuticals' surveillance data. An HIV-negative female with an intrauterine pregnancy of 32 weeks' gestation who had acyclovir-resistant HSV encephalitis and retinitis was treated with foscarnet 60 mg/kg IV every 8 hours for 17 days. A healthy baby was delivered at term. In the second case, an HIV-positive female with an intrauterine pregnancy of 25 weeks' gestation was treated with foscarnet 40 mg/kg IV every 8 hours beginning at week 29-30 of pregnancy; no further follow-up data are available.

A 29-year-old woman in week 22 of her first pregnancy presented with a recurrence of genital HSV-2 lesions. A 7-day course of intravenous foscarnet (40 mg/kg) three times daily caused complete clearing, and HSV cultures were negative 6 days after starting therapy. At 32 weeks' gestation, she again tested positive for HSV-2. A second course of foscarnet was administered. Subsequent HSV cultures were negative, and the lesions cleared. After an emergency cesarean section during gestational week 39, the infant died from progressive respiratory failure from acute hemorrhagic pneumonia and hyaline membrane disease. No signs of HSV infection were present. The role of foscarnet in the death of the infant is unknown (Beasley et al. 1997).

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3.7. Pamidronate

3.7.1. Current Labeling-US

There are no adequate and well-controlled studies in pregnant women. Bolus intravenous studies conducted in rats and rabbits determined that Aredia produces maternal toxicity and embryo/fetal effects when given during organogenesis at doses of 0.6 to 8.3 times the highest recommended human dose for a single intravenous infusion. As it has been shown that Aredia can cross the placenta in rats and has produced marked maternal and nonteratogenic embryo/fetal effects in rats and rabbits, it should not be given to women during pregnancy.

3.7.2. NDA review: no studies were reviewed in the NDA (20-036).

3.7.3. Literature Review of Animal Studies

Aside from the studies published by the sponsor (Graepel et al. 1992), no preclinical reproductive toxicity studies of pamidronate were found in the literature. The article describes Segment I-III oral studies in rats and Segment II studies in rabbits. Segment II studies by iv administration were also conducted in both species. Doses administered and fetal effects are summarized in Table 3 in Section 4 of this review. The authors conclude that there was no evidence of teratogenicity in any of the studies conducted. Renal effects in rats as noted in the table were considered by the authors not to be evidence of teratogenic potential but to be a consequence of the known nephrotoxic effect of pamidronate.

3.7.4. Literature Review of Human Case Reports

A 24 year old female with malignant hypercalcemia received 30 mg pamidronate over a 2 hour infusion 2 weeks before giving birth. Aside from transient hypocalcemia in the infant, no other adverse effects were reported (Dunlop et al., 1990). A second patient with metastatic breast cancer was given pamidronate in the third trimester with no adverse effects on the fetus reported (Illidge et al., 1996).

3.8. Risedronate

3.8.1 Current Labeling-US

Survival of neonates was decreased in rats treated during gestation with oral doses $\geq$ 16 mg/kg/day (approximately 5.2 times the 30 mg/day human dose based on surface area, mg/m ^{2}). Body weight was decreased in neonates from dams treated with 80 mg/kg (approximately 26 times the 30 mg/day human dose based on surface area, mg/m^2). In rats treated during gestation, the number of fetuses exhibiting incomplete ossification of sternebrae or skull was statistically significantly increased at 7.1 mg/kg/day (approximately 2.3 times the 30 mg/day human dose based on surface area, mg/m^2). Both incomplete ossification and unossified sternebrae were increased in rats treated with oral doses $\geq$ 16 mg/kg/day (approximately 5.2 times the 30 mg/day human dose based on surface area, mg/m^2). A low incidence of cleft palate was observed in fetuses from female rats treated with oral doses $\geq$ 3.2 mg/kg/day (approximately 1 time the 30 mg/day human dose based on surface area, mg/m^2). The relevance of this finding to human use of ACTONEL is unclear. No significant fetal ossification effects were seen in rabbits treated with oral doses up to 10 mg/kg/day during gestation (approximately 6.7 times the 30 mg/day human

dose based on surface area, mg/m^2). However, in rabbits treated with 10 mg/kg/day, 1 of 14 litters were aborted and 1 of 14 litters were delivered prematurely.

Similar to other bisphosphonates, treatment during mating and gestation with doses as low as 3.2 mg/kg/day (approximately 1 time the 30 mg/day human dose based on surface area, mg/m^2) has resulted in periparturient hypocalcemia and mortality in pregnant rats allowed to deliver.

There are no adequate and well-controlled studies of ACTONEL in pregnant women. ACTONEL should be used during pregnancy only if the potential benefit justifies the potential risk to the mother and fetus.

3.8.2. Literature Review of Animal Studies

No preclinical reports were found in the literature.

3.9. Tiludronate

3.9.1. Current Labeling-US

In a teratology study in rabbits dosed during days 6-18 of gestation at 42 mg/kg/day and 130 mg/kg/day (2 and 5 times the 400 mg/day human dose based on body surface area), there was dose-related scoliosis likely attributable to the pharmacologic properties of the drug.

Mice receiving 375 mg/kg/day tiludronic acid (7 times the 400 mg/day human dose based on body surface area, mg/m^2) for days 6-15 of gestation showed slight maternal toxicity (decreased body weight gain), increased postimplantation loss, decreased number of fetuses/dam, and decreased fetus body weight. Uncommon malformations of the paw (shortened or missing digits, blood blisters between or in place of digits) were present in six fetuses at 375 mg/kg/day, all from the same litter.

Maternal toxicity (decreased body weight) was also observed in a teratology study in rats dosed during days 6-18 of gestation at 375 mg/kg/day tiludronic acid (10 times the 400 mg/day human dose based on body surface area, mg/m^2). There were reduced percent implantations, increased postimplantation loss, and increased intra-uterine deaths in the rats. There were no teratogenic effects on fetuses.

Protracted parturition and maternal death, presumably due to hypocalcemia, occurred at 75 mg/kg/day tiludronic acid (two times the 400 mg/day human dose based on body surface area, mg/m^2) when rats were treated from day 15 of gestation to day 25 postpartum.

There are no adequate and well-controlled studies in pregnant women. SKELID should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

3.9.2. Literature Review of Animal Studies

No preclinical reports were found in the literature.

3.10. Zoledronate

3.10.1. Current Labeling-US

In female rats given subcutaneous doses of zoledronic acid of 0.01, 0.03, or 0.1 mg/kg/day beginning 15 days before mating and continuing through gestation, the number of stillbirths was increased and survival of neonates was decreased in the mid- and high-dose groups (0.2 times the human systemic exposure following an intravenous dose of 4 mg, based on an AUC comparison). Adverse maternal effects were observed in all dose groups (with a systemic exposure of =0.07 times the human systemic exposure following an intravenous dose of 4 mg, based on an AUC comparison) and included dystocia and periparturient mortality in pregnant rats allowed to deliver. Maternal mortality may have been related to drug-induced inhibition of skeletal calcium mobilization, resulting in periparturient hypocalcemia. This appears to be a bisphosphonate class effect.

In pregnant rats given a subcutaneous dose of zoledronic acid of 0.1, 0.2, or 0.4 mg/kg/day during gestation, adverse fetal effects were observed in the mid- and high-dose groups (with systemic exposures of 2.4 and 4.8 times, respectively, the human systemic exposure following an intravenous dose of 4 mg, based on an AUC comparison). These adverse effects included increases in pre- and post-implantation losses, decreases in viable fetuses, and fetal skeletal, visceral, and external malformations. Fetal skeletal effects observed in the high-dose group included unossified or incompletely ossified bones, thickened, curved or shortened bones, wavy ribs, and shortened jaw. Other adverse fetal effects observed in the high-dose group included reduced lens, rudimentary cerebellum, reduction or absence of liver lobes, reduction of lung lobes, vessel dilation, cleft palate, and edema. Skeletal variations were also observed in the low-dose group (with systemic exposure of 1.2 times the human systemic exposure following an intravenous dose of 4 mg, based on an AUC comparison). Signs of maternal toxicity were observed in the high-dose group and included reduced body weights and food consumption.

In pregnant rabbits given subcutaneous doses of zoledronic acid of 0.01, 0.03, or 0.1 mg/kg/day during gestation (0.5 times the human intravenous dose of 4 mg, based on a comparison of relative body surface areas), no adverse fetal effects were observed. Maternal mortality and abortion occurred in all treatment groups (at doses =0.05 times the human intravenous dose of 4 mg, based on a comparison of relative body surface areas). Adverse maternal effects were associated with, and may have been caused by, drug-induced hypocalcemia.

There are no adequate and well-controlled studies in pregnant women. Zometa should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

3.10.2. Literature Review of Animal Studies

No preclinical reports were found in the literature.

4. Summary

Table 3 contains a summary of the reported effects of the bisphosphonates and foscarnet in animals. This information is based on the drug labels and published reports. Adverse effects primarily include skeletal anomalies, cleft palate, delays in parturition, hypocalcemia, dental and renal abnormalities, decreased implantations and fetal death.

Table 3. Summary of bisphosphonate effects in developmental toxicity studies.

Drug	Species	Dose	Finding
alendronate	rat	0.1 mg/kg sc	Decreased diaphyseal length, increased diaphyseal bone volume and decreased bone marrow volume
	rat	5, 10, 25 mkd po	5 mkd: incomplete ossification
	rat	5, 10, 15 mkd po	5 mkd: maternal death; dead full-term pups; hypocalcemia; 10 mkd: delay and failure of delivery; death of dam; fetal death
	rat	1, 2, 5 mkd po	1 mkd: decreased pup weight gain; 2 mkd: decreased post implantation survival; 5 mkd: increase in supernumerary ribs
	rat	0.5, 1.25, 5 mkd po	0.5 mkd: protracted parturition
	rat	0.5, 1, 2.5 mkd	0.5 mkd: incisor deformation
	rabbit	3.5, 10, 35 mkd po	35 mkd: possible skeletal malformation/variation
etidronate	mice	200 mg/kg ip single injection	Exencephalia, internal hemorrhage in the cranial suture region, nose and eyelid, cleft palate and cleft lip, dental abnormalities
	rat	300 mg/kg oral	300 mg/kg: skeletal abnormalities
	rat	0.1, 0.5% diet	No fetal effects (see text for comment)
	rabbit	25, 50, 100 mkd diet	No fetal effects
foscarnet	rat	75, 150 mg/kg sc	Studies inadequate to address teratogenicity
	rabbit	75 mg/kg sc	
pamidronate		25, 60, 150 mkd po	No maternal toxicity at any dose in Seg II study; 60 mkd: ↓ mean number live pups, pup viability; 150 mkd: delay or prolongation in parturition; skeletal maturation and ossification delayed; increased renal cavitation (LD and HD only)
		6, 9, 12, 15 mkd iv	6 mkd: dose-dependent decrease in food consumption in dams (amt not stated); skeletal maturation and ossification delayed; 9 mkd: dose-dependent decrease in body weight gain in dams (amt not stated); 12 mkd: ↑ in early and late resorptions, embryonic and fetal deaths; shortening of scalpula, humerus, ulna, radius or femur; cleft palate with local edema in 1 fetus at this dose
		1, 3, 6 mkd iv	Some dams in all groups sacrificed in distress or for humane reasons (hypocalcemia suspected); 1 mkd: displaced testes; nasal cavity dilated 3 mkd: renal pelvic cavitation; kinked ureter; dilated ureter; displaced testes; fetal hematoma; placental hemorrhage; 6 mkd: subcutaneous hemorrhage outer surface of cerebellum; tarsal flexure; extra cleft in median lobe of liver
	rabbit	12.5, 25, 50 po	No maternal or fetal effects.
	rabbit	0.25, 0.75, 1.5 mkd iv	1.5 mkd: Increased intrauterine deaths

risedronate	rat	3.2, 16, 80 mkd po	3.2 mkd: cleft palate, periparturient hypocalcemia and mortality of mothers; 16 mkd: decreased survival of neonates, incomplete ossification and unossified sternebrae; 80 mkd: Decreased body weight of neonates
	rat	7.1 mkd po	Incomplete ossification of sternebrae or skull
	rabbit	10 mkd po	Spontaneous abortion, premature delivery
tiludronate	mice	375 mkd	Decreased maternal and fetal weight gain, increased postimplantation loss, decreased number of fetuses, shortened or missing digits, blood blisters between or in place of digits
	rat	375 mg/kg/d	No teratogenic effects
	rat	75 mg/kg/d	Protracted parturition and maternal death
	rabbit	42, 130 mkd	42 mkd: scoliosis
zolendronate	rat	0.1, 0.2, 0.4 mkd sc	0.1 mkd: skeletal variations; 0.2 mkd: pre and post implantation losses, decreased viable fetuses, fetal skeletal, visceral and external malformation; 0.4 mkd: skeletal anomalies, reduced lens, rudimentary cerebellum, reduction or absence of liver lobes, reduction of lung lobes, vessel dilation, cleft palate and edema
	rabbit	0.01, 0.03, 0.1 mkd sc	0.01 mkd: maternal mortality and abortion; no fetal effects

References -

1. Alvarez-McLeod A, Havlik J, Drew KE. Foscarnet treatment of genital infection due to acyclovir-resistant herpes simplex virus type 2 in a pregnant patient with AIDS: case report. Clin Infect Dis 1999; 29:937-8.

2. Beasley KL, Cooley GE, Kao GR et al. Herpes simplex vegetans: atypical genital herpes infection in a patient with common variable immunodeficiency. J Am Acad Dermatol 1997; 37:860-3.

3. Caracatsanis M, Fouda N, Hammarstrom L. Effects of phosphonoformic and phosphonoacetic acids on developing enamel of rat molars. Scand J Dent Res 1989; 97:139-49.

4. Dunlop DJ, Soukop M, McEwan HP. Antenatal administration of aminopropylidene diphosphonate. Ann Rheum Dis 1990; 49:955.

5. Graepel P, Bentley P, Fritz H, Miyamoto M, Slater SR. Reproduction toxicity studies with pamidronate. Arzneimittelforschung 1992; 42:654-67.

6. Illidge TM, Hussey M, Godden CW. Malignant hypercalcemia in pregnancy and antenatal administration of intravenous pamidronate. Clin Oncol (R Coll Radiol) 1996; 8: 257-8.

7. McKenzie AF, Budd RS, Yang C, Shapiro B, Hicks RJ. Technetium-99m-methylene diphosphonate uptake in the fetal skeleton at 30 weeks gestation. J Nucl Med 1994; 35:1338-1341.

8. Minsker DH, Manson JM, Peter CP. Effects of the Bisphosphonate, alendronate on parturition in the rat. Toxicol. Appl. Pharmacol 1993; 121:217-223.

9. Nolen GA, Buehler EV. The effects of disodium etidronate on the reproductive functions and embryogeny of albino rats and New Zealand rabbits. Toxicol Appl Pharmacol 1971; 18:548-561.

10. Okazaki A, Matsuzawa T, Takeda M, York R et al. Intravenous reproductive and developmental toxicity studies of cimadronate (YM175), a novel bisphosphonate, in rats and rabbits. J Toxicol SC 1995; 20:1-13.

11. Patlas N, Golomb G, Yaffe P et al. Transplacental effects of bishosponates on fetal skeletal ossification and mineralization in rats. Teratology 1999; 60:68-73.

12. Porras AG, Holland SD, and Gertz BJ. Pharmacokinetics of alendronate. Clin Pharmacokinet 1999; 36:315-328.

13. REPROTOX: Alendronate. REPROTEXT® Database. MICROMEDEX, Englewood, Colorado (Revised December 2000).

14. Sakiyama Y, Tada I, Yamamoto H, Nakanishi T, Yasuda Y, Soeda Y, Oda M. The effect of ethane-1-hydroxy-1, 1-diphosphonate (EHDP) on fetal mice during pregnancy – with emphasis on implantation and fetal weight. J. Osaka Dent Univ 1985; 19: 87-90.

15. Sakiyama Y, Yamamoto H, Soeda Y, Tada I, Oda M, Nagasawa S, Ikeo T. The effect of ethane-1-hydroxy-1, 1-diphosphonate (EHDP) on fetal mice during pregnancy. J. Osaka Dent Univ 1986; 20:91-100.

16. TERRIS. Etidronate. http://www.tomes.com, reviewed in 1999.

Appendix I - **Zoledronic Acid**

Segment II (Teratology) Study in Rats
Study No: 936059

Site and testing facility: CIBA Pharmaceuticals, Preclinical Safety, Stamford Lodge, Cheshire, UK

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 7-100%. 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 4.6% to 64%. 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, ti tibia shortened, fi 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:

CGP 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, CGP 42 446 was teratogenic in the rat at doses ≥ 0.2 mg/kg.

Appendix II Risedronate sodium

Segment II study rat (C4A/B)
Doses 0 3.2 16 80 mkd (Females treated on Gest Days 6-17; C-section on Gest Day 20)
Examined F0, F1, F2

Effects in F0:
Decreased BW gain (-75%) in HD (Day 6-9), and decreased BW gain in MD and HD (-4%) on Day 15.
Decreased FC (5-15%) in MD and HD during period Day 9-17.
Drug-related periparturient mortality in F0 allowed to deliver. One death of 1 F0 animal during lactation.

Reproductive effects in F0:
Fertility index (% f gravid) reduced in HD (81% vs. 94% in control).
No abortions/early delivery.
No effects on # of corpora lutea/implantations/resorptions in gravid animal
Reduced number of litters (11-10-8-4) mostly due to periparturient mortality.

Effects in F1:
1. C-sectioned animals
Fetal BW decreased in HD (C-section group)
No significant effects on incidence of soft tissue/ skeletal/ external malformations (However, 2 fetuses from 2/22 litters with cleft palate in LD group).
Skeletal variations:
Incidence of unossified 5th and 6th sternebrae: increased in HD
Incidence of incompletely ossified 5th sternebrae: decreased in HD
Incidence of incompletely ossified 4th sternebrae: increased in MD and HD
Incidence of incomplete skull ossification: decreased in LD (sign), increased in HD (ns)
2. Delivering animals
(Note: The small # of HD litters make the F1 findings from litters delivered questionable)
Neonates:
BW of neonates increased in LD, MD, decreased in HD group
Reduced # of neonates (< pp day 4)in MD (?) and HD groups, due to (A) less litters and (B) reduced # viable pups/litter in MD, HD
Reduced survival of neonates from pp day 4-21 in MD, HD
Pups from MD and HD dams had clinical signs. In HD group pups were missing (cannibalized). After weaning on pp day 21 all HD pups died or were euthanized.
Time to pinna detachment: increased in HD; Time to incisor eruption increased in MD and HD (2x in HD).

Reproductive effects in F1:
All HD neonates dead before they could be mated: no results on this group.
Fertility reduced in LD- and MD-derived F1 (90% in control, 70% in LD and MD)
Number of fetuses/litter and birth weight not affected by treatment (in LD and MD)

Maternal NOAEL 3.2 mkd (systemic tox at higher dose: BW/FC)
Developmental NOAEL 3.2 mkd
F1 maternal NOAEL not determined (< 3.2 mkd)

Appendix IV Etidronate

Reproduction and Teratology Studies

A. Rats

1. Three generations of rats (22/s/group) were fed diets containing 0, 0.1 or 0.5% of EHDP continuously from time of weaning, or 0, 0.1 or 0.5% of EHDP on days 6-15 of gestation only. Rats (F_0 generation) were bred three times. Twenty pairs of f16 generation naturally born were selected for the second generation breeding stock and bred twice. F_{1a} and F_{2a} generations naturally born were sacrificed at weaning and F_{1c} and F_{2b} generations were surgically removed from uterus on day 13 and 21 of gestation to evaluate teratogenic effects.

Results

a. There was a significant reduction in the number of live pups born to dams fed 0.5% EHDP during organogenesis in the F_{1a} phase and an increase in the number of still born in the F_{1b} litters.

b. In the second generation females, formation of corpora lutea in dams fed 0.5% EHDP continuously, and the number of live fetus born to mothers treated with 0.5% EHDP during gestation were decreased.

2. Rats (20^f/group) were fed diets containing 0 or 1% EHDP on days 6-15 of gestation. The parent rats (F_0 generation) were bred twice. Pups of the F_{1a} generation were sacrificed at weaning, and those of the F_{1b} generation were surgically removed from uterus on day 13 or 21 of gestation. Both groups were evaluated for teratogenic effects.

Results

EHDP fed at 1% dietary level on days 6-15 of gestation had no effect on maternal clinical findings, reproduction and teratogenic parameters.

Appendix V -Alendronate

REPRODUCTIVE TOXICITY
ORAL RANGE-FINDING IN NON-PREGNANT RABBITS (VOL 11)
TT #88-722-2
TREATMENT: Six groups of female rabbits were given for 13 days orally by gavage in 0.5% methylcellulose, 0, 1.5, 5, 15, 50, or 150 mkd.
RESULTS: There was mortality at 50 and 150 mkd. These groups were killed early; there were no findings in other groups.

ORAL RANGE-FINDING IN PREGNANT RABBITS (VOL 11)
TT #89-701-1. January 1989. Merck, West Point, PA
Lot: #008
TREATMENT: Four groups of New Zealand White rabbits (8 months old; 10/g) were given 0, 4, 10, or 25 mkd orally by gavage in deionized water, days 6 through 18 of gestation. Rabbits were ovulated with 25 USP units of HCG iv and artifically inseminated.
RESULTS
MORTALITY: none
BW/FC/PHYSICAL SIGNS/HEMATOLOGY/SERUM BIOCHEMISTRY: no effects
EMBRYO SURVIVAL/LIVE FETAL WT/EXTERNAL FETAL EXAM: no effects

ORAL RANGE-FINDING IN PREGNANT RABBITS (VOL 11)
TT #89-701-2. February 1989. Merck, West Point, PA
Lot: #008
TREATMENT: Four groups of New Zealand White rabbits (6 months old; 10/g) were given 0, 35, 50, or 75 mkd orally by gavage in deionized water, days 6 through 18 of gestation. Rabbits were ovulated with 25 USP units of HCG iv and artifically inseminated.
RESULTS
MORTALITY: 75 mkd group killed due to excessive wt loss
BW/FC: Wt loss MD (vs gain control)
PHYSICAL SIGNS: decreased water consumption M and HD
HEMATOLOGY: no effects

SERUM BIOCHEMISTRY (day 19 of gestation):

urea nitrogen:	13.5; 14.9, 17.3,	[22 (day 16)]
creatinine:	1.1; 1.2, 1.5,	[1.7 (" ")]
protein	5.0; 4.6*, 4.5*	
albumin,	3.7; 3/3*, 3.2*	[2.6
A/G:	2.9; 2.7, 2.5*	[2.1
calcium:	13.6; 12.9*, 12.7*	[10.7
phosphorus:	4.3; 3.9, 4.1	[3.8
potassium:	4.0; 3.9, 3.4*	[3.4

EMBRYO SURVIVAL: no effects
LIVE FETAL WT: decreased M and HD (13%; $p<0.05$)
EXTERNAL FETAL EXAM: one MD fetus had enlarged hemorrhagic eye
"Maternotoxicity at all doses" p. c-229

EXPLORATORY URINE DRUG LEVEL IN PREGNANT RABBITS (day 8)
TT #89-701-3 February 1989. Merck, West Point, PA
Lot: #008
TREATMENT: Pregnant rabbits (4/g) were given doses of 0, 5, 35, and 75 mkd by gavage in deoinized water on day 8 of gestation. A catheter was inserted into the bladder and urine collected 30 min prior to dosing and 0-3 and 3-6 hours after. No food or water were available, but 100 ml water was intubated 30 min before and 3 hours post dose. Samples were frozen; later thawed, acidified to pH 1, and analyzed.

EXCRETION (0-6 HOURS POSTDOSE)

DOSE (mg/kg)	ug (0-6 h)	PER CENT RECOVERY
5	30	0.2
35	300	0.2
75	800	0.3

ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS
TT #89-701-0. April 1989. Merck, West Point, PA Lot: 008
TREATMENT: Four groups of to New Zealand White rabbits (6 months old; 18/g (ovulated with 25 USP units of HCG iv and artifically inseminated) were given 0, 3.5, 10, or 35 mkd orally by gavage in deionized water, days 6 through 18 of gestation. Rabbits were sacrificed day 28 of gestation. Thorax and viscera of dams examined in gross necropsy.

ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS (0,3.5,10,35mkd)
RESULTS
MORTALITY: Two "treatment-related" at 35 mkd (one non-pregnant and one pregnant); both ate very little and lost weight
NECROPSY (rabbits that died):
#0327: LUNGS: Generalized congestion, edema, red discoloration
histology: diffuse congestion
#0334: STOMACH: edematous and hemorrhagic with surface erosions
"(erosive gastritis = COD)"
ABORTIONS: One (control) and 3 (3.5 mkd)

BODY WEIGHT/FOOD CONSUMPTION:
BW: wt gain decreased at 35 mkd (pregnant) with compensatory gain postdose days 19-28
wt loss at 35 mkd (non-pregnant)
FC (day 19): 135; 140, 121, 121 g/day (other days similar)

LAPAROTOMY DATA	0	3.5	10	35 mkd
LIVE/PREGNANT:	14	15	15	10
LIVE/NOT PREGNANT:	3	0	3	6
DEAD NOT PREGNANT	0	0	0	1
DEAD PREGNANT	0	0	0	1
LIVE FETUSES:	98	95	106	66
IMPLANTS/PREGNANT FEMALE:	7.9	7.4	7.4	7.0
% PREIMPLANTATION LOSS:	15	32	25	25
LIVE FETUSES/PREGNANT FEMALE	7.0	6.3	7.1	6.6

NO EFFECTS ON: fetal wt

FETAL EXAM
EXTERNAL
1/15 litter with malformations (MD) 7% spina bifida
1/15 litter with variations (LD) 7% hematoma

VISCERAL: no drug-related effects
SKELETAL (per litter): 14;15,15,10 litters
Caudal vertebra malformation 1; 2, 0, 3 (7;13,0,30%)
Sternebral malformation 0; 0, 0, 1 (0; 0,0,10%)
Sternebral variation 0; 0, 0, 1 (0; 0,0,10%)
FETAL OSSIFICATION
Fetuses examined 98 95 106 66
% with incomplete oss. 32 31 36 35

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ORAL RANGE-FINDING STUDY IN PREGNANT RATS
TT #89-702-1. January 1989. Merck, West Point, PA
Lot #: L-670,452-0057008
TREATMENT: Six groups of Sprague-Dawley CD rats (10 weeks old; 10/g) were given by gavage in deionized water, 0, 0.5, 1.5, 5.0, 10, and 25 mg/kg/day days 6 through 17 of gestation. Females were sacrificed on day 20 of gestation.

RESULTS
MORTALITY: none
ABORTION: none
PHYSICAL SIGNS: none treatment-related
BODY WEIGHT CHANGES (days 6-18):
18% gain (25 mkd) vs 29% (controls) (4/9 transient wt loss)
HEMATOLOGY: no drug effects
SERUM BIOCHEMISTRY (*p<0.05):
Phosphorous: 6.0; 6.0, 6.1, 6.5, 6.9*, 7.4* mg/dl
ALT: 48; 53, 54, 55, 54, 59* U/L
EMBRYO SURVIVAL (p<0.05)
implants/pregnant female: 17% decrease (25 mkd)
live fetuses/" " : 16% decrease (25 mkd)
LIVE FETAL WEIGHT: 7% decrease (not significant at 25 mkd)
3.3 g vs 3.5g for rest

EXTERNAL EXAM FETUSES: no drug effects

ORAL DEVELOPMENTAL TOXICITY IN PREGNANT RATS (VOL 12)
TT #89-702-C. March 1989. Merck, West Point, PA
Lot #: L-670,452-005Y008
TREATMENT: Six groups of Sprague-Dawley CD rats (12 weeks old; 25/g) were given by gavage in deionized water, 0, 5, 10, and 25 mg/kg/day days 6 through 17 of gestation. Females were sacrificed on day 20 of gestation. The 5 mkd group dosing solution was 55% of expected on the second sampling (week two). All fetuses were examined externally and skeletally; 1/3rd examined viscerally.
RESULTS
MORTALITY: none
ABORTION: none
PHYSICAL SIGNS: rales day 15 gestation (25 mkd)
BODY WEIGHT GAIN: decreased about 9% M and HD
FOOD CONSUMPTION: decreased M and HD (10 and 15%)

MATERNAL NECROPSY:
2 controls: lung mottled/red (pneumonia)
1 control: lung autolyzed
1 HD : lung mottled, edema
1 HD : distended intestines "tx related"

LAPAROTOMY DATA (*p<0.05)
live fetal weight (g; males) 3.6; 3.5, 3.6, 3.4*
(g; females) 3.4; 3.4, 3.6, 3.2

NOT SIGNIFICANT ACCORDING TO MERCK BUT CLEAR TREND:
% preimplantation loss 5.7; 7.3, 7.5, 10.5

EXTERNAL EXAM 25; 24,24,25 litters
litters with malformations 0; 0, 1, 1
VISCERAL EXAM
litters with malformations 0; 1, 0, 2
SKELETAL EXAM

SUMMARY OF FETAL OSSIFICATION DATA (Litter)

	Control	5	10	25 mkd
# LITTERS EXAMINED	25	24	24	25
# WITH SITES OF INCOMPLETE OSSIFICATION	7	11 (p=0.175)	13 (p=0.032)	14 (p=0.016)

SITES OF INCOMPLETE OSSIFICATION				p value
INCOMP. OSS. CERVICAL VERTEBRA				
0	2	1	11	not provided
INCOMP. OSS. THORACIC VERTEBRA				
1	1	6	9	" "
INCOMP. OSS. LUMBAR VERTEBRA				
0	1	0	5	" "
INCOMP. OSS. SKULL BONE				
1	4	1	9	" "
INCOMP. OSS. RIB				
0	3	2	8	" "
INCOMP. OSS. STERNEBRA				
7	10	21	37	p<0.05 trend
INCOMP. OSS. PELVIC BONE				
2	15	5	18	N.S. trend