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APPLICATION NUMBER: 020835

PHARMACOLOGY REVIEW(S)

JAN 9 1998

Drug: NE-5095

Name: Risedronate sodium (Actonel).

NDA SUBMITTED: March 31, 1997

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Sponsor: Procter & Gamble Pharmaceuticals
 Sharon Woods Technical Center
 11450 Grooms Road
 Cincinnati, OH 45242-1434

Category: Bisphosphonate

Indication: Paget's disease of bone.

Proposed clinical dose: 1 x 30 mg capsule/day for 2 months (18.5 mg/m²)

Related Submissions: Efficacy Supplement for Osteoporosis (expected in 1998).

Medical Officer: G. Troendle/S. Dutta

Chemist: S. Markofsky

Pharmacologist: D. Coleman/G. Kuijpers

C. S. O.: R. Hedin

Reviewer Recommendation Code: AP (pending labeling)

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Concurrence:

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1/9/98

CC:

HFD-510
 HFD-510/ColemanD/Kuijpers/Steigerwalt/Hedin/Lutwak/Troendle



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SUMMARY REVIEW OF BONE QUALITY STUDIES:

The following bone quality studies were conducted throughout the course of the

Vol	Study#	Spec.	Rte.	Dose		Study Title:	Result
				Actual	mg/kg/d equiv.		
11	F1 (44448)	Rat	oral			Effects of Experimental Compounds on Longitudinal Growth, Mineralization and Trabecular Bone Density in Weanling Rats (Modified Schenk Procedure)	In 8-wk old rat, Risedronate increased BMD of proximal tibia at doses \geq 0.5 mkd.
11	F2 (40352)	Rat	oral	for 28 days		Subchronic Oral Dose Regimens of NE-58095 in Rat Tibia Metaphyseal Bone	Risedronate increased tibial BMD and trabecular bone volume at all doses used. There was no effect on longitudinal bone growth and epiphyseal plate width at any dose.
11	F3A/B (40376)	Rat (OVX)	oral	on 3-7/28 days for 12 weeks, starting 3 wks after OVX		Pulse Dosing of NE-58095: Effects on Skeletal Metabolism in Oophorectomized Rats (STUDY PART B = F3A)	Risedronate increased tibial BMD at the highest dose used. This dose is equivalent to a total of 10.5 mg/kg/84 days, or a 0.125 mkd oral dose.
11	F4A (40350)	Rat	SC	for 4 days		Acute Pharmacologic Potency of NE-58019 in the TPTX Model and the Schenk Rat Models (TPTX Model)	Risedronate inhibited PTH-induced bone resorption and Ca release at all doses.
11	F4B (40350)	Rat	SC	for 7 days		Acute Pharmacologic Potency of NE-58019 in the TPTX Model and the Schenk Rat Models (TPTX Model)	Risedronate increased tibial BMD and trabecular volume throughout dose range. At doses \geq 0.05 mkd it decreased longitudinal growth rate, and at 5 mkd it decreased epiphyseal plate width.
1	F5 (44573)	Rat	SC	for 7 days		Effects of Experimental Compounds on Longitudinal Growth, Mineralization and Trabecular Bone Density in Weanling Rats (Modified Schenk Procedure)	Risedronate increased tibial BMD and trabecular volume throughout dose range. At doses \geq 0.005 mkd, it decreased longitudinal bone growth, and doses \geq 0.5 mkd it decreased growth plate width.
11	F44 (45301)	Rat (OVX)	SC	91-98 days, starting 1-7 days after OVX (estrogen dose 0.03 mg/90d)		Characterization of the Skeletal Effects of Estrogen Depletion Produced by Ovariectomy and Treatment with Bone Active Agents in the Young and Aged (??) Rat	Risedronate dose-dependently increased vertebral, mid-femoral, proximal tibial BMD at all doses, up to above intact control levels. Mid- and distal tibial BMD were not significantly affected. OVX decreased all parameters. Estrogen had no significant effect on BMD.
11	F6 (44572)	Rat (OVX)	SC	0.015 mg/kg on 1/14 days for 5, 10 or 15 wks, starting 1 wk after OVX; observation at 0,5,10 wks after dose cessation	0.001 mkd	Evaluation of the Bone Effects Following Cessation of Antiresorptive Therapy in the Oophorectomized Rat Model	Risedronate completely reversed the decrease in tibial BMD caused by OVX, estrogen did partially. Effect of Risedronate appeared to be maintained after dose cessation. Prior estrogen dosing did not impair subsequent BMD-increasing action of Risedronate.
11	F7A (44571)	Rat (OVX)	SC	0.005 mg/kg on 2/7 days for 5, 10 or 15 weeks, starting 4 wks after OVX; PTH 0.08 mg/kg on 6/7 days	0.0014 mkd	Effects of Transient Estrogen and Diphosphonate Treatment on Bone Loss in Ovariectomized Rats	Risedronate prevented OVX-induced decrease in cancellous bone mass and OVX-induced increase of bone turnover seen after 5 wks of OVX/treatment. It reversed strength of femoral neck and vertebrae to intact control levels (ns). PTH increased cancellous and cortical bone mass and strength, and increased bone turnover. Risedronate did not affect anabolic effect of PTH.

Vol	Study#	Spec.	Rte.	Dose		Study Title:	Result
				Actual	mg/kg/d equiv.		
11	F7B (44571)	Rat (OVX)	SC	0.005 mkd on 2/7 days for 6 months, starting day after OVX; observation at 0, 35, 180, 360 days after termination of dosing	0.0014 mkd	Effects of Transient Estrogen and Diphosphonate Treatment on Bone Loss in Ovariectomized Rats	Risedronate protected against OVX-induced bone loss and increased bone formation. Bone protection was sustained for a period of 6-12 mo after dose cessation. Estrogen therapy also prevented OVX-induced bone loss but effect was lost within 35 days after treatment cessation.
12	F8 (44601)	Rat (OVX)	SC	0.005 mg/kg on 7/28 days for 52 wks, starting 1 day after OVX; estradiol 0.01 mg/kg on 5/7 days	0.00125 mkd	Direct Stereological Estimation of 3-D Connectivity in Rat Vertebrae: Effect of Estrogen, Etidronate, and Risedronate Following Ovariectomy	Risedronate and estradiol completely prevented vertebral bone loss that occurred after OVX. Risedronate reversed trabecular number and connectivity to above intact control value, estradiol reversed these parameters to control value.
12	F9 (45302)	Ferret (OVX)	SC	0.0075 mkd for 12 wks, starting 10 days after OVX		Evaluation of the Effects of Ovariectomy on Ferrets and Response to Known Bone Agents as a Model for Osteoporosis	Risedronate increased tibial, femoral and vertebral bone mass, and increased tibial trabecular volume and number in OVX animals (sign). Risedronate also increased vertebral and femoral neck strength of OVX animals (ns). OVX itself did not have an effect on any of these parameters.
13	F10 (44948)	Dog (Intact)	oral	0.1, 0.5, 2.5 mg/kg on 7/28 days for 12 wks		Relative Effects of One Cyclic Intermittent Dosing Regimen with Varying Dosages of NE-58095 Administered Orally on Bone Remodeling Dynamics in the Canine	Dose-range-finding study. Risedronate did not decrease distal radial BMD at any dose. However, doses of 0.5-2.5 mg/kg inhibited various bone turnover parameters in iliac crest.
	F11 (45291)	Dog (Intact)	oral	0.08, 0.32, 2.5 mg/kg on 5/7 days for 6 months		Comparative Effects of Various Bisphosphonates on Cancellous Bone Remodeling in the Dog	Risedronate or other bisphosphonates had no effect on vertebral BMD or iliac crest trabecular architecture. Risedronate depressed iliac crest cancellous bone resorption and formation, and resulted in positive bone balance. Risedronate was more potent than other bisphosphonates.
13	F12 (45327)	Dog (Intact)	oral	0.2, 0.5, 2.0 mg/kg on 7/7 days, OR 0.5, 2.0, 8.0 mg/kg on 7/28 days for 6 months		Assessment of Pharmacologic Effects of Oral NE-58095 in Beagle Dogs	Risedronate caused a dose-dependent increase in vertebral BMD, and a decrease in bone turnover in rib and iliac crest. Effect was dependent on cumulative dose, not on dosing regimen.
13	F13 (44218)	Dog (Intact)	oral	0.2, 0.5, 2.0 mg/kg on 7/7 days, OR 0.5, 2.0, 8.0 mg/kg on 7/28 days for 2 years		Effects of NE-58095 on the Biomechanical and Morphological Properties of Bone from Dogs Treated for Two Years	Risedronate caused a small increase in core vertebral and proximal femoral bone mass, but had no clear effect on vertebral trabecular architecture. No effect on cortical bone mass. No effect on biomechanical strength of whole vertebrae, distal femoral core, or mid-femur.
13	F15 (44066)	Pig (OVX)	oral	0.5 mg/kg on 7/28 days for 224 days, starting 2 wks after OVX	0.125 mkd	Comparative Effects of Etidronate and Risedronate on Bone Mass and Biomechanical Strength in Calcium Restricted Ovariectomized Pigs	OVX nor Risedronate had any effect on vertebral BMD. Also, no significant effects of either one on biomechanical strength/other parameters of vertebral cores. OVX increased serum TRAP (bone resorption), Etidronate and Risedronate decreased it.

Vol	Study#	Spec.	Rte.	Dose		Study Title:	Result
				Actual	mg/kg/d equiv.		
13	F14 (44164)	Dog (Intact)	oral	0.5, 2.0 mg/kg on 7/7 days, OR 8.0 mg/kg for 7/28 days, all for 2 years	OR 2 mkd	Effects of Risedronate (NE-58095) on Repair of Microdamage in Dogs	Risedronate slightly increased cortical and trabecular bone volume. Activation frequency was decreased in trabecular and especially in cortical bone. The number of microcracks detected (per animal and per dose group) was too small to determine effect of treatment or microdamage repair.
14	F16A/B (44901)	Mouse	oral	0.1, 9.8 mg/kg/day for 14 days		Developing Collagen-Induced Arthritis (CIA) Model in Mice (Pilot Study)	Dosing efficacy screen. Both doses of Risedronate, and other bisphosphonates, produced thickening of primary spongiosa and growth plate alteration (unspecified what alteration). The 9.8 mkd was selected.
14	F16A/B (44901)	Mouse	oral	9.8 mkd for ≥74 days		Developing Collagen-Induced Arthritis (CIA) Model in Mice	Risedronate or other bisphosphonates had no effect on the development, incidence or severity of collagen-induced arthritis in mice.
14	F17B (44826)	Rat	oral	for 4-5 wks		Comparative Efficacy of Experimental Compounds in the PA-III Rat Model of Metastatic Bone Disease	S.C. injection of prostate adenocarcinoma cells over the calvarium of rats results in local bone tumors and osteolytic lesions. Risedronate produced a decrease in size and incidence of skull perforations at doses ≥0.5 mkd. Effectiveness was increased by dosing prior to tumor cell injection.
14	F17B (44826)	Rat	oral	mkd for 7-30 days		Comparative Efficacy of Experimental Compounds in the PA-III Rat Model of Metastatic Bone Disease	Risedronate produced a dose-dependent decrease in size and incidence of skull perforations. Delayed dosing reduced efficacy.
14	F18 (40351)	Rat	SC	for 5 days		NE-58095 Treatment of Hypercalcemia & Hypercalciuria in the Rat Leydig Cell Tumor Model	Risedronate decreases tumor-cell-induced hypercalcemia and -calciuria, and may thus protect against humoral hypercalcemia of malignancy (HHM).
14	F19 (43131)	Rat	SC	19 days		The Assessment of Disease Progression and NE-58095 Alone or in Combination with Indomethacin in Treating Adjuvant Rats: The Use of a Quantitative One-Dimensional Magnetic Resonance Imaging Technique.	Risedronate inhibited paw swelling and inflammation induced by Modified Freund's Adjuvant (MFA). It also inhibited bone resorption (radiography method).
14	F20 (44574)	Rat	SC	0.15 mkd for ca. 28 days		The Effects of Bisphosphonate Treatment on Bone Resorption in Metastatic Sites in the BD-IV Rat Mammary Adenocarcinoma Model System	Ventricular injection of mammary adenocarcinoma cells causes bone metastases and osteolytic lesions. Risedronate inhibited initiation and severity of bone metastases after injection, and inhibited tumor-induced bone resorption.
14	F21 (45317)	Guinea Pig	SC	0.15 mg/kg for 2 wks daily, then on ½ days for 4 or 6 months	0.075 mkd	The Potential of NE-58095, NE-10216, and EHDP to Alter or Prevent Osteoarthritis (Osteoarthrosis) in the Hartley Guinea Pig	Guinea pig develops osteoarthritic lesions with age. Risedronate decreased size and severity of tibial cartilage lesions in the guinea pig.
14	F22 (45318)	Guinea Pig	SC	0.01 mg/kg on 5/7 days for 6 wks, then 30 days off, for total of 6 or 12 mo		The Potential of EHDP, NE-58095, and Indomethacin to Alter or Prevent Osteoarthritis (Osteoarthrosis) in the Hartley Guinea Pig	After 12 months, Risedronate produced moderate but significant decrease in size and severity of arthritic lesions in medial tibial cartilage.

Vol	Study#	Spec.	Rte.	Dose		Study Title:	Result
				Actual	mg/kg/d equiv.		
14	F23 (44824)	Rat	SC	0.015 mg/kg on 1/7 or 1/14 days for 28 days, NaF 0.8 mkd oral		Evaluation of the Bone Effects of Fluoride and Bisphosphonates in a Combination Therapy in Rats	Risedronate increased tibial and vertebral bone mass. NaF itself had no effects on bone and did not affect the activity of Risedronate.
14	F24 (44210)	Rat (OVX)	SC	0.005 mg/kg on 2/7 days for 5 weeks; estrogen 0.01-0.03 mg implant	0.0014 mkd	Evaluation of the Apparent Synergy Occurring When Low-Dose Estrogen and Bisphosphonates are Dosed Concurrently	Both estrogen and Risedronate inhibited OVX-induced decrease in tibial/vertebral BMD and OVX-induced increase in vertebral bone turnover. Effects of estrogen + Risedronate were larger than effect of either agent alone, ie, estrogen did not block anti-resorptive effect of Risedronate.
14	F25 (44212)	Rat (OVX)	SC	mg/kg on 1/14 days for 6 weeks; estrogen 0.01-0.03 mg implant		Evaluation of the Bone Effects of Combined Estrogen and NE-58095 Treatment in the Oophorectomized Rat Model	Estrogen and Risedronate protected against OVX-induced proximal tibial decrease in BMD. Effects of the agents were additive.
14	F26 (44211)	Rat (OVX)	SC	0.005 mg/kg on 2/7 days for 12 weeks	0.0014 mkd	Evaluation of the Apparent Synergy Occurring When Low-Dose Estrogen and Bisphosphonates are Dosed Concurrently	Both estrogen and Risedronate protected (almost) completely against OVX-induced tibial or vertebral bone loss. Effects of the agents combined was about the same as the effects when used separate (not additive).

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A. EFFECTS RELATED TO PRIMARY ACTIVITY

1. Young growing rat model

Study	Age (wk)	Dose Route	Duration (days)	Dose Range (mg/kg/day)	LED (mg/kg/day)	Dose Affecting GPW	Ratio
F4B	3-4	sc	7		0.0015	>5.0	>3000
F5	3-4	sc	7		0.0015	>5.0	>3000
F1	8	oral	7		0.5	Not determined	-
F2	8	oral	28		0.008	>0.8	>100

LED = lowest effective dose assessed from lowest dose producing a significant increase in BMD.
 GPW = growth plate width, a measure of effect on mineralization.
 Ratio = lowest effective dose/dose affecting GPW.

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Dose (mg/kg/day)	BMD of Tibia (mg/cm)		GPW of Tibia (µm)	
	Study F4B	Study F5	Study F4B	Study F5
Control	87 ± 12	81 ± 7	474 ± 26	574 ± 34
0.0005		91 ± 5		562 ± 78
0.0015	88 ± 17*	96 ± 3*	489 ± 31	555 ± 40
0.005	102 ± 8*	111 ± 7*	485 ± 53	525 ± 29
0.05	129 ± 7*	129 ± 14*	482 ± 33	529 ± 28
0.5		158 ± 13*		472 ± 44*
5.0	140 ± 12*	143 ± 4*	289 ± 60	454 ± 60*

Values are mean ± standard deviation; *p<0.05 compared to control
 BMD= bone mineral density; GPW = growth plate width, a measure of effect on mineralization.
 In both studies, animals 3-4 wk old at initiation; 7 days treatment subcutaneously.

Conclusions

- Risedronate inhibits bone resorption in young growing rats:
 - Lowest effective SC dose is 0.0015 mkd
 - Lowest effective oral dose is generally
- Risedronate does not inhibit mineralization at S.C. doses up to 5 mkd. This conclusion is based on the finding that there is no increase in the width of the growth plate in growing rat bone. The relevance of the actually observed decrease of the plate width is unclear.
- Therapeutic index (effective dose/toxic dose) is >3000. The toxic dose causing mineralization inhibition was actually not established, and was higher than the highest dose used.

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NOTES:

- Assuming an oral bioavailability of . means that an S.C. dose can be multiplied by . to yield the comparable oral dose.
- To obtain the equivalent oral dose in humans, an oral dose in animals is multiplied by: 6x (rat), 5x (ferret), 2x (dog), 1x (minipig). The intended therapeutic dose in humans for Paget's disease is 30 mg/day, or 0.5 mg/kg/day.

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2. General bone loss model

Summary of Studies in Bone Loss Models							
Study	Model	Age at Start	Dose Duration	Dose (mg/kg/dose)	Dose Route	Dose Regimen	Parameters
F4A	TPTX rat	NA	4 days		sc	daily	Serum calcium
F25	OVX rat	10 wk	8 wk		sc	1d/2wk	BMD; histo
F24	OVX rat	10 wk	6 wk	0.005	sc	2d/4wk	BMD; histo
F44	OVX rat	11 wk	1, 12 or 14 wk		sc	varied	BMD
F26	OVX rat	8 mo	12 wk	0.005	sc	2d/4wk	BMD; histo
F9	OVX rat	10 wk	5, 10 or 15 wk	0.015	sc	1d/2wk	BMD
F7A	OVX rat	12 wk	5, 10 or 15 wk	0.005	sc	2d/4wk	Histo; biomech
F7B			6 mo	0.005	sc	2d/4wk	BMD; histo
F3B	OVX rat	>12 mo	12 wk		ip	varied	BMD; histo
F3A			12 wk		oral	varied	BMD
F8	OVX rat	3 mo	52 wk	0.005	sc	7d/26d	Connectivity; histo
F9	OVX ferret	4 mo	12 wk	0.0075	sc	daily	BMD; histo; biomech
F15	OVX minipig	2-4 yr	8 mo	0.5	oral	7d/26d	BMD; biomech

histo-histomorphometry; biomech-biomechanics; NA-not applicable
Intermittent/cyclical dose regimens given as dose days/cycle (cycle is total days on and off dosing)

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Summary of Studies in Ovariectomized Rats						
Study	Age at Start	Dose Duration	Dose Route	Dose (mg/kg/dose)	Dose Regimen	Other
F25	10 wk	8 wk	sc		1d/2wk	Dose immediately post OVX
F24	10 wk	6 wk	sc	0.005	2d/4wk	Dose immediately post OVX
F44	11 wk	1, 12 or 14 wk	sc		varied	Dose immediately or 1 wk post OVX
F26	8 mo	12 wk	sc	0.005	2d/4wk	Dose immediately post OVX
F6	10 wk	5, 10 or 15 wk	sc	0.015	1d/2wk	Start dosing 1 or 8 wk post OVX
F7A	12 wk	5, 10 or 15 wk	sc	0.005	2d/4wk	Start dosing 4 wk post OVX
F7B		6 mo	sc	0.005	2d/4wk	Study up to 360 d post dosing
F3B	>12 mo	12 wk	ip		varied	Cyclic dosing regimens
F3A			oral		varied	Cyclic dosing regimens
F8	3 mo	52 wk	sc	0.005	7d/26d	Trabecular connectivity

Intermittent/cyclical dose regimens given as dose days/cycle (cycle is total days on and off dosing)

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Bone loss models studied include the PTH-treated TPTX (thyroparathyroidectomized) rat, and the OVX rat, OVX ferret and OVX minipig (latter two are remodeling species). Bone resorption is increased in these models, causing increased serum Ca (TPTX model) or decreased BMD (ovx models). Parameters examined were serum Ca in the TPTX model, and BMD/ histomorphometry/ biomechanics in OVX animals.

Conclusions

1. Risedronate inhibits increased bone resorption in animal bone loss models. The lowest effective S.C. dose in the rat varies between
2. In the ovx rat, Risedronate can completely inhibit ovx-induced loss of bone mass and trabecular connectivity. In this model, Risedronate can also prevent the ovx-induced decrease of femoral neck strength.
3. In the ovx rat, Risedronate can inhibit ovx-induced bone loss for extended period after discontinuation of dosing. This is probably related to long half-life and accumulation of substance in bone compartment.
4. In the ovx rat, Risedronate is effective in preventing (further) bone loss when dosing starts immediately after ovx, or when dosing is delayed and bone loss is already established.
5. In the ovx rat, the effectiveness of Risedronate is related to total (cumulative) dose.
6. In the intact rat, Risedronate also inhibits bone resorption and increases BMD.
7. In OVX ferrets (3-month study), Risedronate can increase BMD, improve trabecular architecture, and increase vertebral bone strength, even though OVX has no effect on these parameters.
8. By exception, in OVX minipigs (8-month study), OVX and Risedronate have no effect on BMD or vertebral strength.

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3. Effects on bone activation and bone balance

Summary of Studies on Bone Activation and Bone Balance							
Study	Species	Age at Start	Dose Duration	Dose Route	Dose (mg/kg/dose)	Dose Frequency	Equivalent Daily Dose (mg/kg/day)
F25	OVX rat	10 wk	6 wk	sc		1d/2wk	0.0011*
F24	OVX rat	10 wk	6 wk	sc	0.005	2d/wk	0.0014
F7A	OVX rat	12 wk	5, 10 or 15 wk	sc	0.005	2d/wk	0.0014
F7B			6 mo	sc	0.005	2d/wk	0.0014
F11	Intact dog	4-8 yr	6 mo	oral	0.03 and 0.32	5d/wk	0.057 and 0.29
F10	Intact dog	9-18 mo	84 days	oral	0.1, 0.5, 2.5	7d/28d cycle	0.025 to 0.625
F12	Intact dog	8-11 mo	8 mo or 2 yr	oral	0.2, 0.5, 2.0	daily	0.2 to 2.0
				oral	0.5, 2.0, 8.0	7d/28d cycle	0.2 to 2.0

* Note: bone activation, turnover and balance not measured at the 0.005 mg/kg dose level in Study F25. Equivalent daily dose calculated from the dose level and dose frequency.

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Two types of studies addressed this issue:

1. Studies in OVX rats on bone formation, resorption and turnover
2. Studies in intact dogs on bone formation, resorption and turnover, and on bone remodeling

Conclusions

Risedronate inhibits bone resorption and formation, and bone turnover, in both intact dogs and ovx rats. Bone turnover markers evaluated include resorption (=erosion) depth, activation frequency, mineral apposition rate, % mineralizing surface, bone formation rate, resorption period.

In the ovx rat, Risedronate can completely prevent the trabecular bone loss and the increased bone turnover caused by ovx.

In the ovx rat, Risedronate is effective in preventing bone loss and suppressing trabecular turnover (by ca. 80%) at a dose equivalent to the therapeutic human dose of 30 mg

In the intact dog, Risedronate causes a decrease in bone turnover, and an increase in BMD after prolonged treatment. Suppression of trabecular bone turnover is suboptimal (ca. 60%) at the 30 mg-equivalent dose. At higher doses a >80% suppression can be achieved.

In the intact dog, Risedronate decreases osteoclast and osteoblast activity, while maintaining a positive bone balance.

In the intact dog, at high doses, Risedronate also suppresses cortical bone turnover.

Risedronate does not cause osteoid accumulation.

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4. Maintenance of bone quality

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Summary of Studies Examining Bone Quality							
Measure of Bone Quality	Study	Model	Age	Dose Duration	Dose (mg/kg/dose)	Dose Route	Dose Regimen
Connectivity	F8	OVX rat	3 mo	52 wk	0.005	sc	7d/28d
Mineralization	F49	Growing rat		7 days	0.0015 - 8.0	sc	daily
	F9	Growing rat		7 days	0.00015 - 8.0	sc	daily
	F2	Growing rat	6 wk	28 days	0.008 - 0.8	oral	daily
	F10	Intact dog		84 days	0.1, 0.5, 2.5	oral	7d/28d
	F12	Intact dog		6 mo or 2 yr	0.2, 0.5, 2.0	oral	daily
Biomechanics	F7A	OVX rat	12 wk	5, 10 or 15 wk	0.005	sc	2d/wk
	F9	OVX ferret	4 mo	12 wk	0.0075	sc	daily
	F15	OVX minipig	2-4 yr	8 mo	0.5	oral	7d/28d
	F13	Intact dog		2 yr	0.2, 0.5, 2.0	oral	daily
Spontaneous Fractures	B16	Intact rat	6 wk	1 yr	0.1, 0.5, 4.0	oral	daily
	B17	Intact dog		6 mo or 2 yr	0.2, 0.5, 2.0	oral	daily
	B19				0.5, 2.0, 8.0	oral	7d/28d
Microfractures	F14	Intact dog		2 yr	0.5, 2.0	oral	daily
					8.0	oral	7d/28d

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Conclusions

Risedronate increases trabecular number and connectivity in ovx rats, as compared to ovx and intact control values, while it reverses the ovx-induced decrease in bone volume to intact control values.

Risedronate maintains or increases bone biomechanical strength, in parallel with effects on bone mass, in ovx rats, ferrets and minipigs.

Risedronate does not cause spontaneous fractures in bone of intact rats or dogs, at pharmacologically effective doses.

It is unclear whether Risedronate has an effect on incidence or size of microfractures in femoral trabecular bone in intact dogs.

Bone formed in the presence of Risedronate appears to be of normal quality. However, explicit data on the histology of bone formed in the presence of Risedronate (lamellar or woven) were not submitted.

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B. EFFECTS RELATED TO SECONDARY ACTIVITY

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Summary of Studies on Secondary Activity						
Study	Animal	Model	Dose (mg/kg/dose)	Dose Route	Dose Duration	Dose Regimen
Tumor Models						
F18	Rat	Leydig cell tumor	0.005-0.05	sc	5 days	daily
F17B	Rat	PA-III tumor	0.0005-0.16	sc	7, 11, 28 or 30 days	daily
F17A			0.0005-5.0	oral	28, 30 or 35 days	daily
F20	Rat	Mammary adenocarcinoma	0.15	sc	1 mo	daily
Arthritis Models						
F16B	Micase	Collagen-induced arthritis	0.8	oral	10 wk	daily
F18	Rat	Adjuvant arthritis	0.025	sc	18 days	daily
F22	Guinea pig	Spontaneous osteoarthritis	0.01	sc	6/12 mo	5 days/wk for 6 wk then 30 days off
F21	Guinea pig	Spontaneous osteoarthritis	0.15	sc	4/8 mo	daily for 2 wk alternate days

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"Secondary activity" is activity in disease models that involve secondary bone effects, in particular excessive osteoclast-mediated bone resorption. The models are of: (1) tumor-induced hypercalcemia and osteolysis, and (2) arthritis.

Conclusions

In animal tumor models, Risedronate suppresses hypercalcemia and osteolysis. The suppression results from inhibition of osteoclast resorption and perhaps also inhibition of tumor cell growth. In some of the animal arthritis models studied, Risedronate has a beneficial effect on arthritic lesions. The effect occurs at high doses relative to anti-resorptive doses.

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C. EFFECTS OF COMBINATION WITH OTHER THERAPIES (DRUG INTERACTIONS)

Summary of Studies of Combination Therapies					
Study	Animal	Concomitant Therapy	Concomitant Drug Dose (mg or mg/kg)	Risedronate Dose (mg/kg/dose)	Dose Duration
F25	OVX rat	Estrogen	0.01 or 0.03 mg over 90 days	0.005/0.015 (1d/2wk)	6 wk
F24	OVX rat	Estrogen	0.01 or 0.03 mg over 90 days	0.005 (2d/wk)	6 wk
F28	OVX rat	Estrogen	0.01 or 0.03 mg over 90 days	0.005 (2d/wk)	12 wk
F8	OVX rat	Estrogen	0.03 mg over 90 days	0.015 (1d/2wk)	6 wk estrogen 5 or 10 wk RIB
F7A	OVX rat	PTH	0.08 mg/kg/day, 6 days/wk	0.005 (2d/wk)	5, 10, 15 wk
F23	Intact rat	Fluoride	0.8 mg/kg/day for 26 days	0.015 (1d/wk or 1d/2wk)	26 days
F19	Intact rat	Indomethacin	0.1 mg/kg/day	0.025 daily	19 days

Note that Study F8 is sequential and not concomitant administration of estrogen and risedronate.
All drugs were given subcutaneously except for fluoride, which was given in the drinking water.
Estrogen was given as slow release implant that released drug at rate of 0.01 or 0.03 mg over 90 days.

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Effects of Risedronate were studied in combination with:

1. Estrogen
2. PTH
3. Fluoride
4. Indomethacin

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Risedronate's anti-resorptive action is maintained when co-administered with estrogen (anti-resorptive agent) or fluoride.

In the presence of an anabolic dose of PTH (0.08 mkd, 6 days/wk), Risedronate has no additional bone effect.

Risedronate does not diminish the anabolic effect of PTH.

Risedronate and Indomethacin have additive effects on paw edema/inflammation in a rat arthritis model.

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In Paget's disease, the primary abnormality is a local increase in osteoclast activity that results in increased bone resorption and bone turnover with formation of structurally fragile osseous tissue at specific sites or lesions. Current therapies are bisphosphonates and Calcitonin. There are no animal models of Paget's disease. The following pharmacology data from animal studies support the use of Risedronate in the treatment of Paget's disease:

In several models of osteoclast-mediated bone resorption and bone loss Risedronate has a dose-dependent inhibitory effect on bone resorption and a concomitant suppression of bone turnover.

In tumor models, Risedronate reduced hypercalcemia and osteolysis, primarily through an anti-osteoclastic action. There were also some beneficial effects in models of arthritis. Effectiveness was observed upon daily or intermittent dosing, in prevention and treatment paradigms, and was sustained for a considerable amount of time after discontinuation of dosing.

Risedronate did not appear to have deleterious effects on mineralization, spontaneous fracture incidence, or mechanical bone strength.

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SUMMARY REVIEW OF PHARMACOLOGY SCREENING PANEL

The following toxicity screening experiments were conducted
Norwich Eaton Pharmaceuticals between July and November of 1990. All experiments used the same lot #
10447-075A.

Vol:	Study#:	Spec.	Route	Dose	Study Title:	Result:
15	F27A/B (42411)	Mouse 20	oral fasted	236 mg/kg	Pharmacology Screen - Neuropharmacological Profile of NE-58095 in the Mouse	No anticonvulsant activity vs. electric shock.
15	F28 (42412)	Mouse 20	oral fasted	236 mg/kg	Pharmacology Screen - The Effects of NE-58095 on Spontaneous Motor Activity in (SMA) Mice	No effect.
15	F29 (42509)	Mouse 20	oral fasted	236 mg/kg	Pharmacology Screen - Pentylentetrazol (Metrazol)- Induced Seizures in Mice	No effect.
15	F30 (42510)	Mouse 20	oral fasted	236 mg/kg	Pharmacology Screen - Acetic Acid Writhing Assay in Mice	No effect.
15	F31 (42508)	Mouse 20	oral fasted	236 mg/kg	Pharmacology Screen - Barbiturate Sleep Time	No effect.
15	F32 (42512)	Mouse 20	oral fasted	236 mg/kg	Pharmacology Screen - Gastrointestinal Propulsion Assay in Male Mice	No effect.
15	F33 (42513)	Rat 40	oral fasted	236 mg/kg	Pharmacology Screen - The Effects of NE-58095 Upon Hemostatic Parameters	No effect.
15	F34 (42416)	Rat 12	oral fasted	236 mg/kg	Pharmacology Screen - The Effect of NE-58095 on Liver Function in Rats	No effect.
15	F35 (42610)	Rat 20	oral fasted	236 mg/kg	Pharmacology Screen - The Effect of NE-58095 on Choleresis in the Rat	No effect.
15	F36 (42415)	Rat 40	oral fasted	236&30 mg/kg	Pharmacology Screen - Diuretic Assay of NE-58095 in the Rat	No effect at 30 mg/kg.
15	F37 (42514)	Rat 20	oral fasted	236 mg/kg	Pharmacology Screen - Carrageenan-Induced Rat Paw Edema Assay	No effect.
15	F38 (42413)	Rat 20	oral fasted	236 mg/kg	Pharmacology Screen - The Effect of NE-58095 Upon Body Temperature in Rats	No effect.
15	F39 (42511)	Rat 20	IV	3 mg/kg	Pharmacology Screen - Secretory Screen in Pyloric Ligated Rats	No effect.
15	F40 (42414)	Dog 4	IV	1 mg/kg	Pharmacology Screen - Acute Hemodynamic Effects of Intravenous Infusion of NE-58095 in the Open-Chest Anesthetized Dog	No effect.
15	F41 (42608)	Rat 8	Bath	10 ug/ml	Pharmacology Screen - Evaluation of NE-58095 for Effects on the Isolated Uterus in Pregnant and Non- Pregnant Rats	No effect.
15	F42 (42609)	Guinea Pig (8)	Bath	10 ug/ml	Pharmacology Screen - Evaluation of NE-58095 for Effects on the Isolated Guinea Pig Right Atrium	No effect.
15	F43 (42607)	Guinea Pig (12)	Bath	10 ug/ml	Pharmacology Screen - Evaluation of NE-58095 for Effects on Spontaneous Motility and Antagonism to Acetylcholine, Histamine and Barium Chloride in the Isolated Guinea Pig Ileum	No effect.

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SUMMARY AND CONCLUSIONS:

These experiments did not detect any significant tendency of Actonel to induce any acute toxicity or response in these specific assays.

SUMMARY REVIEW OF SPECIAL TOXICITY STUDIES:

Special toxicity studies were conducted to attempt to determine the toxic potential of Risedronate in the GI tract (where toxicity has been noted for this entire class of drugs). For manufacturing occupational safety purposes additional tests were conducted to determine eye and skin irritation potential. Antigenicity was also determined in guinea pigs. The following special toxicity studies were conducted throughout the course of the sponsor and by several contract labs:

Vol:	Study#:	Spec.	Route	Dose	Study Title:	Result:
49	Q1A/B/C (45322)	Rat (M) 6/8/8	Oral fasted	30-300 mg/kg	Gastric Safety of Bisphosphonates in the Fasted Rat (STUDY A, B and C)	Minimal increase in gastric irritation in Naprosen & Indomethacin pretreated rats.
49	Q2 (44938)	Rat (M) 8	Oral fasted	30 mg/rat	Evaluation of the Effects of Bisphosphonates on Gastric Acid Secretion in the Rat	All significantly increased volume and acidity of stomach contents.
49	Q3 (44863)	Rat (M)	Intra- colonic fasted	300-800 mg/kg	Evaluation of the Colonic Irritant Properties of Bisphosphonates	No effect of Risedronate or other amino-bisphosphonates but Pamidronate was positive.
49	Q4 (45069)	Guinea Pig	SC	4.2 mg 4 x 1 wk	Antigenicity Study of NE-58095 in Guinea Pigs	Negative
49	Q5 (45091)	Rabbit 2M, 1F	topical	500 mg	A Primary Irritation of the Skin Study Using NE-58095	Minimal skin irritation
49	Q6 (45204)	Rabbit 2M, 1F.	intra ocular	7.7 mg	A Low Volume Eye Irritation Study in Rabbits	Slight irritation and significant increase in opacity.
	Q7 (43684)	Rabbit 3 F	tissue bath	800 mg	Evaluation of the Effect of Risedronate (NE-58095) and Other Diphosphonates on the Colonic Mucosa of Female New Zealand White Rabbits: Interim Report	No effect of any treatment on colonic mucosa.
50	Q8 (44583)	Rabbit 3 F	tissue bath	60 mg	Evaluation of the Effect of Risedronate (NE-58095) and Other Diphosphonates on the Colonic Mucosa of Female New Zealand White Rabbits: Final Report	All treatments resulted in mild irritation of colonic mucosa
50	Q9 (43165)	Dog 3 M / dose	Intra articular	0.1-2.5 mg	Two Week Repeated Dose Toxicity Study of Risedronate(NE-58095) when Administered Intra-articularly in Dogs	Irritation of joints and synovial necrosis, NOEL: 0.1 mg per injection.

SUMMARY AND CONCLUSIONS:

Initial examination of gastric irritation in rats pre-exposed to NSAIDs indicated significant gastric irritation in response to all bisphosphonates tested. Alendronate was the most damaging in this model but Risedronate also induced significant irritation. The physiologic relevance of comparisons based on this model is unclear.

Several additional assays of gastric irritation were either negative or showed slight irritation in response to several bisphosphonates tested, including Risedronate.

Risedronate caused slight ocular and skin irritation but was not antigenic in guinea pigs.

In an unusual assay, dogs showed sensitivity to intra articularly injected Risedronate.

In conclusion, Risedronate appears similar to other bisphosphonates in its potential to irritate the GI system directly. While this effect of Risedronate does not seem to be more severe or less severe than other bisphosphonates at this time, the question has not been conclusively answered by these investigations. The mechanism(s) of this effect remains unknown.

SUMMARY REVIEW OF ACUTE TOXICITY STUDIES:

The following acute toxicity studies were conducted between 1987 and 1990, except the rabbit dermal study which was conducted in 1996. All experiments were conducted by Norwich-Eaton.

All studies included male and female animals.

Vol:	Study#:	Spec.	Route	Dose	Study Title:	Result:
16	A1A/B (40384)	Rat & Mouse n=16x2	Oral fasted	0.5,1,2,4 g/kg	Preliminary Acute Toxicity of NE-58095 Given Perorally to Rodents	All HD animals died within 7 days. Lower doses were well tolerated. Dead rats had distended stomachs. Dead mice had pale livers.
16	A2A/B (42226)	Rat & Mouse n=16x2	IV	6,12,25,50 mg/kg	An Acute Up/Down Study of NE-58095 in Rodents Following Intravenous Administration	All HD animals died within 7 days. Lower doses were well tolerated. MLD= 50 mg/kg, MTD= 25 mg/kg Drug related effects on rat and mouse STOMACHS at 50 mg/kg
16	A3 (40368)	Rat 5/sex/d	Oral not fasted	10 doses between 1-8 g/kg	Maximum Tolerated Dose (MTD) Finding Study (NE-58095)	LD-50 estimated to be 1400 mg/kg for females and 2300 mg/kg for male rats. LD-10 only 50% lower. NOT FASTED
16	A4 (40287)	Rabbit 1/sex/d	Oral fasted	100, 250, 500, 1000 2000 mg/kg	Acute Toxicity of NE-58095 Given Perorally to Rabbits	Both HD, 1 1000 mg and 1 250 mg rabbit died within 7 days. Dead animals exhibited red lungs, pale kidneys and gastric damage.
16	A5 (42253)	Rabbit 1/sex/d	IV	3,6,12,25, 50&100 mg/kg	An Acute Up/Down Study of NE-58095 in Rabbits Following Intravenous Administration	MTD = 3 mg/kg MLD = 6 mg/kg One 6 mg and one 12 mg rabbit died.
	A6 (40301)	Dog 1/sex/d	Oral fasted	50, 75 and 100 mg/kg	Acute Toxicity of NE-58095 Given Perorally to Dogs	Minimum acute emetic dose was 75 mg/kg. Higher doses were vomited. Both 75 mg/kg dogs died of pneumonia. (Gavage or drug related?)
16	A7 (42298)	Dog 1/sex/d	IV	1.6, 4, 9, 25 mg/kg	An Acute Up/Down Study of NE-58095 in Dogs Following Intravenous Administration	All 9 and 25 mg/kg dogs died. Others had diarrhea and anorexia. MTD 4 mg/kg, MLD 9 mg/kg.
16	A8 (45203)	Rabbit 5/sex	Dermal	2 g/kg	An Acute Dermal Toxicity Study in Rabbits with NE-58095	For manufacturing occupational safety. This dose irritated but was not lethal

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SUMMARY AND CONCLUSIONS:

Single oral doses (~2 g/kg) were tolerated by rats and mice (dogs vomited doses greater than 50 mg/kg). On a mg/m² basis the multiple of human (0.5 mg/kg) exposure is 666 (for rats) and 333 (for mice). Higher doses were lethal. Rabbits appeared to be more sensitive to Risedronate but were not extensively tested.

Single IV doses, (3 mg/kg in rabbits, 4 mg/kg in dogs, and 25 mg/kg in mice and rats) were well tolerated. Higher doses were lethal. These IV doses are about 1% of the maximum tolerated oral doses. This is predicted due to an expected absorption of only about 1% of the oral dose.

Drug related gastric effects were noted in dead dogs, rats and rabbits given drug IV. In addition, liver and kidneys were noted as possible target organs in many of the acute studies.

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SUMMARY OF SUBCHRONIC TOXICITY STUDIES < 6 MONTHS:

The following chronic toxicity studies were conducted throughout the course of the by Norwich Eaton:

Vol:	Study#:	Spec.	Route	Dose	Study Title:	Result: (all effects mild - moderate unless otherwise noted).
17	B1 (43744)	Mouse 10/s/d	Oral fasted	1/3/10/32 mg/kg	Subchronic (13 Week) Oral Toxicity of NE-58095 in Mice	No deaths. All doses showed significantly increased trabecular bone but no adverse effects.
18	B2 (39635)	Rat 4M, 6F	Oral fasted	6 doses 0.008-8 mg/kg	Subchronic (4-week) Oral Toxicity of NE-58095 in Rats: A Pilot Study	No deaths. Pharmacological effects on bone but no drug related adverse effects except mild vacuolation of stomach epithelial cells and mild renal degeneration at 8 mg/kg.
18- 19	B3 (40395)	Rat 10/s/d	Oral fasted	0/0.8/2.5/8/ 16 mg/kg	Subchronic (13-week) Oral Toxicity of NE-58095 in Rats	No deaths. Pharmacological effects on bone but no drug related adverse effects except slightly increased SGOT at 16 mg/kg.
20- 22	B4 (45255)	Rat 20/s/d	Oral fasted	0/4/8/16/ 32/64 mg/kg	A 13-Week Oral Toxicity Study of NE-58095 in the Albino Rat Followed by a 6- Week Recovery	64 mg/kg; 9M & 2F died, severe gastritis, severely increased neutrophils, WBC, BUN & thyroid wt, decreased prostate and seminal vesicle weight and hematuria. 32 mg/kg; 1F died. Moderate gastritis. Decreased thymus wt 16 mg/kg and higher doses resulted in reduced calcium, decreased wt. gain & food consumption, smaller teeth increased ALT, AST, testicular atrophy. All doses had decreased alkaline phosphatase and hypertrophy of primary spongiosa
23	B5 (40036)	Rat 4M/d	IV 2 days fasted	1.2/3.5/ 12.5 mg/kg	Comparative IV Toxicity of Bisphosphonates (NE-58019) in Rats: A Screen	No deaths. 12 mg/kg IV for 2 days caused renal damage (Dec. creatinine, nitrogen and kidney wt) and gastric edema. 3.5 mg/kg caused only renal damage. Low dose caused hypocalcemia and hypophosphatemia
23- 24	B6 (42294)	Rat 10/s/d	IV not fasted	0.025/0.1/ 0.4/1.6 mg/kg	28-Day Intravenous Toxicity Study of NE-58095 in the Albino Rat	No drug related deaths. All doses produced hypocalcemia and hypophosphatemia as well as hypertrophy of the primary spongiosa. HD 1.6 mg/kg also caused increases in SGOT and SGPT and minimally reduced liver wt.
	B7 (40394)	Dog 3/s/d	Oral fasted	0.08/0.8/8 mg/kg	Subchronic (4 Week) Oral Toxicity of NE-58095 in Dogs	8 mg/kg; 1 M died. All doses produced hypocalcemia and hypophosphatemia as well as pharmacological effects on the primary spongiosa. HD 8 mg/kg also increased SGOT and AST and reduced liver wt.
26- 27	B8 (40396)	Dog 7/s/d	Oral fasted	0.5/2/4/8 mg/kg	Subchronic (13-Week) Peroral Toxicity of NE-58095 in Dogs	No drug related deaths. The HD 8 mg/kg caused liver atrophy and increased liver enzymes renal cortical cell necrosis and mild-severe spermatid arrest and testicular lesions in addition to expected pharmacological effects at lower doses.
28- 29	B9 (45268)	Dog 4/s/d	Oral fasted	6/8/12 mg/kg for 13 weeks	A Subchronic Study to Evaluate Hepatotoxicity in the Dog When Administered via Capsule	No drug related deaths. HD 12 mg/kg induced mild testicular degeneration and oligospermia and >50% increased ammonia and bile acids, increased serum globulin, AST (400%) and ALT (50%) These effects were slightly less severe at 8 mg/kg.
30	B10 (42658)	Dog 4/s/d	Oral fasted	0.1/0.5/2 mg/kg w. Prednisone	A 13-Week Oral Toxicity Study on NE-58095 in Combination with Prednisone in Beagle Dogs	No deaths. No adverse effects of Risedronate or potentiation of Prednisone effects
31	B11 (42297)	Dog 1/s/d	IV not fasted	0.1/0.3/1 mg/kg	Subacute (7 Day) Toxicity of NE-58095 Administered by Intravenous Infusion in Dogs: a Dose-Ranging Pilot	No deaths. No significant adverse effects, only some pharmacological effects of Risedronate. However an unexpectedly high number of cases of small prostates mirrors the previous finding of a small prostate in 1 dog at 8 mg/kg seen in B8 and in rats in B4.
32	B12 (42299)	Dog 6/s/d	IV not fasted	.05/0.3/1.5 mg/kg	Subacute (14-Day) Toxicity of NE-58095 Administered by Intravenous Infusion in Dogs	No deaths. 1.5 mg/kg HD increased SGOT 4-fold and SGPT 50%. Mild Liver atrophy, Renal necrosis and Moderate Spermatid maturation block in addition to expected pharmacological actions at lower doses.
33	B13 (42296)	Dog 8/s/d	IV not fasted	0 and 0.3	A 4-Week Intravenous Toxicity Study on NE-58095 in Beagle Dogs	No deaths. No adverse effects at 0.03 or 0.1 mg/kg. HD 0.3 mg/kg caused (100%) increase in SGOT and 30% decrease in BUN in addition to pharmacological effects which were seen at lower doses.
34	B14 (43427)	Mouse 10/s/d	Oral fasted	0/16/24/64 mg/kg	Oral (Gavage) Range Finding Study in the Mouse:	0/2/3/8 deaths per group. Weight loss and GI distention only in HD group. 20 week interim report

SUMMARY:

Fourteen repeated dose toxicity studies of less than 6 month duration were conducted with Risedronate in mice rats and dogs. Although several of these studies (B1, B2, B3, B10, B11) did not demonstrate any clear toxic effects and were conducted at levels below the NOEL, all of these studies taken together show the time and dose dependence of the toxic effects of Risedronate in these three species.

Dogs were tested with oral Risedronate in seven studies of 1 - 13 week duration. Three were IV and one was at too low a dose range. In the remaining three studies 8 mg/kg/d consistently was the lowest dose at which toxicity was observed. The most sensitive indicator of toxicity to oral Risedronate was moderately increased AST, ALT and SGOT. This 8 mg/kg dose also resulted in moderate liver atrophy, renal necrosis and signs of testicular degeneration such as atrophy and spermatid arrest (more severe at higher doses).

Rats were tested over a wide range of doses for 13 weeks in study B-4. 8 mg/kg is the NOAEL in this study (although the sponsor claims a NOAEL of 16 mg/kg/d). At 16 mg/kg/d evidence of several toxicities seen at higher doses; moderate hematuria, testicular atrophy and decreased food consumption were evident. 32 mg/kg/d resulted in severe increases in AST, ALT, weight loss and decreased food consumption, gastritis, decreased thymus weight and renal tubular necrosis. 64 mg/kg resulted in additional toxicities including severely increased neutrophils and WBC, BUN and thyroid/parathyroid weight along with decreased prostate, seminal vesicles and testes weights and severe gastritis and enteritis. In other studies, doses below 16 mg/kg/d and less than 13 weeks were not toxic in rats.

Mice were the least clearly characterized species; in a 13 week study, a 32 mg/kg/d HD had no toxicity, but in a 20 week dose ranging study a 16 mg/kg/d LD killed 2 mice and caused weight loss and gastric distention in all groups. This suggests that the long-term NAEL is slightly below 16 mg/kg/d

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CONCLUSION:

The NOAELs for oral Risedronate in dogs, rats and mice are 4, 8 and 8 mg/kg/d in studies of less than 6-month duration, corresponding to 80, 48 and 24 mg/m² respectively. Liver toxicity was seen in all species (moderately increased AST, ALT, SGOT and moderate atrophy in rats dogs and mice at > 4 mg/kg). Gastric effects have been noted in all species but in these particular studies no adverse gastric effects were noted in dogs. Mild to severe gastritis and mild vacuolation of stomach epithelial cells was noted in rats at > 4 mg/kg, GI distention was observed in mice > 16 mg/kg. In dogs and rats mild-moderate renal necrosis was noted at 8 mg/kg and higher. Testicular toxicity (atrophy and spermatid arrest) was also observed in both dogs and rats at 8-16 mg/kg. Some dog and rat studies also noted smaller prostates in the HD groups (64 mg/kg oral rat, 1.5 mg/kg IV dog and one 8 mg/kg oral dog).

Oral Toxicity Studies:				Fold-recommended human dose:		
Species:	NOAEL (mg/kg/d)	NOAEL (mg/m ²)	Principal Target Organs:	mg/kg	mg/m ²	AUC
Dog < 6-month	4	80	liver, kidney, testes	8 X	4.3 X	ND
Rat < 6-month	8	48	stomach, liver, kidney, testes	16 X	2.6 X	ND
Mouse < 6 mo.	8	24	stomach, liver	16 X	1.3 X	ND

Recommended human dose = 30 mg/60 kg = 0.5 mg/kg = 18.5 mg/m².

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**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
A 26-Week Oral Toxicity Study of NE-58095 in the Albino Rat.
Study # B15 (45351)**

PURPOSE:

To assess the toxicity of Risedronate in rats when administered orally for six months.

EXPERIMENTAL DESIGN:

Testing Facility:

Study #: 15 (45351)

Study Initiated: 6/7/95

Study Completed: 10/18/96.

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Dose & Formulation: 0, 4, 8, 16, 32 mg/kg/day,
oral gavage, daily, in Water, fasted 4 h before ,2 h after.

Batch of drug: lot 13427-070B

Food:

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GLP statement: Included.

Animals: 30 rats/sex/group
Sprague-Dawley Cri:CD (SD) BR rats from Charles River (Canada).

<u>Group:</u>	<u>Dose (mg/kg):</u>	<u># of Animals:</u>
1	0	30 males + 30 Females
2	Risedronate 4	30 males + 30 Females
3	Risedronate 8	30 males + 30 Females
4	Risedronate 16	30 males + 30 Females
5	Risedronate 32	30 males + 30 Females

Dose Selection:

Dose selection was based on the results of the 3-month toxicity trial. A 3-month toxicity trial in rats resulted in significant toxicity at doses of 32 mg/kg. In that 3-month study, 16 mg/kg was the NOEL. 32 mg/kg was selected as the maximum dose for this 6-month study in order to assure that toxicity would be observed.

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RESULTS:**OBSERVED EFFECTS:**

Increased respiratory findings (sounds and shallow breathing) in the 16 and 32 mg/kg groups. Other findings were confined to the 32 mg/kg group; Dehydration, decreased body temperature, thin, reduced feces, urogenital staining.

MORTALITY:

12 males and 7 females were found dead or euthanized during the study as follows:

Group:	Dose (mg/kg/d)	Dead males/total	Dead females/total	Time of death (week):
1	0	0/30	1/30	25
2	4	0/30	0/30	NA
3	8	1/30	1/30	19 & 18
4	16	1/30	0/30	9
5	32	10/30	5/30	throughout

BODY WEIGHT:

Significant reductions in weight and weight gain were noted in males and females in groups 4 and 5 and in the male half of group 3 in the second half of the study.

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FOOD CONSUMPTION:

Food consumption was significantly lower in the 16 and 32 mg/kg groups than in control groups.

EYE EXAMINATION:

No findings were evident.

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HEMATOLOGY:

In male and female rats in the 32 mg/kg HD group; differential and absolute segmented neutrophils were significantly increased from week 12 onward. Severely increased segmented neutrophils were also noted at most time points after week 12 in the 16 mg/kg group.

Variations in RBC, hemoglobin, mean corpuscular volume and red cell size distribution were noted at several time points in several treated groups but were not dose and time dependent and were within normal physiological ranges.

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BLOOD CHEMISTRY:

Group 2 and 3 (4 and 8 mg/kg) animals showed no consistent significant changes in blood chemistry. Group 4 and 5 (16 and 32 mg/kg) rats consistently, after week 6, had elevated ALT and AST

Variations in several additional parameters were noted at several time points in several treated groups but were not dose and time dependent and were within normal physiological ranges.

URINALYSIS:

No significant findings were evident.

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BONE BIOCHEMISTRY MARKERS:

Not measured.

ORGAN WEIGHTS:

No dose dependent, significant changes in absolute or relative organ weights were noted in the 4, 8 or 16 mg/kg groups.

Significant absolute and relative increases in male and female thyroid, and decreases in spleen weights, were noted in HD (32 mg/kg) rats.

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GROSS and HISTOPATHOLOGY:

There were no significant histopathologic changes observed in the 4 and 8 mg/kg groups in this study. Treatment-related microscopic lesions were observed in; lung, trachea, stomach and nasal cavities spleen and thymus in higher dose animals. Most of these lesions were noted in 32 mg/kg animals but some (in lungs, trachea and stomach) were noted also in 16 mg/kg animals.

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SUMMARY and DISCUSSION:

16 and 32 mg/kg/day of Risedronate was shown to result in the death of several rats in these groups within six months. These doses caused moderate thymic and lymphatic atrophy, increases in AST and ALT, weight loss and gastric irritation as well as moderate lesions in nasal cavities and lungs and severe increases in neutrophils as well as moderate changes in bone consistent with pharmacological action.

While all doses tested caused significant lengthening of the primary spongiosa, this is considered evidence of the pharmacological effect of the drug. 8 mg/kg/d, or less, of Risedronate had little toxic effect on the rats. There was no clear evidence of a sex based difference in sensitivity to the drug.

8 and 4 mg/kg/d of Risedronate achieved pharmacodynamic (bone) responses similar to the higher doses but did not cause any of the toxic responses.

Pharmacokinetic measurements of blood levels of Risedronate in these animals indicated that at 8 mg/kg/d the drug began to saturate the elimination pathways and blood levels rose dramatically with increasing doses. This may partially explain the small range of doses between the NOAEL of 4 mg/kg/d and the lethal dose of 16 mg/kg/d.

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CONCLUSIONS:

4 mg/kg/d may be considered the NOAEL for oral Risedronate in rats for 6-months as the sponsor suggests but I did not note any clear evidence of toxicity at 8 mg/kg/d and I suggest that the NOAEL for oral Risedronate in rats for 6-months may be 8 mg/kg/d. The sponsor calls 8 mg/kg/d a no toxic effect level.

Toxicity of Risedronate appears to be; hepatotoxicity and lesions in the lung, trachea, stomach and nasal cavities, spleen and thymus. Poor food consumption was observed at 16 and 32 mg/kg/d.

Toxic effects appear above the dose of 8 mg/kg/d where pharmacokinetics become nonlinear.

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**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
Two Year Oral Toxicity of NE-58095 in Dogs:
Chronic (6-Month) Toxicity of NE-58095 Study # B17 (42735)
Two year Oral Toxicity of NE-58095 Study # B19 (43659)**

PURPOSE:

To assess the toxicity of Risedronate in dogs when administered orally on a daily schedule, or orally on an intermittent schedule, for 6 months and 2 years.

EXPERIMENTAL DESIGN:

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ON ORIGINAL

Testing Facility:

Study #: 42735 and 43659

Study Initiated: 3/7/89
Study Completed: 10/7/92.

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Dose & Formulation: 0, 0.2, 0.5, & 2.0 mg/kg/day oral Risedronate daily, **fasted**.
0.5, 2.0, 8.0 mg/kg/day daily for 7 days, then placebo for 21 days.

Batch of drug: several lots.

Food: CERTIFIED CANINE DIET

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ON ORIGINAL

GLP statement: Included.

Animals: 140 purebred beagle dogs

<u>Group:</u>	<u>Dose (mg/kg):</u>	<u># of Animals:</u>	<u>6-Month Study:</u>	<u>2-year Study:</u>
1	0		4 M + 4 F	6 M + 6 F
2	Risedronate	0.2	4 M + 4 F	6 M + 6 F
3	Risedronate	0.5	4 M + 4 F	6 M + 6 F
4	Risedronate	2.0	4 M + 4 F	6 M + 6 F
5	Risedronate	0.5 I	4 M + 4 F	6 M + 6 F
6	Risedronate	2.0 I	4 M + 4 F	6 M + 6 F
7	Risedronate	8.0 I	4 M + 4 F	6 M + 6 F

I = intermittent dosing (1 week on, 3 weeks off)

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Dose Selection:

In a previous 13-week oral toxicity trial in dogs; mild testicular effects (atrophy spermatid arrest and lesion) were noted in 1 dog each at 2 and 4 mg/kg and were mild-severe in all 3 dogs at 8 mg/kg/d. The sponsor used this level (2 mg/kg) as a minimal toxicity level from the shorter term assay and selected it as the highest dose for this 2-year study. Intermittent dosing groups received the same accumulated dose over time by receiving 4-fold higher doses for 1 of 4 weeks per month (and no drug for the remaining 3 of 4 weeks) throughout the study.

In hindsight these doses were too low.

RESULTS:

OBSERVED EFFECTS:

No adverse effects attributable to exposure to drug at any dose level or schedule.

MORTALITY:

No adverse effects attributable to exposure to drug at any dose level or schedule.

BODY WEIGHT:

No adverse effects attributable to exposure to drug at any dose level or schedule.

FOOD CONSUMPTION:

No adverse effects attributable to exposure to drug at any dose level or schedule.

EYE EXAMINATION:

No findings were evident.

APPEARS THIS WAY
ON ORIGINAL

HEMATOLOGY and COAGULATION:

No adverse effects attributable to exposure to drug at any dose level or schedule.

BLOOD CHEMISTRY:

No adverse (or any) effects attributable to exposure to drug at any dose level or schedule.

URINALYSIS:

No adverse (or any) effects attributable to exposure to drug at any dose level or schedule.

ORGAN WEIGHTS:

No effects attributable to exposure to drug at any dose level or schedule.

GROSS and HISTOPATHOLOGY:

No adverse effects attributable to exposure to drug at any dose level or schedule. Microscopic findings were limited to effects on bone. All doses produced expected pharmacological effects on bone. Effects of intermittent doses were possibly greater than the same accumulated dose on a daily schedule, but these comparisons were inconclusive.

APPEARS THIS WAY
ON ORIGINAL

SUMMARY and DISCUSSION:

Oral Risedronate (2 mg/kg/day) did not result in any toxicity over a 2 year period. This 2 mg/kg was the maximal dose at which toxicities were not observed in shorter term (<6-month) studies. This helps support the idea that there is no unexpected cumulative effect of long term exposure to the drug. I believe the testicular atrophy seen in one 2 mg/kg dog in the 13-week study was a random event and that the minimally toxic level in the 13-week study was 4 mg/kg. As a result I would have liked to have seen a 4 mg/kg top dose in this study. The 1-year dog study was conducted at much higher doses.

APPEARS THIS WAY
ON ORIGINAL

CONCLUSIONS:

- The "High Dose" of 2 mg/kg/day of Risedronate caused expected pharmacological effects on bone in dogs but no adverse effects and can be considered a NOAEL.
- Similar results were obtained with the 8 mg/kg/d intermittent dosing arm of this study, resulting in the same total exposure as 2 mg/kg/d.

**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
An Oral Chronic (1 Year) Toxicity Study of NE-58095 in the Rat:
Study # B16 (42734)**

PURPOSE:

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ON ORIGINAL

To assess the toxicity of Risedronate in rats when administered by gavage daily for one year.

EXPERIMENTAL DESIGN:

Testing Facility:

Study #: 16 (42734)

Study Initiated: 2/13/89

Study Completed: Feb. 18, 1991

Dose & Formulation: 0, 0.1, 0.6, & 4.0 mg/kg/day,
Oral via gavage.

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ON ORIGINAL

Batch of drug: lot 11661-JKL-84B

Food: certified rodent chow # 5002

GLP statement: Included.

Animals: 160 Cr:CD(SD)BR Rats from

<u>Group:</u>	<u>Dose (mg/kg):</u>	<u># of Animals/sex/group:</u>
0	0	20
1	Risedronate 0.1	20
2	Risedronate 0.6	20
3	Risedronate 4.0	20

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ON ORIGINAL

Dosing was 4 h after and 2 h before food.

All rats in this "toxicity subgroup" of the Carcinogenicity study were killed after 1 year.

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ON ORIGINAL

Dose Selection:

Rationale was not provided, however, in a 6-month rat toxicity study no significant toxicity was noted at doses of 8 mg/kg, and 4 mg/kg was selected by the sponsor as the NOAEL. Never the less the 4 mg/kg dose was selected as the HD for this study. This is probably because the study was planned as part of a 2 year carcinogenicity study.

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ON ORIGINAL

RESULTS:**OBSERVED EFFECTS:**

No treatment related clinical signs.

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ON ORIGINAL

MORTALITY:

There was no drug related mortality although there were several deaths that may have been gavage related.

Group:	Dose (mg/kg/d)	Dead males/total	Dead females/total
1	0	1/20	0/20
2	0.1	2/20	2/20
3	0.6	1/20	0/20
4	4	2/20	1/20

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ON ORIGINAL

BODY WEIGHT:

There was no significant drug related effect on body weight or weight gain although HD males and females were slightly (~10%) lighter (body weight) than other groups at the end of the study.

FOOD CONSUMPTION:

No significant changes in food consumption were noted although HD males and females consumed slightly (~10%) less food than other groups throughout the study.

EYE EXAMINATION:

No treatment related findings were evident.

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ON ORIGINAL

HEMATOLOGY and COAGULATION:

No biologically meaningful toxic effect was noted. RBC were slightly (< 10 %) and statistically significantly lower in 0.6 and 4 mg/kg animals than in controls. This was associated with slightly decreased HCT in males and slightly increased MCV and MCH in females. These values were still within the normal range and considered not biologically meaningful.

BLOOD CHEMISTRY:

No biologically significant treatment related effects. Although slightly increased sodium (M and F) and decreased phosphorous (M and F) and decreased calcium (F) was observed these effects were not biologically meaningful.

URINALYSIS:

No treatment related findings were evident.

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ON ORIGINAL

ORGAN WEIGHTS:

No treatment related changes were evident.

GROSS and HISTOPATHOLOGY:

No treatment related changes were evident except in the histopathological evaluation of the sternbrae and tibia. There was a dose related increase in the incidence and severity of hypertrophy of the primary spongiosa in these bones. This effect was more severe in the tibia. This is evidence of excessive pharmacological activity of the drug resulting in abnormal bone remodeling. Females clearly appear to be more sensitive to this effect.

M/F n=20	Control	0.1 mg/kg	0.6 mg/kg	4 mg/kg
Sternum, Slight	0/0	0/3	3/18	17/20
Sternum, Mild	0/0	0/0	0/0	3/0
Tibia, <severe	0/0	0/8	12/11	13/1
Tibia, Severe	0/0	0/0	0/9	7/19

APPEARS THIS WAY
ON ORIGINAL

SUMMARY and CONCLUSION:

There were no significant adverse effects of the drug in any group.

There were no adverse effects at all at the low dose. At the mid dose there were slight effects on hematology but they were not considered biologically significant. At this mid-dose there was also a significant number of incidence of mild lengthening of the primary spongiosa in the tibia and sternum. These effects were also not considered adverse.

At the high dose, there were statistically significant effects on RBC and some other hematological and serum biochemical parameters. These effects were not considered biologically significant due to the small magnitude of the effect. We would anticipate however that these effects would be more severe at higher doses. The effect of this drug on the primary spongiosa was more severe at this dose than at the mid-dose but is considered an excessive pharmacological action.

The 4 mg/kg HD is considered the NAEL in this 1 year rat study.

Higher doses would have provided a more informative outcome. On a mg/m² basis, this dose is only 2-fold the human dose!

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ON ORIGINAL

APPEARS THIS WAY

**PHARMACOLOGY AND TOXICOLOGY REVIEW OF
52-Week Repeated Dose Chronic Oral Toxicity Study of NE-58095
Administered Via Capsule to Dogs
Study # B-18 (45323)**

PURPOSE:

To assess the toxicity of oral Risedronate in dogs for one year.

EXPERIMENTAL DESIGN:**Testing Facility:**

Study #: 995.09.00-CC (45323)

Study Initiated: 2/25/94.
Report Dated: 10/10/96.

Dose & Formulation: 0, 4, 8, 16, 32 mg/kg/day.
oral, daily capsules.

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ON ORIGINAL

Batch of drug: lot 14241-22B

Food: CERTIFIED CANINE DIET
Fresh filtered water.

GLP statement: Included.

Animals: 40 purebred beagle dogs 6 mo. old.

<u>Group:</u>	<u>Dose (mg/kg):</u>	<u># of Animals:</u>
1	0	4 Males + 4 Females
2	Risedronate 4	4 Males + 4 Females
3	Risedronate 8	4 Males + 4 Females
4	Risedronate 16	4 Males + 4 Females
5	Risedronate 32	4 Males + 4 Females

Dose Selection:

A 24-month toxicity trial in dogs resulted in minimal toxicity at doses of 2 mg/kg daily and 8 mg/kg intermittently (7/28 days). At these doses there were significant pharmacological effects on bone. The 16 and 32 mg/kg/d doses were included in this one year study to increase the likelihood of overt toxicity and to characterize the toxic effects. This objective was certainly obtained. All 32 mg/kg dogs died within 18 days and all 8 mg/kg dogs died within 43 days. The low dose 4 mg/kg dogs experienced minimal adverse effects, as expected.

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ON ORIGINAL

RESULTS:

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ON ORIGINAL

OBSERVED EFFECTS:

Dehydration, prostration, hunched posture, muscle twitching sensitivity to touch and decreased skin temperature were seen in all treated animals and was dose dependent. These effects were primarily observed in the group 4 and 5 (16 and 32 mg/kg) dogs but were also noted in the two lower doses (4 and 8 mg/kg).

MORTALITY:

All animals in groups 4 and 5 were euthanized or found dead and two females in the 8 mg/kg/d group were euthanized during the course of the study.

Group:	Dose (mg/kg/d)	Dead males/total	Dead females/total	Time of death (day):
1	0	0/4	0/4	NA
2	4	0/4	0/4	NA
3	8	0/4	2/4	39 & 138
4	16	4/4	4/4	18-43
5	32	4/4	4/4	11-18

BODY WEIGHT:

Significant reductions in weight) were noted in groups 4 and 5 and in the female half of group 3 in the week(s) prior to death. Surviving females in group 3 were consistently significantly 30% lighter than both control and group 2 females. Conversely, group 2 and 3 males grew faster than controls during the first six months, and were significantly ~10% heavier than controls after 6 months but were no longer statistically significantly heavier than controls at the end of the study.

FOOD CONSUMPTION:

Food consumption was generally lower in all treated groups than in control groups. The results for this measurement were highly variable and achieved statistical significance sporadically.

NEUROLOGIC EXAMINATION:

No findings were evident.

APPEARS THIS WAY
ON ORIGINAL

CARDIOLOGIC EXAMINATION:

No findings were evident.

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EYE EXAMINATION:

No findings were evident.

HEMATOLOGY:

In males- Platelet counts were significantly (50%) reduced in group 3 (8 mg/kg) males throughout the study and euthanized Group 4 (16 mg/kg) and 5 (32 mg/kg) males. Group 3 males also had significantly (12%) reduced hemoglobin and hematocrit levels at the end of the study. Although mean corpuscular hemoglobin was transiently slightly reduced in group 2 and 3 males at 181 days, these levels returned to normal by the end of the study. Group 2 (4 mg/kg) males showed no other significant differences from controls.

In females- Group 2 and 3 females had nearly twice the control polymorphonuclear leukocytes at day 181 and 366. Additionally, the two surviving Group 3 females had decreased RBC, HGB, HCT, MCV, MCH,

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and MCHC at day 181 and 366. Although Group 2 females showed a transient 10% decrease in hemoglobin and 40% decrease in LYM and ALYM, these differences may have been due to random fluctuations in these measurements (controls were elevated). Euthanized high dose females also exhibited increased APLY and decreased platelets and lymphocytes.

COAGULATION:

APTT was significantly (~20%) elevated in male and female Group 3 (8 mg/kg) dogs at days 181 and 366 and even more (50%) elevated in euthanized dogs. Prothrombin time was significantly (<10%) increased in Group 3 females and euthanized group 5 males. These effects may be secondary to hepatic effects.

BLOOD CHEMISTRY:

Group 2 (4 mg/kg) animals showed no consistent significant changes in blood chemistry. Group 3 (8mg/kg) male and female dogs consistently, through the study, had elevated total bile acid (TBA), ammonia (AMM), AST, and CK, and decreased BUN and Phosphate. Similar effects were noted in euthanized (16-32 mg/kg) animals except that these animals also exhibited severe hypocalcemia and severely increased total bile acid, AST, TBA, CK and Ammonia.

At Day 366 or Euth.	AST (U/L)	GGT (U/L)	Ammonia (ug/dL)	CK (U/L)	BUN (mg/dl)	Phos- phate	Ca (mg/dl)	Total Bile Acid
M, Control	37	6	32	158	15	3.7	9.9	8
F, Control	31	5	25	108	15	4	10	6
M, 4 mg/kg	60*	6	31	345*	15	3.5	10	7
F, 4 mg/kg	55	7	16	225*	12	3	10	7
M, 8 mg/kg	186*	6	95*	274	9*	3.3	10	84
F, 8 mg/kg	120*	4	143	178	11	3	10	156
M, 16 mg/kg	655	24	262	1007	15	2.7	7.2	130
F, 16 mg/kg	4020	15	280	1243	26	3.8	7.3	115
M, 32 mg/kg	2143	13	216	951	15	2.7	7.3	105
F, 32 mg/kg	3856	11	253	927	20	4.0	5.6	56

n=3-5

URINALYSIS:

No significant findings were evident.

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ON ORIGINAL

BONE BIOCHEMISTRY MARKERS:

Osteocalcin, Urinary Pyridinoline, Urinary Deoxypyridinoline, and Alkaline Phosphatase levels were significantly lower in all treated groups, reflecting the pharmacological action of the drug to inhibit bone resorption.

ORGAN WEIGHTS:

No significant changes in absolute or relative organ weights were noted in the 4 mg/kg LD Group 2. Significant absolute and relative increases in male and female kidney (25%) and brain (25%) weights were noted in Group 3 (8 mg/kg) dogs. Male spleen (50%) and relative prostate (20%) weights were also increased in this group as were female relative heart, lung, pituitary and salivary gland weights (all about 20%). Results of other groups were too variable to detect significant changes.

GROSS and HISTOPATHOLOGY:

There were no significant histopathologic changes observed in the 4 mg/kg LD Group 2 in this study. A wide variety of treatment-related microscopic lesions were observed in; lung, liver, lymph nodes, kidney, esophagus, stomach, small intestine, large intestine, acinar pancreas, and testes in higher dose animals. Most of these lesions were noted in euthanized, moribund animals but some were noted also in surviving Group 3 animals.

Lungs: 3/24 dogs in groups 3-5 had some engorged blood vessels, increased numbers of blood vessels and hemorrhage in the lungs.

Thymus: Lymphoid atrophy was observed in half the animals in groups 3-5.

Lymph nodes: Lymphoid atrophy and hemorrhage were observed in half the females and most of the males in groups 3-5.

Liver: Hepatocellular degeneration was observed in the majority of group 3 animals and in all animals in groups 4 and 5. The degree of degeneration was moderate - marked and dose dependent. Chronic inflammation was also noted in 1/4 group 3 males, 2/4 group 3 females and 2/4 group 5 females.

Gallbladder: Microscopic hemorrhage was observed in 1/4 group 4 females and 2/4 group 5 females.

Esophagus: Esophageal erosion characterized by epithelial atrophy was observed in 1-3 females and 3-4 males/group in groups 3-5. Hemorrhage was observed in 3/8 females in groups 4 and 5. These effects were moderate - marked and dose dependent.

Stomach: Gastropathy, edema and erosions were observed in 2-3 animals in each of groups 3-5. These effects were moderate - marked and dose dependent.

Small and Large Intestine: Enteropathy, hemorrhage, or congestion were noted in 1-2 males and females in each of groups 3-5.

Pancreas: Interlobular edema, inflammation, and/or fat necrosis were observed in half the animals in groups 3-5.

Kidney: Nephropathy, hyperplasia, hemorrhage, cysts, and tubular necrosis were observed in about half of the animals in groups 3-5. These effects were mild - moderate and dose dependent.

Testes: Increased tubular degeneration was moderate in groups 3 and 4. It is likely that Group 5 did not survive long enough to develop these effects.

SUMMARY and DISCUSSION:

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ON ORIGINAL

16 and 32 mg/kg/day of Risedronate was shown to result in the death of all dogs in these groups in about a month. This was apparently due to a combination of hypocalcemia, hepatotoxicity, localized tissue irritation accompanied by increased hemorrhaging due to decreased clotting secondary to liver damage.

8 mg/kg/d of Risedronate had similar but less severe effects on the dogs resulting in the deaths of 2/4 of the female dogs at this dose. There was no clear evidence of a sex based difference in sensitivity to the drug that would account for the deaths of two females and no males at this dose.

4 mg/kg/d of Risedronate achieved biochemical pharmacodynamic responses similar to the higher doses but did not cause any of the toxic responses except for a small increase in AST and CK.

Pharmacokinetic measurements of blood levels of Risedronate in these animals indicated that at 8 mg/kg/d the drug began to saturate the elimination pathways and blood levels rose dramatically with increasing doses. This may partially explain the small range of doses between the NOAEL of 4 mg/kg/d and the lethal dose of 16 mg/kg/d.

CONCLUSIONS:

APPEARS THIS WAY
ON ORIGINAL

4 mg/kg/d may be considered the NOAEL for Risedronate in dogs for a year.

Toxicity of Risedronate appears to be: Hypocalcemia, Hepatotoxicity, Renal tubular nephropathy and hypertrophy, testicular degeneration, diminished coagulation, erosion of the GI tract from the esophagus to the large intestine and inflammation of lungs, acinar pancreas, and lymph nodes.

PHARMACOLOGY AND TOXICOLOGY REVIEW

Reviewers Overall Summary and Conclusions of Toxicity Studies:

Acute toxicity:

Single oral doses (~2 g/kg) were tolerated by rats, mice and rabbits (dogs vomited doses greater than 50 mg/kg). On a mg/m² basis this is 666 (for rats) and 333 (for mice) times the proposed human exposure. Higher doses were lethal. Rabbits appeared to be more sensitive to Risedronate but were not extensively tested.

Single IV doses, (3 mg/kg in rabbits, 4 mg/kg in dogs, and 25 mg/kg in mice and rats) were well tolerated. Higher doses were lethal. These IV doses are about 1% of the maximum tolerated oral doses. This is predicted due to an expected absorption of only about 1% of the oral dose.

Liver and kidneys were noted as possible target organs in many of the acute studies. In addition, drug related gastric effects were noted in dead dogs, rats and rabbits given drug IV as well as orally. Gastric edema was also noted in the 2-day IV study in rats. It is interesting to note that evidence of gastric irritation was noted not only in oral studies but also in IV studies even though there is no direct contact of drug with the luminal side of the digestive system (ADME studies show no evidence of biliary secretion) after IV exposure.

Repeated dose (<6-month) toxicity:

14 repeated dose toxicity studies were conducted with Risedronate in mice rats and dogs. Although several of these studies were conducted at levels below the NOEL, all of these studies taken together show the time and dose dependence of the toxic effects of Risedronate in these three species.

In dogs tested with oral Risedronate 8 mg/kg/d consistently was the lowest dose at which toxicity was observed. The most sensitive indicator of toxicity to oral Risedronate was increased AST, ALT and SGOT. 8 mg/kg also resulted in liver atrophy, renal necrosis and signs of testicular degeneration such as atrophy and spermatid arrest.

Rats were tested over a wide range of doses for 13 weeks in study B-4. 8 mg/kg is the NOAEL in this study (although the sponsor claims a NOAEL of 16 mg/kg/d). At 16 mg/kg/d evidence of several toxicities seen at higher doses; hematuria, testicular atrophy and decreased food consumption were evident. 32 mg/kg/d resulted in severe increases in AST, ALT, weight loss and decreased food consumption, gastritis decreased thymus weight and renal tubular necrosis. 64 mg/kg resulted in additional toxicities including severely increased neutrophils and WBC, BUN and thyroid/parathyroid weight along with decreased prostate, seminal vesicles and testes weights and severe gastritis and enteritis. In studies of less than 13-week duration, doses below 16 mg/kg/d were not toxic.

Mice were the least clearly characterized species; in a 13 week study, a 32 mg/kg/d HD had no toxicity, but in a 20 week dose ranging study a 16 mg/kg/d LD killed 2 mice and caused weight loss and gastric distention in all groups. This suggests that the long-term NOAEL in mice is slightly below 16 mg/kg/d

6-Month and 1-Year Rat Oral Toxicity:

In the 6-month study 16 and 32 mg/kg/day of Risedronate was shown to result in the death of several rats in these groups. These doses caused thymic and lymphatic atrophy, increases in AST and ALT, weight loss and gastric irritation as well as lesions of the nasal cavities and lungs and moderate changes in bone consistent with pharmacological action.

8 and 4 mg/kg/d of Risedronate achieved pharmacodynamic (bone) responses similar to the higher doses but did not cause any of the toxic responses. There was no clear evidence of a sex based difference in sensitivity to the drug.

Pharmacokinetic measurements of blood levels of Risedronate in these animals indicated that at 8 mg/kg/d the drug began to saturate the elimination pathways and blood levels rose dramatically with increasing doses. This may partially explain the small range of doses between the NOAEL of 8 mg/kg/d and the lethal dose of 16 mg/kg/d.

The 1 year rat study was conducted at doses which were too low (HD=2 mg/kg). There were no significant adverse effects of the drug in any group. However, this does help to confirm that there is no increase in toxicity as time of exposure is increased from 6-months to 1-year in the rat.

6-Month, 1 and 2-year Oral Dog Studies:

16 and 32 mg/kg/day of oral Risedronate was shown to result in the death of all dogs in these groups in about a month. This was apparently due to a combination of hypocalcemia, hepatotoxicity, localized tissue irritation accompanied by increased hemorrhaging due to decreased clotting secondary to liver damage.

8 mg/kg/d of Risedronate had similar but less severe effects on the dogs resulting in the deaths of 2/4 of the female dogs at this dose. There was no clear evidence of a sex based difference in sensitivity to the drug that would account for the deaths of two females and no males at this dose.

4 mg/kg/d of Risedronate achieved biochemical pharmacodynamic responses similar to the higher doses but did not cause any of the toxic responses except for a small increase in AST and CK in the 1-year study.

Oral Risedronate (2 mg/kg/day) did not result in any toxicity over a 2 year period in dogs. This helps support the idea that there is no unexpected cumulative effect of long term exposure to the drug. This 2 mg/kg was the maximal dose at which toxicities were not observed in shorter term (<6-month) studies.

Special Toxicity:

Initial examination of gastric irritation in rats pre-exposed to NSAIDs indicated significant gastric irritation in response to all bisphosphonates tested. Alendronate was the most damaging in this model but Risedronate also induced significant irritation. The physiologic relevance of comparisons based on this model is inconclusive.

Several additional assays of gastric irritation were either negative or showed slight irritation in response to several bisphosphonates tested, including Risedronate.

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CONCLUSION:

The NOAELs for oral Risedronate in dogs, rats and mice are summarized in the following table.

Oral Toxicity Studies:				Fold-recommended human dose:		
Species:	NOAEL (mg/kg/d)	NOAEL (mg/m ²)	Principal Target Organs:	based on mg/kg	based on mg/m ²	based on AUC
Dog < 6-month	4	80	stomach, liver, kidney, testes	8 X	4.3 X	
Dog 1 year	4	80	stomach, liver, kidney, testes	8 X	4.3 X	15.3 X
Dog 2 year	>2	>40	doses too low	>4 X	> 2.1 X	
Rat < 6-month	8	48	stomach, liver, kidney	16 X	2.6 X	
Rat 6 month	8	48	stomach, liver, kidney	16 X	2.6 X	7 X
Rat 1-year	> 4	>24	doses too low	>8 X	>1.3 X	
Mouse < 6 mo.	8	24	stomach, liver	16 X	1.3 X	

Recommended human dose = $30 \text{ mg}/60 \text{ kg} = 0.5 \text{ mg}/\text{kg} = 18.5 \text{ mg}/\text{m}^2$.

The toxicities seen in all species were hepatic toxicity (increased AST, ALT...lesions and atrophy) and gastric irritation and gastric distress leading to poor food consumption. In dogs and rats testicular toxicity, severely increased neutrophils, renal tubular nephropathy and necrosis were evident. Erosion of the GI tract from the esophagus to the large-intestine and inflammation of lungs, acinar pancreas, and lymph nodes was also observed in dogs.

Risedronate appears similar to other bisphosphonates in its potential to irritate the GI system directly. While this effect of Risedronate does not seem to be more severe or less severe than other bisphosphonates at this time, the question has not been conclusively answered by these investigations. The mechanism(s) of this effect remains unknown.

Risedronate also caused slight ocular and skin irritation but was not antigenic in guinea pigs.

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PHARMACOLOGY AND TOXICOLOGY REVIEW SUMMARY REVIEW AND CONCLUSIONS OF GENETIC TOXICITY STUDIES.

The following genetic toxicity screening experiments were conducted by

Some final reports were revised (minor) by P&G.

Vol:	Study#:	Spec.	Route	Dose	Study Title:	Result:
56	D1 (40366)	Rat	oral not fasted		Cytogenicity Study - Rat Bone Marrow In Vivo (NE-58095).	Negative
56	D2 (40017)	Sal.	NA		Salmonella/Mammalian-Microsome Mutagenicity Assay (Ames Test)	Negative
56	D3 (42933)	E. Coli	NA		NE-58095 Escherichia Coli Reverse Mutation Assay	Negative
56	D4 (40139)	CHO	NA		CHO/HGPRT Mutation Assay	Negative
56	D5 (40243)	Rat Hepat.	NA		Test Chemical Induction of Unscheduled DNA Synthesis (UDS) in Primary Cultures of Rat Hepatocytes (NE-58095)	Negative
56	D6 (40016)	CHO	NA		Cytogenicity Study - Chinese Hamster Ovary (CHO) Cells In Vitro	Positive clastogenicity for all doses +/- S-9 but survival was 6-7% for all doses.
56	D7 (43104)	CHO	NA		Cytogenicity of NE-58095 in Chinese Hamster Ovary (CHO) Cells In Vitro (for Japanese Submission)	Negative clastogenicity +/- S-9 Survival was > 29% for all doses.

SUMMARY AND CONCLUSIONS:

These experiments did not detect any significant tendency of Actonel to induce any genetic toxicity or response in these specific assays except in the cytogenicity assay in CHO cells at concentrations of Risedronate that severely limited cell survival.

- The Cytogenicity Study of chromosomal aberrations in bone marrow cells of unfasted rats given 1336 mg/kg acutely or 500 mg/kg for 5 days by gavage showed no effect of drug. Exposure was not demonstrated by toxicity or blood levels, but a separate LD₅₀ study showed that 1336 mg/kg is approximately half the LD₅₀ in unfasted rats.
- The Ames Assay was conducted with TA98, TA100, TA1535, TA1537, and TA1538, with and without Aroclor induced rat liver microsomes. No significant increase in reverse mutation frequency was noted. No toxicity was noted up to the "maximum workable concentration" of 8000 ug/plate.
- The Reverse mutation assay was conducted with WP2-uvrA strain of E. coli, with and without Aroclor induced rat liver microsomes. No significant increase in reverse mutation frequency was noted. No toxicity was noted up to the "maximum concentration" of 5000 ug/plate.
- The CHO-HGPRT Assay was conducted with and without Aroclor induced rat liver microsomes. No significant increase in mutant colonies was noted without S-9 at up to 950 ug/ml or with S-9 at up to 25 ug/ml. Cloning efficiency dropped to 12% at the 950 ug/ml maximum concentration tested without S-9. In the presence of S-9 the survival of dividing cells dropped below 60% at the highest concentration tested (25 ug/ml).
- In the Test of Chemical Induction of Unscheduled DNA Synthesis (UDS) in Primary Cultures of Rat Hepatocytes, Risedronate at concentrations up to 4 mg/ml did not increase the rate of UDS.
- The Cytogenicity test of Risedronate in CHO cells was positive in the presence and in the absence of S-9 at concentrations of _____ . At this concentration the cloning efficiency was _____ . At lower concentrations _____ where the cloning efficiency was greater than 29%, there was no significant increase in aberrant cells in the presence or absence of S-9.

**PHARMACOLOGY AND TOXICOLOGY REVIEW
PERINATAL AND POSTNATAL STUDY IN RATS- SEGMENT III**

Study Nr. C8 (Accession No. 45500).

Project No. 995.09.00-AL

Study Facility:

(Study No : 331-NE-001-94)

Study period: January 15 - June 26, 1994.

Final Report: October 11, 1996.

Signed GLP statement provided.

Lot Nr. 14241-022B (09B).

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PURPOSE - Determine effects of test compound on latter stage of fetal development, labor and parturition, lactation, and on growth and development of newborn.

PROCEDURES -

Presumed pregnant female Sprague-Dawley rats (n=25/dose group, N=100), initial weight 230-310 g, were dosed orally, by gavage, with 0, 0.5, 2, 8 mg/kg/day, beginning on Gestation Day 15 through Postpartum (PP) Day 21 (10 ml/kg). Vehicle was Sterile Water for Injection, USP. Females were allowed to deliver naturally and rear the F1 progeny. From each litter, 4 male and 4 female rats, if possible, were selected for developmental and behavioral testing. Standardization (culling) to n=8 neonates/litter was performed on PP Day 4. Females that did not deliver were sacrificed on Gestation Day 26. On PP Day 21 all F1 neonates were sacrificed except one male and one female from each litter who were retained as parents for the F2 generation. The F1 females were mated with males from different litters but within same dose group, when at least 13 weeks of age. A C-section was carried out on each mated F1 female on Day 20 of presumed gestation to examine F1 uterus and F2 fetuses.

RESULTS -

1. F0 generation

F0 Survival -

Gestation: In HD, 8 animals (all gravid) died before parturition between Day 21 and Day 23, and 3 HD f were killed moribund (one on Day 21, two on Day 22).

Lactation: No deaths

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F0 Clinical Signs -

Gestation: In LD 3 dams with signs (alopecia, chromodacryorrhea). In MD 1 dam had decreased activity and flaccid body tone. In HD 11 dams had signs of systemic toxicity (piloerection, ptosis, decreased activity, flaccid body tone, body and head tremors), and 1 dam had alopecia.

Lactation: In LD 2 dams with clinical signs (alopecia, soft stool), in HD 2 dams with signs (alopecia, ataxia, ptosis, tremors)

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F0 Body Weight --

Gestation: No effects

Lactation: No effects

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F0 Food Consumption -

Gestation: No effects

Lactation: No effects

F0 Reproductive Performance - (control-LD-MD-HD)

Evidence of mating - In N = 25-25-25-25

Fertile matings - No significant change (92%-96%-96%-100%)

Animals gravid - 23-24-24-25

Gestation length - Small but significant decrease in HD (21.9 - 21.9 - 21.8 - 21.6 days)

Abortion or early delivery - Not observed

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Animals killed/died before delivery - 0-0-0-11

Number of litters delivered - 23-24-24-14

Total number of neonates - 328-357-368-199; total viable 326-353-364-190; total stillborn 2-3-2-9 (from 2-3-1-3 dams)

Number of neonates per litter at delivery - 14.3 - 14.9 - 15.3 - 14.2 (litter means)

Number of live births - 14.2 - 14.7 - 15.2 - 13.6 (litter means)

Number of stillbirths - 0.1 - 0.1 - 0.1 - 0.6 (litter means)

Litters with stillborn progeny - Increase in HD (2-3-1-3 dams/litters, ie, 9%-13%-4%-21%) APPEARS THIS WAY
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F0 Progeny Findings -

Survival - No effects up to PP Day 21

Clinical observations - No treatment-related effects

Body weight - On PP Day 0 increase in body weight in LD m and f. On Day 4 decrease in body weight in LD, MD, HD (m and f). On Day 7 decreased BW in LD, MD, HD m. No effects seen on Days 14 and 21.

Physical development - Dose-related increase in time to incisor eruption in MD, HD. Also, eye opening delayed in both MD and HD. Pinna detachment delayed in HD.

Behavioral development - No effects

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Neonate findings -

Skeletal malformations - None in any group.

Skeletal variations - There were (0-2-2-0) pups with malformations among ones that died during first 3 PP days (this does not say anything). These consisted of incomplete ossifications of 5th sternebrae (2 pups in LD, 2 in MD) or of xiphoid process (1 pup in MD). No dose-relation observed.

Terminal necropsy findings - Males

Females??

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2. F1 generation

F1 Clinical Signs - No treatment-related effects before or after standardization. No clinical signs in F1 dams selected for C-section (males??)

F1 Reproductive Performance -

Evidence of mating - N=22/22- 23/23- 22/24- 5/8

Fertile matings - No effects (100%-81.8%-100%-100%)

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F1 Progeny Findings -

No f2 fetal deaths. One fetus in control group had abnormal pathology.

CONCLUSIONS -

F0 generation:

Systemic toxicity was seen at 2 and 8 mkd (MD and HD), and death before parturition at 8 mkd (HD). NOAEL for reproductive toxicity was 0.5 mkd, LOAEL 2 mkd.

At 8 mkd there was an increase in % stillborn pups (number stillborn pups/total nr pups) and in % dams that delivered with stillborns.

F1 generation:

Body weight of F1 pups was decreased in LD, MD and HD up till PP Day 7, recovered thereafter. Physical development (incisor, eye) was delayed in MD and HD.

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PHARMACOLOGY AND TOXICOLOGY REVIEW
Summary Review and Conclusions of
Reproductive Toxicity Studies:

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Reproductive Toxicity Studies With Endpoints						
Study	Species and Strain	No. and Sex Per Group	Route	Dose Range (mg/kg/Day)	Dosage Form	F ₀ Treatment Days
C2A/B	Rat Sprague-Dawley	30/group (Sex I)	Oral gavage	0, 0.2, 7.1, 16, 80/40*	Aqueous suspension	14: 20-20 pp P: 10-20 - 20 pp
C1	Rat Sprague-Dawley	25/group (Sex I -Japan)	Oral gavage	0, 2.5, 10, 40, 160/80*	Aqueous suspension	14: 20-20 pp P: 14-20 - 7 pp
C4A/B	Rat Sprague-Dawley	25 Female (Sex II)	Oral gavage	0, 2.5, 10, 80	Aqueous suspension	P: 0 pp - 17 pp
C3	Rat Sprague-Dawley	6 Female (Sex II -Japan)	Oral gavage	0, 0.5, 0.12, 0.5, 0.5	Aqueous suspension	P: 7 pp - 17 pp
C5	Rabbit New Zealand White	16 Female (Sex II)	Oral gavage	0, 2, 10, 50	Aqueous suspension	P: 0 pp - 16 pp
C6	Rat Sprague-Dawley	25 Female (Sex II)	Oral gavage	0, 0.5, 2, 0	Aqueous suspension	P: 10 pp - 21 pp
C7	Rat Sprague-Dawley	5 Female (Sex II -Japan)	Oral gavage	0, 0.1, 0.25, 1, 2.5	Aqueous suspension	P: 10 pp - 7 pp
C8	Rat Sprague-Dawley	6 Female (Sex II -Japan)	Oral gavage	0, 0.5, 12.5, 25, 50, 50	Aqueous suspension	P: 10 pp - 4 pp

4d = days ante-partum (prior to mating)
 pp = days post-partum (after mating)
 14d = days post-partum (during lactation)
 *C Doses will replace from T, B, Y doses for study.

SUMMARIES OF INDIVIDUAL STUDIES

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Segment I study rat (C1, Japan)

Doses 0 2.5 10 40 160/80 mkd (Females treated through Gestation Day 7; C-section Gestation Day 20)
 Examined F0, F1

Effects in F0:

In males, decreased BW and FC in HD. Clinical toxicity signs and mortality in HMD and HD. Histopathology showed changes of reproductive organs in HMD and HD (testis and epididymis atrophy/inflammation).

In females, mortality observed in LMD, HMD and HD (4-12-12%)

Reproductive effects in F1:

Fertility index decreased in HD pairs (unclear why: male or female fertility, since no sperm parameters measured)
 Weight of fetuses in HD group reduced by ca. 10%
 NOAEL 10 mkd

Segment I study rat (C2A/B)

Doses 0 3.2 7.1 16 mkd (f) (Females treated through Gestation Day 20; C-section Gestation Day 20)
 0 3.2 16 80/40 mkd (m)
 Examined F0, F1, F2

Effects in F0:

In males, BW and FC reduced in MD and HD. Drug-related clinical signs of systemic toxicity in MD and HD, and mortality in HD. Increased relative weight of testis, prostate, epididymis and seminal vesicles in HD.

In females, some decrease in FC in HD. No clinical signs during gestation, but adverse signs and mortality (dystocia)-occurred during periparturient period in all dose groups. This effect was probably related to drug-induced periparturient hypocalcemia. The hypocalcemia was measured in F0 dams right before C-section (serum Ca reduced non-dose-dependently in all dose groups by

Reproductive effects in F0:

(# Litters examined from C-sectioned animals: 20-19-20-14, from delivering animals: 13-7-7-3)

Fertility index decreased by ca. 30% in HD groups of F0

Corpora lutea reduced in HD, and # implantations reduced in MD and HD f. No effect on # or % pre- or postimplantation loss, or # resorptions.

Effects in F1:

Reduced survival of neonates in HD (pp day 0-4). No effect on survival from pp day 4-21.

Skeletal variations:

Incidence of unossified 5th and 6th sternbrae: decreased in LD and MD (sign), and HD (ns)

Incidence of incompletely ossified 5th sternbrae: increased in MD only (ns)

Incidence of incompletely ossified 4th sternbrae: decreased in LD (sign), and in MD and HD (ns)

Effects were not dose-related.

Cleft palate in LD and MD (#affected: 1 fetus in 1/19 litters; 1 fetus each in 2/20 litters).

BW of pups slightly increased in HD group through pp day 21.

Development/behavior: Time for grasp/holding increased in all treated groups. Time for surface righting decreased in MD and HD, time for pinna detachment decreased in MD and HD, and time for eye opening decreased in all treated.

Reproductive effects in F1:

Conclusions not possible due to small sample size (# mated females of F1: 10-5-5-2)

NOAEL males 3.2 mkd

NOAEL females 7.1 mkd (excluding peri-parturient effect)

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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.

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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 sternbrae: increased in HD

Incidence of incompletely ossified 5th sternbrae: decreased in HD

Incidence of incompletely ossified 4th sternbrae: 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)

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Segment II study rabbit (C5)

Doses 0 2 10 50 mkd (Females treated on Gest Days 6-18, C-section on Gest Day 29)
Examined F0, F1

Effects in F0:

Decreased FC and BW gain in HD.
Dose-related clinical signs (diarrhea, feces minimal, flaccidity) in MD, HD.
Drug-dose-related mortality (spontaneous or euthanasia) in F0 in all dose groups (LD, MD, HD: 17-17-100%). LD and MD deaths were due to gavage accidents. All HD deaths occurred before Day 24. HD mortality was not due to parturition, but according to Sponsor related to esophageal toxicity.
1 premature delivery and 1 abortion in MD.
Reduction in # animals carrying to term (100-85-71-0%)
Histopathology: Dose-related esophageal ulceration in MD and HD. Bronchio-alveolar inflammation in all treated.
Myofiber degeneration in some rabbits (dose groups??)

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Reproductive effects in F0:

No effect of treatment on #corp lutea, implantation sites, viable fetuses, resorptions, pre/post-implantation loss in LD, MD.
In LD, MD: no effects on pre/postimplant loss, # resorptions, # viable fetuses.

Effects in F1

No significant effects on incidence of soft tissue/ skeletal/ external malformations or skeletal variations in LD, MD (HD not examined)

NOAEL maternal < 2 mkd developmental 10 mkd

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Segment III study rat (C8)

Doses 0 0.5 2 -8 mkd (Females treated on Gest Day 15 - Lact Day Day 21)
Examined F0, F1, F2

Effects in F0

No effects on FC, BW

Dose-related clinical signs of systemic toxicity in 1 MD, and many HD.

Drug-related mortality (spontaneous or euthanasia) in F0 in HD. HD mortality occurred between Gest Day 21-23 (periparturient). Number of litters examined: 23-24-24-14.

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Reproductive effects in F0:

No effect on #neonates/litter

Increase in #stillborns/litter in HD, and #litters with stillborns

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Effects in F1:

No significant effects on incidence of external malformations. Skeletal results inconclusive.

Reproductive effects in F1:

Number of animals that mated reduced in HD (100-100-92-63%)

No effect on #fertile matings, # viable fetuses, corpora lutea, resorptions, pre/postimplantation loss.

Effects in F2: None

NOAEL 0.5 mkd (maternal and fetal)

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SUMMARY AND CONCLUSIONS

In the rat, treatment with Risedronate reduced fertility by inhibiting ovulation and implantation at 16 mg/kg/day (equivalent to 5.5 times the 30-mg human dose on the basis of surface area comparison, mg/m²). It is unclear from the data whether there was a reduction in male rat fertility at doses of 40 mg/kg/day or higher. When dams were treated with 16 or 80 mg/kg/day

) there was a reduction in viability of neonates. However, at these doses (16 and 80 mg/kg/day) maternal toxicity, ie, reduced food consumption and body weight gain, was also observed. At maternal doses of , the incidence of incompletely ossified or unossified fetal sternbrae or skull was decreased. At doses of 16 mg/kg/day and higher the incidence of incompletely ossified or unossified sternbrae or skull was increased. Cleft palate was observed in fetuses from dams treated with 3.2 and 7.1 mg/kg/day. At doses of 3.2 mg/kg/day and higher there was periparturient mortality possibly related to hypocalcemia occurring around parturition time. This effect was also seen when treatment was discontinued before parturition. When dams were treated throughout parturition, there was an increased number of stillborn fetuses at 8 mg/kg/day. Rabbits treated with 50 mg/kg/day all died, probably due to esophageal ulceration and resulting systemic toxicity. Abortion and premature delivery occurred at 10 mg/kg/day. No fetal malformations were noted at doses up to 10 mg/kg/day

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PHARMACOLOGY AND TOXICOLOGY REVIEW

SUMMARY REVIEW OF PHARMACOKINETIC & ADME STUDIES:

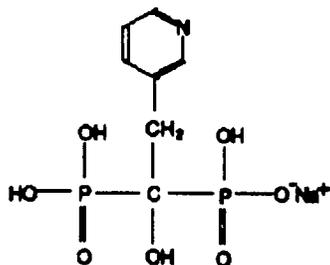
Introduction:

Numerous animal pharmacokinetic and ADME studies were carried out by the sponsor in house and in several contract labs. Approximately half of the studies were not "in compliance with GLP" however all of the studies appear to have been carefully executed. I have tabulated and summarized the findings of these studies in groups according to the main objectives of each study.

Methods:

The principal animal models used in the pharmacokinetic evaluation of Risedronate were rat and dog. Oral dose levels studied included those approximating the anticipated Page's dose of 30 mg for a 60 kg human (i.e., 0.5 mg/kg), to 64 mg/kg used in repeated dose toxicity studies. Due to the low bioavailability of Risedronate (about 1%), the intravenous doses studied were adjusted accordingly (e.g., a 5 ug/kg iv dose was approximately equivalent to a 0.5 mg/kg oral dose).

Chemical Characteristics of Risedronate Relevant to PK:



Structure of Risedronate (NE-58095)

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Risedronate ([1-hydroxy-2-(3-pyridinyl)ethylidene]bis[phosphonic acid] monosodium salt; (NE-58095), MW of 305.10, is a bisphosphonate compound with potent anti-resorptive activity on bone. Risedronate has 5 pKa values corresponding to the initial dissociable protons of the phosphate groups, the pyridinyl nitrogen, and the remaining dissociable protons of the phosphate groups, and has a net negative charge over the pH encountered in the intestine (about 5-8) and blood (about 7.4). It is highly water soluble (>65 mg/mL), possessing excellent stability, both as a solid and in solution. ¹⁴C-Risedronate, used in some animal studies, also is radiochemically stable, with approximate degradation rates of 0.10% and 0.02% per week when stored as a solid or solution at -70°C. Risedronate is known to form both soluble and insoluble complexes with Ca⁺⁺ (and presumably other divalent metal cations) and binds to metal and non-silanized glass surfaces. As a result, precautions were taken in all studies to limit exposure of drug to these surfaces.

Overview:

Risedronate is poorly absorbed due in part to its highly polar state; however, absorption occurs rapidly (T max ; about 30 min to 1 hr). About 60% of an absorbed dose distributes to bone, with less distribution to soft tissue. The remainder of an absorbed dose is excreted rapidly in urine. Drug bound to bone can either be released or intercalated into the bone matrix during formation of new bone. Bound drug is only able to dissociate once this new bone has been resorbed. This process is likely responsible for the very long estimates of Risedronate half-life on bone in rats. After reaching T max, systemic Risedronate levels decrease rapidly in a

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multi-exponential manner. The initial exponential phase represents both the distribution of drug to bone and renal elimination of drug. The apparent terminal phase is representative of the early rapid elimination of Risedronate, with about 90% of the drug not bound to bone excreted in the first 8 hr after dosing. Since a large fraction of an absorbed dose binds to bone, the actual terminal half-life of drug in serum is probably reflective of the very slow release of this compound from bone. Risedronate is not metabolized systemically and shows no cytochrome P-450 induction potential. Evaluations of several repeated dose pharmacokinetic studies indicate steady-state exposure is reached by at least Day 14 in rats and dogs at doses < 8 mg/kg/day with little or no systemic accumulation. At 8 mg/kg/d there is some accumulation and nonlinearity in the AUC vs. dose relationship. This dose is 15-times the human dose (0.5 mg/kg), but, on a mg/m² basis, for dogs it is only 8-times the human dose, and for rats it is only 2.6-times the human dose. At higher doses a great deal of systemic accumulation is seen over time, being more significant in rats than dogs. Where possible, analysis of dose proportionality at steady-state indicates linear kinetics at doses below 8 mg/kg/day for rats, becoming nonlinear at higher doses. No sex differences in exposure are seen in rats and dogs after single and multiple doses.

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Summary Review of Absorption and Elimination Studies:

Study	Species	Route	Duration of Treatment	Title: APPEAR THIS WAY ON ORIGINAL	Doses (mg/ kg/ day)
G1A	Rat	oral	1 d (single dose)	Pharmacokinetics of NE-58095 in Rats	0.100, 0.101, 0.304, 0.309, 1.17, 4.45, 16.1
G3A	Rat	oral	1 d (single dose)	Absorption, Distribution and Excretion of ¹⁴ C in the Rat Following Intravenous and Oral Administration of NE-58095- ¹⁴ C	0.5
G4A	Rat	oral	1 d (single dose)	Biliary Excretion of Risedronate in Rats	1.0
G6	Rat	oral	7 d (daily)	Investigation of the Possible Transfer of [¹⁴ C]Risedronate from Feces to Urine after Multiple Oral Doses in Rats	16
G1B	Rat	iv	1 d (single dose)	Pharmacokinetics of NE-58095 in Rats	1.90, 6.78, 23.6, 101, 305 µg/ kg
G4B	Rat	iv	1 d (single dose)	Biliary Excretion of Risedronate in Rats	0.01, 0.04
G3B	Rat	iv	1 d (single dose)	Absorption, Distribution and Excretion of ¹⁴ C in the Rat Following Intravenous and Oral Administration of NE-58095- ¹⁴ C	0.5
G7A	Dog	oral	1 d (single dose)	Pharmacokinetics of NE-58095 in Dogs	0.108, 0.302, 0.899, 2.66, 7.87
G8A	Dog	oral	1 d (single dose)	Dose Proportionality of Risedronate Pharmacokinetics in Dogs	0.2, 0.8, 4.0, 16, 64
G9A	Dog	oral	1 d (single dose)	Absorption, Distribution and Excretion of ¹⁴ C in the Beagle Following Intravenous and Oral Administration of NE-58095- ¹⁴ C	0.5
G10	Dog	oral	1 d (single dose)	A Bioavailability Study to Compare the Effect of Food on the Absorption of NE-58095 in Dogs	10
G7B	Dog	iv	1 d (single dose)	Pharmacokinetics of NE-58095 in Dogs	3.68, 11.2, 33.4, 94.3, 295 µg/ kg
G8B	Dog	iv	1 d (single dose)	Dose Proportionality of Risedronate Pharmacokinetics in Dogs	0.04
G9B	Dog	iv	1 d (single dose)	Absorption, Distribution and Excretion of ¹⁴ C in the Beagle Following Intravenous and Oral Administration of NE-58095- ¹⁴ C	0.5

Absorption:

Risedronate is poorly absorbed due in part to its highly polar state; however, absorption occurs rapidly (T_{max}; about 30 min to 1 hr). Absorption of Risedronate was estimated in animals by comparison of radioactivity recovered in urine and bone after intravenous and oral dosing of ¹⁴C-Risedronate, or by the relative area under the Risedronate concentration (blood, plasma, or serum)-time curve (AUC) after oral and intravenous administration. Since this drug is not systemically metabolized (see Metabolism section of this summary), measured radioactivity in the ¹⁴C-Risedronate experiments represented the parent compound.

Bioavailability of oral ¹⁴C-Risedronate to fasted rats was As dose increased, the cumulative sum of drug excreted in urine and drug remaining in bone after 72 hr increased as a linear function of dose, suggesting that absorption was proportional with dose. T max was 0.5 hr, indicating absorption was rapid.

In fasted dogs given oral ¹⁴C-Risedronate) absorption was low and ranged from The higher estimates obtained based on urinary data for rats and dogs) were possibly due to contamination of urine with feces that could not be ruled out, indicating that estimates based on data from blood, plasma, serum, and bone) were more reliable. In later studies, precaution was taken to minimize cross-contamination of feces and urine. In dogs, cumulative amounts of drug excreted in urine, and drug remaining in bone 72 hr post-dose were also linear with dose. Tmax was , indicating absorption was rapid in dogs.

Two studies were conducted in dogs with unlabeled Risedronate to confirm the absolute bioavailability seen in radiolabeled studies and to investigate the effect of food on absorption.

In the first study, dogs were given Risedronate IV at 0.04 mg/kg, and orally over a range At dose levels where kinetics were linear (0.2, 0.8 mg/kg), bioavailability ranged from in agreement with the estimates obtained after administration of ¹⁴C-Risedronate. Dogs fed 30 min before dosing had a statistically significant 3.5-fold lower absorption of Risedronate by comparison to dogs fasted overnight for a period including 4 hr after dosing

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Elimination:

About 60% of an absorbed dose is distributed to the bone. The remainder of an absorbed dose (about 40%) is excreted rapidly in urine. After single intravenous administration of 0.5 mg/kg ¹⁴C-Risedronate to rats, blood ¹⁴C drug levels decreased rapidly in a multi-exponential fashion, to nearly 0 within 8 hr. ¹⁴C was excreted predominately in urine and increased linearly with dose from About 40% of the dose was excreted in the urine. The terminal phase of elimination had a t1/2 of 20.9 hr. Due to the long residence time of drug on bone, the true terminal t1/2 could not be measured. The 60 % of drug not excreted initially in urine was presumed bound to bone (based on results from tissue distribution studies) and released very slowly (possibly over a period of years) to be excreted by the kidney.

Very small amounts of ¹⁴C were excreted in feces after intravenous dosing , possibly due to ingestion of radioactive urine during preening activity or gastrointestinal secretion of drug; cross-contamination of feces and urine was also a possibility. Biliary excretion was negligible in rats, with the cumulative amount excreted after an intravenous dose of representing less than 0.03% of the dose. Apparent volume of distribution was very large (22.8 L/kg).

PK in dogs was similar to the results in rats following a single intravenous dose ranging from ¹⁴C-Risedronate, or a 0.04 mg/kg unlabeled dose of Risedronate. Most of the excreted ¹⁴C was recovered in urine), with minor recovery in feces), suggesting some gastrointestinal secretion of Risedronate or cross-contamination of feces and urine. As for rats, the volume of distribution was very large . The terminal phase of the systemic disappearance of drug in plasma was characterized by t1/2 values ranging from r and did not change over the doses examined. Kinetic parameters based on serum concentration-time data were similar to those calculated from plasma data.

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Summary Review of Distribution Studies:

Study	Species	Route	Duration of Treatment	Title:	Doses (mg/ kg/ day)
G11	Mouse	iv	1 d (single dose)	Tissue Distribution of Intravenously Administered ¹⁴ C-NE-58095 in the Mouse	5
G12A	Rat	oral	1 d (single dose)	Distribution of ¹⁴ C in the Rat Following Single and Multiple-Dose Oral Administration of NE-58095- ¹⁴ C	0.5
G12B	Rat	oral	4 or 7 d (daily)	Distribution of ¹⁴ C in the Rat Following Single and Multiple-Dose Oral Administration of NE-58095- ¹⁴ C	0.5
G13A	Rat (M)	oral	1 or 21 d (daily)	[¹⁴ C]Risedronate: Tissue Distribution in the Rat	0.8
G13B	Rat (F)	oral	1, 7, 14 or 21 (daily)	[¹⁴ C]Risedronate: Tissue Distribution in the Rat	0.8
G13C	Rat pregnant	oral	1 or 7 (daily)	[¹⁴ C]Risedronate: Tissue Distribution in the Rat	0.8
G13D	Rat lactating	oral	1 d (single dose)	[¹⁴ C]Risedronate: Tissue Distribution in the Rat	0.8
G15A	Rat	iv	1 d (single dose)	[¹⁴ C]-Risedronate: Quantitative Whole-Body Autoradiography in the Rat (Pilot Study)	1.67, 8.33 µg/kg
G15B	Rat	iv	14 (daily)	[¹⁴ C]-Risedronate: Quantitative Whole-Body Autoradiography in the Rat (Pilot Study)	8.33 µg/kg
G16A	Rat	iv	1, 14, or 28 (daily)	[¹⁴ C]-Risedronate: Quantitative Whole-Body Autoradiography Following Intravenous Administration in the Rat	5 µg/kg
G16B	Rat	iv	7 d (daily)	[¹⁴ C]-Risedronate: Quantitative Whole-Body Autoradiography Following Intravenous Administration in the Rat	5 µg/kg
G17A	Rat	in vitro	3 hr incubation	Plasma Protein Binding of NE-58095 in the Rat, Dog, and Human	0.01, 0.05, 0.75, 10 µg/mL
G17B	Dog	in vitro	3 hr incubation	Plasma Protein Binding of NE-58095 in the Rat, Dog, and Human	0.01, 0.10, 1.0, 10 µg/mL
G17C	Human	in vitro	3 hr incubation	Plasma Protein Binding of NE-58095 in the Rat, Dog, and Human	0.01, 0.05, 0.25, 1.0, 10 µg/mL

About 60% of an absorbed dose of Risedronate distributes to bone, with very low levels in soft tissue compared to bone.

Tissue distribution of ¹⁴C-Risedronate in mice and rats after single and multiple daily doses was determined

Mice given 5 mg/kg ¹⁴C-Risedronate IV showed extensive and long lasting distribution to bone. There was a higher relative concentration seen in kidney, reflective of the renal excretion of drug, and in the gastrointestinal tract (1 and 3 hr sampling times only, and possibly indicative of some mucosal secretion of drug, since biliary excretion is negligible). When x-ray film exposure time was lengthened (to maximize detection of radioactivity in soft tissues) distribution to other soft tissues was seen, but traces of radioactivity were very faint and not quantifiable.

WBA studies in rats dosed IV with 5 mg/kg ¹⁴C-Risedronate as single, 14, or 28 once-daily doses showed significant distribution of radioactivity to bone, with peak levels generally occurring at _____ after completion of the dosing regimen. Drug was discretely localized on bone, with highest concentrations seen in regions undergoing active bone growth; lesser amounts were seen in the medullary cavity region of bone. Much lower radioactivity concentrations were seen in soft tissues, consistent with findings in the mouse. Once bound, ¹⁴C-Risedronate was slowly released from bone, with estimates of half-life values ranging from _____ after single doses and from _____ after 28 daily doses, indicating a slower release of drug from bone after multiple doses. Based on the pharmacological action of Risedronate to inhibit bone resorption, thereby decreasing bone turnover, it was not surprising that multiple doses of Risedronate slowed its own release from bone. Uptake of ¹⁴C-Risedronate into bones was largely proportional to the number of administered doses; however, a less than proportional uptake was seen for regions undergoing active bone growth, and bone surface

areas associated with the periosteum, possibly indicating some small degree of saturation of binding in these regions. Continued bone growth was maintained in all treatment groups following completion of dosing. A preliminary WBA study in rats receiving single intravenous doses of 1.67 or 8.33 mg/kg indicated concentration of radioactivity in bone was approximately proportional to the administered dose.

Negligible distribution to pigmented tissue was also demonstrated in this study after 14 daily intravenous doses of 8.33 mg/kg ¹⁴C-Risedronate to Lister Hooded (pigmented) rats, suggesting no binding of drug to melanin.

Additional tissue distribution studies were conducted using tissue dissection and analysis by scintillation spectrometry in (a) male, female, pregnant, and lactating rats given single or multiple-daily (7, 14, or 21 days) oral doses of 0.8 mg/kg ¹⁴C-Risedronate, or (b) male rats given single, 4 or 7 daily oral doses of 0.5 mg/kg ¹⁴C-Risedronate. Results from these studies agreed with those obtained from the WBA studies, with respect to distribution to bone and soft tissues. Very low levels of drug were found in soft tissues after single or multiple doses. Some soft tissue accumulation was seen after multiple dosing, but concentrations decreased to low levels at 72 hr post-dose, indicating little drug persistence in these tissues. The data also did not support any distribution to erythrocytes. No major differences were noted in the drug distribution pattern when comparing male and female, or normal and pregnant animals. Although radioactivity in fetuses from pregnant animals given ¹⁴C-Risedronate was not detectable by scintillation counting, autoradiographs of these fetuses showed some radioactivity, indicating some placental transfer of drug occurred in rats. ¹⁴C-Risedronate was below the level of analytical sensitivity in the milk of lactating rats given 0.8 mg/kg orally, but very low levels (<1 ng equiv/g tissue) were detected in whole neonates, indicating some lacteal transfer.

In vitro plasma protein binding of Risedronate was about 95% and 86% for rats and dogs, respectively, and was independent of Risedronate concentration over the . . . Human plasma protein binding of drug was about 93% from . . . progressively decreasing from . . . as . . . concentration increased from . . .

Percent Risedronate Plasma Protein Binding:

Concentration (mg/ mL)	0.01	0.05	0.10	0.25	0.75	1.0	10
Rat:	94.2± 4.66	95.1 ± 0.69	ND	ND	95.2 ±0.39	ND	96.4 ± 0.50
Dog:	84.8± 1.14	ND	85.8 ±1.28	ND	ND	86.7 ± 1.23	87.2 ± 1.62
Human:	93.7± 0.56	90.3 ±3.01	ND	95.0 ± 0.61	ND	83.6 ± 4.46	67.6 ± 4.31

Values are mean percent bound ± standard deviation (n = 3- 7); ND = not determined

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Note added in proof:

The sponsor has FAXed a statement that an amendment is being submitted which significantly changes the plasma protein binding. This study has not been received or reviewed yet. The new values are approximately 98% for rats, 35% for dogs and 25% for humans.

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Summary Review of Metabolism Studies:

Study	Species	Route	Duration of Treatment	Title:	Doses (mg/ kg/ day)
G18A	Rat	oral	1 d (single dose)	Assessment of the Metabolism of NE-58095-14C in the Rat	2.0
G19	Rat	oral	14 d (daily)	The Effects of Risedronate (NE-58095) on Hepatic Enzyme Activities in Rats after 2-Weeks of Treatment and after a 2-Week Recovery Period	0.1, 0.8, 4, 16
G20	Rat	oral	21 d (daily)	Assessment of the In Vivo Biotransformation of 14C-NE-58095 in Urine, Bone and Feces from a 21 Day Rat Study	0.8
G18B	Rat	iv	1 d (single dose)	Assessment of the Metabolism of NE-58095-14C in the Rat	0.2
G21	Rat	iv	1 d (single dose)	Assessment of the Metabolism of NE-58095-14C in the Rat. Intravenous Administration and Urine Collection Via Ureter Cannulation	0.2
G22A	Rat urine & plasma	in vitro	8, 24, or 48 hr urine; 20 or 24 hr plasma incubation	Assessment of the Biotransformation In Vitro of NE-58095-14C in Rat and Dog Urine and Plasma	6 mg/mL (plasma)
G23A	Rat liver slices	in vitro	8 or 24 hr incubation	Assessment of the In Vitro Metabolism of NE-58095-14C by Liver Slices Obtained From the Livers of Humans, Dogs, and Rats	100, 1000 mg/mL
G24A	Rat fecal flora cultures	in vitro	0, 6, 12, or 18 hr incubation	Assessment of the In Vitro Metabolism of NE-58095-14C: Samples Generated by Fecal Flora of Humans, Dogs, and Rats	0.2, 0.4, 1.0 mg/mL suspension
G25A	Dog	oral	1 d (single dose)	Assessment of the Metabolism of NE-58095-14C in the Dog	2.0
G25B	Dog	iv	1 d (single dose)	Assessment of the Metabolism of NE-58095-14C in the Dog	0.2
G22B	Dog urine & plasma	in vitro	16, 24, or 96 hr urine; 20 or 24 hr incubation	Assessment of the Biotransformation In Vitro of NE-58095-14C in Rat and Dog Urine and Plasma	6.0 mg/mL urine 6.0 mg/mL plasma
G23B	Dog liver slices	in vitro	8 or 24 hr incubation	Assessment of the In Vitro Metabolism of NE-58095-14C by Liver Slices Obtained From the Livers of Humans, Dogs, and Rats	100, 1000 mg/mL
G24B	Dog fecal flora cultures	in vitro	0, 6, 12 or 18 hr incubation	Assessment of the In Vitro Metabolism of NE-58095-14C: Samples Generated by Fecal Flora of Humans, Dogs, and Rats	0.2, 0.4, 1.0 mg/mL suspension
G26	Human urine, plas. & serum.	in vitro	8, 24, or 48 hr (urine, plasma, & serum)	Assessment of the Biotransformation In Vitro of NE-58095-14C in Control Human Urine and Plasma	6.0 mg/ mL (urine, plasma, or serum)
G23C	Human liver	in vitro	8 or 24 hr incubation	Assessment of the In Vitro Metabolism of NE-58095-14C by Liver Slices Obtained From the Livers of Humans, Dogs, and Rats	100, 1000 mg/mL
G24C	Human fecal flora cultures	in vitro	0, 6, 12, or 18 hr incubation	Assessment of the In Vitro Metabolism of NE-58095-14C: Samples Generated by Fecal Flora of Humans, Dogs, and Rats	0.2, 0.4, 1.0 mg/mL suspension

Risedronate is not metabolized systemically and shows no cytochrome P-450 induction potential. Metabolites were not detected in bone or plasma samples collected from rats or dogs dosed with Risedronate. No products other than Risedronate were detected in urine continuously collected by ureter-cannulation (pre-bladder urine) from rats dosed IV with Risedronate. Risedronate did not degrade when incubated with liver tissue, flora of the gut, or plasma from rat, dog or human. However, two known chemical degradation products, 1-oxo-2-(3-pyridinyl)ethylphosphonic acid (keto) and 3-pyridyl acetic acid (3-PAA), were found in rat and dog urine samples collected after oral and intravenous dosing and were produced when Risedronate was incubated in fresh urine. Similarly, urine collected from rats given Risedronate IV did generate the keto degradant upon incubation of the