# CENTER FOR DRUG EVALUATION AND RESEARCH

**APPLICATION NUMBER: 020819**

## PHARMACOLOGY REVIEW(S)

FEB - 5 1998

**NDA # 20,819**
**Pharmacology Review**

**Reviewed: February 4, 1998**
**Initial NDA Submission**

| | |
|---|---|
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| **DRUG:** | Ab 122358, 19-nor Vitamin $D_2$ analog |
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| **CATEGORY:** | Vitamin D analog |
| **INDICATIONS:** | Renal osteodystrophy.<br>Secondary hyperparathyroidism. |
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| **NDA Filing Meeting:** | March 5,1997 |
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**REVIEWER RECOMMENDATION CODE: AP (pending labeling revisions).**

/S/

2/4/98

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Concurrence: /S/

2/5/98

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# PHARMACOLOGY AND TOXICOLOGY REVIEW OF Three Month Intravenous Toxicity Study of Paracalcin in Dogs Study # TB94-345

## PURPOSE:

To assess the toxicity of Paracalcin in dogs when administered IV 3 times per week for three months. Calcitriol (0.3 ug) was given as a positive control.

## EXPERIMENTAL DESIGN:

**Testing Facility:** Drug Safety Evaluation Division. Abbott Labs, Abbott Park, IL.

**Study #:** TB94-345

**Study Initiated:** 2/6/95.
**Study Completed:** 9/27/95.

**Dose & Formulation:** 0, 0.02, 0.1, & 0.3 ug/kg/day Paracalcin, or 0.3 ug/kg/day Calcitriol, IV, 3 times/wk, in 20% EtOH, 30% Propylene Glycol / Water.

**Batch of drug:** lot 85-552-je

**Food:** CERTIFIED CANINE DIET,
0.5% Calcium, 0.4% Phosphorous, 0.8 IU/g Vitamin D.

**GLP statement:** **Included.**

**Animals:** 40 purebred beagle dogs

| Group: | | Dose (ug/kg): | | # of Animals: |
|---|---|---|---|---|
| | 0 | | 0 | 4 males + 4 Females |
| | 1 | Paracalcin | 0.02 | 4 males + 4 Females |
| | 2 | Paracalcin | 0.1 | 4 Males + 4 Females |
| | 3 | Paracalcin | 0.3 | 4 males + 4 Females |
| | 4 | Calcitriol | 0.3 | 4 males + 4 Females |

All dogs in group 4 were killed on day 29 due to poor health. All dogs in group 3 were killed on day 57 due to poor health All other animals were killed after 3 months.

**Dose Selection:**

A prior 1-month toxicity trial in dogs resulted in significant toxicity at doses of 0.3 ug/kg and higher. In this study a diet containing minimal calcium phosphorus and Vitamin D in order to reduce the effect of hypercalcemia and simulate the clinical setting. 0.3 ug/kg was selected as the highest dose. The high dose of Paracalcin was matched with an equal (0.3 ug/kg) dose of Calcitriol. This dose is 6x (comparisons on a mg/kg basis) the maximum recommended dose of Calcitriol in humans (0.05 ug/kg) and just above the proposed maximum recommended dose for Paracalcin (0.24 ug/kg). On a $mg/m^2$ basis, the maximal doses tested is actually below the maximal doses recommended for patients. This is reasonable because the toxic effects in normals are part of the therapeutic actions in patients.

## RESULTS:

**OBSERVED EFFECTS:**

Emaciation, dehydration and abnormal stools were significant in the Calcitriol group and they were sacrificed on day 29 due to poor general condition.

Emaciation, dehydration and abnormal stools became significant in the HD (0.3 ug/kg) Paracalcin group during the second month. On days 42 and 50 animals in this group were killed in moribund condition and the remainder of the group was sacrificed on day 57 due to poor general condition.

No drug related observed effects were noted in the MD and LD groups but injection site reactions were noted in all groups (including controls).

**MORTALITY:**

8 Calcitriol animals were killed after 28 days.

2 HD (0.3 ug/kg) Paracalcin dogs were sacrificed on days 42 and 50.

The remainder of the HD (0.3 ug/kg) Paracalcin dogs were sacrificed on day 57.

**BODY WEIGHT:**

Significant reductions in weight were noted in the HD group (19% decrease in M, 15% decrease in F) and Calcitriol group (30% M, 35% F) during the first month. After the eighth week the males in the MD (0.1 ug/kg) group were significantly lighter than control males (17 % M, 10% F).

| Body Weights (kg): | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Group (8/group)** | **C** | | **0.02 Paracalcin** | | **0.1 Paracalcin** | | **0.3 Paracalcin** | | **0.3 Calcitriol** | |
| **Sex (4/sex)** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| **Body weight (kg, day 28)** | 12 | 10.4 | 12.1 | 10.5 | 11.7 | 10.3 | **9.7*** | **8.9*** | **8.4*** | **6.7*** |
| **Body weight (kg, day 91)** | 13 | 11 | 13.2 | 10.6 | **10.8*** | 10 | **Dead** | **Dead** | **Dead** | **Dead** |

*=P<0.05, n=4.

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**FOOD CONSUMPTION:**

The weight loss changes above corresponded with changes in food consumption. The Calcitriol and HD Paracalcin groups had statistically significantly reduced food consumption _____ decrease in M and F) at 3-6 weeks. This effect occurred 2-3 weeks sooner in the Calcitriol group than the HD Paracalcin group. No other significant differences in food consumption were noted.

**EYE EXAMINATION:**

No findings were evident.

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**HEMATOLOGY and COAGULATION:**

The only biologically meaningful toxic effect was a slight prolongation of the APPT in the Calcitriol and HD (0.3 ug/kg) Paracalcin groups.

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**BLOOD CHEMISTRY:**

No treatment related effects except (the expected pharmacological actions) decreased PTH levels and hypercalcemia (greater than 13 mg/dl) noted in all drug treated animal groups. This effect was dose and time dependent. From one month onward it was noted in the Calcitriol and HD Paracalcin groups. After 2 months it was frequently noted in the MD Paracalcin group. And one male exhibited consistent

hypercalcemia in the LD group in the second half of the study. The hypercalcemia tended to be worse in males.

All groups had PTH levels lower than controls but this effect was less marked in the LD group. The HD group also had decreased ALP. The mechanism for this is unknown.



**URINALYSIS:**

The hypercalcemia lead to calciuria despite the fact that fractional excretion of calcium was reduced. From one month onward calciuria was noted in the Calcitriol and HD Paracalcin groups. After 2 months calciuria was noted in the MD Paracalcin group.



**ORGAN WEIGHTS:**

Prostate and thymus were slightly reduced (not significant) in size in the Calcitriol group and the HD group. This is believed to be an effect of weight loss and inanition.



**GROSS and HISTOPATHOLOGY:**

Numerous pathological findings were noted in the HD Paracalcin and the Calcitriol groups. These findings were of similar severity (mild-moderate) in both groups, except weight loss which was severe in both groups. When these findings were noted in the MD Paracalcin group they were generally milder and of lower incidence.

| Incidence of Pathological findings:: | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Group (8/group)** | **C** | | **0.02 Paracalcin** | | **0.1 Paracalcin** | | **0.3 Paracalcin** | | **0.3 Calcitriol** | |
| **Sex (4/sex)** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| **Abnormal Aortic Surface** | | | | | | | 4 | 3 | 4 | 2 |
| **Aortic Mineralization** | | 1 | | | | | 3 | 2 | 4 | 3 |
| **Myocardial Discoloration** | | | | | | | | | 1 | 1 |
| **Discolored Intestine** | | | | | | | 3 | 2 | 3 | 4 |
| **Discolored Kidney** | | | | | | 2 | | | | 2 |
| **Thin** | | | | | 2 | 1 | 4 | 4 | 4 | 4 |
| **Renal tub. Degeneration** | | | | | 2 | 1 | 4 | 4 | 4 | 4 |
| **Renal tub. Regeneration** | | | | | 4 | 3 | 4 | 4 | 4 | 4 |
| **Renal Tub. Dilatation** | | | | | 4 | 3 | 4 | 4 | 4 | 4 |
| **Renal tub. Mineralization** | | | | | 1 | 1 | 4 | 4 | 4 | 3 |
| **Renal Tub Fibroplasia** | | | | | 3 | 1 | 2 | 2 | 2 | 2 |
| **Renal Tub. Inflammation** | | | | | 4 | 3 | 4 | 4 | 4 | 4 |
| **Small Thymus** | | | | | | | 4 | 3 | 1 | 4 |
| **Small or degen. Testes** | 1 | | | | | | 1 | | 3 | |
| **Small Prostate** | | | | | | | 2 | | 1 | |
| **Parathyroid Atrophy** | | | | | 4 | 3 | 4 | 4 | 4 | 4 |

## SUMMARY TABLE

(of all statistically significant findings. See main text for details.)

| Effect/dose | 0 | 0.02 Paracalcin | 0.1 Paracalcin | 0.3 Paracalcin | 0.3 Calcitriol |
|---|---|---|---|---|---|
| CLINICAL SIGNS | | | | Dehydration<br>Emaciation<br>Abnormal Stool | Dehydration<br>Emaciation<br>Abnormal Stool |
| TERMINATED EARLY: | | | | 8/8 (2 Mo.) | 8/8 (1 Mo.) |
| WEIGHT LOSS: | | | Decreased (3 Mo.) | Decreased (2 Mo.) | Decreased (1 Mo.) |
| FOOD CONSUMPTION: | | | | Decreased | Decreased |
| HEMATOLOGY: | | | | ⇑ APPT | ⇑ APPT |
| BLOOD CHEMISTRY: | | ⇓PTH,⇑Ca (3 Mo.) | ⇓PTH,⇑Ca (3 MO.) | ⇓PTH,⇑Ca (2 Mo.) | ⇓PTH,⇑Ca (1 Mo.) |
| URINALYSIS: | | ⇑ Ca (1 male) | ⇑ Ca | ⇑ Ca | ⇑ Ca |
| HISTO-PATHOLOGY: | see table above | | | | |

## SUMMARY and DISCUSSION:

At 0.02 ug/kg 3 times per week, Paracalcin caused Hypercalcemia and decreased levels of PTH with resulting increased fractional calcium excretion. The effects on PTH and serum calcium are expected pharmacological actions of the drug. At doses equal to or greater than 0.1 ug/kg, physiologically significant toxicities were seen in response to IV Paracalcin 3 times/week in dogs. At this middle dose level renal cortical mineralization was noted in most dogs and contributed to renal inflammation, dilatation and degeneration. Although this response was significant at 0.1 ug/kg, at higher doses the effect was even more severe. These effects are secondary to the hypercalcemia. Dogs at this dose level also lost weight slightly by the end of the 3 month study.

At doses of 0.3 ug Paracalcin/kg, 3 times per week, numerous physiologically significant toxicities were seen in dogs, in addition to the effects noted above. At this high dose level, soft tissue mineralization was noted in most dogs in kidney, testicles, heart, intestines and aorta. Dogs also suffered dehydration and marked weight loss. These effects contributed to renal and testicular degeneration. The weight loss was probably primarily responsible for the atrophy of the thymus and prostate, and the direct effect of the drug combined with hypercalcemia contributed to Parathyroid atrophy. APTT was also significantly increased. After two months two dogs had died and the group had to be terminated early.

0.3 ug/kg Paracalcin 3 times per week is just slightly above (on a mg/kg basis) the highest (0.24 ug/kg) initial dose of Paracalcin recommended for patients. 0.3 ug/kg Calcitriol is 6 times the highest typical dose of Calcitriol for patients (on a mg/kg basis). Not surprisingly, this dose of Calcitriol caused a similar spectrum of effects in the dogs. These effects were all more severe and developed more rapidly. The group was prematurely terminated after only one month.

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## CONCLUSIONS:

- 0.02 ug of Paracalcin / kg, 3 times per week IV caused expected therapeutic effects in dogs and can be considered a NOAEL.
- 0.1 ug of Paracalcin / kg, 3 times per week IV caused excessive expected pharmacological activity resulting in hypercalcemia and renal mineralization.
- 0.3 ug of Paracalcin / kg, 3 times per week IV caused very excessive expected pharmacological activity resulting in the death of two dogs within two months and the early termination of the group. All of the effects appear to have a primary cause related to the drug induced hypercalcemia.
- 0.3 ug Calcitriol / kg, 3 times per week IV resulted in a similar spectrum of toxicities seen at 0.3 ug Paracalcin / kg, but was more severe and rapid. Paracalcin was less toxic than Calcitriol at the same dose level (on a mg/kg basis) - But the recommended clinical doses of Paracalcin are about 6 times higher than those used for Calcitriol. At six fold higher Paracalcin levels (1.8 u/kg) we would expect significantly greater toxicity, possibly even more than seen in the Calcitriol group. The relative rates of metabolism of these two analogs are quite similar (T1/2 ~6h) but has not been directly compared in dogs.

# PHARMACOLOGY AND TOXICOLOGY REVIEW OF Six Month Intravenous Toxicity Study of Paracalcin in Rats Study # TA95-193

## PURPOSE:

To assess the toxicity of Paracalcin in rats when administered IV 3 times per week for six months.

## EXPERIMENTAL DESIGN:

**Testing Facility:** Drug Safety Evaluation Division. Abbott Labs, Abbott Park, IL.

**Study #:** TA95-193



**Study Initiated:** 7/95.
**Study Completed:** 6/12/96.

**Dose & Formulation:** 0, 0.1, 0.5, & 3.0 ug/kg/day,
IV, 3 times/wk, in 20% EtOH, 30% Propylene Glycol / Water.



**Batch of drug:** lot 95-0417

**Food:** Rat diet, _____
0.5% Calcium, 0.4% Phosphorous, 1 IU/g Vitamin D.

**GLP statement:** **Included.**

**Animals:** 200 Crl:CD(SD)BR Rats _____
150-227 g

| Group: | | Dose (ug/kg): | | # of Animals/sex/group: |
|---|---|---|---|---|
| | 0 | | 0 | 20 + 5 satellite rats |
| | 1 | Paracalcin | 0.1 | 20 + 5 satellite rats |
| | 2 | Paracalcin | 0.5 | 20 + 5 satellite rats |
| | 3 | Paracalcin | 3.0 | 20 + 5 satellite rats |



All rats were killed after 6 months.

**Dose Selection:**

In 1-month and 3-month toxicity studies significant toxicity was noted at doses of 3 ug/kg and higher. All of these toxicities result from excessive pharmacological action of the drug. In this study (as in the 3 month study) a diet containing minimal calcium phosphorus and Vitamin D in order to reduce the effect of hypercalcemia and simulate the clinical setting. 3 ug/kg was selected as the highest dose. Rats do not seem to be as extraordinarily sensitive to the hypercalcemic effects of Vitamin D as dogs. The high dose of Paracalcin was matched with an equal (3 ug/kg) dose of Calcitriol in the three month dog study. This arm was dropped in the current rat study.

## RESULTS:

### OBSERVED EFFECTS:

The delay to onset of the development of ocular Keratopathy (common in rats) was reduced in proportion to the dose of Paracalcin in the 6-month rat study (see ophthalmology).

No drug related observed effects were noted in the MD and LD groups but injection site reactions were noted in all groups (including controls).

### MORTALITY:

There was no drug related mortality.



### BODY WEIGHT:

There was no significant drug related effect on body weight or weight gain.

### FOOD CONSUMPTION:

No significant changes in food consumption were noted.



### EYE EXAMINATION:

An increase in the incidence and decrease in the age of first appearance of mild ocular keratopathy was noted in this 6-month rat study.

| Dose: (ug/kg) | **Males with "Superficial Punctate Keratopathy"/Rats examined:** | **Females with "Superficial Punctate Keratopathy"/Rats examined:** |
|---|---|---|
| 0 | 5/20 | 2/20 |
| 0.1 | 0/20 | 1/20 |
| 0.5 | 5/20 | 1/20 |
| 3.0 | 12/20* | 6/20* |

*=P<0.05, n=20. No other significant findings were evident.



### HEMATOLOGY and COAGULATION:

No biologically meaningful toxic effect was noted. HCT, HB and RBC were slightly (< 10 %) but statistically significantly lower in all treated females than in controls. A slight prolongation of the APPT in the HD (3.0 ug/kg) males was also considered not biologically meaningful.



### BLOOD CHEMISTRY:

No treatment related effects except (the expected pharmacological actions) dramatically decreased PTH levels, hypercalcemia and elevated phosphate (slightly but significantly increased at the end of the study) noted in all drug treated animal groups. This effect was dose and time-dependent. The hypercalcemia tended to be worse in males.

All groups had PTH levels lower than controls.



**Blood levels at the end of the study: (M/F)**

| Dose: (ug/kg) | **Calcium: (mg/dl)** | **Phosphorous: (mg/dl)** | **PTH: (pg/dl)** |
|---|---|---|---|
| 0 | 10.1/10/5 | 5.9/4.7 | 249/220 |
| 0.1 | 10.9*/10.8* | 7.1*/5.8* | 69*/86* |
| 0.5 | 11.5*/10.8* | 8.1*/6.0* | 13*/59* |
| 3.0 | 12.6*/11.5* | 7.9*/6.5* | 6*/12* |

*=P<0.05, n=20.

**URINALYSIS:**

The majority (16/20 M and 17/20 F) of the HD rats and 6/20 male and 6/20 female MD rats developed aciduria. This result is considered drug related and a consequence of hypercalcemia induced nephropathy.



**ORGAN WEIGHTS:**

Slight, statistically significant increases in weights of adrenal glands, and kidneys in HD and MD groups were too small to be of toxicological significance when taken alone. However; the effect in the adrenals may reflect a hypercalcemia induced hyperplasia reported to occur in rats and the effect in the kidneys may reflect the nephropathy and calcification of the kidney. Because body weights were similar in all dose groups (within each sex) absolute weights are reported. In addition, because statistical comparisons of percentages (of body or brain weight) are invalid the % of brain or body weight do not provide additional information and are not presented.

Organ Weights:

| Group (40/group) | C | | 0.1 Paracalcin | | 0.5 Paracalcin | | 3.0 Paracalcin | |
|---|---|---|---|---|---|---|---|---|
| Sex (20/sex) | M | F | M | F | M | F | M | F |
| Adrenal Gland (mg) | 54.6 | 68 | 58.7 | **77.1*** | **61.1*** | **78.1*** | **66.5*** | **83.7*** |
| Kidney (g) | 3.95 | 2.17 | 3.97 | 2.18 | 3.91 | 2.26 | 4.19 | **2.41*** |
| Liver (g) | 18.2 | 8.79 | 18.4 | 8.59 | 18.2 | 8.9 | 20.2 | 9.5 |

* = P<0.05. n=20



**GROSS and HISTOPATHOLOGY:**

Numerous pathological findings were noted in the HD Paracalcin group. These findings were mild-moderate. When these findings were noted in the MD or LD Paracalcin group they were more likely to be mild in addition to being of lower incidence.

Incidence of Pathological findings:

| Group (40/group) | C | | 0.1 Paracalcin | | 0.5 Paracalcin | | 3.0 Paracalcin | |
|---|---|---|---|---|---|---|---|---|
| Sex (20/sex) | M | F | M | F | M | F | M | F |
| Mineralization of Heart | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |
| Mineralization of Bladder | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| Nephrocalcinosis | 1 | 10 | 13 | 16 | 13 | 17 | 19 | 20 |
| Renal Tubular Dilatation | 0 | 1 | 1 | 1 | 6 | 12 | 8 | 13 |
| Progressive Nephrosis | 1 | 0 | 5 | 0 | 5 | 1 | 4 | 0 |
| Tubular Basophilia | 3 | 0 | 1 | 0 | 5 | 0 | 10 | 0 |
| Mild Hyperostosis of the Distal Femur | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 |



## SUMMARY TABLE

(of all statistically significant findings. Toxicologically meaningful effects are in bold. See main text for details.)

| Effect/dose | 0.1 Paracalcin | 0.5 Paracalcin | 3.0 Paracalcin |
|---|---|---|---|
| CLINICAL SIGNS | 0 | ⇑ Rate of Keratopathy | |
| HEMATOLOGY: | ⇓(Hct, Hb, RBC) in F at all doses, | | ⇑ APTT in HD M |
| BLOOD CHEMISTRY: | ⇓PTH,⇑Ca, P | ⇓PTH,⇑Ca, P | ⇓PTH,⇑Ca, P |
| URINALYSIS: | 0 | Aciduria | Aciduria |
| ORGAN WEIGHTS | ⇑ Adrenal weight | | ⇑ Adrenal and Kidney weight |
| HISTO-PATHOLOGY: | See table above. | | |

## SUMMARY and DISCUSSION:

At the lowest dose tested, 0.1 ug/kg 3 times per week, Paracalcin caused hypercalcemia and phosphatemia and decreased levels of PTH. In female rats there was also an increase in adrenal gland weight (possibly secondary to hypercalcemia) and slight anemia (⇓RBC, Hct., Hb.). There was evidence of nephrocalcinosis and progressive nephrosis (also secondary to hypercalcemia). None of the secondary effects were severe enough to be considered evidence of toxicity at this dose level.

At doses equal to or greater than 0.5 ug/kg, physiologically significant toxicities were seen in response to IV Paracalcin 3 times/week in rats. In addition to the effects noted above, at this middle dose level, renal cortical mineralization was noted in most rats and contributed to renal dilatation and nephrosis resulting in increased incidence of aciduria. Although this response was significant at 0.1 ug/kg, at higher doses the effect was even more severe. These effects are secondary to the hypercalcemia.

At doses of 3.0 ug Paracalcin/kg, 3 times per week, numerous physiologically significant toxicities were seen in rats in addition to the effects noted above. At this high dose level, soft tissue mineralization was noted in most rats' kidneys (perhaps causing a slight increase in average kidney weight), and in 3-4/40 rats in heart and urinary bladder. HD male rats also showed signs of hyperostosis of the distal femur and aciduria resulting from renal damage. 12/20 M and 6/20 F rats developed "Superficial Punctate Keratopathy" which is normally seen in older rats. The HD tested in this experiment (3 ug/kg)is just slightly above 10 times (on a mg/kg basis) the highest (0.24 ug/kg) initial dose of Paracalcin recommended for patients and was clearly toxic in these rats. On a mg/m$^2$ basis the maximal (3 ug/kg) dose tested in these rats (18 ug/m$^2$) is only 2-fold the maximum recommended dose in patients (9 ug/m$^2$). The LD (0.1 ug/kg) in rats (0.6 ug/m$^2$) is 15-fold **below** the highest recommended human dose.



## CONCLUSIONS:

0.1 ug of Paracalcin / kg, 3 times per week IV caused expected pharmacodynamic effects in rats and can be considered a NOAEL.

0.5 ug of Paracalcin / kg, 3 times per week IV caused excessive expected pharmacodynamic activity resulting in hypercalcemia and renal mineralization.

3 ug of Paracalcin / kg, 3 times per week IV caused very excessive pharmacodynamic activity resulting in soft tissue mineralization, renal nephrosis, hyperostosis and increase in number and acceleration of onset of ocular keratopathy. All of the effects appear to arise from the drug induced hypercalcemia.

# PHARMACOLOGY AND TOXICOLOGY REVIEW OF
# Six Month Intravenous Toxicity Study of Paracalcin in Dogs
# Study # TB95-194

## PURPOSE:

To assess the toxicity of Paracalcin in dogs when administered IV, 3 times per week, for six months.

## EXPERIMENTAL DESIGN:

**Testing Facility:** Drug Safety Evaluation Division. Abbott Labs, Abbott Park, IL.

**Study #:** TB95-194

**Study Initiated:** 7/31/95.
**Study Completed:** 5/23/96.

**Dose & Formulation:** 0, 0.02, 0.06, & 0.2 ug/kg/day,
IV, 3 times/wk, in 20% EtOH, 30% Propylene Glycol / Water.

**Batch of drug:** lot 95-0419

**Food:** CERTIFIED CANINE DIET,
0.5% Calcium, 0.4% Phosphorous, 0.5 IU/g Vitamin D.

**GLP statement:** **Included.**

**Animals:** 40 purebred beagle dogs

| Group: | | Dose (ug/kg): | | # of Animals: |
|---|---|---|---|---|
| | 0 | | 0 | 5 males + 5 Females |
| | 1 | Paracalcin | 0.02 | 5 males + 5 Females |
| | 2 | Paracalcin | 0.06 | 5 Males + 5 Females |
| | 3 | Paracalcin | 0.2 | 5 males + 5 Females |

**Dose Selection:**

A 1-month toxicity trial in dogs resulted in significant toxicity at doses of 0.3 ug/kg and higher. In the 3-month study, dogs were fed a diet containing minimal calcium phosphorus and Vitamin D in order to reduce the effect of hypercalcemia and simulate the clinical setting. 0.3 ug/kg was selected as the highest dose and again resulted in significant toxicity (weight loss, calcification) resulting in the death and early termination of dogs in the group. The high dose of Paracalcin was lowered to 0.2 ug/kg for this study. This dose is **below** the maximum recommended dose of Paracalcin in humans on a mg/kg basis. The exposure in these dogs is even lower when compared to the human exposure on a $mg/m^2$ basis.

## RESULTS:

### OBSERVED EFFECTS:

Emaciation and dehydration became significant in the HD (0.2 ug/kg) Paracalcin group beginning in the third month and corresponded to a decrease in food consumption (see table below).

No drug related observed effects were noted in the MD and LD groups but mild injection site reactions were noted in all groups (including controls).



### MORTALITY:

No spontaneous deaths were reported. Four HD (0.2 ug/kg) dogs were terminated early due to extreme (>30%) weight loss, on days 97, 125, 139, and 157 respectively.



### BODY WEIGHT:

Significant (~30%) reductions in weight were noted in the HD group during the third month and continuing until the end of the study. No significant body weight differences from control animals was noted in the MD and LD (0.06 and 0.02 ug/kg) groups.

**Body Weights and Food Consumption:**

| Group: | C | | 0.02 Paracalcin | | 0.06 Paracalcin | | 0.2 Paracalcin | |
|---|---|---|---|---|---|---|---|---|
| **Sex:** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| **Weight (Kg) day 182** | 13.9 | 11.3 | 13.5 | 11.4 | 12.5 | 12.3 | **8.5*** | **8.6*** |
| **Food Consumed (g) d 123** | 398 | 267 | 306 | 338 | 287 | 321 | **20*** | **66*** |

* = P<0.05. n=5 except HD = 3M, 1F.



### FOOD CONSUMPTION:

The weight loss changes above corresponded with changes in food consumption (also shown above for correlation). The HD Paracalcin groups had dramatically and statistically significantly reduced food consumption in the third fourth and fifth month. No other significant differences in food consumption were noted.

### EYE EXAMINATION:

No findings were evident.



### HEMATOLOGY and COAGULATION:

Toxicological effects on hematology were confined to the HD group, were minimal in importance and are probably secondary to extreme weight loss although direct effects of the vitamin D analog cannot be ruled out. These dose dependent effects were; slightly reduced lymphocyte, neutrophil, and total leukocyte counts in males and females and decreased RBC (not anemia) in males and slightly prolonged APTT in females. Vitamin D analogs have been shown to affect differentiation of various blood cell lines. The effect on APTT may be a direct effect on production of some of the clotting factors in the liver. Very similar hematological findings were reported in other rat and dog toxicity studies.

### BLOOD CHEMISTRY:

No treatment related effects except (the expected pharmacological actions) decreased PTH levels and hypercalcemia (greater than 13 mg/dl) noted in all HD drug treated animal groups, and corresponding hypercalcemia induced increased $CO_2$ and BUN. The hypercalcemia tended to be worse in males.

HD and MD groups had PTH levels lower than controls but this effect was less marked in the LD group. The HD group also had numerous other slight and physiologically unimportant changes in blood chemistry related to hypercalcemia and weight loss.

| Blood levels on day 178: (M/F) | | | | |
|---|---|---|---|---|
| Dose: (ug/kg) | **Calcium: (mg/dl)** | **$TCO_2$ (mmol/L)** | **BUN: (mg/dl)** | **PTH: (pg/dl)** |
| 0 | 11.1/9.5 | 24.6/24 | 14.0/11.8 | 14/16 |
| 0.02 | 10.8/9.7 | 25.1/25 | 16.2/17.8 | 29/16 |
| 0.06 | 11.8/10.9 | 25.0/26 | 18.8/18.2 | **3*/3*** |
| 0.2 | **16.4*/13.6*** | **29.4*/28*** | **29.5*/29.0*** | **1*/1*** |

*=P<0.05, n=2-5.

**URINALYSIS:**

The hypercalcemia lead to slightly increased fractional calcium excretion which reached a statistically significant level only in the MD females and was not of physiological importance.

**ORGAN WEIGHTS:**

In the HD (0.2 ug/kg) group decreases in absolute organ weights and increases in relative organ weights were all clearly related to weight loss and not pathology of any particular organ. No other significant changes in organ weights were noted.

**GROSS and HISTOPATHOLOGY:**

Numerous pathological findings were noted in the HD Paracalcin group. These findings were minimal-mild. When these findings were noted in the MD Paracalcin group they were more likely to be minimal in addition to being of much lower incidence.

| Incidence of Pathological findings: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Group (10/group)** | **C** | | **0.1 Paracalcin** | | **0.5 Paracalcin** | | **3.0 Paracalcin** | |
| **Sex (5/sex)** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| Kidney, discoloration | | | | | 1 | | 4 | 2 |
| Kidney, nephrosis | | | | | 2 | 1 | 5 | 5 |
| Kidney, Glomerular atrophy | | | | | 1 | | 4 | 4 |
| Aorta, mineralization | | | | | | | 1 | 2 |
| Aorta, plaque | | | | | | | 1 | 3 |
| Parathyroid, atrophy | | | | | | | 3 | 2 |

## SUMMARY TABLE

(of all statistically significant findings. Toxicologically meaningful effects are in bold. See main text for details.)

| **Effect/dose (ug/kg)** | **0.02 Paracalcin** | **0.06 Paracalcin** | **0.2 Paracalcin** |
|---|---|---|---|
| **Early terminations:** | 0 | 0 | **4/10** |
| **CLINICAL SIGNS** | 0 | 0 | **⇓Food Consumption**<br>**⇓Body Weight** |
| **HEMATOLOGY:** | 0 | 0 | ⇑APTT<br>⇓Lymphocytes & RBC |
| **BLOOD CHEMISTRY:** | 0 | **⇓PTH** | **⇓PTH, ⇑Ca, ⇑$TCO_2$, ⇑BUN** |
| **HISTO-PATHOLOGY:** | **See table above.** | | |

## SUMMARY and DISCUSSION:

At 0.2 ug/kg (HD) 3 times per week, Paracalcin caused hypercalcemia and decreased levels of PTH. Dogs appear to be extremely sensitive to hypercalcemia, responding with dramatically decreased food consumption and weight loss. In 4 HD animals the weight loss was so severe that they were terminated early. This hypercalcemia also resulted in mineralization of kidneys and the aorta (seen in previous studies and in rats) demonstrating the dangers of hypercalcemia. The effects of hypercalcemia on the kidney also resulted in some mild nephropathy and mildly increased $TCO_2$ and BUN. Parathyroid atrophy may have been a direct effect of the drug or could have been secondary to the hypercalcemia. Reduced neutrophil and lymphocyte counts and slightly prolonged APTT was also noted.

At doses below 0.2 ug/kg there were no significant effects except to decrease levels of PTH and a few incidence of mild renal nephrosis (at 0.06 ug/kg).

## CONCLUSIONS:

0.02 ug of Paracalcin / kg, 3 times per week IV caused no toxic effects in dogs over six months and can be considered a NAEL.

0.06 ug of Paracalcin / kg, 3 times per week IV for six months caused some renal toxicity in dogs and can be considered a minimally toxic level.

0.2 ug of Paracalcin / kg, 3 times per week IV caused very excessive expected pharmacological activity (hypercalcemia and reduced PTH levels) The hypercalcemia induced extreme weight loss and premature euthanasia of 4/10 dogs, calcification of kidneys and aorta, and some evidence of nephropathy. Hypercalcemia is known to decrease the glomerular filtration rate. This resulted in some changes in serum chemistry (increased $CO_2$ and BUN). All of the toxic effects appear to result from the drug induced hypercalcemia.

# PHARMACOLOGY AND TOXICOLOGY REVIEW OF One Year Intravenous Toxicity Study of Paracalcin in Dogs Study # TB95-207

## PURPOSE:

To assess the toxicity of Paracalcin in dogs when administered IV, 3 times per week, for one year.

## EXPERIMENTAL DESIGN:



**Study #:** TB95-207,_____126-074

**Study Initiated:** 7/31/95.
**Study Completed:** 12/11/96.

**Dose & Formulation:** 0, 0.02, 0.06, & 0.2 ug/kg/day,
IV, 3 times/wk, in 20% EtOH, 30% Propylene Glycol / Water.

**Batch of drug:** lot 96-016-JE

**Food:** CERTIFIED CANINE DIET
0.5% Calcium, 0.4% Phosphorous, 0.5 IU/g Vitamin D.

**GLP statement:** **Included.**

**Animals:** 40 purebred beagle dogs

| Group: | | Dose (ug/kg): | | # of Animals: | |
|---|---|---|---|---|---|
| | 0 | | 0 | | 5 males + 5 Females |
| | 1 | Paracalcin | 0.02 | | 5 males + 5 Females |
| | 2 | Paracalcin | 0.06 | | 5 Males + 5 Females |
| | 3 | Paracalcin | 0.2 | | 5 males + 5 Females |



**Dose Selection:**

A 1-month toxicity trial in dogs resulted in significant toxicity at doses of 0.3 ug/kg and higher. In the 3-month study, dogs were fed a diet containing minimal calcium phosphorus and Vitamin D in order to reduce the effect of hypercalcemia and simulate the clinical setting. 0.3 ug/kg was selected as the highest dose and again resulted in significant toxicity (weight loss, calcification) resulting in the death and early termination of dogs in the group. The high dose of Paracalcin was lowered to 0.2 ug/kg for the 6-month study. This dose is **below** the maximum recommended dose of Paracalcin in humans (on a mg/kg basis). Despite the fact that 4/10 HD dogs were prematurely terminated in the 6-month study due to excessive weight loss, the 0.2 ug/kg HD was maintained for the 1-year study.

## RESULTS:

### OBSERVED EFFECTS:

Emaciation and dehydration became significant in the HD (0.2 ug/kg) Paracalcin group from the third month onward and corresponded to a decrease in food consumption. Lack of stools, shivering, hunched posture and decreased activity were also noted in the HD group.

No drug related observed effects were noted in the MD and LD groups but injection site reactions were noted in all groups (including controls).



### MORTALITY:

No spontaneous deaths were reported. Seven HD (0.2 ug/kg) dogs were terminated early due to extreme (>30%) weight loss, between days 64 and 306.



### BODY WEIGHT:

Significant reductions in weight were noted in the HD group from the third month and continuing until the end of the study. 3 M and 4 F were sacrificed early due to extreme weight loss. The HD values presented here reflect the weight of the 2 M and 1 F dogs surviving at the end of the study. Some weight loss was noted in the MD females in the last three months of the study. No significant body weight differences from control animals was noted in the MD and LD (0.06 and 0.02 ug/kg) groups.

Body Weights and Food Consumption:

| Group | C | | 0.02 Paracalcin | | 0.06 Paracalcin | | 0.2 Paracalcin | |
|---|---|---|---|---|---|---|---|---|
| Sex | M | F | M | F | M | F | M | F |
| Weight (Kg) day 363 | 12.2 | 11.7 | 12.7 | 12.4 | 12.8 | 11.2 | 7.9* | 6.8* |
| Avg. Food Cons. (g/kg/day) day 1-363 | 28.6 | 30 | 31.7 | 27 | 27.3 | 27.9 | 22.3[a] day 1-188 | 24.9[b] day 1-132 |

* = P<0.05. n=5 except HD = 2M, 1F. a = calculated from day 1-188, b = calculated from day 1-132.

### FOOD CONSUMPTION:

The weight loss changes shown above corresponded with reported observed changes in food consumption. The HD Paracalcin groups had dramatically reduced food consumption from the third month onward. Accurate measurements of the weight of food consumed could not be made, and could not be presented above, because the food was wetted to encourage consumption. No other significant differences in food consumption were noted.

### EYE EXAMINATION:

No findings were evident.



### HEMATOLOGY and COAGULATION:

Toxicological effects on hematology were confined to the HD group, were minimal in importance and are probably secondary to extreme weight loss, although direct effects of the vitamin D analog cannot be ruled out. These dose dependent effects were; slightly reduced lymphocyte, neutrophil, and total leukocyte counts in males and females and slightly decreased RBC in males and slightly prolonged APTT in females. Vitamin D analogs have been shown to affect differentiation of various blood cell lines. The effect on APTT may be a direct effect on production of some of the clotting factors in the liver. Very similar hematological findings were reported in other rat and dog toxicity studies.

### BLOOD CHEMISTRY:

No treatment related effects except (the expected pharmacological actions) decreased PTH levels and hypercalcemia (greater than 13 mg/dl) noted in all HD drug treated animal groups, and corresponding hypercalcemia induced increased BUN (there have been reports in the literature of hypercalcemia induced decreases in glomerular filtration resulting in increased BUN). The hypercalcemia tended to be worse in males.

HD and MD groups had PTH levels lower than controls but this effect was not significant in the LD group. The HD group also had decreased phosphorous and numerous other slight and physiologically unimportant changes in blood chemistry related to hypercalcemia and weight loss.

Blood levels on day 178: (M/F)

| Dose: (ug/kg) | Calcium: (mg/dl) | Phosphorous: | BUN: (mg/dl) | PTH: (pg/dl) |
|---|---|---|---|---|
| 0 | 9.7/9.6 | 4.5/4.1 | 12/13 | 32/37 |
| 0.02 | 9.8/9.5 | 4.9/4.5 | 12/13 | 27/31 |
| 0.06 | 10.6/10.0 | 5.5/4.7 | 15/14 | **2.7/3.5*** |
| 0.2 | **14.6*/14.2*** | 4.5/3.9 | **32*/20** | **1.2*/1.5*** |

*=P<0.05, n=2-5.



**URINALYSIS:**

The hypercalcemia lead to slightly increased fractional calcium excretion which reached a statistically significant level only in the HD males and MD females and was not of physiological importance. HD animals also tended to have slightly acidic urine.



**ORGAN WEIGHTS:**

In the HD (0.2 ug/kg) group decreases in absolute organ weights and increases in relative organ weights were all clearly related to weight loss and not pathology of any particular organ, except the increased relative adrenal gland weight which could be partially due to hypercalcemia induced adrenal hyperplasia. No other significant changes in organ weights were noted.

**GROSS and HISTOPATHOLOGY:**

Numerous pathological findings were noted in the HD Paracalcin group. These findings were mild-moderate. When these findings were noted in the MD Paracalcin group they were more likely to be mild in addition to being of much lower incidence.

Incidence of Pathological findings:

| Group (10/group) | C | | 0.1 Paracalcin | | 0.5 Paracalcin | | 3.0 Paracalcin | |
|---|---|---|---|---|---|---|---|---|
| Sex (5/sex) | M | F | M | F | M | F | M | F |
| Kidney, discoloration | | | | | | | 3 | 2 |
| Kidney, hyperplasia | | | | | | 1 | 4 | 4 |
| Kidney, tub. degeneration | | | | | 4 | 3 | 5 | 5 |
| Kidney, mineralization | | | | | 2 | | 4 | 5 |
| Pul. Artery Mineralization | | | | | | 1 | | |
| GI Mucosa Mineralization | | | | | | 1 | 1 | |
| Parathyroid, atrophy | | | | | | | 4 | 5 |



## SUMMARY TABLE

(of all statistically significant findings. Toxicologically meaningful effects are in bold. See main text for details.)

| Effect/dose (ug/kg) | 0.02 Paracalcin | 0.06 Paracalcin | 0.2 Paracalcin |
|---|---|---|---|
| **Early terminations:** | 0 | 0 | **7/10** |
| **CLINICAL SIGNS** | 0 | **⇓Body Weight (F)** | **⇓Food Consumption**<br>**⇓Body Weight** |
| **HEMATOLOGY:** | 0 | 0 | ⇑APTT<br>⇓Lymphocytes & RBC |
| **BLOOD CHEMISTRY:** | 0 | **⇓PTH, ⇑Ca** | **⇓PTH, ⇑Ca, ⇑$PO_4$, ⇑BUN** |
| **HISTO-PATHOLOGY:** | **See table above.** | | |

## SUMMARY and DISCUSSION:

At 0.2 ug/kg (HD) 3 times per week, Paracalcin caused hypercalcemia and decreased levels of PTH. Dogs appear to be extremely sensitive to hypercalcemia, responding with dramatically decreased food consumption and weight loss. In 7 HD animals the weight loss was so severe that they were terminated early. This hypercalcemia also resulted in mineralization of kidneys (seen in previous studies and in rats) demonstrating the dangers of even mild hypercalcemia one female had mineralization of the pulmonary artery and 1 male had mineralization of the gastric mucosa. The effects of hypercalcemia on the kidney also resulted in increased aciduria and BUN. Parathyroid atrophy may have been a direct effect of the drug or could have been secondary to the hypercalcemia.

At 0.02 ug Paracalcin/kg 3 times per week for a year, there were no significant effects at all.



## CONCLUSIONS:

0.02 ug of Paracalcin / kg, 3 times per week IV for a year caused no effects in dogs over a year and can be considered a NOEL.

0.06 ug of Paracalcin / kg, 3 times per week IV caused some renal toxicity in dogs and can be considered a minimally toxic level.

0.2 ug of Paracalcin / kg, 3 times per week IV caused very excessive expected pharmacological activity resulting in extreme weight loss and premature euthanasia of 7/10 dogs. All of the effects appear to result from the drug induced hypercalcemia.

# PHARMACOLOGY AND TOXICOLOGY REVIEW
## Reviewers Overall Summary and Conclusions of Toxicity Studies:

### SUMMARY and DISCUSSION:

In the acute IV studies conducted in rats and mice (reviewed previously by G.K.) no adverse effects were detected up to doses of 16 ug/kg.

In long term IV studies conducted in dogs and rats the typical spectrum of toxicities began to appear in rats at doses of 3 ug/kg. Dogs were much more sensitive and demonstrated significant toxicity at 0.6 ug/kg. These toxicities result directly or secondarily from the expected pharmacodynamic activity of a Vitamin D; decreases in serum PTH, hypercalcemia, and mineralization of soft tissues, especially kidneys, aorta, GI tissues and heart. Dogs and rats that became hypercalcemic showed hypercalcemia induced weight loss. Dogs also had increased levels of liver enzymes in some studies but these changes were not large enough to be physiologically important. 6-month and 1-year studies in dogs uncovered some longer term effects of Paracalcin. Parathyroid atrophy was noted, but this would be considered a therapeutic effect in patients. Other effects; aciduria, increased BUN, renal nephropathy and anemia, were secondary to long term hypercalcemia and weight loss.

In 6-month studies the rat NOAEL was 0.1 ug/kg/dose. In dogs the NOAEL was 0.02 ug/kg/dose in 6-month and 1-year studies. These doses are **lower** (on a mg/kg basis) than the highest recommended human dose (0.24 ug/kg). In other words, the highest recommended human dose would have toxic effects (on a mg/kg basis) in rats and dogs over six months. This is acceptable because human patients will be hypocalcemic, with renal failure, and will be less sensitive to the toxic effects of this drug, namely hypoparathyroidism and hypercalcemia due to increased calcium absorption in the gut and reabsorption in kidneys. The patients will need to be monitored for hypercalcemia since this is the expected and most sensitive clinical indicator of toxicity. In addition dogs seem to be extraordinarily sensitive to the hypercalcemic effects of Vitamin D.

| Comparison of NOAEL across species: | ug/kg | ug/m$^2$ |
|---|---|---|
| NOAEL in 6-Mo Rat Study | 0.10 ug/kg | 0.6 ug/m$^2$ |
| NOAEL in 6-Mo Dog Study | 0.06 ug/kg | 1.2 ug/m$^2$ |
| NOAEL in 12-Mo Dog Study | 0.02 ug/kg | 0.4 ug/m$^2$ |
| Maximum Recommended Human Dose: | 0.24 ug/kg | 8.9 ug/m$^2$ |

In all three studies the lowest effective dose (suppressed PTH levels - the desired clinical endpoint) also caused hypercalcemia. This clearly demonstrates that there is little "margin of safety" between the dose needed for efficacy (suppression of PTH) and the dose where toxicity (hypercalcemia) develops in healthy animals. This emphasizes the need to titrate the dose carefully to achieve efficacy without hypercalcemia. None of these experiments was designed to (or fortuitously demonstrated) a "therapeutic index" for this drug or compared the "margin of safety" for this drug to any other drug's "margin of safety" However, this question was addressed in a clinical trial.

None of the toxicity studies examined bone histology specifically. There were no significant reports of adverse effects on bones in any of the studies.



### CONCLUSIONS:

- 0.02 ug of Paracalcin / kg, 3 times per week IV can be considered a NOAEL in dogs for one year.
- 0.1 ug of Paracalcin / kg, 3 times per week IV can be considered a NOAEL in rats for six months.
- All of the significant toxicities seen in these experiments were secondary to hypercalcemia.
- The toxic effects of hypercalcemia can be quite severe over time, including; nephropathy, testicular degeneration ,soft tissue mineralization (including heart and aorta), weight loss and death.
- The hypercalcemic effect of Paracalcin is known to occur at slightly higher doses than required to suppress PTH levels. Serum calcium levels should be carefully monitored to obtain a therapeutic dose while avoiding hypercalcemia.

# PHARMACOLOGY AND TOXICOLOGY REVIEW

## Ames Test of 19-nor 1 alpha, 25 dihydroxyvitamin $D_2$ Bacterial reverse mutation assay. (TX 94-310)

**PURPOSE:**

To examine the mutagenic potential of Paracalcin in the "Ames Assay"; to induce reverse mutations in TA1535, TA1537, TA100, TA98, and WP2uvrA-, with and without metabolic activation with Aroclor induced rat liver microsomes (S-9).



**EXPERIMENTAL DESIGN:**

| | |
|---|---|
| **Testing Facility:** | Abbott Laboratories, No address given. |
| **Date of experiment:** | 2/95 |
| **Study Report Written:** | 8/1/95 |
| **GLP statement, Q/A:** | **GLP statement included,** |
| **Dose & Formulation:** | 1,3,10,30,100,300,1000,3000 ug/plate.<br>Lot 96-016-JE<br>Dissolved in DMSO. |
| **Dose Selection:** | No rationale provided. Top dose is beyond limit of solubility. |

**RESULTS:**

The maximum concentration tested was determined by the solubility of the compound. There was some precipitation of the drug at the highest concentration tested. Paracalcin did not induce any increase in the number of revertant colony counts in the presence or absence of S-9. Negative and positive controls assured that the test was valid. The results constitute a negative result for the in vitro mutagenicity test in the bacterial/microsomal activation assay.

**CONCLUSIONS:**

**The Ames assay does not indicate a mutagenic potential for Paracalcin.**

# PHARMACOLOGY AND TOXICOLOGY REVIEW

## L5178Y/TK $^{+/-}$ Mouse Lymphoma Mutagenesis Assay of 19-nor 1-alpha, 25-dihydroxyvitamin $D_2$. (TX 95-202)



**PURPOSE:**

To examine the mutagenic potential of Paracalcin at the TK locus by using the Mouse Lymphoma mutagenesis assay with and without activation with Aroclor induced S-9.



**EXPERIMENTAL DESIGN:**

| | |
|---|---|
| **Testing Facility:** | |
| **Date of experiment:** | 8/17/95 |
| **Study Report Written:** | 11/27/95 |
| **GLP statement, Q/A:** | **GLP statement included,** |
| **Dose & Formulation:** | 6.3, 12.5, 25, 50, 75, 100, 200 ug unactivated. (no toxicity in any sample)<br>6.3, 12.5, 25, 50, 100, 200, 500, 1000 ug unactivated (Some toxicity observed above 100 ug)<br>1.6, 3.1, 6.3, 12.5, 25, 50, 75, 100 ug activated (>50% toxicity at 75 & 100 ug).<br>Lot 96-016-JE<br>Dissolved in DMSO. |
| **Dose Selection:** | No rationale provided. After an initial test to determine which concentration gave > 50% toxicity (100 ug with activation), the maximum dose was set at 200 ug. |



**RESULTS:**

Paracalcin did not induce any increase in the number of mutant colony counts in the presence or absence of S-9. Negative and positive controls assured that the test was valid. The results constitute a negative result for the Mouse Lymphoma Mutagenesis assay for mutagenicity. No attempt was made to size the colonies in order to evaluate clastogenic potential of the test compound.



**CONCLUSIONS:**

**The Mouse Lymphoma assay does not indicate a mutagenic potential for Paracalcin. The potential of this assay to identify clastogens (by sizing colonies) was not exploited.**

# PHARMACOLOGY AND TOXICOLOGY REVIEW

## Chemical Induction of Chromosome Aberration in Cultured Human Lymphocytes with and without Metabolic Activation TX 94-309.

### Purpose:

To assess the potential for Paracalcin to induce chromosome aberrations in cultured human peripheral blood lymphocytes, with and without exogenous activation by Aroclor-induced rat liver post mitochondrial supernatant (S9 fraction).

### Experimental design:

| | | |
|---|---|---|
| **Testing Facility:** | | |
| **Study #:** | 94-309 | |
| **Study Initiated:** | 3/23/95 | |
| **Study Completed:** | 6/7/95 | |
| **GLP statement** | Included. | |
| **Dose & Formulation:** | 0.1,0.3,1,3,10,30,60,100 ug/ml (+/- act.) | |
| | 0.1, 1, 10 ug/ml - act were scored. | |
| | 30, 60 100 ug/ml + act. were scored. | |
| | Dissolved in DMSO. | |
| **Batch of drug:** | lot 96-016-JE | |
| **Conditions:** | **- Activation** | RPMI-1640 + 10% FBS |
| | **+ Activation** | RPMI-1640 + S-9 |

Lymphocytes were grown for 48 h in culture medium. Medium was changed to + or - activation medium containing Paracalcin for 4 or 22 h respectively and then replaced with fresh culture medium for the remaining 20 or 2 h respectively. Cultures were then grown for 2 more h with 0.1 ug/ml

### Results:

Without activation toxicity (>50% decrease in mitotic index) was seen at concentrations 10 ug/ml and higher. With activation (>50%) inhibition of mitosis was seen at 100 ug/ml.

In the presence and absence of metabolic activation, no significant increase in frequency of chromosomal aberrations was observed at any concentration of Paracalcin.

### Conclusions:

The Human Lymphocyte assay indicates that Paracalcin does not induce chromosomal damage in the presence or absence of metabolic activation with S-9.

# PHARMACOLOGY AND TOXICOLOGY REVIEW

## Mouse Micronucleus Test, *In Vivo*. TD 95-077

### Purpose:

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

### Experimental design:

| | |
|---|---|
| **Testing Facility:** | Abbott Laboratories |
| **Report #:** | TD 95-077 |
| **Study Initiated:** | May, 1995. |
| **Study Completed:** | 7/13/95 |
| **Dose & Formulation:** | 20, 40, 60 ug/kg IV in 20% EtOH, 30% propylene glycol. |
| **Batch of drug:** | lot not specified??? |
| **Mice:** | male Crl:CD-1 (ICR) BR mice<br>5/group. |
| **GLP statement** | Included. |

The doses were selected based on the appearance of (vehicle related, unspecified) toxicity at higher doses. Animals were killed 30 hours after the second daily i.v. bolus and bone marrow was harvested from the femur. The positive control substance was Cyclophosphamide, 40 mg/kg oral. The negative control was vehicle.

### Results:

None of the groups treated with Paracalcin had significantly ($P < 0.05$, Chi-Square-Test) more micronucleated polychromatic erythrocytes (PCEs). There was a highly significant increase in the number of micronucleated PCEs in the positive control samples.

### Conclusions:

Paracalcin was not clastogenic *in vivo* in the rat micronucleus assay under the conditions described.

# PHARMACOLOGY AND TOXICOLOGY REVIEW

## MTD studies for CAC:
## (Reviewed previously by GK).

**Three Month S.C. MTD in Rats:**
Study # TA94-370, 1995.
Lot # 95-0135.
3X per week; 0, 0.1, 0.5, 3.0 ug/kg.

Major findings:
- Drug related effects stronger and more common in males. Of these males, those with some symptoms tended to have many symptoms while others had none.
- Ocular opacity in 3 HD males
- Ca and P increased (dose dependent) in all drug groups.
- PTH decreased (dose dependent) in all groups.
- Urinary Ca increased (dd) in all groups.
- Hyperostotis (6/9 m, 1 f) in HD group.
- Mineralization of Heart, Kidney, Lung ... in HD and some MD rats.

**Three Month S.C. MTD in Mice:**
Study # TA94-381, 1995.
Lot # 95-0135.
3X per week; 0, 0.1, 0.5, 3.0 10 ug/kg.

Major findings:
- Moderate dd decrease in WBC count in MHD and HD m and all f dose groups.
- Ca (dd) and P (all) increased
- PTH decreased from ~10 to 1 pg/ml in all groups at the end of the study.
- Inflammation (m 0-1-1-5-5) (f 0-1-1-0-1) and mineralization (m 0-1-1-5-8) (f 0-2-0-1-1) of the kidney.

**CAC Recommendations:**

The CAC met on March 19 1996 and concurred with the suggested doses:
0.15, 0.5 1.5 ug/kg, S.C., 3X/wk in rats and
1,3, and 10 ug/kg, S.C., 3X/wk in mice.

**Current Status:**

Rat and mouse 2-year carcinogenicity studies are under way. NDA will be reviewed prior to finding the outcome of the studies.

Review of CAC report is attached as an appendix.