

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-087

PHARMACOLOGY REVIEW



**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-087
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	27-DEC-07
PRODUCT:	Silks Ointment
INTENDED CLINICAL POPULATION:	Patients with psoriasis
SPONSOR:	Galderma Laboratories, L.P.
DOCUMENTS REVIEWED:	All
REVIEW DIVISION:	Division of Dermatologic and Dental Drug Products (HFD-540)
PHARM/TOX REVIEWER:	Norman A. See, Ph.D.
PHARM/TOX SUPERVISOR:	Barbara Hill, Ph.D.
DIVISION DIRECTOR:	Susan Walker, M.D.
PROJECT MANAGER:	Margo Owens

Date of review submission to Division File System (DFS): 30-JUN-2008

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	6
2.6.1 INTRODUCTION AND DRUG HISTORY	6
2.6.2 PHARMACOLOGY	9
2.6.2.1 Brief summary	9
2.6.2.2 Primary pharmacodynamics	9
2.6.2.3 Secondary pharmacodynamics	10
2.6.2.4 Safety pharmacology	10
2.6.2.5 Pharmacodynamic drug interactions	10
2.6.3 PHARMACOLOGY TABULATED SUMMARY	10
2.6.4 PHARMACOKINETICS/TOXICOKINETICS	10
2.6.4.1 Brief summary	10
2.6.4.2 Methods of Analysis	11
2.6.4.3 Absorption	11
2.6.4.4 Distribution	11
2.6.4.5 Metabolism	11
2.6.4.6 Excretion	11
2.6.4.7 Pharmacokinetic drug interactions	11
2.6.4.8 Other Pharmacokinetic Studies	11
2.6.4.9 Discussion and Conclusions	11
2.6.4.10 Tables and figures to include comparative TK summary	13
2.6.5 PHARMACOKINETICS TABULATED SUMMARY	13
2.6.6 TOXICOLOGY	14
2.6.6.1 Overall toxicology summary	14
2.6.6.2 Single-dose toxicity	17
2.6.6.3 Repeat-dose toxicity	17
2.6.6.4 Genetic toxicology	46
2.6.6.5 Carcinogenicity	48
2.6.6.6 Reproductive and developmental toxicology	72
2.6.6.7 Local tolerance	83
2.6.6.8 Special toxicology studies	84
2.6.6.9 Discussion and Conclusions	84
2.6.6.10 Tables and Figures	89
2.6.7 TOXICOLOGY TABULATED SUMMARY	89
OVERALL CONCLUSIONS AND RECOMMENDATIONS	89
APPENDIX/ATTACHMENTS	92

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: The product is approvable with respect to nonclinical concerns.

B. Recommendation for nonclinical studies: None.

C. Recommendations on labeling: It is recommended that section 8.1 (Pregnancy) and section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility) of the label be modified to the statements indicated below:

b(5)

b(4)

b(4)

II. Summary of nonclinical findings

The primary sign of toxicity observed in studies that involved repeated administrations of calcitriol was perturbation of calcium homeostasis, including elevated concentrations of calcium in the serum and urine, microscopic evidence of stimulation of bone formation, and mineralization of the kidney. However, little transdermal absorption of calcitriol occurs, and if treated animals are prevented from ingesting the applied material then little systemic exposure occurs and consequently little or no toxicity is observed. In a nine-month topical study in which minipigs were treated with calcitriol ointment six hours per day, under a dressing, and the residual material removed at the end of the treatment period to prevent ingestion, little toxicity was observed.

Calcitriol is an endogenous compound, and as such, extensive evaluation for genetic toxicity was judged to be unnecessary. However, calcitriol was evaluated in the mouse lymphoma TK locus assay, and was confirmed to be non-mutagenic.

Calcitriol was evaluated in a two-year carcinogenicity study in which it was orally administered to rats. The incidence of benign pheochromocytomas was significantly increased in female rats. No other significant differences in tumor incidence were observed.

A two-year topical carcinogenicity study was conducted in which calcitriol ointment was applied to the skin of mice. No statistically significant differences in tumor incidence were observed in this study.

Calcitriol ointment was evaluated for activity as a cocarcinogen with UV light in a 12-month study with hairless mice. The vehicle for the test material was identical to the vehicle of the clinical formulation of Silkis ointment. The median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed was slightly, but statistically significantly, reduced for both males and females that were treated with the vehicle of the product, relative to untreated animals. Materials that contained calcitriol did not reduce latency to formation of the first tumor greater than or equal to 1.0 mm in diameter relative to vehicle alone. These data suggest that the vehicle of calcitriol ointment slightly enhances UV-induced skin tumor formation (possibly by enhancing UV penetration into the skin), but calcitriol per se does not enhance photo-induced carcinogenesis.

Calcitriol was evaluated for effects upon reproduction. Calcitriol had no effects on fertility of male or female rats. Calcitriol exhibited no developmental toxicity when orally administered to pregnant rats. When topically applied to pregnant rabbits, calcitriol induced fetal toxicity which included elevated mean post-implantation loss and an increased incidence of minor skeletal abnormalities due to retarded ossification of the pubic bones. A slightly increased incidence of skeletal variations was also observed (extra 13th rib, reduced ossification of epiphyses). These effects were likely secondary to maternal toxicity. No effects were observed on the incidence of major fetal abnormalities, or of minor external or visceral abnormalities. When assessed for effects on peri-natal or post-natal development, calcitriol had no remarkable effects on any parameter, including survival, behavior, body weight, litter parameters, or the ability of female rats to nurse or rear pups.

In a series of nonclinical and clinical studies, Silkis ointment was found to be essentially non-irritating to the skin or eyes, non-sensitizing, non-phototoxic, and non-photosensitizing.

Appears This Way
On Original

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-087

Review number: 1

Sequence number/date/type of submission: N-000/21-DEC-2007

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Galderma Laboratories, LP

Manufacturer for drug substance: _____

b(4)

Reviewer name: Norman A. Sec, Ph.D.

Division name: Division of Dermatologic and Dental Products

HFD #: 540

Review completion date: 25-JUN-2008

Drug:

Trade name: Silkis ointment

Generic name: Calcitriol

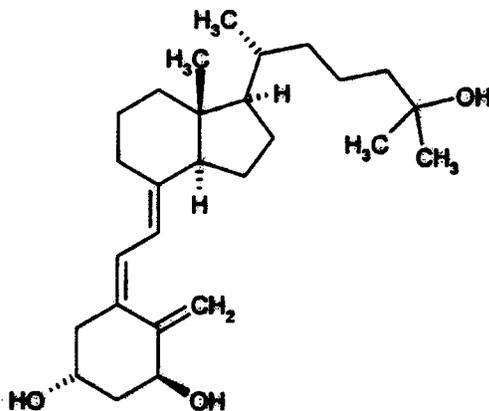
Code name: 1,25-dihydroxycholecalciferol; 1,25-dihydroxyvitamin D₃; 1,25-DHCC

Chemical name: (1 α ,3 β ,5Z,7E)-9,10-Secosteroid-5,7,10(19)-triene-1,3,25-triol

CAS registry number: 32222-06-3

Molecular formula/molecular weight: C₂₇H₄₄O₃/416.64.

Structure:



Relevant INDs/NDAs/DMFs: NDA 18-044 (Rocaltrol capsules, Roche, approved 17-AUG-1978); NDA 18-874 (Calcijex, Abbott Labs., approved 25-SEP-1986); NDA 21-068 (Rocaltrol Oral Solution, Roche, approved (20-NOV-1998). Note: These applications are mentioned for the sake of completeness only; NDA 22-087 does not reference these applications. NDA 22-087 was developed under IND 62,151.

Drug class: Vitamin D analog

Intended clinical population: Patients with plaque-type psoriasis vulgaris

Clinical formulation (topical ointment):

<u>Compound</u>	<u>Amount (percent w/w)</u>
Calcitriol.....	0.0003
White soft paraffin.....	b(4)
Liquid paraffin.....	b(4)
α-Tocopherol.....	b(4)

Notes: "white soft paraffin" is a synonym for white petrolatum. "Liquid paraffin" is a synonym for mineral oil. "α-Tocopherol" is a member of the "vitamin E" family of compounds, and is added to the product as _____ 0.0003 % w/w is equivalent to 3 µg/g and 3 ppm.

b(4)

Route of administration: Topical to the skin. The proposed use of the product (application to areas of the skin that are affected by psoriasis) may involve application to a large percentage of the body surface area. The material would be applied twice daily for an indefinite period, resulting in chronic exposure to the product. The label states that not more than _____ of product should be applied per day to a given patient.

b(4)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless otherwise indicated.

Studies reviewed within this submission: Some of the studies which support this NDA were reviewed under review formats that were in use at the time the data were originally submitted. Reviews of those studies are included in this NDA in the format under which those studies were originally reviewed and signed off.

Repeat-Dose Toxicology:

1. Subchronic 28-day dermal toxicity with calcitriol vaseline by daily administration (5 days in 7) in the rat, study No. H.141.408.
2. Calcitriol - 13 week dermal preliminary toxicity study in the mouse. Study No. 913/080. Sponsor reference No. RDS.03.SRE.12242.

3. Calcitriol - 13 week oral (gavage) dose range finding toxicity study in the rat. Study No. 913/093. Sponsor reference No. RDS.03.SRE.12336.
4. Thirteen-week topical range-finding study of calcitriol in hairless mice, with or without simulated sunlight. Study No. 1207-017. Sponsor reference No. RDS.03.SRE.12332.
5. Calcitriol ointment - A 13 week dermal toxicity study in minipigs. Study No. 38094. Sponsor reference No. RDS.03.SRE.12241.
6. Calcitriol ointment (Silkis) - 13 week toxicity study by cutaneous administration in the beagle dog. Study No. 913/097. Sponsor reference No. RDS.03.SRE.12297.
7. Calcitriol ointment - A 26-week dermal study in rats. Study No. 51981. Sponsor reference No. RDS.03.SRE.12388.
8. Calcitriol ointment - A 9 month dermal toxicity study in minipigs. Study No. 51104. Sponsor reference No. RDS.03.SRE.12394.

Genetic Toxicology:

1. Calcitriol: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre fluctuation technique. Study No. 1867/30.

Carcinogenicity:

1. 12-month topical study to determine the influence of calcitriol on photocarcinogenesis in hairless mice. Study No. 1207-018. Sponsor reference No. RDS.03.SRE.12333.
2. Calcitriol ointment - 104 week dermal carcinogenicity study in the mouse. Study No. 913/118. Sponsor reference No. RDS.03.SRE.12299.
3. Calcitriol - 104 week oral (gavage) carcinogenicity study in the rat. Study No. 913/119. Sponsor reference No. RDS.03.SRE.12318.

Reproductive Toxicology:

1. Calcitriol - Fertility study by the oral route (gavage) in the rat (Segment I). Study No. 913/124. Sponsor reference No. RDS.03.SRE.12400.
2. Calcitriol - Embryo toxicity study by the oral route (gavage) in the rat (Segment II) with systemic exposure evaluation. Study No. 913/123. Sponsor reference No. RDS.03.SPR.12319.
3. Dermal (occluded) rabbit developmental toxicity study, study No. H.141.418.

4. Calcitriol - Pre- and post-natal development study by the oral route (gavage) in the rat (Segment III). Study No. 913/125. Sponsor reference No. RDS.03.SRE.12393.

Local Tolerance Studies:

Primary irritation study of calcitriol vaseline to the eye of male rabbits, study No. H.141.407.

Special Toxicology Studies:

1. Sensitization study with calcitriol in guinea pigs (maximization test). Study No. H.141.405.

Studies not reviewed within this submission: The submission contained a number of photocopies of journal articles that were not specifically summarized in this review because they were judged to add no useful information to the database that was captured in the review. In addition, some studies were not reviewed because they were judged to be inferior to the studies that were reviewed (listed above), and to add nothing of consequence to the database (they were primarily pilot, preliminary, or dose-ranging studies).

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Calcitriol is an agonist of the vitamin D receptor. After binding, the receptor-ligand complex influences the activity of vitamin D-responsive genes, thereby altering protein synthesis. The pharmacologic effect of interest in the treatment of psoriasis is an inhibition of keratinocyte differentiation and proliferation within psoriatic lesions. The precise mechanism through which calcitriol effects keratinocyte differentiation and proliferation is unclear. Vitamin D receptor agonists are also involved in modulation of calcium metabolism, including stimulation of bone formation, and induce synthesis of intestinal calcium transport proteins.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Calcitriol binds to vitamin D receptors and the receptor-ligand complex modulates the activity of certain genes, leading to inhibition of keratinocyte differentiation and proliferation within psoriatic lesions. The precise mechanism through which calcitriol affects keratinocyte differentiation and proliferation is unknown.

Drug activity related to proposed indication: Calcitriol inhibits proliferation of keratinocytes within psoriatic lesions, resulting in reduced skin cell turn over.

2.6.2.3 Secondary pharmacodynamics

Vitamin D receptor agonists, such as calcitriol, are involved in modulation of calcium metabolism, and induce synthesis of intestinal calcium transport proteins. The net effect is to increase levels of calcium within the body.

2.6.2.4 Safety pharmacology

Neurological effects: None known that are relevant to the proposed clinical use.

Cardiovascular effects: None known that are relevant to the proposed clinical use, although calcitriol at high systemic levels is capable of altering cardiovascular function through modulation of calcium metabolism.

Pulmonary effects: None known that are relevant to the proposed clinical use.

Renal effects: None known that are relevant to the proposed clinical use.

Gastrointestinal effects: None known that are relevant to the proposed clinical use, although calcitriol at high systemic levels is capable of enhancing intestinal absorption of calcium through induction of intestinal calcium transport proteins.

Abuse liability: None known.

Other: None

2.6.2.5 Pharmacodynamic drug interactions

None known that are relevant to the proposed clinical use.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not available.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Calcitriol is an endogenous compound. In the body, synthesis of calcitriol begins with conversion of 7-dehydrocholesterol to vitamin D₃ (cholecalciferol) in the skin, a reaction that is dependent upon energy from UV light which strikes the skin. Cholecalciferol is metabolized in the liver to form 25-hydroxy-D₃ (calcifediol), which is further metabolized in mitochondria of the kidneys to yield 1,25-dihydroxy-D₃ (calcitriol).

2.6.4.2 Methods of Analysis

Please see Clinical Pharmacology and Biopharmaceutics reviews of NDA 22-087 for a description of methods by which systemic exposure to calcitriol have been assessed in humans. Some of the nonclinical studies included use of radiolabeled calcitriol (^3H -calcitriol), systemic levels of which were measured through use of a liquid scintillation counter.

2.6.4.3 Absorption

When topically applied, the percentage of calcitriol that is systemically absorbed in nonclinical studies is apparently low. Plasma levels of calcitriol were apparently close to, or below, the limit of detection in the majority of nonclinical studies that involved the clinically relevant route of administration, even though the detection limit for calcitriol in most of the studies was in the pg per mL range.

2.6.4.4 Distribution

Calcitriol is highly (approximately 99.9%) bound in blood, and is transported by an alpha-globulin vitamin D binding protein. Calcitriol is widely distributed throughout the body.

2.6.4.5 Metabolism

In man, the primary metabolic pathway for calcitriol involves a five-step conversion to calcitroic acid; the first step involves hydroxylation of the side-chain at the 24 position to form 1,24,25-trihydroxyvitamin D_3 . A second, minor, metabolic pathway begins with hydroxylation of position 23 of calcitriol, eventually leading to calcitriol-26,23-lactone. Data concerning metabolism in non-humans is limited.

2.6.4.6 Excretion

The primary end metabolite of calcitriol, calcitroic acid, is largely excreted in bile, and enterohepatic circulation of calcitriol and its metabolites may occur.

2.6.4.7 Pharmacokinetic drug interactions

None known.

2.6.4.8 Other Pharmacokinetic Studies

Not applicable.

2.6.4.9 Discussion and Conclusions

Transdermal absorption of topically applied calcitriol is limited. Calcitriol is transported in blood bound to a specific vitamin D binding protein. Calcitriol is widely distributed throughout the body. Calcitriol is primarily metabolized to calcitroic acid. Some metabolism to calcitriol-26,23-lactone also occurs. Calcitriol and/or metabolites thereof are primarily excreted in the feces, and may undergo enterohepatic circulation.

The submission included information concerning a clinical study that was conducted in adult patients with psoriasis that applied Silkis ointment to evaluate pharmacokinetic parameters (study No. SRE.40005). That study is briefly summarized below (quoted from the submission, with slight paraphrasing in the interest of brevity). For definitive review of this information please see the appropriate Clinical Pharmacology and Biopharmaceutics review of NDA 22-087.

"Study SRE.40005 enrolled 23 male and female subjects with psoriasis, 18 years of age or older. During the treatment phase, each subject applied 15 grams of calcitriol ointment 3 µg/g (the to-be-marketed formulation of Silkis ointment) twice daily to 35% of their body surface area (BSA) for 3 weeks. This area included all skin lesions (at least 25% BSA was specified as an inclusion criterion at Baseline) and additional uninvolved skin, if necessary, to obtain 35% BSA treated. To ensure homogeneity in daily calcium intake, a 500 mg calcium tablet was taken daily for the duration of the study. Blood samples to determine calcitriol plasma levels and serum levels of calcium, phosphorus, albumin and creatinine were drawn on Days -8, -1, 0, and 21 (at 1, 2, 3, 4, 6, 9, 12, 16, and 24 hours) and on Day 14 (at 0, 1, 2, 3, 4, 6, 9, and 12 hours). The concentrations of calcitriol were determined in plasma using a radioimmunoassay method for total vitamin D₂/D₃. The limit of quantification was 5 pg/mL. The pharmacodynamic parameters (serum albumin adjusted calcium, serum phosphorus, urinary calcium and urinary phosphorus) were used for the assessment of any effects of the topical application of calcitriol ointment 3µg/g on calcium homeostasis. The duration of the study was based on expected steady state of calcitriol levels following two weeks of treatment with an elimination half-life of less than 10 hours. Therefore, plasma levels of calcitriol were measured after two weeks and three weeks of treatment in this study. The values of C_{12h} (trough concentration), C_{max}, T_{max}, and AUC_(0-12h) are provided in the table below (mean±SD):

Pharmacokinetic Parameters	Calcitriol Ointment 3µg/g N=23									
	Day -8		Day -1		Day 0		Day 14		Day 21	
C_{12h} (pg/mL)										
Mean SD	44.3	20.5	41.1	22.5	42.2	17.8	53.3	20.8	54.1	18.9
C_{max} (pg/mL)										
Mean SD	58.1	28.3	58.2	28.6	58.8	28.6	72.3	25.6	75.3	27.3
T_{max} (h)										
Mean SD	3.8	4.0	2.3	2.7	4.5	3.8	5.4	4.3	2.6	2.0
AUC_(0-12h) SD (pg·h/mL)										
Mean SD	538.9	270.2	519.7	271.5	544.5	231.7	604.8	282.4	725.6	281.0

Steady-state appears to have been achieved by Day 14 with no significant differences in PK parameters between Day 14 and Day 21. The mean values of C_{12h} , C_{max} , and $AUC_{(0-12h)}$ on Day 21 were (respectively) 27%, 32%, and 37% higher than the corresponding mean baseline values (mean values for Days -1 and -8)."

These data suggest that use of Silkis ointment by patients under the conditions which appear to approximate the maximum exposure that would be consistent with the proposed label of the product may result in a small but measurable increase in systemic exposure to calcitriol. The submission states that there were no clinically meaningful changes in albumin-adjusted calcium values, no statistically significant or clinically meaningful changes in the mean 24-hour urine calcium value over the course of this study, and that hypercalcemia was not observed at any time during the study.

A similar study (SRE.18102) that involves psoriasis patients under the age of 18 is currently ongoing. Preliminary results from that study, which include data from 11 subjects, aged 12-17 years, who applied calcitriol ointment 3 µg/g twice daily to between 10% and 35% of the BSA for 8 weeks, were included in the submission. No effects on plasma levels of calcitriol or levels of calcium or phosphorus were observed in this study. No serious adverse events were reported.

Several studies were conducted in which calcitriol ointment that had been spiked with 3H -calcitriol was applied to human skin. In one such study (H.141.605), a single 1 g dose of white petrolatum that contained 14.5 µg calcitriol and 45.8 µCi 3H -calcitriol (specific activity 3160 µCi/mg calcitriol) was applied over 300 cm² of the lower back of healthy male volunteers. The remaining ointment was removed 12 hours following application. Blood, urine, and fecal samples were obtained at various time points. Of the applied activity, 49% to 60% was removed from the skin at the 12 hour point. Approximately 4.4% and 8.5% of the activity was recovered in urine and feces, respectively.

Again, the reader is cautioned to consult the appropriate Clinical Pharmacology and Biopharmaceutics review for definitive information concerning the clinical pharmacokinetics associated with NDA 22-087.

2.6.4.10 Tables and figures to include comparative TK summary

Not available, since plasma levels in nonclinical studies were generally below the limit of detection of the assays that were validated for use with those species.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not available.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: The primary sign of toxicity observed in studies that involved repeated exposures to calcitriol was perturbation of calcium homeostasis, including elevated concentrations of calcium and phosphorus in the serum and urine, microscopic evidence of stimulation of bone formation, and mineralization of the kidney and other tissues. However, calcitriol is apparently not well absorbed through the skin, and if topically treated animals can be prevented from ingesting the applied material then little systemic exposure occurs and consequently little or no toxicity is observed.

Studies in mice and rats that involved 13 to 26 weeks of dosing with calcitriol via gavage or topical application to shaved skin indicated that, at sufficient dosage, toxicity representative of the pharmacodynamic effects of calcitriol was observed. These effects included effects on calcium and phosphorus metabolism, stimulation of bone growth, and mineralization/calcification of various tissues throughout the body. Severe perturbations of calcium metabolism can be incompatible with life, and in a number of the rodent studies reduced body weight gain and/or reduced survival was observed. It should be noted that rodents, while grooming themselves, lick from the skin and ingest materials that are topically applied. Therefore, systemic toxicity observed in topical rodent studies reflects systemic exposure that occurs via both the transdermal and the oral routes. Also, rodent species tend to be highly sensitive to the pharmacodynamic actions of vitamin D analogs (relative to nonrodents), even when plasma levels of the drug substance are below the limit of quantitation. For these reasons, it is often impossible to meaningfully compare dosages from rodent studies with vitamin D analogs to clinical dosages of topical vitamin D products. Topical nonclinical models that involve nonrodent species (discussed below) are much more reflective of clinically relevant effects.

Repeat-dose toxicology studies conducted with calcitriol in nonrodent species that have been submitted to NDA 22-087 include 13 week studies with minipigs and dogs, and a 9-month study with minipigs. Each of these studies included the proposed clinical formulation (3 ppm calcitriol in the same vehicle proposed for marketing), and involved dosing via the route proposed for clinical use (topical application to the skin). The studies with minipigs also included enriched formulations, that contained a higher concentration of calcitriol than does the proposed clinical formulation.

In a 13-week study with minipigs, formulations that contained 0 (vehicle control), 0.3, 1, 3, or 9 ppm calcitriol were applied daily to shaved skin, 6 hours per day under a non-occlusive dressing, at a rate of 2 mL/kg/day. Parameters monitored included clinical pathology and full histopathology. Essentially no toxicity was observed, although the mean weight of the kidney was significantly increased in animals of both genders in the high-dose group (9 ppm material). Under the conditions of this study ointment that contained 3 ppm calcitriol was a NOAEL, and 9 ppm ointment induced slight toxicity.

A similar study was conducted with beagle dogs that received daily topical applications of formulations that contained 0 (vehicle control), 0.3, 1, or 3 ppm calcitriol, applied to shaved skin for 13 weeks, 6 hours per day under a non-occlusive dressing. All the test materials were applied at a rate of 2 mL/kg/day; material that contained 3 ppm was also applied at a rate of 4 mL/kg/day (as a means of achieving an accelerated level of exposure in lieu of using an enriched formulation). Parameters monitored included clinical pathology and full histopathology. All animals survived to scheduled sacrifice, and there were no effects on general clinical signs or hematology. Treatment-related effects (reflective of the pharmacodynamics of calcitriol) were apparent primarily among animals at the two highest exposure levels (those that received calcitriol 3 ppm ointment at either 2 or 4 mL/kg/day), and included elevated levels of calcium in the serum and urine, slightly increased mean kidney weight (likely a reactive effect of increased excretion of calcium and phosphorus), and minimal to moderate mineralization of various tissues.

In a study in which minipigs received 9 consecutive months of daily topical applications of formulations that contained 0 (vehicle control), 1, 3, 9, or 15 ppm calcitriol, 6 hours per day under a non-occlusive dressing, at a rate of 2 mL/kg/day, the test materials were generally well tolerated. Signs of toxicity (primarily observed only in the highest dosage group) included reduced mean weight gain (females only), slightly reduced erythrocytic parameters (significant in females only), increased mean weight of the kidney, and histological evidence of inflammation of the kidneys (females at 9 ppm and 15 ppm and males at 15 ppm), hypertrophy of the zona glomerulosa of the adrenals (females at 15 ppm only), and hyperostosis of bone (females at 15 ppm only). Under the conditions of this study ointment that contained 3 ppm calcitriol (the same formulation proposed for marketing) was a NOAEL, and 9 ppm ointment induced only slight toxicity.

Genetic toxicology: Calcitriol is an endogenous compound, and as such, extensive evaluation for genetic toxicity was judged to be unnecessary. However, calcitriol was evaluated in the mouse lymphoma TK locus assay, and was confirmed to be non-mutagenic.

Carcinogenicity: A two-year carcinogenicity study was conducted in which calcitriol solution was orally administered (via gavage) daily to rats. Dosages of approximately 0.005, 0.03, and 0.1 µg/kg/day were investigated. The study included both a vehicle-treated control group (which received Neobee oil M5) and a second control group which received water. Survival rates did not differ significantly between groups; terminal sacrifice of all groups occurred following 104 weeks of treatment. The incidence of benign pheochromocytomas was significantly increased in female rats (pairwise p-value of 0.0001; trend value of 0.0036). No other tumor incidence data differed according to the Haseman-Lin-Rahman criteria.

A two-year topical carcinogenicity study was conducted in which calcitriol ointment was applied to the skin of mice. Materials that contained calcitriol at concentrations of 0 (vehicle), 0.3, 0.6, and 1.0 ppm were evaluated. The vehicle for the test material was

identical to the vehicle of the clinical formulation of Silkis ointment. No statistically significant differences in tumor incidence were observed in this study.

Calcitriol ointment was evaluated for activity as a cocarcinogen with UV light in a 12-month study with hairless mice. The vehicle for the test material was identical to the vehicle of the clinical formulation of Silkis ointment. The median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed was slightly, but statistically significantly, reduced for both males and females that were treated with the vehicle of the product, relative to untreated animals. Materials that contained calcitriol did not reduce latency to formation of the first tumor greater than or equal to 1.0 mm in diameter relative to vehicle alone. These data suggest that the vehicle of calcitriol ointment slightly enhances UV-induced skin tumor formation (possibly by enhancing UV penetration into the skin), but calcitriol per se does not enhance photo-induced carcinogenesis.

Reproductive toxicology: Calcitriol was evaluated for effects upon the fertility of male and female rats, effects upon developmental toxicity of rats and rabbits, and for effects upon pre-natal and perinatal development of rats.

To evaluate calcitriol for effects on fertility, calcitriol was orally administered to male and female rats (males dosed beginning 29 days prior to pairing and beyond and females dosed from 15 days prior to pairing and continuing until day 7 of gestation) at dosages of 0, 0.1, 0.3, and 0.6 µg/kg/day. Male and female reproductive performances were unimpaired in this study, including no effects on the percentages of animals that copulated or became pregnant, latency to mating, pre- or post-implantation losses, numbers of corpora lutea, or numbers of viable embryos. There were no effects on sperm concentration or motility. A dosage of 0.6 µg/kg/day was a NOAEL for reproductive parameters under the conditions of this study, although evidence of mild general toxicity was observed at 0.3 and 0.6 µg/kg/day.

To evaluate calcitriol for effects upon developmental toxicity in rats, calcitriol was orally administered to pregnant female rats on days 6 through 17 of gestation at dosages of 0, 0.1, 0.3, and 0.9 µg/kg/day. No effects were observed on C-section data, including numbers of live or dead fetuses, post-implantation loss, early or late resorptions, or mean fetal body weight, and there were no effects on the visceral or skeletal malformations or variations of fetuses. A dosage of 0.9 µg/kg/day was a NOAEL for reproductive parameters under the conditions of this study, although evidence of mild general toxicity was observed at 0.3 and 0.9 µg/kg/day.

To evaluate calcitriol for effects upon developmental toxicity in rabbits, calcitriol ointment was topically applied under conditions of occlusion. Exposure was modulated through varying the percentage of the body-surface area that was treated. Evidence of systemic exposure included reduced maternal survival (high-dose group), reduced maternal body weight gain (mid and high-dose groups), and elevated serum calcium and inorganic phosphorus levels in all maternal treatment groups. The mean plasma

concentration of Calcitriol was significantly elevated in mid and high-dose animals on day 18. Evidence of fetal toxicity included a significantly elevated mean post-implantation loss in the high-dose group, and an increased incidence of minor skeletal abnormalities among fetuses from the mid and high-dose groups due to retarded ossification of the pubic bones. A slightly increased incidence of skeletal variations was also observed in the high-dose group (extra 13th rib, reduced ossification of epiphyses). These effects were likely secondary to maternal toxicity. No effects were observed on the incidence of major fetal abnormalities, or of minor external or visceral abnormalities.

To evaluate calcitriol for effects upon pre-natal and perinatal development in rats, calcitriol was orally administered to pregnant rats from gestation day 6 through day 20 postpartum at dosages of 0, 0.1, 0.3, and 0.6 µg/kg/day. No effects were observed on maternal survival, mean duration of gestation, mean litter size, mean numbers of pups born alive or dead, physical development of F1 animals, behavioral development of F1 animals, or reproduction of F1 animals, and no effects on F2 animals were observed during necropsy of F1 dams. 0.6 µg/kg/day was an apparent NOAEL for effects on pre and post-natal development under the conditions of this study.

Special toxicology: Calcitriol ointment was found to be essentially non-irritating to the skin or eyes and to not induce sensitization.

2.6.6.2 Single-dose toxicity

No acute toxicology data are available that are relevant to the proposed clinical use.

2.6.6.3 Repeat-dose toxicity

2.6.6.3.1 Study Title: Subchronic 28-day dermal toxicity with calcitriol vaseline by daily administration (5 days in 7) in the rat

Key Study Findings: Treatment-related adverse observations included reduced weight gain, significantly reduced plasma inorganic phosphorus (males only), significantly increased plasma calcium in all treatment groups, urinary calcium and phosphate loss, increased mean relative kidney and adrenal weights, and mineralization in the heart and kidney.

Study No: H.141.408

Amendment #, Vol #, and page #: Mod 4, vol. 11

Conducting laboratory and location: _____

Date of study initiation: 8-JAN-1991

GLP compliance: Yes

QA- Report Yes (X) No ()

Methods:

Dosing:

- species/strain: Rat/Sprague-Dawley

b(4)

- #/sex/group or time point: 20 in control group, 10 in treatment groups
- age: 6 weeks at initiation
- weight: At initiation of treatment, females 132g to 178g, males 166g to 213g
- satellite groups used for toxicokinetics or recovery: No
- dosage groups in administered units: Vehicle treated control (white petrolatum), 2.5 µg/g calcitriol in white petrolatum, 8.75 µg/g calcitriol in white petrolatum, and 15 µg/g calcitriol in white petrolatum. Treatment consisted of once daily application of the assigned test material to shaved dorsal skin representing 10% of the body surface area (approximately 35 cm² in males and 28 cm² in females) at a rate of 1 g per 100 cm² (or 0.35 g/day in males and 0.28 g/day in females), 5 days per week for four weeks. The ointment was removed after six hours by wiping with dry tissue. The animals were restrained throughout the treatment period.
- route, form, volume, and infusion rate: Topically to dermis, ointment, 1 g per 100 cm²
- Drug, lot#, radiolabel, and % purity: Calcitriol, lot No. and purity not specified.
- Formulation/vehicle: Ointment (apparently just calcitriol in white petrolatum; note that this is somewhat different from the clinical formulation).

Observations and times:

- Clinical signs: Yes (once daily)
- Application site observation: Weekly
- Body weights: Yes (weekly)
- Food consumption: Yes (weekly)
- Ophthalmoscopy: Yes
- EKG: No
- Hematology: Yes, including differential
- Clinical chemistry: Yes
- Urinalysis: Yes
- Organ weights: Yes
- Gross pathology: Yes
- Organs weighed: Adrenals, brain, heart, kidneys, liver, pituitary, ovaries, spleen, testes, thymus
- Histopathology: Yes (control and high-dose animals, plus heart and kidneys only in low and mid-dose groups)
- List of tissues histologically examined: Adrenals, heart, kidneys, liver, ovaries, spleen, testes, skin (treated and untreated), and gross lesions
- Toxicokinetics: Blood samples obtained on day 28 or 29 at two hours after treatment, and analyzed for calcitriol level.
- Other: None

Results:

- Survival: 4 control males, one mid-dose male, and one low-dose female were found dead while under restraint. The deaths were considered to be the result of restraint, and not related to exposure

to calcitriol.

- Clinical signs: Emaciated appearance in 2/10 high-dose males during week 4.
- Application site observations: No remarkable observations.
- Body weights: Mean body weights of animals at termination are summarized below; body weight in high-dose males was significantly reduced:

Group	Mean Body Weight of Females (g)	Mean Body Weight of Males (g)
Vehicle Control	194±18	249±20
Low-Dose	191±13	233±25
Mid-Dose	193±14	232±22
High-Dose	180±15	203±23*

*p<0.01

- Body weight gain: Mean body weight gain values at termination are summarized below:

Group	Mean Body Weight Gain of Females (%)	Mean Body Weight Gain of Males (%)
Vehicle Control	23±7	27±7
Low-Dose	21±7	21±8
Mid-Dose	22±6	21±12
High-Dose	17±6*	7±7**

*p<0.05; **p<0.01

- Food consumption: Slightly, but significantly reduced in both males and females of high-dose group only.
- Ophthalmoscopy: No remarkable findings.
- Electrocardiography: Not performed.
- Hematology: Increased RBCs, hematocrit, and hemoglobin in high-dose females only.
- Clinical chemistry: Significantly reduced plasma inorganic phosphorus in all male treatment groups. Significantly increased plasma calcium in all treatment groups (both male and female).
- Urinalysis: Treatment groups of both males and females exhibited, roughly in proportion to exposure to calcitriol, decreased specific gravity, decreased pH, creatinine concentration, and decreased concentrations of sodium and chloride, and increased urinary volume, increased calcium concentration and total calcium spillage, and increased phosphate spillage.
- Organ Weights: In relation to body weight, the mean kidney and adrenal weights were significantly increased in both males and females. A few other significant differences were observed, but those were probably incidental.
- Gross pathology: No remarkable observations.

- Histopathology: Minimal vascular mineralization in the heart observed in 1 male and 3 females in the mid-dose group and 4 males and 2 females in the high-dose group. Inflammation and mineralization of the kidneys was observed in all treatment groups, but the incidence and severity tended to increase with exposure to calcitriol.
- Toxicokinetics: (AUC values not available, since only one time point examined):

Group	Serum Calcitriol Levels of Females (pg/mL±SD)	Serum Calcitriol Levels of Males (pg/mL±SD)
Vehicle Control	83±46	72±20
Low-Dose	359±138	693±377
Mid-Dose	789±313	1792±898
High-Dose	729±349	1706±994

2.6.6.3.2 Study Title: Calcitriol ointment - 13 week dermal preliminary toxicity study in the mouse.

Key study findings: One MD female, one HD female, and one HD male died as a result of treatment. Mean body weight and body weight gain were significantly reduced in HD males and females. Plasma calcium was significantly elevated in all treatment groups, both male and female. Mineralization was observed in a variety of tissues in all treatment groups. The incidence and severity of the mineralization increased with increased exposure to calcitriol. A NOAEL was not observed in this study. Useful toxicokinetic data were not obtained in the study.

Study No.: 913/080

Document #, Volume #, and Page #: Mod 4, vol. 8

Conducting laboratory and location: _____

Date of study initiation: 21-FEB-2001

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol ointment 0.0001% (1 ppm), Lot 9002001.8, and 0.0003% (3 ppm), Lot 100004.8, 100%.

Formulation/vehicle:

<u>Compound</u>	<u>Amount (percent w/w)</u>
Calcitriol.....	0.0003
White soft paraffin.....	✓
Liquid paraffin.....	✓
α-Tocopherol.....	✓

b(4)

b(4)

Presumably the 0.0001% formulation was similar but contained slightly more white paraffin.

Methods (unique aspects):

Dosing:

Species/strain: Mouse —CD-1 (ICR) BR
#/sex/group or time point (main study): 12/sex/group; housed 1 per cage
Satellite groups used for toxicokinetics or recovery: yes, for toxicokinetics
Age: At least 5 weeks at start of dosing
Weight: At start of dosing: males, 26-37 g, females, 18-26 g
Doses in administered units: 0 (vehicle control); 1 ppm, 25 μ L/day; 1 ppm, 50 μ L/day; and 3 ppm, 25 μ L/day; these dosages equate to approximately 1, 2, and 3 μ g/kg/day if a BW of 25 g is assumed.
Route, form, volume, and infusion rate: Topical, 25-50 μ L/day (see above), once per day for 13 consecutive weeks (91 administrations for males, 92 administrations for females). Note: The assigned material was applied to shaved skin on the back (approximately 10% of the body surface area), measured with a syringe, and gently rubbed into the treatment site with a gloved finger "to give a thin uniform layer". The treated area was not covered, but was washed approximately six hours after application with water and dried with paper.

b(4)

Observations and times:

Clinical signs: Animals observed daily for clinical signs; full clinical exam once weekly

Body weights: Weekly

Food consumption: Weekly

Ophthalmology: No

EKG: No

Hematology: Six main-study animals per sex at termination (where possible)

Clinical chemistry: Six main-study animals per sex at termination (where possible)

Urinalysis: No

Gross pathology: All main-study animals.

Organs weighed: Adrenals, epididymis, heart, kidneys, liver, lungs, ovaries.

Histopathology: A standard list of tissues from animals in high-dose and control group killed after 13-weeks of treatment. The following tissues were examined from the mid-dose and low-dose groups: skin at treatment site, aorta, colon, duodenum, femoral bone and joint, heart, kidneys, lungs with bronchi, stomach, trachea and larynx.

Toxicokinetics: Blood samples drawn from three animals per sex per treatment level (satellite animals) on days 1 and 85 at 3, 6, 9, 12, and 24 hours post-application for toxicokinetic analysis.

Results:

- Mortality: One MD female and one HD female died on day 76. One HD male sacrificed on day 78. All described as having a "thin appearance". These deaths

were ascribed to treatment. One LD female died on day 50 "as a result of traumatic injury", this death was not ascribed to treatment.

- Clinical signs: Thin appearance in 2 HD males and 5 HD females and 1 MD female. Essentially no signs of local irritation, edema, or erythema.
- Body weights: Mean body weight and weight gain significantly reduced in HD males and females:

Mean body weights of unfasted males, day 90 (mean±SD):

Vehicle controls 36.8±2.8g

Low dose 35.0±1.6g

Mid dose 36.4±2.1g

High dose 30.9±3.9g**

Mean body weights of unfasted females, day 90 (mean±SD):

Vehicle controls 29.5±2.5g

Low dose 29.5±1.6g

Mid dose 28.8±4.0g

High dose 23.1±4.8g**

- Body weight gains:

Mean body weight gains of males, days 0-90 (mean±SD):

Vehicle controls 6.5±1.4g

Low dose 5.4±1.8g

Mid dose 5.7±2.2g

High dose -0.2±4.9g***

Mean body weight gains of females, days 0-90 (mean±SD):

Vehicle controls 6.7±2.0g

Low dose 6.6±1.6g

Mid dose 5.8±4.5g

High dose 0.5±5.1g**

**p≤0.01

***p≤0.001

- Food consumption: Tended to be slightly reduced in HD animals, achieving statistical significance at some time intervals.
- Ophthalmology: NA
- EKG: NA
- Hematology: Some isolated statistically significant differences, but no effects that appear to be related to treatment.
- Clinical chemistry: Mean serum calcium level was significantly increased in all treatment groups:

Mean serum calcium of males, day 91 (mg/L)

Vehicle controls 95±3
Low dose 108±4**
Mid dose 124±9**
High dose 132±11**

Mean serum calcium of females, day 92 (mg/L)
Vehicle controls 101±6
Low dose 124±12**
Mid dose 117±5**
High dose 135±12*

*p≤0.05 relative to water control group

**p≤0.01

***p≤0.001

A trend toward increased phosphorus concentration was apparent. No other remarkable observations.

- Urinalysis: NA

- Organ Weights: The absolute mean weights of a number of organs were lowest in HD animals, although in most cases no difference was apparent after normalizing the data to body weight, so the effect was presumably secondary to the general reduction in mean body weight mentioned above. The mean weights of the ovaries and uteri were significantly reduced in HD females, both absolute and relative, suggesting a genuine effect. No other remarkable observations.

- Gross pathology: Isolated pale area observed in diaphragm of one female form both the MD and HD groups; histopathology revealed these lesions to be due to mineralization. No other remarkable observations.

- Histopathology: Minimal to marked mineralization observed in various organs in all treatment groups, including the heart, vasculature, larynx, trachea, lungs, bronchi, stomach, duodenum, colon, kidneys, diaphragm, joints, and periosteum of the femur. The incidence and severity of the mineralization increased with increased exposure to calcitriol. A no-adverse-effect-level (NOAEL) was not observed. Moderate mineralization of some control tissues was also observed. No other remarkable observations.

- Toxicokinetics: The following TK data were submitted:

Week 13:

Control males: Below Limit of Quantification (BLQ, 200 pg/mL) at all time points.

LD males: BLQ all time points

MD males: BLQ all time points

HD males: 290 pg/mL at 3 hrs, BLQ all other time points

Control females: BLQ all time points

LD females: 250 pg/mL at 3 hrs, BLQ all other time points
MD females: 260 pg/mL at 3 hrs, BLQ all other time points
HD females: 420 pg/mL at 3 hrs, BLQ all other time points

2.6.6.3.3 Study Title: Calcitriol - 13 week oral (gavage) dose range finding toxicity study in the rat.

Key study findings: Mean serum calcium levels were increased, as was urinary calcium excretion. The mean kidney weight was significantly increased in high-dose males (as a percentage of body weight). The incidence of myocarditis was increased. The incidence and severity of renal mineral deposits increased in proportion to dosage in both genders.

Study No.: 913/093

Document #, Volume #, and Page #: Mod 4, vol. 15

Conducting laboratory and location: _____

Date of study initiation: 08-FEB-2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol, Lots PA202710141 and PA202710142, 100%.

Formulation/vehicle: Calcitriol was dissolved in ethanol (1000 µg/mL), then diluted in Neobee oil M5 to final concentrations of 0.002, 0.02, and 0.06 µg/mL. Note: Neobee M5 is a medium chain triglyceride, _____

_____ respectively. _____

b(4)

b(4)

Methods (unique aspects):

Dosing:

Species/strain: Rat/Wistar (Ico - WI (IOPS AF/Han)IGS)

#/sex/group or time point (main study): 10/sex/group; housed 3-5 per cage

Satellite groups used for toxicokinetics or recovery: yes, for toxicokinetics

Age: Approx. 7 weeks at start of dosing

Weight: At start of dosing: males, 157-203 g, females, 141-172 g

Doses in administered units: 0 (vehicle control (Neobee oil M5)); 0 (water control); 0.01 µg/kg/day; 0.1 µg/kg/day; and 0.3 µg/kg/day.

Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg, once per day for 13 consecutive weeks (90 administrations for males, 91 administrations for females).

Observations and times:

Clinical signs: Animals observed daily for clinical signs; full clinical exam once weekly

Body weights: Weekly
Food consumption: Weekly (per cage)
Ophthalmology: All animals pre-test; vehicle control group and high-dose animals during week 13
EKG: No
Hematology: All main-study animals at termination
Clinical chemistry: All main-study animals at termination
Urinalysis: All main-study animals at termination
Gross pathology: All main-study animals.
Organs weighed: Adrenals, brain, epididymis, heart, kidneys, liver, lungs, ovaries.
Histopathology: Standard list from all main-study animals in vehicle control group, water control group, and high-dose group killed after 13-weeks of treatment.
Toxicokinetics: Four untreated (control) animals per gender and 6 additional (satellite) animals per gender in each of the calcitriol-treatment groups had blood samples drawn on the first day of treatment and on the 65th day of treatment at times 0, 1, 2, 4, 8, and 24 hours post-dosing for toxicokinetic analysis.

Results:

- Mortality: No unscheduled deaths
- Clinical signs: Hair loss and hypersalivation; both effects ascribed to vehicle.
- Body weights: Trends toward reduced mean weight and weight-gain observed in males, but differences not significant. No effect on females:

Mean body weights of unfasted males, day 89 (mean±SD; none significant):

Vehicle controls 449.1±41.1g
Water controls 446.7±38.5
Low dose 428.3±43.0g
Mid dose 423.0±41.7g
High dose 418.48±29.7g

Mean body weights of unfasted females, day 89 (mean±SD; none significant):

Vehicle controls 285.2±12.3g
Water controls 288.8±16.4
Low dose 294.3±21.4g
Mid dose 291.1±24.5g
High dose 288.7±15.2g

- Body weight gains:

Mean body weight gains of males, days 0-89 (mean±SD; none significant):

Vehicle controls 256.0±39.4g

Water controls 261.7±43.1
Low dose 242.4±36.0g
Mid dose 236.7±37.7g
High dose 230.6±28.0g

Mean body weight gains of females, days 0-89 (mean±SD; none significant):

Vehicle controls 127.2±11.7g
Water controls 131.7±16.3
Low dose 134.6±19.0g
Mid dose 133.6±16.5g
High dose 128.5±12.5g

- Food consumption: Slightly reduced in all treatment groups and vehicle control group relative to water control group.
- Ophthalmology: No remarkable observations.
- EKG: NA
- Hematology: No remarkable observations.
- Clinical chemistry: Mean serum calcium level was increased in all treatment groups (not significant in low-dose males):

Mean serum calcium of males, day 90 (mg/L)

Vehicle controls 104±2
Water controls 104±4
Low dose 106±4
Mid dose 111±3**
High dose 116±3***

Mean serum calcium of females, day 91 (mg/L)

Vehicle controls 104±4
Water controls 103±3
Low dose 108±4*
Mid dose 113±5***
High dose 114±4***

*p≤0.05 relative to water control group

**p≤0.01

***p≤0.001

No other remarkable observations.

- Urinalysis: Urinary volume was slightly reduced in all treatment groups and vehicle control group relative to water control group, and urinary specific gravity was correspondingly elevated in those groups relative to water controls. Urinary calcium excretion was increased relative to the water-treated control value for all

treatment groups; excretion of phosphorus also tended to be increased in treatment groups:

Relevant Urine Parameters in Male Animals, day 90

Group	Volume (mL)	Calcium Concentration (mg/L)	Calcium Excreted (mg)	Phosphorus Concentration (mg/L)	Phosphorus Excreted (mg)
Vehicle Control	10.4±2.4***	98±57	0.98±0.58	368±144	3.82±1.84***
Water Control	16.4±2.4	67±32	1.08±0.52	501±160	8.10±2.39
Low Dose (0.01 µg/kg/day)	11.3±3.5***	235±99***	2.63±1.22**	641±287	6.98±3.11
Mid Dose (0.1 µg/kg/day)	11.9±1.5***	786±264***	9.19±2.82***	955±506**	11.01±5.30
High Dose (0.3 µg/kg/day)	12.2±2.5**	993±323***	11.90±3.68***	1293±450***	15.55±5.85*

*p≤0.05 relative to water control group

**p≤0.01

***p≤0.001

Relevant Urine Parameters in Female Animals, day 91

Group	Volume (mL)	Calcium Concentration (mg/L)	Calcium Excreted (mg)	Phosphorus Concentration (mg/L)	Phosphorus Excreted (mg)
Vehicle Control	5.8±2.2***	229±116	1.21±0.56**	991±560*	5.13±2.54
Water Control	11.2±1.9	216±63	2.45±0.90	476±42	5.36±1.19
Low Dose (0.01 µg/kg/day)	8.0±2.2**	560±231***	4.28±1.47*	1000±649*	7.43±4.14
Mid Dose (0.1 µg/kg/day)	8.7±2.2*	1253±450***	10.62±3.56***	1599±619***	13.60±5.73**
High Dose (0.3 µg/kg/day)	7.8±1.7**	1607±412***	12.27±3.50***	2172±310***	16.64±3.38***

No other remarkable observations.

- **Organ Weights:** Mean kidney weight (relative to body weight) was significantly increased in high-dose males relative to water control males (0.635±0.029 vs. 0.563±0.045, respectively, expressed as a percentage of total body weight). No other remarkable observations.

- **Gross pathology:** No remarkable observations.

- **Histopathology:** Treatment-related findings were observed in the heart and kidneys of both genders. In the heart, the incidence of minimal or slight myocarditis was increased in all male treatment groups and in mid and high-dose females. Mineralization of the heart was not observed. In the kidney, the incidence and severity of renal mineral deposits increased in proportion to dosage in both genders. No other remarkable observations.

- Toxicokinetics: With the exception of a few values in high-dose males, and one mid-dose-male sample, all samples were below the limit of quantification in the study (200 pg/mL). In samples from high-dose males that were obtained at two hours post-dosing during study week 10, calcitriol levels ranged from 210 to 370 pg/mL. These data are not adequate to permit meaningful comparison to clinical PK data or calculation of AUC values, although they suggest that systemic exposure to calcitriol increased with increasing dose and that males may have experienced higher blood levels of calcitriol at a given dose than did females.

2.6.6.3.4 Study Title: Thirteen-week topical range-finding study of calcitriol in hairless mice, with or without simulated sunlight

Key study findings: Under the conditions of this study a dosage of 2 µg/kg/day induced substantial toxicity, including unscheduled deaths, reduced body weight gain, elevated serum calcium, and mineralization of the aorta and kidney. A dosage of 1 µg/kg/day induced some toxicity but was reasonably well tolerated.

Note: This study was conducted as a dose range-finding study to select exposure levels for a 12 month photocarcinogenesis study.

Study No.: 1207-017

Document #, Volume #, and Page #: Mod 4, vol. 10

Conducting laboratory and location: _____

b(4)

Date of study initiation: 10-APR-2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol ointment placebo, formulation No. 9000, batch No. 006*01; calcitriol ointment 0.3 ppm, formulation No. 9001, batch No. 007*01; calcitriol ointment 1.0 ppm, formulation No. 9002, batch No. 008*01, 100%.

Formulation/vehicle: The vehicle for the test materials was the same as for the clinical formulation of Silkis ointment.

Methods (unique aspects):

Dosing:

Species/strain: Mouse/SKH1-hrBR (albino hairless)

b(4)

#/sex/group or time point (main study): 6/sex/group; housed 1 per cage

Satellite groups used for toxicokinetics or recovery: No

Age: Approximately 55-60 days at initiation

Weight: At start of dosing: males, 25-35 g, females, 21-27 g

Doses in administered units: 0 (untreated); 0 (vehicle control); 0.3 ppm, 25 µL/day; 0.3 ppm, 50 µL/day; 1.0 ppm, 25 µL/day; 1.0 ppm, 50 µL/day. For each exposure level, one group of 6 animals was maintained for 13 weeks of treatment without exposure to UV light and an identical group of animals received daily treatments for 13 weeks with exposure to 600 RBU of UV light

per week (120 RBU daily on Monday through Friday, with the test material applied prior to UV exposure on M, W, and F, and post-UV exposure on T and R). The test material applications resulted in exposure to approximately 0, 0.3, 0.6, 1, and 2 $\mu\text{g}/\text{kg}/\text{day}$ if a BW of 25 g is assumed, or, more accurately (since the density of the ointment was 0.85 g/mL) 0, 0.26, 0.51, 0.85, and 1.7 $\mu\text{g}/\text{kg}/\text{day}$. UV light was generated by a 6.5 kW xenon long arc lamp with definitive output in both the UVA (320 nm to 400 nm) and UVB (280 nm to 320 nm) ranges.

Route, form, volume, and infusion rate: Topical, 25-50 $\mu\text{L}/\text{day}$ (see above), once per day M-F for 13 consecutive weeks. Note: The assigned material was applied to the back and sides (approximately 25 cm^2) of the mice.

Observations and times:

Clinical signs: Animals observed twice daily for viability and weekly for general appearance. Clinical signs and local skin reactions weekly. Skinfold thickness measured during weeks 0 (prior to first application), 4, 8, and 13.

Body weights: Weekly

Food consumption: No

Ophthalmology: No

EKG: No

Hematology: NA

Clinical chemistry: At termination (serum calcium and phosphorus levels only)

Urinalysis: No

Gross pathology: All animals

Organs weighed: Liver, brain, spleen, thymus, pituitary, adrenal

Histopathology: All animals, limited to kidneys and heart

Toxicokinetics: No

Results:

- **Mortality:** Two HD (1.0 ppm, 50 $\mu\text{L}/\text{day}$) females that were not exposed to UV light died on study (days 68 and 79). Both animals were emaciated and dehydrated. No other unscheduled deaths.

- **Clinical signs:** All test materials were well tolerated. "Barely perceptible" erythema and flaking, mild edema, and wrinkling were observed in a few animals, but incidence and severity did not seem to increase with exposure in either irradiated or non-irradiated mice. Skin-fold thickness tended to be slightly reduced in HD animals, achieving statistical significance at a few time points.

- **Body weights:** Mean body weights and weight gains tended to be reduced in proportion to exposure in both presence and absence of UV irradiation in both males and females:

Body weight gains:

Mean body weight gains of males, no irradiation, days 0-93 (mean \pm SD):
Untreated 6.7 \pm 2.0g

Vehicle controls 5.7±2.1g
Low dose (0.3 µg/kg/day) 5.3±2.2g
Mid dose (0.6 µg/kg/day) 2.7±2.8g
2nd Mid dose (1.0 µg/kg/day) 4.3±2.0
High dose (2.0 µg/kg/day) -1.8±4.4g**

Mean body weight gains of males, with irradiation, days 0-93 (mean±SD):

Untreated 5.5±2.4g
Vehicle controls 5.2±1.5g
Low dose (0.3 µg/kg/day) 3.5±1.9g
Mid dose (0.6 µg/kg/day) 3.8±1.2g
2nd Mid dose (1.0 µg/kg/day) 2.7±2.0
High dose (2.0 µg/kg/day) -2.7±6.8g*

Mean body weight gains of females, no irradiation, days 0-92 (mean±SD):

Untreated 4.7±1.6g
Vehicle controls 5.0±1.8g
Low dose (0.3 µg/kg/day) 3.3±1.2g
Mid dose (0.6 µg/kg/day) 4.0±2.0g
2nd Mid dose (1.0 µg/kg/day) 2.5±2.2
High dose (2.0 µg/kg/day) 1.5±3.9g

Mean body weight gains of males, with irradiation, days 0-92 (mean±SD):

Untreated 3.5±1.4g
Vehicle controls 3.3±1.4g
Low dose (0.3 µg/kg/day) 3.7±1.4g
Mid dose (0.6 µg/kg/day) 3.2±1.9g
2nd Mid dose (1.0 µg/kg/day) 2.3±2.6
High dose (2.0 µg/kg/day) -2.8±2.1g**

*p≤0.05 compared to matched untreated control

**p≤0.01

- Food consumption: NA
- Ophthalmology: NA
- EKG: NA
- Hematology: NA
- Clinical chemistry: Mean serum calcium level increased slightly with increasing exposure, the difference from untreated controls achieving statistical significance in HD (2.0 µg/kg/day) males and females only. A trend toward increased phosphorus concentration was also apparent.
- Urinalysis: NA
- Organ Weights: The absolute mean weights of a number of organs were lowest in HD animals, although in some instances no difference was apparent after

normalizing the data to body weight or brain weight, so the effect was presumably secondary to the general reduction in mean body weight mentioned above.

- Gross pathology: No remarkable observations.
- Histopathology: Treatment-related histopathology observations were limited to high-dose animals, and included mineralization of the aorta at the base of the heart and of renal tubules of the outer medulla.
- Toxicokinetics: NA

2.6.6.3.5 Study Title: Calcitriol ointment - a 13 week dermal toxicity study in minipigs.

Key study findings: Essentially no toxicity was observed in this study. Mean kidney weight was significantly increased in animals in the high-dose group (9 ppm material). Under the conditions of this study ointment that contained 3 ppm calcitriol (approximately 5.1 µg/kg/day) was a NOAEL, and 9 ppm ointment (approximately 15.3 µg/kg/day) induced slight toxicity.

Study No.: 38094

Document #, Volume #, and Page #: Mod 4, vol. 20

b(4)

Conducting laboratory and location: _____

Date of study initiation: Treatment of animals initiated 09-AUG-2000

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol placebo ointment (0 ppm), Lot 900001.8; calcitriol ointment 0.3 ppm, Lot 9001001.8; calcitriol ointment 1 ppm, Lot 9002001.8; calcitriol ointment 3 ppm, Lot 0100004.8; calcitriol ointment 9 ppm, Lot 9003001.8; 100%.

Formulation/vehicle:

<u>Compound</u>	<u>Amount</u>
Calcitriol.....	0.3, 1, 3, or 9 ppm
White soft paraffin.....	_____ % w/w
Liquid paraffin.....	_____ % w/w
α-Tocopherol.....	_____ % w/w

b(4)

Methods (unique aspects):

Dosing:

- Species/strain: Gottingen SPF minipigs
- #/sex/group or time point (main study): 3/sex/group; housed 1 per pen
- Satellite groups used for toxicokinetics or recovery: No
- Age: At least 3 months at start of dosing
- Weight: At start of dosing: Approx. 5.5-8 kg
- Doses in administered units: 0 (vehicle control); 0.3 ppm; 1 ppm; 3 ppm; 9 ppm.
- These dosages equate to approximately 0, 0.5, 1.7, 5.1, and 15.3 µg/kg/day (2

mL/kg/day applied, ointment density is 0.85 g/mL) applied to the skin, although it is unclear how much calcitriol was removed and discarded when the dressing was removed and the site washed.

Route, form, volume, and infusion rate: Topical, 2 mL/kg/day, once per day for at least 91 consecutive days. The assigned material was applied to shaved skin on the back (approximately 10% of the body surface area) and gently rubbed into the treatment site for one minute. The treated area was covered with a non-occlusive dressing for 6 hours. At the end of the treatment period each day the site was washed with mild soap.

Observations and times:

Clinical signs: Animals observed daily for clinical signs, including skin reactions (local effects)

Body weights: Weekly

Food consumption: Daily

Ophthalmology: Yes, during weeks 5 and 13

EKG: Yes, during weeks 5 and 13

Hematology: Yes, before start of treatment and week 13

Clinical chemistry: Yes, before start of treatment and week 13

Urinalysis: Yes

Gross pathology: All animals.

Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries.

Histopathology: Standard list from all animals.

Toxicokinetics: Blood samples drawn from all animals on days 1 and 91 pre-treatment and at 2, 4, 8, 10, 12, 16, and 24 hours post-application for toxicokinetic analysis.

Results:

- Mortality: No unscheduled deaths
- Clinical signs: No remarkable observations, including skin at treatment site
- Body weights: No remarkable observations
- Food consumption: No remarkable observations
- Ophthalmology: No remarkable observations
- EKG: No remarkable observations
- Hematology: No remarkable observations
- Clinical chemistry: No remarkable observations
- Urinalysis: No remarkable observations. Note: A trend toward a small increase in mean urinary calcium concentration was apparent, but the difference was not statistically significant.
- Organ Weights: The mean weight of the kidneys was significantly increased in both males and females in the HD group (79.96 ± 3.51 versus 63.91 ± 8.13 in controls and 90.81 ± 7.08 versus 64.34 ± 6.56 in controls in males and females, respectively, mean weight in g \pm SD). No other remarkable observations.
- Gross pathology: No remarkable observations
- Histopathology: Slight evidence of inflammation at the application site was

observed in all groups. This did not appear to be dose-limiting. No other remarkable observations.

- Toxicokinetics: The concentration of calcitriol in all TK samples was below the LOQ (100 pg/mL).

2.6.6.3.6 Study Title: Calcitriol ointment (Silkis) - 13 week toxicity study by cutaneous administration in the beagle dog.

Key study findings: Toxicity was observed primarily at exposures of 5.1 µg/kg/day and above, and included reduced body weight gain, elevated serum calcium, increased calcium excretion, increased mean kidney weight, mineralization of various tissues, hyperplasia of the treatment site, and cortical tubular nephropathy. A NOAEL was not observed in the study, but exposures of 1.7 µg/kg/day and below appeared to be well tolerated under the conditions of this study.

Study No.: 913/097

Document #, Volume #, and Page #: Mod 4, 18

Conducting laboratory and location: _____

b(4)

Date of study initiation: 18-MAR-2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol placebo ointment (0 ppm), Batch No. 006.01; calcitriol ointment 0.3 ppm, Batch No. 007.01; calcitriol ointment 1 ppm, Batch No. 008.01; calcitriol ointment 3 ppm, Batch No. 010.01; 100%.

Formulation/vehicle: Note: The formulation was not clearly specified, other than as "calcitriol ointment" and "Silkis". Presumably the formulation was:

<u>Compound</u>	<u>Amount</u>	
Calcitriol.....	0, 0.3, 1, or 3 ppm	
White soft paraffin.....	_____	w/w (varies slightly) b(4)
Liquid paraffin.....	_____	n/w
α-Tocopherol.....	_____	w/w

Methods (unique aspects):

Dosing:

Species/strain: Dog/Beagle

#/sex/group or time point (main study): 4/sex/group; housed 1 per pen

Satellite groups used for toxicokinetics or recovery: No

Age: At least 5-6 months at start of dosing

Weight: At start of dosing: Males: 7-10 kg; Females: 6-9 kg

Doses in administered units: 0 (vehicle control), 2 mL/kg/day; 0.3 ppm, 2 mL/kg/day; 1 ppm, 2 mL/kg/day; 3 ppm, 2 mL/kg/day; 3 ppm, 4 mL/kg/day.

These dosages equate to approximately 0, 0.51, 1.7, 5.1, and 10.2 $\mu\text{g}/\text{kg}/\text{day}$ (ointment density is 0.85 g/mL) applied to the skin, although it is unclear how much calcitriol was removed and discarded when the dressing was removed and the site washed.

Route, form, volume, and infusion rate: Topical, 2 mL/kg/day or 4 mL/kg/day (see above), once per day for 91 consecutive days in males, 92 in females. The assigned material was applied to shaved skin on the back (approximately 10% of the body surface area) and spread uniformly with a spatula. The treated area was covered with a non-occlusive dressing for 6 hours. The animals were fitted with Elizabethan collars during the treatment period. At the end of the treatment period each day the site was washed with mild soap.

Observations and times:

Clinical signs: Animals observed daily for clinical signs. Local skin reactions assessed weekly.

Body weights: Weekly

Food consumption: Daily

Ophthalmology: Yes, pretreatment and week 13

EKG: Yes, pretreatment and during weeks 4 and 13

Hematology: Yes, before start of treatment and weeks 4 and 13

Clinical chemistry: Yes, before start of treatment and weeks 4 and 13 (serum calcium measured every two weeks)

Urinalysis: Yes, before start of treatment and weeks 4 and 13 (urine calcium measured every two weeks)

Gross pathology: All animals.

Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries.

Histopathology: Standard list from all animals.

Toxicokinetics: Blood samples drawn from control and HD groups on days 1, 27, and 89 pre-treatment and at 1, 3, 6, 9, and 24 hours post-application for toxicokinetic analysis.

Results:

- Mortality: No unscheduled deaths
- Clinical signs: No treatment-related effects on general clinical condition. Slight to well-defined erythema observed at treatment site, increasing slightly with increased exposure to calcitriol. No edema. Product scored as "slightly irritant".
- Body weights: Mean body weights on day 91 tended to be lower in treated animals than in controls, but the differences were not statistically significant. Body weight gain data are presented below:

- Body weight gains:

Mean body weight gains of males, days 0-91 (mean \pm SD):

Vehicle controls 1.3 \pm 0.5 kg

Low dose (0.51 $\mu\text{g}/\text{kg}/\text{day}$) 1.2 \pm 0.5 kg

First Mid dose (1.7 $\mu\text{g}/\text{kg}/\text{day}$) 0.3 \pm 0.7 kg

Second Mid dose (5.1 µg/kg/day) 0.9±0.4 kg
 High dose (10.2 µg/kg/day) 0.7±0.3 kg

Mean body weight gains of females, days 0-91 (mean±SD):

Vehicle controls 1.7±0.6 kg
 Low dose (0.51 µg/kg/day) 1.7±0.8 kg
 First Mid dose (1.7 µg/kg/day) 1.6±0.4 kg
 Second Mid dose (5.1 µg/kg/day) 1.1±0.7 kg
 High dose (10.2 µg/kg/day) 0.1±0.5 kg*
 *p≤0.05

- Food consumption: Reduced in HD animals, particularly females, achieving statistical significance at some time points.
- Ophthalmology: No remarkable observations
- EKG: No remarkable observations
- Hematology: No remarkable observations
- Clinical chemistry: Serum calcium levels were increased in both genders at exposures of 5.1 µg/kg/day and above:

Mean serum calcium levels of males, day 88 (mean±SD):

Vehicle controls 111±3 mg/L
 Low dose (0.51 µg/kg/day) 116±2 mg/L
 First Mid dose (1.7 µg/kg/day) 116±3 mg/L
 Second Mid dose (5.1 µg/kg/day) 140±22 mg/L*
 High dose (10.2 µg/kg/day) 162±11 mg/L*

Mean serum calcium levels of females, day 88 (mean±SD):

Vehicle controls 111±3 mg/L
 Low dose (0.51 µg/kg/day) 111±1 mg/L
 First Mid dose (1.7 µg/kg/day) 111±5 mg/L
 Second Mid dose (5.1 µg/kg/day) 144±5 mg/L*
 High dose (10.2 µg/kg/day) 168±12 mg/L*
 *p≤0.05

No other remarkable observations.

- Urinalysis: Daily urine volume and calcium excretion increased in proportion to exposure to calcitriol:

Relevant Urine Parameters in Male Animals, day 88

Group	Volume (mL)	Calcium Concentration (mg/L)	Calcium Excreted (mg)	Phosphorus Concentration (mg/L)	Phosphorus Excreted (mg)
Vehicle Control	78.8±28	45±32	3.2±2.0	1690±522	128±36
Low Dose (0.51)	87.5±28	161±119	13±7.7*	1733±461	150±52

$\mu\text{g}/\text{kg}/\text{day}$)					
First Mid Dose (1.7 $\mu\text{g}/\text{kg}/\text{day}$)	100 \pm 8.2	116 \pm 65	12 \pm 6.5*	1745 \pm 277	173 \pm 15*
Second Mid Dose (5.1 $\mu\text{g}/\text{kg}/\text{day}$)	288 \pm 140	161 \pm 82	49 \pm 41*	905 \pm 349*	230 \pm 96
High Dose (10.2 $\mu\text{g}/\text{kg}/\text{day}$)	360 \pm 99*	177 \pm 84	647 \pm 33*	743 \pm 200*	254 \pm 30*

*p \leq 0.05 relative to water control group

Relevant Urine Parameters in Female Animals, day 88

Group	Volume (mL)	Calcium Concentration (mg/L)	Calcium Excreted (mg)	Phosphorus Concentration (mg/L)	Phosphorus Excreted (mg)
Vehicle Control	95.0 \pm 21	63 \pm 28	6.1 \pm 3.2	2428 \pm 416	225 \pm 26
Low Dose (0.51 $\mu\text{g}/\text{kg}/\text{day}$)	95.0 \pm 17	103 \pm 96	9.4 \pm 7.6	2053 \pm 173	195 \pm 39
First Mid Dose (1.7 $\mu\text{g}/\text{kg}/\text{day}$)	103 \pm 13	90 \pm 28	9.4 \pm 3.4	1670 \pm 351*	168 \pm 20
Second Mid Dose (5.1 $\mu\text{g}/\text{kg}/\text{day}$)	350 \pm 41*	143 \pm 45	51 \pm 21*	700 \pm 100*	246 \pm 50
High Dose (10.2 $\mu\text{g}/\text{kg}/\text{day}$)	273 \pm 134*	157 \pm 51	43 \pm 25*	670 \pm 139*	172 \pm 66*

*p \leq 0.05 relative to water control group

No other remarkable observations.

- Organ Weights: The mean weight of the kidneys tended to be increased in both males and females at 5.1 $\mu\text{g}/\text{kg}/\text{day}$ and above (achieving statistical significance in some instances):

Mean kidney weight of males, day 91 (mean \pm SD):

Vehicle controls 47.8 \pm 3.6 g
 Low dose (0.51 $\mu\text{g}/\text{kg}/\text{day}$) 51.6 \pm 7.3 g
 First Mid dose (1.7 $\mu\text{g}/\text{kg}/\text{day}$) 45.5 \pm 4.4 g
 Second Mid dose (5.1 $\mu\text{g}/\text{kg}/\text{day}$) 56.1 \pm 6.7 g
 High dose (10.2 $\mu\text{g}/\text{kg}/\text{day}$) 54.9 \pm 1.4 g*

Mean kidney weight of females, day 92 (mean \pm SD):

Vehicle controls 40.6 \pm 4.7 g
 Low dose (0.51 $\mu\text{g}/\text{kg}/\text{day}$) 41.5 \pm 3.3 g
 First Mid dose (1.7 $\mu\text{g}/\text{kg}/\text{day}$) 39.8 \pm 3.1 g
 Second Mid dose (5.1 $\mu\text{g}/\text{kg}/\text{day}$) 47.8 \pm 1.9 g*

High dose (10.2 µg/kg/day) 46.7±5.3 g*

*p≤0.05

No other remarkable observations.

- Gross pathology: No remarkable observations
- Histopathology: Minimal to moderate mineralization was observed in a number of tissues, including the vasculature, cecum, jejunum, kidneys, salivary glands, and stomach, primarily at exposures of 5.1 µg/kg/day and above. A dosage-dependent "minimal to moderate diffuse epithelial hyperplasia of the skin" was observed at the application site. The mineral deposition in the kidneys was associated with cortical tubular nephropathy characterized by vacuolation and single-cell death of the tubular epithelium, tubular dilation, tubules lined by flattened or basophilic (regenerating) epithelium, fibrosis, and interstitial mononuclear cell infiltration. One HD female exhibited ureteritis, diffuse cystitis and minimal diffuse hyperplasia of the epithelium of the urinary bladder, in association with calculi. No other remarkable observations.
- Toxicokinetics: Neither individual animal data nor group mean data were submitted. The concentration of calcitriol was below the LOQ (50 pg/mL, in this instance) in all control samples and (apparently) most of the HD samples. The submission states: "Calcitriol was quantifiable in one sample at least in all animals [at 10.2 µg/kg/day] except in one female. In most cases, calcitriol concentrations were quantifiable at 9 h postdose only...irrespective of the duration of treatment (day 0, Week 4, Week 13). The concentrations amounted to up to 357 pg/mL. They did not show a tendency to be dependent on the sex".

2.6.6.3.7 Study Title: Calcitriol ointment - A 26-week dermal study in rats.

Key study findings: This study evaluated the affects of topical application of calcitriol to the skin of rats for 26 weeks; concentrations of calcitriol in ointment base ranged from 0.3 ppm to 9 ppm, resulting in theoretical dosages of 0.51, 1.7, 5.1, and 15.3 µg/kg/day. The higher dosages were not tolerated under the conditions of this study, resulting in 100% mortality. Reduced mean body weight gain was observed at all dosages. Evidence of pharmacodynamic effects of calcitriol included elevated levels of calcium in the serum and urine, mineralization of various tissues, and hyperostosis. A NOAEL was not observed under the conditions of this study.

Note: Calcitriol was not well tolerated under the conditions of this study. However, rodents are known to be more sensitive to vitamin D-related compounds than are nonrodent species, including primates. Even a concentration of 0.3 ppm, one-tenth the concentration of the clinical formulation, produced clear toxicity, although the toxic effects were extensions of the pharmacodynamic effects of calcitriol. In view of the availability of chronic toxicology data from a species that is more relevant to humans (the minipig), as well as chronic toxicology data that were obtained during a topical two-year

study conducted in mice and a two-year oral study in rats (which confirmed a lack of general toxicity other than those due to the well known pharmacodynamic actions of calcitriol), I consider the total database to adequately describe the chronic toxicity of the drug substance.

Study No.: 51981 (Sponsor reference No. RDS.03.SRE.12388)

Document #, Volume #, and Page #: Mod 4, vol. 12

Conducting laboratory and location: _____

b(4)

Date of initiation of treatment: 02-JUN-2003

Date of final report: 02-MAR-2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol ointment vehicle control, batch No. 9000/006*01; Calcitriol ointment 0.3 ppm, batch No. 9001/007*01; Calcitriol ointment 1 ppm, batch No. 9002/008*01; Calcitriol ointment 3 ppm, batch No. 100/010*01; Calcitriol ointment 9 ppm, batch No. 9003/011*01, assumed 100%.

Formulation/vehicle: Calcitriol ointment; vehicle same as vehicle for clinical formulation

Methods (unique aspects):

Dosing:

Species/strain: Rat/Sprague Dawley (Tac:SPRD)

#/sex/group or time point (main study): 20/sex/group; housed 1 per cage

Satellite groups used for toxicokinetics or recovery: yes, 6/sex/group for toxicokinetics

Age: Approx. 5-6 weeks at start of dosing

Weight: At start of dosing: 77-110 g

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA). Applied once daily, 7 days per week, for 26 weeks, 2 mL/kg/day.

Doses in administered units: 0 (vehicle control), 2 mL/kg/day; 0.3 ppm, 2 mL/kg/day; 1 ppm, 2 mL/kg/day; 3 ppm, 2 mL/kg/day; 9 ppm, 2 mL/kg/day. These dosages equate to calcitriol exposures of approximately 0, 0.51, 1.7, 5.1, and 15.3 µg/kg/day (ointment density is 0.85 g/mL, meaning that 1.7 g of material was applied per kg per day), respectively, applied to the skin. The application sites were not covered or washed. The material was spread uniformly over the application site with a gloved hand and massaged into the skin until no excess was visible.

Study overview:

Group	Estimated Calcitriol Exposure (µg/kg/day)	Number/sex in Main Study	Number/sex in TK Group

1. Control (Vehicle)	0	20	6
2. Low Dose (0.3 ppm)	0.51	20	6
3. Mid Dose 1 (1 ppm)	1.7	20	6
4. Mid Dose 2 (3 ppm)	5.1	20	6
5. High Dose (9 ppm)	15.3	20	6

Observations and times:

Clinical signs: Animals observed daily for clinical signs

Skin reaction at application site: Daily just prior to application for first 4 weeks, then weekly (scale of 0-4 for erythema and edema)

Body weights: Weekly for first 13 weeks, then every 4 weeks

Food consumption: Weekly for first 13 weeks, then every 4 weeks

Ophthalmology: Baseline and termination (10/sex/group)

EKG: No

Hematology: Yes, at 3 months and termination (10/sex/group)

Clinical chemistry: Yes, at 3 months and termination (10/sex/group)

Urinalysis: Yes, at 3 months and termination (10/sex/group)

Gross pathology: All main-study animals.

Organs weighed: Adrenals, brain, epididymis, heart, kidneys, liver, ovaries, spleen, testes, thymus, uterus.

Histopathology: Standard list from all main-study animals.

Toxicokinetics: Blood samples obtained from animals in satellite groups on day 1 at 0.5, 1, 3, 6, 9, and 24 hours post-dosing (3/sex/group) and (to the extent possible) during weeks 4 and 26 at the same time points for toxicokinetic analysis.

Results:

- **Mortality:** All animals in groups 4 and 5 died or were sacrificed in extremis prior to scheduled sacrifice. All group 5 animals died by day 22. In group 4, deaths in males occurred between days 22 and 135, and in females between days 15 and 170. In group 3, unscheduled deaths were observed in both genders beginning on day 44, although the majority survived for more than 100 days, and some survived to scheduled sacrifice (2/20 males and 8/20 females in group 3 survived to scheduled sacrifice). In both genders, 1/20 animals in both groups 1 and 2 died on study (19/20 animals of both genders in both groups 1 and 2 survived to scheduled sacrifice); the death of the group 2 female, on day 178, was thought to possibly be related to treatment, while the other unscheduled deaths in groups 1 and 2 were thought to be incidental.

- **Clinical signs:** Treatment-related clinical signs (that increased in incidence and severity with increasing dosage) included:

Group 1: Small wounds due to shaving; red nodules.

Group 2: Treatment-relates signs commenced day 107, affected most animals by end of study, included thin appearance, ungroomed, inactive, reduced grip response, poor balance, abnormal gait (not all signs observed in all animals).

Group 3: Signs commenced day 18, all animals in group eventually affected, included all those seen in group 2, but greater severity. Exthalmia, passivity, no or reduced righting reflex.

Group 4: Signs commenced day 11, signs similar to group 3.

Group 5: Signs commenced day 9, primary signs thin appearance, dehydration, inactivity, group terminated day 22 for humane reasons.

- Skin reactions at treatment site: No remarkable observations.

- Body weight gain: Reduced mean weight-gain observed in all treatment groups:

Group	Estimated Calcitriol Exposure (µg/kg/day)	Approx. Mean BW Gain of Males, over Study Days 1-183 (grams)	Approx. Mean BW Gain of Females, over Study Days 1-183 (grams)
1. Control (Vehicle)	0	406.6±41.3 (n=19)	173.4±17.8 (n=19)
2. Low Dose (0.3 ppm)	0.51	301.5±55.0** (n=19)	130.1±29.5** (n=19)
3. Mid Dose 1 (1 ppm)	1.7	184.0 (n=2)	86.3±38.6** (n=8)
4. Mid Dose 2 (3 ppm)	5.1	NA (n=0)	NA (n=0)
5. High Dose (9 ppm)	15.3	NA (n=0)	NA (n=0)

**p<0.01

- Food consumption: Food consumption was reduced in groups 3-5, but was comparable to controls among group 2 animals.

- Ophthalmology: No remarkable observations.

- EKG: NA

- Hematology: Erythrocytic parameters (Hb, RBCs, HCT) were slightly, but statistically significantly, reduced in males in group 2, but were unaffected in group 2 or 3 females (data not available for group 3 males or groups 4 and 5 due to mortality).

- Clinical chemistry: At month 3, mean serum calcium was significantly elevated (circa 120% control value) in all treatment groups (statistical analysis not possible in group 5 or in group 4 females due to mortality). At month 6, calcium and phosphorus were elevated in group 2 males and in group 2 and 3 females (data not available for group 3 males or groups 4 and 5 due to mortality); no other remarkable observations.

- Urinalysis: At month 3, mean urine calcium was significantly elevated in all treatment groups (circa 15-20 times control value; statistical analysis not possible in group 5 or in group 4 females due to mortality). At month 6, urinary

volume tended to be slightly reduced, calcium was substantially elevated, and phosphorus levels largely unaffected in group 2 males and in group 2 and 3 females (data not available for group 3 males or groups 4 and 5 due to mortality):

Relevant Urine Parameters in Male Animals, 6 months

Group	Volume (mL)	Calcium Concentration (mmol/L)	Phosphorus Concentration (mmol/L)
1. Control (Vehicle)	16.5±2.6 (n=10)	0.79±0.31	42.0±10.9
2. Low Dose (0.3 ppm)	13.3±2.6 (n=9)	17.09±5.75**	48.3±21.1
3. Mid Dose 1 (1 ppm)	10.0 (n=1)	9.90	54.9
4. Mid Dose 2 (3 ppm)	NA (n=0)	NA	NA
5. High Dose (9 ppm)	NA (n=0)	NA	NA

**p≤0.01

Relevant Urine Parameters in Female Animals, 6 months

Group	Volume (mL)	Calcium Concentration (mmol/L)	Phosphorus Concentration (mmol/L)
1. Control (Vehicle)	10.2±2.3 (n=9)	2.09±0.82	40.8±7.1
2. Low Dose (0.3 ppm)	9.7±3.1 (n=10)	15.69±4.79**	44.9±13.6
3. Mid Dose 1 (1 ppm)	7.8±2.0 (n=6)	16.95±5.65**	48.8±19.4
4. Mid Dose 2 (3 ppm)	NA (n=0)	NA	NA
5. High Dose (9 ppm)	NA (n=0)	NA	NA

**p≤0.01

No other remarkable observations.

- Organ Weights: Mean organ weights among group 2 males and group 2 and 3 females tended to be reduced, apparently as a consequence of reduced body weight. Some statistically significant differences were observed in mean absolute and relative organ weights, but these appeared to be only indirectly related to treatment. Data not available for group 3 males or groups 4 and 5 due to mortality.
- Gross pathology:

Animals found dead or sacrificed in extremis: Treatment-related findings recorded in the kidneys, lungs, liver, and reproductive organs; included red discoloration, grey white foci, and small size (groups 3, 4, 5).
 Animals killed at terminal sacrifice: Observations similar to those described for early decedents, but incidence of foci in kidneys was higher.

- Histopathology: Primary finding was mineralization of various tissues and hyperostosis, which increased with increasing time of treatment and increasing dosage. Mineralization observed in kidney tubules, arterial walls, and interstitial tissue of various organs.
- Kidneys: Tubular basophilia/dilation, proteinaceous casts, cellular infiltration, urothelial hyperplasia, pelvic dilation, and interstitial fibrosis.
- Arterial walls: Mineralization, destruction of elastic fibers, dilation, granulomatous inflammation, fibrosis.
- Tracheal mucosa and respiratory epithelium: Mineralization, granulomatous inflammation, replacement of epithelial cells with squamous cells.
- Bone: Hyperostosis (increased growth and ossification) of all bones examined (sternum, femur, patella and vertebrae). Increased trabecular bone with consequently reduced marrow space was particularly pronounced in sternum. Mineralization, ossification, and deformity of cartilage in trachea, larynx, and articular cartilage.
- Lungs: Mineralization and granulomatous inflammation of interstitial tissue of terminal bronchioles. Alveolar macrophage accumulation and edema.
- Non-glandular stomach: Diffuse hyperplasia and hyperkeratosis.
- Skin at application site: Diffuse hyperplasia and hyperkeratosis.
- Thyroid: Hyperplasia of C-cells.
- Liver: Cellular infiltration, extramedullary hematopoiesis, single cell necrosis, hyaline droplets (groups 2 and 3, terminal sacrifice).
- Spleen: Extramedullary hematopoiesis (groups 2 and 3, terminal sacrifice).
- Adrenal medulla: Focal hyperplasia (groups 2 and 3, terminal sacrifice).
- Parathyroid: Diffuse condensation and increased basophilia of nuclei.
- Vagina/cervix/mammary gland: Fewer females in estrus/increased mucous secretion of epithelium, atrophy of mammary tissue, considered sign of reduced estrogen level, possibly secondary to weight loss (groups 2 and 3, terminal sacrifice).
- Toxicokinetics: The submission lists group mean pharmacokinetic parameters for day 1, and limited data for day 24. Apparently data from males and females were combined, and data from individual animals are not included. The following values were calculated for day 1:

Group (ppm)	C _{max} (ng/mL)	t _{max} (hr)	AUC (hr·ng/mL)	t _{1/2} (hr)
2 (0.3 ppm)	0.266	1	1.17	3.67
3 (1 ppm)	0.685	1	4.58	3.07
4 (3 ppm)	4.11	1	16.1	2.71
5 (9 ppm)	10.6	1	41.3	1.98

These data, limited to day 1 of treatment, have little utility in the interpretation of chronic toxicology data, but are captured here for completeness. These data suggest that systemic exposure to calcitriol increased with increasing dose.

2.6.6.3.8 Study Title: Calcitriol ointment - A 9 month dermal toxicity study in minipigs

Key study findings: Ointments that contained up to 15 ppm calcitriol were applied to minipigs for 9 months. The materials were well tolerated. Signs of toxicity (primarily observed only in the highest dosage group) included reduced mean weight gain (females only), slightly reduced erythrocytic parameters (significant in females only), increased mean weight of the kidney, and, histologically, evidence of inflammation of the kidneys (females at 9 ppm and 15 ppm and males at 15 ppm), hypertrophy of the zona glomerulosa of the adrenals (females at 15 ppm only), and hyperostosis of bone (females at 15 ppm only). Under the conditions of this study ointment that contained 3 ppm calcitriol (approximately 5.1 µg/kg/day) was a NOAEL, and 9 ppm ointment (approximately 15.3 µg/kg/day) induced only slight toxicity.

Study No.: 51104 (sponsor reference No. RDS.03.SRE.12394)

Document #, Volume #, and Page #: Mod 4, vol. 21

Conducting laboratory and location

b(4)

Date of study initiation: Treatment of animals initiated 24-FEB-2004

Date of study completion: 05-AUG-2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol placebo ointment (0 ppm), Lot 90000/011*03; calcitriol ointment 1 ppm, Lot 9002/062*03; calcitriol ointment 3 ppm, Lot 0100/056*03; calcitriol ointment 9 ppm, Lot 9003/057*03; calcitriol ointment 15 ppm, Lot 9035/063*03; 100%.

Formulation/vehicle:

<u>Compound</u>	<u>Amount</u>
Calcitriol.....	1, 3, 9, or 15 ppm
White soft paraffin.....	_____ % w/w _____
Liquid paraffin.....	_____ % w/w
α-Tocopherol.....	_____ % w/w

b(4)

Methods (unique aspects):

Dosing:

Species/strain: Gottingen SPF minipigs
#/sex/group or time point (main study): 4/sex/group; housed 1 per pen
Satellite groups used for toxicokinetics or recovery: No
Age: At least 3 months at start of dosing

Weight: At start of dosing: Approx. 5.6-9.6 kg

Doses in administered units: 0 (vehicle control); 1 ppm; 3 ppm; 9 ppm; 15 ppm.

These dosages equate to approximately 0, 1.7, 5.1, 15.3, 25.5 µg/kg/day (2 mL/kg/day applied, ointment density is 0.85 g/mL) applied to the skin, although it is unclear how much calcitriol was removed and discarded when the dressing was removed and the site washed.

Route, form, volume, and infusion rate: Topical, 2 mL/kg/day, once per day for approximately 9 months. The assigned material was applied to shaved skin on the back (approximately 10% of the body surface area) and gently rubbed into the treatment site for 30-60 seconds. The treated area was covered with a non-occlusive dressing for 6 hours, 7 days per week. At the end of the treatment period each day the site was washed with mild soap.

Study overview:

Group	Estimated Calcitriol Exposure (µg/kg/day)	Number/sex
1. Control (Vehicle)	0	4
2. Low Dose (1 ppm)	1.7	4
3. Mid Dose 1 (3 ppm)	5.1	4
4. Mid Dose 2 (9 ppm)	15.3	4
5. High Dose (15 ppm)	25.5	4

Observations and times:

Clinical signs: Animals observed daily for clinical signs

Skin reactions at application site: observed daily for first 4 weeks, then weekly (erythema and edema rated on scale of 0-4)

Body weights: Weekly

Food consumption: Daily

Ophthalmology: Yes, baseline and termination

EKG: Yes, baseline and termination

Hematology: Yes, baseline and at weeks 13 and 26 and at termination

Clinical chemistry: Yes, baseline and at weeks 13 and 26 and at termination

Urinalysis: Yes, baseline and at weeks 13 and 26 and at termination

Gross pathology: All animals.

Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid, uterus.

Histopathology: Standard list from all animals in groups 1 and 5 (control and high-dose), plus skin from the treatment site and untreated site, kidneys, and gross

lesions from all groups, plus all tissues that were considered abnormal in group 5 animals ("target tissues") were examined from all intermediate groups; this included the trachea, lung, glandular stomach, prostate, adrenals, sublingual gland, submandibular gland, thoracic vertebrae, bone, and sternum.

Toxicokinetics: Blood samples drawn from all animals on day 1, in week 13, and at termination pre-treatment and at 1, 3, 6, 9, and 24 hours post-application for toxicokinetic analysis.

Results:

- Mortality: No unscheduled deaths; all animals survived to scheduled sacrifice on day 274.
- Clinical signs: No remarkable observations in groups 1-4. One male and one female in group 5 exhibited signs that may have been related to treatment, including reduced appetite, thinness, passivity, and pain upon palpitation of the abdomen.
- Skin reactions at treatment site: Occasional erythema and edema (grade 1-2) observed in all groups (including control); erythema (but not edema) more severe in group 5 (7 instances of grade 3 and 2 instances of grade 4).
- Body weight gain: Mean weight gain was significantly reduced in group 5 females; no effect in males:

Group	Estimated Calcitriol Exposure (µg/kg/day)	Approx. Mean BW Gain of Males, over Study Days 1-274 (kg)	Approx. Mean BW Gain of Females, over Study Days 1-274 (kg)
1. Control (Vehicle)	0	15.5±2.4	18.6±2.8
2. Low Dose (1 ppm)	1.7	15.9±2.7	15.7±2.1
3. Mid Dose 1 (3 ppm)	5.1	12.7±3.4	15.6±2.5
4. Mid Dose 2 (9 ppm)	15.3	16.7±2.4	16.7±1.6
5. High Dose (15 ppm)	25.5	15.8±3.2	11.4±2.6**

**p<0.01

- Food consumption: The one male and one female in group 5 that exhibited clinical signs (see above) ate less than 100% of the offered food during the periods when those signs were observed; no other remarkable observations.
- Ophthalmology: No remarkable observations
- EKG: No remarkable observations
- Hematology: Group 5 animals exhibited a small reduction in erythrocytic parameters (Hb, RBC, HCT) at week 39 (but not week 13 or 26); the difference

achieved statistical significance in females only. Small but statistically significant increases in eosinophil levels observed in group 5 animals (both genders) at some time points. It is unclear if these effects were genuine or incidental. No other remarkable observations.

- **Clinical chemistry:** At week 39, serum calcium appeared to be somewhat elevated in group 5 females (3.7 ± 0.55 mmol/L, compared to 2.88 ± 0.07 for control females), although the difference was not statistically significant. No other remarkable observations.

- **Urinalysis:** Statistically significant differences were observed in some parameters at some time points, but the data do not appear to indicate any effects that were clearly related to treatment.

- **Organ Weights:** The mean absolute weight of the kidneys was significantly increased ($p < 0.05$) in both males and females in group 5 (146.5 ± 31.68 versus 100.5 ± 6.57 in controls and 147.6 ± 31.49 versus 101.7 ± 10.22 in controls in males and females, respectively, mean weight in $g \pm SD$). No other remarkable observations.

- **Gross pathology:** Discoloration (paleness) of the kidneys was observed in three group 5 females and one female from group 4.

- **Histopathology:** Treatment-related changes were observed in groups 4 and 5; most of the effects were graded minimal to moderate, and tended to be more common in females.

The observations included:

Kidneys: Increased incidence of fibrosis, tubular dilation, tubular basophilia, inflammatory cell infiltration, and focal mineralization (group 4 and 5 females and group 5 males).

Adrenal cortex: Hypertrophy of the zona glomerulosa of the adrenals (minimal to slight), group 5 females only.

Bone: Hyperostosis of the femur, sternum, and vertebrae; observed in 2/4 group 5 females only (one group 5 female graded slight, one graded marked).

Other: One group 5 female exhibited cystic granulomatous inflammation (including mineralization) of the stomach and cystic dilated ducts of the mammary gland; it is unclear if these lesions were related to treatment. No other remarkable observations.

- **Toxicokinetics:** The concentration of calcitriol in most of the samples obtained on day 267 were below the LOQ (50 pg/mL). Therefore, useful TK statistics could not be calculated.

2.6.6.4 Genetic toxicology

2.6.6.4.1 Study title: Calcitriol: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre fluctuation technique.

Key findings: Calcitriol was not mutagenic under the conditions of this assay.

Study No: 1867/30

Study Type: In vitro point mutation assay

Volume # and Page #: Mod 4, vol. 23

Conducting Laboratory: _____

b(4)

Date of Study Initiation: 25-MAR-2002

GLP Compliance: Yes

QA Reports Yes (X) No ()

Drug, lot #, radiolabel, and % purity: Calcitriol, lot PA2027J0151, 101%

Formulation/vehicle: Dissolved in DMSO at concentrations up to 250 mg/mL

Methodology:

- Strains/Species/Cell line: L5178Y tk⁺ mouse lymphoma cells
 - Dose Selection Criteria: Cytotoxicity
 - Basis of dose selection: Cytotoxicity in range-finding studies
 - Range finding studies: Examined concentrations of calcitriol in culture medium ranging from 1.25 to 2500 µg/mL, with and without S9
 - Test Agent Stability: Chemical analysis of the test material formulations used in this study were apparently not performed, but test article solutions were used within 1.5 hours of preparation.
 - Metabolic Activation System: Aroclor 1254-induced S9 (supernatant of the post-mitochondrial 9000 g fraction from adult male SD rats)
 - Controls:
 - Vehicle: DMSO in culture medium
 - Negative Controls: Vehicle
 - Positive Controls: 4-nitroquinoline-1-oxide in absence of S9; benzo(a)pyrene in presence of S9
 - Comments: Controls were adequate
 - Exposure Conditions:
 - Incubation and sampling times: 3 hour exposure with S9, 24 hours without S9
 - Doses used in definitive study: 0 µg/mL-60 µg/mL
 - Study design: Following the exposure period, the cells were washed and grown in the presence of TFT (which screens for tk⁺ mutations)
 - Analysis:
 - No. of replicates: Two
 - Counting method: "Identified by eye using background illumination and counted"
 - Criteria for positive results: Mutation frequency at one or more concentrations significantly greater than negative control value and significant dose-relationship observed.
- Summary of individual study findings:
- Study Validity: Acceptable

- Study Outcome: Calcitriol did not increase the incidence of cell survival (colony formation) in medium that contained TFT in either the presence or absence of S9. Appropriate results were obtained with the controls.

2.6.6.5 Carcinogenicity

2.6.6.5.1 Study title: 12-month topical study to determine the influence of calcitriol on photocarcinogenesis in hairless mice

Key study findings: The median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed was slightly, but statistically significantly, reduced for both males and females that were treated with the vehicle of the product, relative to untreated animals. Materials that contained calcitriol did not reduce latency to formation of the first tumor greater than or equal to 1.0 mm in diameter relative to vehicle alone. These data suggest that the vehicle of calcitriol ointment slightly enhances UV-induced skin tumor formation (possibly by enhancing UV penetration into the skin), but calcitriol per se does not enhance photo-induced carcinogenesis.

Study No.: 1207-018 (sponsor reference No. RDS.03.SRE.12333)

Document #, Volume #, and Page #: Mod 4, vol. 24

Conducting laboratory and location: _____

b(4)

Date of study initiation: 08-AUG-2003

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol ointment vehicle (control), batch Nos. 9000/006*01 and 9000/011*03; calcitriol ointment 0.3 ppm, batch Nos. 9001/007*01 and 9001/053*03; calcitriol ointment 0.6 ppm, batch Nos. 9032/012*03 and 9032/054*03; calcitriol ointment 1.0 ppm, batch Nos. 9002/008*01 and 9002/062*03, 100% potency.

Formulation/vehicle:

<u>Compound</u>	<u>Amount</u>
Calcitriol.....	0, 0.3, 0.6, or 1 ppm
White soft paraffin.....	_____ % w/w (varies slightly)
Liquid paraffin.....	_____ % w/w
α-Tocopherol.....	_____ % w/w

b(4)

Methods (unique aspects):

Dosing:

Species/strain: Mouse — SKH1-hrBR (albino hairless)
#/sex/group or time point (main study): 36/sex/group; housed 1 per cage
Satellite groups used for toxicokinetics or recovery: No
Age: Approximately 60 days at initiation

b(4)

Weight: At start of dosing: males, 24-34 g, females, 20-27 g

Doses in administered units:

Group	Approximate Calcitriol Exposure (µg/kg/day)*	Calcitriol Ointment Concentration (ppm)	Volume of Test Material Applied Per Day (µL/mouse)	UVR Exposure (RBU/Week)
1	0	NA**	0	600
2	0	0	25	600
3	0.3	0.3	25	600
4	0.6	0.6	25	600
5	1.0	1.0	25	600
6	0	NA**	0	1200

*Approximate, based upon assumed BW of 25 g, without correction for density of ointment.

**No test material applied to these animals.

The test materials were applied, and the mice irradiated, five days per week (M-F) for up to 40 weeks. The test materials were applied prior to UVR exposure on Mondays, Wednesdays, and Fridays, and following UVR exposure on Tuesdays and Thursdays. The UVR exposure was 120 RBU per day (600 RBU per week) for all groups except group 6, which received 240 RBU per day (1200 RBU per week). UV light was generated by a 6.5 kW xenon long arc lamp with a 1 m^m filter and with definitive output in both the UVA (320 nm to 400 nm) and UVB (280 nm to 320 nm) ranges.

b(4)

All surviving animals were maintained for 12 weeks without treatment (or UVR exposure) following 40 weeks of treatment, with sacrifice during week 52. Mice were sacrificed prematurely if a skin tumor ≥ 10 mm diameter was present. All mice in a given dosage/gender group were killed: a) when survival in that group reached greater than 50%; and b) if more than 50% of the surviving mice had tumors ≥ 4 mm diameter.

Route, form, volume, and infusion rate: Topical, 25 µL/day (see above), once per day M-F for 40 consecutive weeks. The assigned material was applied to the back and sides (approximately 25 cm²) of the mice and distributed with a glass rod.

Observations and times:

Clinical signs: Animals observed twice daily for viability and weekly for general appearance. Clinical signs and local skin reactions (including skin tumors) weekly.

Body weights: Weekly

Food consumption: No

Ophthalmology: No

EKG: No

Hematology: NA

Clinical chemistry: No
 Urinalysis: No
 Gross pathology: All animals
 Organs weighed: None
 Histopathology: No
 Toxicokinetics: No

Results:

- Duration of treatment: Due to treatment-induced toxicity not related to skin tumors, treatment of mice at dosages of 0.6 and 1.0 ppm was stopped prior to week 40, although exposure to UVR was continued. For group 5 (1.0 ppm) animals of both genders, treatment was stopped in week 27. One group 5 male was sacrificed moribund in week 27, and 6 female mice were sacrificed or found dead during weeks 22-27. After this stoppage, and additional 8 group 5 males and 7 females were either found dead or sacrificed for reasons not related to tumor burden between weeks 33-50. The remaining group 5 mice (27 males, 23 females) were sacrificed after meeting the tumor burden criteria. In group 4 (0.6 µg/kg/day), test-article application was stopped during week 35. In or before week 35, 4 males and 3 females in group 4 were sacrificed moribund or found dead due to toxicity not related to tumor burden. After stoppage in week 35, 4 group 4 males and 3 females were sacrificed due to toxicity not related to skin tumors. The remaining group 4 mice (28 males and 30 females) were sacrificed after meeting the tumor burden criteria.

- Mortality: Mortality data are summarized below:

Mortality data for males:

Group	1	2	3	4	5	6
Calcitriol Concentration (ppm)	Untreated	0.0 (Vehicle)	0.3	0.6	1.0	Untreated
UVR Exposure (RBU/Week)	600	600	600	600	600	1200
Found Dead	2	0	3	3	2	1
Moribund Sacrifice (not related to tumor burden)	0	1	2	5**	7**	1
Sacrificed Due to Individual Tumor Burden	13	19	23**	19	21**	32**
Sacrificed Due to Group Tumor Burden	0	16**	8**	9**	6*	2

*p<0.05; **p<0.01 compared to group 1.

Mortality data for females:

Group	1	2	3	4	5	6
Calcitriol Concentration (ppm)	Untreated	0.0 (Vehicle)	0.3	0.6	1.0	Untreated
UVR Exposure (RBU/Week)	600	600	600	600	600	1200
Accidental Death	0	0	1	0	0	0
Found Dead	1	2	0	1	3	0
Moribund Sacrifice (not related to tumor burden)	2	3	5	5	10**	2
Sacrificed Due to Individual Tumor Burden	18	17	16	14	9	21
Sacrificed Due to Group Tumor Burden	0	14**	14**	16**	14**	13**

**p<0.01 compared to group 1.

Survival was greater than 90% in groups 1-5 through week 32, and in group 6 until week 28. All surviving mice in group 6 sacrificed in week 35 due to group tumor burden criteria. All surviving mice in groups 2, 3, 4, and 5 sacrificed in weeks 48, 52, 51, and 50, respectively, due to group tumor burden criteria. Surviving mice in group 1 did not reach criteria for group sacrifice, and were sacrificed in week 53.

- **Clinical signs:** Adverse signs were observed more frequently with increasing exposure to calcitriol, and included emaciation, vocalization, reduced motor activity, and rapid respiration.
- **Skin reactions:** Erythema, edema, and flaking were observed with greater frequency in group 6 animals compared to other groups (due to greater UV exposure), but did not differ between groups 1-5. It was concluded that the test materials did not induce or enhance these skin reactions.
- **Body weights:** Mean body weights and weight gains tended to be slightly reduced with increased exposure to calcitriol, in some instances achieving statistical significance.
- **Gross pathology:** No remarkable observations, with exception of skin tumors (see below).
- **Tumor data analysis:** The median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed was significantly reduced for both genders in group 6 relative to group 1 (untreated animals at 1200 RBU/week compared to untreated animals at 600

RBU/week), confirming that latency to formation of skin tumors decreased with increasing exposure to UVR. The median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed was significantly reduced for both genders in group 2 (vehicle treated animals) relative to group 1, indicating that the vehicle of the product slightly enhanced URV-induced skin tumor development. The median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed did not differ significantly when comparing either gender in groups 3, 4, or 5 (calcitriol plus vehicle) to group 2 (vehicle alone), indicating that material that contained calcitriol did not enhance URV-induced skin tumor development beyond the effect of the vehicle.

Median Number of Weeks on Study at Which First Tumor \geq 1 mm Diameter was Observed:

Group	Calcitriol Concentration (ppm)	UVR Exposure (RBU/Week)	Median Week to Tumor \geq 1 mm, Males	Median Week to Tumor \geq 1 mm, Females	Median Week to Tumor \geq 1 mm, Sexes Combined
1	Untreated	600	40.00	38.00	39.00
2	0 (Vehicle)	600	36.50**	34.00**	36.00**
3	0.3	600	35.00	36.00	35.50
4	0.6	600	33.50	36.00	34.50
5	1.0	600	34.00	40.00	36.00
6	Untreated	1200	22.00*	23.5*	22.50*

*p<0.001 compared to group 1.

**p<0.05 or p<0.01 compared to group 1.

The median time to first tumor greater than or equal to 1 mm did not differ between genders.

2.6.6.5.2 Study title: Calcitriol - 104 week oral (gavage) carcinogenicity study in the rat

Key study findings: A two-year topical carcinogenicity study was conducted in which calcitriol solution was orally administered (via gavage) daily to rats. Dosages of approximately 0.005, 0.03, and 0.1 $\mu\text{g}/\text{kg}/\text{day}$ were investigated. The vehicle for the test material was Neobee oil M5. Neobee oil M5 is a medium chain triglyceride.

The study included both a vehicle-treated control group and a second control group which received water. The protocol for the study, including the dosages used, was discussed by the executive CAC on April 8, 2003; dosage selection was based upon the MTD. Survival rates did not differ significantly between groups; terminal sacrifice of all groups occurred following 104 weeks of

b(4)

treatment. The incidence of benign pheochromocytomas was significantly increased in female rats (pairwise p-value of 0.0001; trend value of 0.0036). No other tumor incidence data differed according to the Haseman-Lin-Rahman criteria.

Adequacy of the carcinogenicity study and appropriateness of the test model: This study was discussed by the executive carcinogenicity assessment committee on 27-MAY-2008. The committee found the study to be valid in all respects, including the dosages that were evaluated.

Evaluation of tumor findings: The Committee found that oral administration of calcitriol for a lifetime resulted in an increased incidence of benign pheochromocytomas in female rats. No evidence of carcinogenesis was observed in male rats.

Study no.: 913/119 (sponsor reference No. RDS.03.SRE.12318)

Document #, Volume #, and Page #: Mod 4, vol. 39

Conducting laboratory and location: _____

Date of study initiation: 21-AUG-2003

Date of study completion: 15-JUN-2006

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Calcitriol, manufacturer batch Nos. WKH01B197; WVC03C197; WKH03F047; approximately 100% purity

CAC concurrence: The protocol for the study was discussed by the exec-CAC on 08-APR-2003. The sponsor proposed oral (gavage) administration of calcitriol to the rat at exposure levels of 0 (water control), 0 (vehicle control), 0.005, 0.025, and 0.1 µg/kg/day. The committee recommended dosages of 0.005, 0.03, and 0.1 µg/kg/day for both males and females.

b(4)

Methods

Study overview/doses administered:

Group	Group Designation	No. of Animals of Each Gender in Main Study	No. of Satellite Animals of Each Gender	Calcitriol Dosage (µg/kg/day)
1	Water Control	60	10	0 (Received Water)
2	Low-Dose	60	10	0.005
3	Mid-Dose	60	10	0.03
4	High-Dose	60	10	0.1
5	Vehicle Control	60	10	0 (Received vehicle)

Basis of dose selection (MTD, MFD, AUC etc.): MTD

Species/strain: Rat/Wistar ~~WI~~ (IOPS AF/Han)

Number/sex/group (main study): 60

Route, formulation, volume: Oral (gavage); Test article dissolved in Neobee oil M5. Neobee M5 is a medium chain triglyceride.

b(4)

b(4)

_____ respectively; approximately 2 mL/kg/day (dosing volume adjusted to latest BW)

Frequency of dosing: Once daily

Satellite groups used for toxicokinetics or special groups: Yes, 10/sex/group used for clinical chemistry and TK analysis with radiolabeled calcitriol

Age: Approx. 5-6 weeks at initiation

Weight: At start of dosing: males, 124-185 g; females, 108-161 g

Animal housing: Groups of 5 per cage

Restriction paradigm for dietary restriction studies: No

Drug stability/homogeneity: Acceptable

Dual controls employed: No

Interim sacrifices: No

Deviations from original study protocol: None remarkable.

Observation times

Mortality: Twice daily

Clinical signs: Observed daily; full exam once every 4 weeks to week 25, weekly thereafter

Body weights: Weekly for first 16 weeks, then monthly

Food consumption (per cage): Weekly for first 16 weeks, then over a one-week interval every 4 weeks thereafter

Hematology: Yes, all main-study animals at termination

Clinical chemistry: Yes, satellite animals only; measured calcium and inorganic phosphorus at week 50

Urinalysis: Yes, satellite animals only; measured calcium and inorganic phosphorus at week 50

Gross pathology: Yes

Organs weighed: None

Histopathology: A standard list of tissues from all animals in groups 1 (water control), 5 (vehicle control), and 4 (high-dose) killed at terminal sacrifice, plus all main-study animals found dead or killed moribund, plus gross lesions from all main-study animals, plus thyroid, stomach, kidneys, aorta, heart, adrenals, and sternum from all main study animals in groups 2 (low-dose) and 3 (mid-dose).

Toxicokinetics: Data from a 13-week study indicated that plasma levels of calcitriol were below the limit of detection (200 pg/mL). Therefore, systemic exposures in the two-year study were estimated through use of ³H-calcitriol.

Animals in the satellite groups were treated on day 398 with materials that contained radiolabeled calcitriol (radiochemical purity 99.0%; 2.17 µCi administered per animal) in addition to 0, 0.005, 0.03, or 0.1 µg/kg of non-labeled calcitriol. Blood samples were obtained from 4 animals/sex/group at 1, 3, 8, 12,

24, and 72 hours post-dosing (control animals at 8 hr only). Activity/unit of plasma was determined using a scintillation counter (subtracting the background value of the control samples); the respective plasma concentrations of calcitriol were estimated, and the data used to calculate C_{max} , T_{max} , and AUC_{0-24h} values. Other: None

Results

Mortality: The survival data are presented below; survival rates did not differ significantly between groups, regardless of whether the water-control data were included in the analyses, although data from females approached statistical significance (please see the associated Biostatistics review for details).

Survival Rates of Rats at Terminal Sacrifice (Day 728)

Group Number	Group Designation	Dosage Level ($\mu\text{g}/\text{kg}/\text{day}$)	Number of Males that Survived to the Terminal Sacrifice	Number of Females that Survived to the Terminal Sacrifice
1	Water Control	0	43/60	33/60
2	Low-Dose	0.005	39/60	39/60
3	Mid-Dose	0.03	34/60	36/60
4	High-Dose	0.1	36/60	27/60
5	Vehicle Control	0	44/60	41/60

Clinical signs: No remarkable differences between groups, including no differences in the incidence of palpable masses.

Body weights: Mean BW gain over the course of the study was significantly reduced in males and females at 0.1 $\mu\text{g}/\text{kg}/\text{day}$ (in comparison to water control group). Mean body weight-gain data are summarized below:

Appears This Way
On Original

Group Number	Group Designation	Dosage Level ($\mu\text{g}/\text{kg}/\text{day}$)	Approx. Mean BW Gain of Males, over Study Days 1-728 (grams)	Approx. Mean BW Gain of Females, over Study Days 1-728 (grams)
1	Water Control	0	515.2 \pm 86.1	274.0 \pm 63.8
2	Low-Dose	0.005	498.8 \pm 72.0	294.4 \pm 55.8
3	Mid-Dose	0.03	479.1 \pm 77.6	272.5 \pm 53.4
4	High-Dose	0.1	457.8 \pm 69.8**	237.5 \pm 35.3**
5	Vehicle Control	0	498.1 \pm 84.3	311.6 \pm 67.7*

* $p < 0.05$; ** $p < 0.01$ in comparison to water control

Food consumption: Food consumption tended to be slightly (approximately 10%) reduced in treated groups and in the vehicle control group relative to the water control group. This was presumably a consequence of the nutritional contribution of the vehicle (Neobee oil).

Hematology: No remarkable observations.

Blood chemistry: At week 50, mean serum calcium and phosphate levels were slightly, but statistically significantly, increased in both genders at 0.03 $\mu\text{g}/\text{kg}/\text{day}$ and above:

Selected Blood Chemistry Parameters, Male Rats, week 50 (n=10):

Group Number	Group Designation	Dosage Level ($\mu\text{g}/\text{kg}/\text{day}$)	Calcium (mmol/L)	Phosphorus (mmol/L)
1	Water Control	0	2.69 \pm 0.07	1.78 \pm 0.17
2	Low-Dose	0.005	2.67 \pm 0.27	1.83 \pm 0.16
3	Mid-Dose	0.03	2.83 \pm 0.06***	2.14 \pm 0.24***
4	High-Dose	0.1	2.82 \pm 0.09**	2.16 \pm 0.16***
5	Vehicle Control	0	2.74 \pm 0.06	1.77 \pm 0.23

** $p < 0.01$; *** $p < 0.001$ in comparison to water control

Appears This Way
On Original

Selected Blood Chemistry Parameters, Female Rats, week 50 (n=10):

Group Number	Group Designation	Dosage Level ($\mu\text{g}/\text{kg}/\text{day}$)	Calcium (mmol/L)	Phosphorus (mmol/L)
1	Water Control	0	2.77 \pm 0.06	1.66 \pm 0.28
2	Low-Dose	0.005	2.76 \pm 0.06	1.62 \pm 0.18
3	Mid-Dose	0.03	2.90 \pm 0.07**	1.95 \pm 0.17*
4	High-Dose	0.1	2.86 \pm 0.07*	1.98 \pm 0.23*
5	Vehicle Control	0	2.77 \pm 0.11	1.59 \pm 0.24

*p<0.05; **p<0.01 in comparison to water control

Urinalysis: At week 50, mean urinary excretion of calcium and phosphate (concentrations adjusted for urine volume) were increased in both genders at 0.03 $\mu\text{g}/\text{kg}/\text{day}$ and above (values indicate mmoles of calcium or phosphorus excreted during a 16 hour collection period); differences in phosphate levels not statistically significant in females:

Excretion of Calcium and Phosphorus, Male Rats, week 50 (n=10):

Group Number	Group Designation	Dosage Level ($\mu\text{g}/\text{kg}/\text{day}$)	Calcium (mmol)	Phosphorus (mmol)
1	Water Control	0	0.03 \pm 0.02	0.28 \pm 0.07
2	Low-Dose	0.005	0.06 \pm 0.03*	0.24 \pm 0.12
3	Mid-Dose	0.03	0.14 \pm 0.05***	0.46 \pm 0.11**
4	High-Dose	0.1	0.22 \pm 0.05***	0.50 \pm 0.16***
5	Vehicle Control	0	0.03 \pm 0.02	0.27 \pm 0.09

*p<0.05; **p<0.01; ***p<0.001 in comparison to water control

Excretion of Calcium and Phosphorus, Female Rats, week 50 (n=10):

Group Number	Group Designation	Dosage Level ($\mu\text{g}/\text{kg}/\text{day}$)	Calcium (mmol)	Phosphorus (mmol)
1	Water Control	0	0.07 \pm 0.02	0.38 \pm 0.12
2	Low-Dose	0.005	0.11 \pm 0.03*	0.43 \pm 0.10
3	Mid-Dose	0.03	0.22 \pm 0.08***	0.52 \pm 0.18
4	High-Dose	0.1	0.24 \pm 0.08***	0.45 \pm 0.10
5	Vehicle Control	0	0.06 \pm 0.02	0.32 \pm 0.12

*p<0.05; ***p<0.001 in comparison to water control

Gross pathology: No remarkable data.

Histopathology:

Non-neoplastic: A number of observations were made that were apparently related to the pharmacodynamic actions of calcitriol; in most instances statistically significant trends toward increasing incidence and/or severity with increasing dosage were apparent. These included:

Mineral deposition: The incidence of observations of mineral deposits in several tissues increased (roughly) with increasing dosage of calcitriol, including the aorta and other major vessels, the stomach and duodenum, eyes, and kidneys.

Bone growth: Increased number and thickness of trabeculae of the sternum and femur were observed with increasing dosage; data concerning the sternum are summarized below:

Sternum group	Males					Females				
	1(*)	2	3	4	5(**)	1(*)	2	3	4	5(**)
Number examined	60	60	60	60	60	60	60	60	58	59
Increase in trabecular bone	0	10	31	57	0	1	8	38	58	0
Chondromucoid degeneration	0	0	0	4	0	0	0	0	1	0
Chondrodystrophy	0	0	0	0	0	2	1	1	1	0

Atrophy of the parathyroid was observed with increasing incidence at 0.03 and 0.1 µg/kg/day. This presumably was related to elevated calcium.

Additional observations made with increased incidence in high-dose animals included myocardial fibrosis (males), interstitial nephritis (females), and extramedullary hematopoiesis in the spleen.

Neoplastic (See associated Biostatistics review of NDA 22-087 for complete information): The incidence of benign pheochromocytomas was significantly increased in female rats (pairwise p-value of 0.0001; trend value of 0.0036). These data are summarized below:

Rat Females	W	V	L	M	H	Pair	Trend
ADRENAL GLANDS							
Benign pheochromocytoma,	0	0	0	2	7	0.0001	0.0036

No other tumor incidence data differed according to the Haseman-Lin-Rahman criteria.

Toxicokinetics: Mean pharmacokinetic parameters, based upon data from rats treated with radiolabeled calcitriol during week 57, indicated that systemic exposure to calcitriol increased roughly in proportion to the quantity administered. The data are summarized below:

Dose (µg/kg)	Sex	C _{max} (pgEq./g)	T _{max} (h)	AUC _(0-72h) (pgEq.h/g)
0.005	Male	24.49	8	815
	Female	18.31	8	631
	Ratio M/F	1.34	NA	1.29
0.03	Male	85.27	8	3449
	Female	80.68	3	2554
	Ratio M/F	1.06	NA	1.35
0.1	Male	204.38	3	8459
	Female	183.09	3	6747
	Ratio M/F	1.12	NA	1.25

2.6.6.5.3 Study title: Calcitriol ointment - 104 week dermal carcinogenicity study in the mouse

Key study findings: A two-year topical carcinogenicity study was conducted in which calcitriol ointment was applied to the skin of mice. Materials that contained calcitriol at concentrations of 0 (vehicle), 0.3, 0.6, and 1.0 ppm were evaluated. The vehicle for the test material was identical to the vehicle of Silkis ointment (NDA 22-087). The protocol for the study, including the test materials to be used, was discussed by the executive CAC on April 8, 2003. Dosage selection was based upon the estimated MTD. The MTD was exceeded in the study. Because of reduced mean weight gain and treatment-related deaths, treatment was suspended for several weeks beginning week 23 and week 29 for groups receiving 0.6 ppm and 1.0 ppm materials, respectively. Treatment of all groups (including those receiving vehicle and 0.3 ppm calcitriol) subsequently was changed to a frequency of three applications per week. Due to reduced survival, and upon recommendation from the executive CAC, males receiving 1.0 ppm calcitriol were sacrificed during week 97 and all groups of females were sacrificed during study week

101. No statistically significant differences in tumor incidence were observed in this study.

Adequacy of the carcinogenicity study and appropriateness of the test model: This study was discussed by the executive carcinogenicity assessment committee on 27-MAY-2008. The committee found the study to be valid in all respects, including the dosages that were evaluated.

Evaluation of tumor findings: The Committee found that no evidence of potential to induce carcinogenesis was obtained in this study, in which calcitriol was applied to the skin of male and female mice over a lifetime.

Study No.: 913/118 (sponsor reference No. RDS.03.SRE.12299)

Document #, Volume #, and Page #: Mod 4, vol. 26

Conducting laboratory and location: _____

Date of study initiation: 14-AUG-2003

Date of study completion: 07-JUN-2006

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol ointment vehicle (control), batch 006*01 and 011*03; calcitriol ointment 0.3 ppm, batch No. 007*01 and 053*03; calcitriol ointment 0.6 ppm, batch No. 012*03 and 054*03; calcitriol ointment 1 ppm, batch No. 008*01 and 062*03; assumed to be 100% pure.

Formulation/vehicle: Calcitriol ointment; vehicle same as vehicle for clinical formulation

b(4)

Methods (unique aspects):

Dosing:

Species/strain: Mouse — CD-1 (ICR) BR

#/sex/group or time point (main study): 60/sex/group

Satellite groups used for toxicokinetics or recovery: Yes (15/sex/group)

Age: Approximately 6 weeks at initiation

Weight: At start of dosing: males, 26-36 g; females, 20-28 g

Study overview/doses administered:

b(4)

Group	No. of Animals of Each Gender in Main Study	No. of Satellite Animals of Each Gender	Calcitriol Exposure (µg/kg/day)*	Calcitriol Ointment Concentration (ppm)	Volume of Test Material Applied Per Day (µL/mouse)
1	60	15	0 (vehicle treated)	0	25
2	60	15	0.3	0.3	25
3	60	15	0.6	0.6	25

4	60	15	1.0	1.0	25
---	----	----	-----	-----	----

*Approximate, based upon assumed BW of 25 g. Note: These were the dosages originally applied per day. Subsequently in the study (see below), the sponsor reduced the dosing frequency to the same dosages, but only three times per week, due to tolerance issues.

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA). Initially applied once daily, 7 days per week. As detailed below, the sponsor reduced the dosing frequency to three times weekly beginning week 23 to 29.

Exec-CAC concurrence/Deviations from original protocol: A draft protocol for this study was discussed by the exec-CAC on 08-APR-2003. The sponsor proposed topical application of several concentrations of calcitriol in ointment base to the skin of mice. As proposed, the study would involve test materials that contained 0 (vehicle control), 0.3 ppm, 0.6 ppm, and 1 ppm calcitriol, applied at a rate of 25 μ L/day, which would equate to exposures of approximately 0.3, 0.6, and 1 μ g/kg/day if a body weight of 25 g was assumed. The proposed study involved application seven days per week for up to 104 weeks. The committee concurred with the dosages. The sponsor initiated the study as outlined. However, without consulting the Agency, the sponsor modified the dosing regimen. Quoting the study report:

"- For Group 4 (1 ppm):

Based on thin appearance of few females given 1 ppm, no body weight gain between study days 91 and 147 and a wide spread mineralization in one female given 1 ppm sacrificed in moribund condition, it was concluded that the dose level of 1 ppm exceeded the maximum tolerated dose and the following modifications to the study design were implemented.

Treatment was stopped from week 23 and animals did not receive any administration for a 19-day wash out period. They received the control item from week 25/26 for 25 days. After this overall treatment-free period of 44 days the treatment at 1 ppm was restarted in week 29 but at a reduced frequency of three times a week on specific days i.e. each Monday, Wednesday and Friday.

- For Group 3 (0.6 ppm):

Based on thin appearance of few females, slightly lower body weight, an increase in serum calcium and inorganic phosphorus concentrations and renal mineralization associated with tubular basophilia and tubular dilation in one male given 0.6 ppm sacrificed in moribund condition, it was concluded that the dose level of 0.6 ppm exceeded the maximal tolerated dose and the following modifications to the study design were implemented.

Treatment was stopped at week 29. These animals were kept without treatment for a 3-day wash out period until day 198 for females, day 199 for males and day

200 for satellites and were treated with the control item only until week 33 three times a week on specific days i.e. each Monday, Wednesday, and Friday. From this date and for the remaining part of the study, the treatment frequency with the test item at 0.6 ppm was reduced to three times a week on specific days i.e. each Monday, Wednesday and Friday.

- For Group 2 (0.3 ppm) and group 1 (control):

As an increase in serum calcium and inorganic phosphorus concentrations was also observed at the dose level of 0.3 ppm and to put control animals in the same treatment conditions, treatment was reduced from week 29 to three times a week on specific days i.e. each Monday, Wednesday and Friday."

Further quoting the study report:

"Treatment frequency:

- Group 4 (1 ppm):

Females: daily until day 155 (156 administrations), then 3 times a week from day 199 to 642 (191 administrations). So they received a total of 347 test item administrations. From day 174 to 198, they received control item daily (25 administrations).

Males: daily until day 156 (157 administrations), then 3 times a week from day 200 to 659 (198 administrations). So they received a total of 355 test item administrations. From day 175 to 199, they received control item daily (25 administrations).

Satellites: daily until day 157 (158 administrations) then 3 times a week from day 201 to 397 (85 administrations). So they received a total of 243 test item administrations. From day 176 to 200, they received control item daily (25 administrations).

- Group 3 animals (0.6 ppm):

Females: daily until day 196 (197 administrations), then 3 times a week from day 227 to 677 (194 administrations). So they received a total of 391 test item administrations. From day 199 to 224, they received control item 3 times a week (12 administrations).

Males: daily until day 197 (198 administrations), then 3 times a week from day 228 to 706 (206 administrations). So they received a total of 404 test item administrations. From day 200 to 225, they received control item 3 times a week (12 administrations).

Satellites: daily until day 198 (199 administrations) then 3 times a week from day 229 to 397 (73 administrations). So they received a total of 272 test item administrations. From day 201 to 226, they received control item 3 times a week (12 administrations).

- Group 1 (control) and 2 animals (0.3 ppm):

Females: daily until day 198 (199 administrations), then 3 times a week from day 199 to 700 or 703 or 705 (215, 216 or 217 administrations). So they received a total of 414 to 416 administrations depending on day of necropsy.

Males: daily until day 199 (200 administrations), then 3 times a week from day 200 to 720 (223 administrations). So they received 423 administrations.

Satellites: daily until day 200 (201 administrations), then 3 times a week from day 201 to 397 (85 administrations). So they received 286 administrations."

The sponsor contacted the Division in a submission dated 12-APR-2005, during week 84 (day 581) of the study, to request permission for early termination of the study due to survival issues.

The Division consulted the exec-CAC and relayed to the sponsor that:

"The study should continue without modification for the present. Dosing of any treatment group (within a gender) should stop if the number of animals in that group should decline to 20, but treatment of other groups would continue. A given treatment group (within a gender) should be terminated and subjected to necropsy if the number of surviving animals in that group declines to 15. If the study has reached at least 100 weeks of treatment by the time a given treatment group declines to 15, then all groups of that gender may be sacrificed at that time (but treatment of the other gender would continue). If no group declines to 15 animals, then the study should continue until the scheduled terminal sacrifice. The sponsor is requested to notify the committee (through the Division) prior to termination of any group."

In a submission dated 29-JUN-2005 (week 96), the sponsor notified the Division that survival in the male and female high-dose groups had reached 16 and 17, respectively, and that sacrifice would be conducted per the instructions of the exec-CAC.

Observations and times:

Morbidity/mortality: Animals observed twice daily.

Clinical signs: Yes, daily for clinical signs; full clinical exam once every four weeks to week 25, then weekly

Body weights: Yes, weekly

Food consumption: Yes, weekly

Ophthalmoscopy: Yes (week 34)

EKG: No

Hematology: Yes, all main-study animals at termination

Clinical chemistry: Yes, satellite animals only; measured calcium and inorganic phosphorus at weeks 28, 33, and 51

Urinalysis: No

Gross pathology: Yes

Organs weighed: None

Histopathology: A standard list of tissues from all animals in control group and high-dose group killed at terminal sacrifice, plus all main-study animals found dead or killed moribund, plus gross lesions from all main-study animals, plus the treatment site (skin), duodenum, kidneys, aorta, eyes, and sternum from all LD and MD animals.

Toxicokinetics: Data from a 13-week topical study in mice indicated that the plasma levels of calcitriol were below the limit of detection (200 pg/mL).

Therefore, systemic exposures in the two-year study were estimated through use of ³H-calcitriol. Animals in the satellite groups were treated on day 400 with formulations that contained radiolabeled calcitriol (radiochemical purity 99.1%; 0.43 µCi applied to each animal) in addition to 0.3, 0.6, or 1.0 ppm of non-labeled calcitriol. Due to mortality, only 12 animals/sex/group were used (instead of the 15/sex/group originally in the satellite groups). Blood samples were obtained from 3 animals/sex/group at 0.5, 1, 6, and 24 hours post-dosing (control animals at 1 hr only). Activity/unit of plasma was determined using a scintillation counter (subtracting the background value of the control samples); the respective plasma concentrations of calcitriol were estimated, and the data used to calculate C_{max}, T_{max}, and AUC_{0-24h} values.

Other: None

Results:

Mortality: The distribution of premature decedents (animals found dead or sacrificed moribund) is presented in the following table:

Group number & label	Dose Level (ppm)	Animals Found Dead or Sacrificed Moribund			
		Males	%	Females	%
1. Vehicle Control	0	34/60	57%	30/60	50%
2. Low	0.3	40/60	67%	34/60	57%
3. Medium	0.6	42/60	70%	42/60	70%
4. High	1	44/60	73%	44/60	73%

The table above excludes animals found dead during the "terminal period" (week 101 for females and weeks 105/106 for males).

A statistically significant trend toward reduced survival with increasing dosage was observed in both genders (see associated Biostatistics review for details). Survival between weeks 52 and 104 is summarized in the following table:

Group/ Week (month)	Group 1		Group 2		Group 3		Group 4	
	M	F	M	F	M	F	M	F
52 (12 months)	57 (95)	56 (93)	56 (93)	56 (93)	52 (87)	54 (90)	57 (95)	53 (88)
60 (14 months)	55 (92)	53 (88)	49 (82)	56 (93)	48 (80)	52 (87)	50 (83)	49 (82)
73 (16 months)	50 (83)	48 (80)	41 (68)	54 (90)	42 (70)	48 (80)	47 (78)	41 (68)
75 (17 months)	49 (82)	46 (77)	38 (63)	53 (88)	37 (62)	44 (73)	39 (65)	34 (57)
80 (19 months)	46 (77)	45 (75)	36 (60)	48 (80)	32 (53)	41 (68)	35 (58)	32 (53)
84 (20 months)	42 (70)	41 (68)	34 (57)	42 (70)	31 (52)	39 (65)	35 (58)	28 (47)
88 (21 months)	41 (68)	38 (63)	31 (52)	40 (67)	29 (48)	32 (53)	26 (43)	25 (42)
92 (21 months)	36 (60)	37 (62)	27 (45)	35 (58)	25 (42)	29 (48)	21 (35)	19 (32)
96 (22 months)	34 (57)	32 (53)	26 (43)	28 (47)	22 (37)	21 (35)	16 (27)	17 (28)
100 (23 months)	32 (53)	30 (50)	24 (40)	26 (43)	22 (37)	18 (30)	-	16 (27)
104 (24 months)	26 (43)	-	20 (33)	-	18 (30)	-	-	-

Clinical signs: The incidence of animals that exhibited corneal opacities (calcification of the eyes) and minor skin lesions (scabs and sores) tended to be increased in both genders in groups 3 and 4.

Mean Body Weight Gain: Mean weight gain over days 0 to 672 (the approximate time of termination of the group 4 animals) was reduced by approximately 10% in the group 4 males and 13% in females, relative to controls

Body Weight Gain over Days 0-672 (g±SD):

Group	Dose Level (ppm)	Change in Weight from Baseline To Day 672			
		Males (g)	% from Control	Females(g)	% from control
1. Vehicle Control	0	13.6±3.5		12.1±3.3	
2. Low	0.3	13.4±3.9	-1.4%	12.2±2.8	0.8%
3. Medium	0.6	12.7±3.7	-6.6%	12.8±3.4	5.8%
4. High	1	12.3±5.3	-9.6%	10.5±3.6	-13.2%

Food consumption: No remarkable observations.

Hematology: No remarkable observations.

Clinical chemistry: At week 51, mean serum calcium and phosphate levels were slightly, but statistically significantly, increased in females in groups 3 and 4, but not in males:

Selected Blood chemistry Parameters, Males, week 51:

Group	Calcium (mmol/L)	Phosphorus (mmol/L)
1 (Control)	2.32±0.10	2.57±0.28
2 (0.3 ppm)	2.26±0.07	2.27±0.14**
3 (0.6 ppm)	2.36±0.17	2.38±0.41
4 (1.0 ppm)	2.35±0.07	2.47±0.25

**p<0.01

Selected Blood chemistry Parameters, Females, week 51:

Group	Calcium (mmol/L)	Phosphorus (mmol/L)
1 (Control)	2.30±0.12	2.16±0.31
2 (0.3 ppm)	2.35±0.13	2.47±0.31*
3 (0.6 ppm)	2.48±0.12**	2.51±0.26**
4 (1.0 ppm)	2.64±0.18***	2.73±0.37***

*p<0.05; **p<0.01; ***p<0.001

Urinalysis: NA

Mean organ weights: NA

Gross pathology: The incidence of gross lesions that were considered to be neoplastic or proliferative in nature did not appear to differ meaningfully between groups. The incidence of mineralized tissues (opacities in the eyes and white deposits of the diaphragm) tended to be increased in treated animals. At the application site, the incidence of sores (local irritation) was increased in animals treated with calcitriol that were found dead or sacrificed on test. No other remarkable observations.

Histopathology:

Non-neoplastic: The incidence of mineralization of various tissues was increased in animals treated with calcitriol, particularly those that were found dead or sacrificed in extremis. The incidence and severity of these lesions tended to increase with increased exposure to calcitriol. Affected tissues included the aorta and other major vessels, eyes, kidneys, brain, bronchi, larynx, lungs, and sciatic nerve. Increased thickness of bone was observed in treated animals:

Incidence of bone changes among animals found dead or sacrificed in extremis:

Group	1		2		3		4	
Treatment	0		0.3		0.6		1	
Sex	M	F	M	F	M	F	M	F
No. of animals examined	34	30	40	34	43	42	46	44
Thickening of trabeculae in sternum	0	0	0	2	3	8	9	15
Thickening of trabeculae in femur	0	0	9	8	8	14	17	21

Incidence of bone changes among animals at terminal sacrifice:

Group	1		2		3		4	
Treatment	0		0.3		0.6		1	
Sex	M	F	M	F	M	F	M	F
Number of animals examined	26	30	20	26	16	17	14	16
Thickening of trabeculae in sternum	1	1	3	8	6	5	6	2
Thickening of trabeculae in femur	1	1	NA	NA	NA	NA	6	1

Neoplastic: (See associated Biostatistics review of NDA 22-087 for complete information): No statistically significant differences in tumor incidence were observed in this study. The report contained the following tables that summarized the incidences of the major neoplastic changes:

Incidence of major neoplastic changes among animals found dead or sacrificed in extremis (see following table):

Appears This Way
On Original

Group	1		2		3		4	
Treatment (ppm)	0		0.3		0.6		1	
Sex	M	F	M	F	M	F	M	F
No. of animals examined	34	30	40	34	43	42	46	44
<u>Systemic neoplasm</u>								
- Malignant lymphoma	1	12	1	4	2	7	3	5
- Histiocytic sarcoma	0	4	1	9	0	1	0	1
Thymic hyperplasia*	1	1	1	4	1	4	1	6
<u>Liver neoplasms</u>								
- M carcinoma	3	0	1	0	1	0	1	0
- B adenoma	4	0	8	0	8	0	7	1
Total	7	0	9	0	9	0	8	1
Percentage	21	0	23	0	21	0	17	2
- Basophilic foci	1	0	4	0	1	0	2	0
- M hemangiosarcoma	1	0	0	0	0	0	1	0
- B hemangioma	0	0	0	0	0	0	1	0
<u>Lungs neoplasms</u>								
- M carcinoma	3	2	2	2	2	0	6	0
- B adenoma	5	3	4	3	4	2	5	4
Total	8	5	6	5	6	2	11	4
Percentage	24	17	15	15	14	5	24	9
- Bronchiolo-alveolar hyperplasia	7	2	2	2	4	1	3	0

(Continued):

Appears This Way
On Original

Group Treatment (ppm)	1 0		2 0.3		3 0.6		4 1	
	M	F	M	F	M	F	M	F
No. of animals examined	34	30	40	34	43	42	46	44
Harderian glands								
- M adenocarcinoma	0	0	0	0	0	1	0	1
- B adenoma	1	3	3	0	2	2	2	1
Total	1	3	3	0	2	3	2	2
Percentage	3	10	8	0	5	7	4	5
Uterus/cervix								
- Adenocarcinoma	NA	0	NA	0	NA	1	NA	0
- Squamous carcinoma	NA	0	NA	0	NA	1	NA	0
- Stromal cell sarcoma	NA	1	NA	0	NA	0	NA	0
- Leiomyosarcoma	NA	0	NA	0	NA	0	NA	1
- Leiomyoma	NA	3	NA	1	NA	4	NA	1
- Endometrial stromal polyp tumor	NA	3	NA	3	NA	2	NA	1
Total	NA	7	NA	4	NA	8	NA	3
Percentage	NA	23	NA	12	NA	19	NA	7

Incidence of major neoplastic changes among animals at terminal sacrifice (see following table):

Appears This Way
On Original

Group	1		2		3		4	
	0		0.3		0.6		1	
Sex	M	F	M	F	M	F	M	F
Treatment (ppm)	26	30	20	26	17	18	14	16
Systemic neoplasm								
- Mast cell tumor	1	0	0	0	0	0	0	0
- Malignant lymphoma	2	0	0	1	1	4	0	2
- Histiocytic sarcoma	1	1	0	0	0	1	0	0
Thymic hyperplasia*	1	9	0	2	0	3	0	5
Liver neoplasms								
Number of animals examined	26	30	3	4	11	3	14	16
- M carcinoma	1	1	0	0	1	0	1	0
- B adenoma	10	0	3	0	7	1	2	0
Total	11	1	3	0	8	1	3	0
Percentage (groups 1 & 4)	42	3	NA	NA	NA	NA	21	0
- Basophilic foci	1	2	1	0	0	0	5	0
- M hemangiosarcoma	0	0	0	0	0	0	1	0
- B hemangioma	1	0	1	0	0	0	0	0
Lungs neoplasms								
- Number of animals examined	26	30	2	2	3	3	14	16
- M carcinoma	6	2	1	0	2	1	1	1
- B adenoma	8	6	1	2	0	3	3	1
Total	14	8	2	2	2	4	4	2
Percentage (groups 1 & 4)	54	27	NA	NA	NA	NA	29	13
- Bronchiolo-alveolar hyperplasia	8	3	0	0	1	0	1	3

(Continued):

Group	1		2		3		4	
Treatment (ppm)	0		0.3		0.6		1	
Sex	M	F	M	F	M	F	M	F
Number of animals examined	26	30	20	26	17	18	14	16
Harderian glands								
Number of animals examined	26	30	1	1	1	2	14	16
- M adenocarcinoma	0	0	0	0	0	0	0	1
- B adenoma	2	2	1	0	0	1	1	1
Total	2	2	1	0	0	1	1	2
Percentage (groups 1 & 4)	8	7	NA	NA	NA	NA	7	13
Uterus/cervix								
Number of animals	NA	30	NA	23	NA	16	NA	16
- Leiomyoma	NA	3	NA	0	NA	1	NA	0
- Endometrial stromal polyp tumor	NA	4	NA	3	NA	1	NA	0
- M-Hemangiosarcoma	NA	1	NA	0	NA	0	NA	0
- B-Hemangioma	NA	0	NA	0	NA	0	NA	1

Toxicokinetics: Mean pharmacokinetic parameters, based upon data from mice treated with radiolabeled calcitriol during week 58, indicated that systemic exposure to calcitriol increased roughly in proportion to the quantity applied. The data are summarized below:

Appears This Way
On Original

Occasion	Concentration (ppm)	Sex	C _{max} (pgEq./g)	T _{max} (h)	AUC _(0-24H) (pgEq.h/g)
Week 58	0.3	Male	234	0.5	3456
		Female	242	1	3586
		Ratio M/F	0.97	NA	0.96
	0.6	Male	332	6	6128
		Female	491	1	6302
		Ratio M/F	0.68	NA	0.97
	1	Male	474	6	9166
		Female	568	6	10015
		Ratio M/F	0.83	NA	0.92

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

2.6.6.6.1 Study title: Calcitriol - Fertility study by the oral route (gavage) in the rat (Segment I).

Key study findings: Calcitriol was orally administered to male and female rats (males dosed beginning 29 days prior to pairing and beyond and females dosed from 15 days prior to pairing and continuing until day 7 of gestation) at dosages of 0, 0.1, 0.3, and 0.6 µg/kg/day. Evidence of toxicity included reduced weight gain (males at 0.6 µg/kg/day and females at 0.3 and 0.6 µg/kg/day) and elevated levels of serum calcium (males at all treatment levels and in females at 0.3 and 0.6 µg/kg/day), and elevated urinary excretion of calcium and phosphorus (males at all treatment levels; urinalysis not conducted in females). Male and female reproductive performances were unimpaired in this study, including no effects on the percentages of animals that copulated or became pregnant, latency to mating, pre- or post-implantation losses, numbers of corpora lutea, or numbers of viable embryos. There were no effects on sperm concentration or motility. A dosage of 0.6 µg/kg/day was a NOAEL for reproductive parameters under the conditions of this study.

Study no.: 913/124 (sponsor reference No. RDS.03.SRE.12400)

Volume #, and page #: Mod 4, vol. 52

Conducting laboratory and location: _____

Date of study initiation: 27-AUG-2003

Date of study completion: 27-OCT-2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Calcitriol, batch Nos. PA2027I0141 and PA2027J0162, 100.2% (assumed to be 100% for dosage calculations)

b(4)

Methods

Doses: 0 (water control), 0 (vehicle control), 0.1, 0.3, and 0.6 µg/kg/day, administered once daily.

Species/strain: Rat/Sprague-Dawley Ico:OFA.SD.(IOPS Caw); males 11 weeks old (363 g-426 g); females 10 weeks (249 g -297 g) at start of treatment.

Number/sex/group: 20

Route, formulation, volume, and infusion rate: Oral (gavage); test article dissolved in Neobee oil M5. Neobee M5 is a medium chain triglyceride, primarily with 8 and 10 carbon side chains (caprylic and capric acid) in proportions of approximately 68% and 32%, respectively.; approximately 2 mL/kg/day (dosing volume adjusted to latest BW)

Satellite groups used for toxicokinetics: No

Study design: F0 males dosed 29 days prior to pairing and continuing until one day prior to termination (males dosed 59 days total at termination). F0 females dosed 15 days prior to pairing and continuing until day 7 of gestation. Animals paired 1:1 within a treatment group. Day of mating (confirmed through presence of sperm in vaginal smear or presence of vaginal plug) was gestation day 0. Females separated from males after confirmed mating. Females euthanized on gestational day 13.

Parameters and endpoints evaluated:

Clinical signs: Yes, daily, plus full clinical exam weekly

Body weight: Yes, males weighed twice weekly; females weighed twice weekly during pre-mating and mating periods, plus on gestational days 0, 4, 8, and 13.

Food consumption: Yes

Clinical pathology: Blood samples obtained from 10 males/group on day 21 (following 21 days of dosing) and at termination (weeks 4 and 9), and from 10 females/group on day 14 (following 14 days of dosing; week 3) for determining serum calcium and phosphorus (from animals not used for TK purposes). Urine samples also obtained from 10 males/group on day 21, analyzed for calcium and phosphorus.

Gross necropsy: Full necropsy of all F0 animals (male and female), including examination of uteri and ovaries. Numbers of embryos, resorptions, implantations, and corpora lutea were determined. Embryo viability was assessed.

Spermatogenic assessment: Yes; all males at termination. Immediately upon

termination, a sperm sample was obtained from the left cauda epididymis and was assessed for sperm motility. Sperm counts were performed using the left testis.

Organ weights: Yes; epididymides, ovaries, testes.

Histopathology: No

Toxicokinetics: Blood samples collected on first day of treatment and on day before pairing (day 14 for females and day 28 for males) from 3 animals/sex/group at 2, 4, 6, 10, and 24 hours post-dosing.

Results

Mortality: No test-article-related deaths (one male in the low-dose group was sacrificed moribund on day 32 following a gavage accident).

Clinical signs: No remarkable observations.

Body weight:

Males: A clear trend toward reduced mean weight gain over the treatment period was apparent among males in the high-dose group of 0.6 µg/kg/day (mean gain of 119.7±32.5 compared to 143.5±33.5 for vehicle control group over days 0-56 of treatment, a reduction of approximately 16%), although statistical significance was not observed.

Females: Mean maternal weight gain during the portion of gestation during which treatment occurred (days 0-7 of gestation) tended to be reduced in all treatment groups, and values for females dosed at 0.3 and 0.6 µg/kg/day were significantly lower than control values over days 0-4 of gestation (17.2±4.6 and 15.3±7.4 g, respectively, compared to 22.7±5.8 for the vehicle control group). The reduction in body weight gain reversed following cessation of treatment, and did not differ from the control value by day 13.

Food consumption: No remarkable observations.

Toxicokinetics: On the day prior to pairing (day 14 for females, day 28 for males), all values at 0.1 and 0.3 µg/kg/day were below the limit of quantitation (BLQ), as were samples from the 0.6 µg/kg/day group at time points later than 6 hours post-dosing for males and 4 hours post-dosing for females, but the following data were obtained:

Group	C _{max} (ng/mL)	t _{max} (hr)	AUC (hr·ng/mL)
Males at 0.6 µg/kg/day	0.495	2	1.44
Females at 0.6 µg/kg/day	0.458	2	0.73

Clinical pathology: Serum calcium was statistically significantly elevated in males at all treatment levels at both week 4 and 9, and in females at 0.3 and 0.6 µg/kg/day at week 3.

Slight elevations of phosphate levels were noted at some time points among treated animals, but did not achieve statistical significance in most instances. Urinary excretion levels of calcium and phosphorus were markedly and statistically significantly elevated in all treatment groups of males (increasing with increasing dosage) at both week 4 and week 9 (urinalysis not conducted in females). No differences were observed in clinical pathology values between the water-treated and vehicle-treated control groups.

Necropsy/Organ weights: No treatment-related effects on gross pathological findings or organ weights for either sex.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Reproductive performance: No treatment-related effects on either male or female reproductive performance, as indicated by the percentages of animals that successfully copulated (mating indices), became pregnant (fertility indices), or latency to mating. No statistical differences in intrauterine survival were observed, including no effects on pre- or post-implantation losses, numbers of corpora lutea, or numbers of viable embryos.

Spermatogenic endpoints:

Sperm number (concentration): No differences between groups on mean number of sperm per testis, or per gram of testis.

Sperm motility: No effects on sperm motility.

Sperm morphology: Not assessed.

Embryofetal development

2.6.6.6.2 Study title: Calcitriol - Embryo toxicity study by the oral route (gavage) in the rat (Segment II) with systemic exposure evaluation.

Key study findings: Calcitriol was orally administered to pregnant female rats on days 6 through 17 of gestation at dosages of 0, 0.1, 0.3, and 0.9 µg/kg/day. Evidence of toxicity included reduced weight gain at 0.3 and 0.9 µg/kg/day over the period of dosing, elevated serum calcium at 0.3 and 0.9 µg/kg/day, and increased urinary excretion of calcium and phosphorus at 0.3 and 0.9 µg/kg/day at day 18. No effects were observed on C-section data, including numbers of live or dead fetuses, post-implantation loss, early or late resorptions, or mean fetal body weight, and there were no effects on the visceral or skeletal malformations or variations of fetuses. A dosage of 0.9 µg/kg/day was a NOAEL for reproductive parameters under the conditions of this study.

Study no.: 913/123 (sponsor reference No. RDS.03.SPR.12319)

Volume #, and page #: Mod 4, vol 55

Conducting laboratory and location: _____

Date of study initiation: 17-OCT-2003

Date of study completion: 29-OCT-2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol, batch Nos. PA202710141, 100.2% (assumed to be 100% for dosage calculations)

Methods

Doses: 0 (water control), 0 (vehicle control), 0.1, 0.3, and 0.9 µg/kg/day, administered once daily.

Species/strain: Rat/Sprague-Dawley Ico:OFA.SD.(IOPS Caw); females 10-13 weeks (200 g -260 g) at start of treatment.

Number/sex/group: 25

Route, formulation, volume, and infusion rate: Oral (gavage); test article dissolved in Neobee oil M5. Neobee M5 is a medium chain triglyceride,

_____ approximately 2

b(4)

mL/kg/day (dosing volume adjusted to latest BW)

Satellite groups used for toxicokinetics: Yes, 6/sex in groups 2-5

Study design: Pregnant rats were dosed on days 6 through 17 of gestation. Main-study dams were killed on day 20 and C-sectioned.

Parameters and endpoints evaluated: Maternal survival and body weight.

Numbers of live, dead, and resorbed fetuses were determined. Live fetuses were weighed and examined for external, visceral, and skeletal anomalies.

Satellite animals were used to assess systemic exposure; blood samples were obtained from satellite animals in the treatment groups on days 6 and 17 of gestation at 2 and 4 hours post-dosing (3/sex/group). Urine samples were collected from satellite animals over gestation days 6-7 and 17-18. Blood and urine samples were analyzed for calcium and phosphorus content. Satellite animals were killed on day 18; they were internally examined for pregnancy status but were not further examined.

Study overview:

Group	Estimated Calcitriol Exposure (µg/kg/day)	Number/sex in Main Study	Number/sex in TK Group
1. Control (Water)	0	25	6
2. Control (Vehicle)	0	25	6
3. Low Dose	0.1	25	6
4. Mid Dose	0.3	25	6
5. High Dose	0.9	25	6

Results:

In-life (maternal) observations:

Maternal Mortality: None

Clinical signs: No remarkable observations.

Maternal body weight: Significantly reduced body weight gain throughout period of dosing at 0.3 µg/kg/day and above:

Group	Estimated Calcitriol Exposure (µg/kg/day)	Mean BW Gain of Females, over Gestation Days 6-18 (g)
1. Control (Water)	0	97.2±15.4
2. Control (Vehicle)	0	92.8±20.1
3. Low Dose	0.1	87.5±16.0
4. Mid Dose	0.3	83.0±14.1*
5. High Dose	0.9	79.1±17.6**

*p<0.05; **p<0.01 compared to vehicle control

Mean maternal weight gain rebounded following cessation of dosing, significantly exceeding control values over gestation days 18-20.

Food consumption: Slightly reduced in all treatment groups during the period of dosing.

Toxicokinetics: Plasma levels of calcitriol were above the limit of detection in animals at 0.3 (mean values of 0.496 ng/mL at 2 hr and 0.346 ng/mL at 4 hr) and 0.9 µg/kg/day (mean values of 1.238 ng/mL at 2 hr and 0.906 ng/mL at 4 hr) on day 6. On day 17, the level was above the limit of detection only in animals dosed at 0.9 µg/kg/day (mean values of 0.606 ng/mL at 2 hr and 0.296 ng/mL at 4 hr).

Clinical pathology (satellites): Serum calcium was slightly, but statistically significantly, increased at 0.3 and 0.9 µg/kg/day; no effect on serum phosphorus (day 17). Urinary excretion of calcium and phosphorus were statistically significantly increased at 0.3 and 0.9 µg/kg/day at day 18.

Terminal and necroscopic evaluations (C-section data): No significant differences in any parameter, including numbers of live or dead fetuses, post-implantation loss, early or late resorptions, or mean fetal body weight.

Offspring (malformations and variations):

Visceral anomalies: No remarkable observations.

Skeletal anomalies: No remarkable observations.

2.6.6.6.3 Study title: Dermal (occluded) rabbit developmental toxicity study

Key study findings: The effects of topically applied calcitriol on developmental toxicity was studied in rabbits. Exposure was modulated through varying the percentage of the body-surface area that was treated. Evidence of systemic exposure included reduced survival (high-dose group), reduced body weight gain (mid and high-dose groups), and elevated serum calcium and inorganic phosphorus levels in all treatment groups. The mean plasma concentration of calcitriol was significantly elevated in mid and high-dose animals on day 18. Evidence of fetal toxicity included a significantly elevated mean post-implantation loss in the high-dose group, and an increased incidence of minor skeletal abnormalities among fetuses from the mid and high-dose groups due to retarded ossification of the pubic bones. A slightly increased incidence of skeletal variations was also observed in the high-dose group (extra 13th rib, reduced ossification of epiphyses). These effects were likely secondary to maternal toxicity. No effects were observed on the incidence of major fetal abnormalities, or of minor external or visceral abnormalities.

Study no.: H.141.418

Volume #, and page #: Mod 4, vol. 57

Conducting laboratory and location: _____

Date of study initiation: Oct-1991

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: 3 µg/g calcitriol ointment, lot No. FLG90K22A, _____ pure (analytical report indicated the test material contained _____)

Formulation/vehicle: Calcitriol ointment (clinical formulation)

b(4)

b(4)

Methods:

Species/strain: Rabbit/New Zealand white

Doses employed: Dosage was based upon percentage of body surface area (BSA) exposed. Areas of 0.4%, 1.6%, and 6.4% were studied (controls received vehicle applied to 6.4% BSA). The quantity of material applied per unit of BSA is not estimated, so it is not possible to estimate exposure to calcitriol in µg/kg/day.

Route of administration: Topical application to shaved skin of dorsal-lumbar region once daily, six hours per day, with occlusion (a lint patch was placed over the site and held in place with _____ tape) and a jacket placed over the tape.

The animals wore Elizabethan collars during the six hour daily treatment period.

Study design: Treated on days 6 through 18 of pregnancy, killed on day 28 and cesarean sectioned. Blood samples obtained from each animal on day 18 (two hours after treatment) and prior to termination on day 28 for analysis of the plasma for calcitriol level and blood chemistry.

Number/sex/group: 16 pregnant females per treatment group.

Parameters and endpoints evaluated: Clinical observations, bodyweight, food consumption, erythema and edema at application site, implantations, resorptions, fetal weights, fetal external abnormalities, visceral anomalies, and skeletal abnormalities.

b(4)

Results:

Mortality: Two treatment-related deaths in high-dose group; both were sacrificed moribund following a period of weight-loss and reduced food consumption, one on day 17 and one on day 23 of gestation. In addition, one high-dose animal was found dead on day 10 of gestation; necropsy revealed "twisted and fluid-filled intestines", and this death was considered to be related to trauma during treatment, but to not be related to exposure to the test material.

Clinical signs: Reduced feces production (due to decreased food consumption) in mid and high-dose groups. Edema and erythema observed in all groups, including controls.

Body weight: Body weight gain significantly reduced in mid and high-dose groups in proportion to dosage:

Group	Mean BW Gain of Females, over Gestation Days 6-18 (kg)
1. Control	0.28±0.10
2. Low Dose	0.25±0.08
3. Mid Dose	0.16±0.09**
4. High Dose	-0.12±0.11***

p<0.01; *p<0.001 compared to vehicle control

Food consumption: Food consumption reduced in all treatment groups during the period of dosing (roughly in proportion to dosage); statistically significant difference from the control value over some time intervals.

Blood chemistry (day 18): Significantly increased serum calcium and inorganic phosphorus in all treatment groups in relation to dosage:

Mean serum calcium levels of females, day 18 (mg%; mean±SD):

Vehicle controls 13.7±0.4

Low dose 14.0±0.5*

Mid dose 14.1±0.5*

High dose 14.6±0.4***

Mean serum phosphate levels of females, day 18 (mg%; mean±SD):

Vehicle controls 5.5±0.5

Low dose 6.3±0.7***

Mid dose 8.4±1.0***

High dose 8.9±0.7***

*p≤0.05; ***p<0.005

Toxicokinetics: Calcitriol level significantly elevated in mid and high-dose animals on day 18 (108.8 ± 45.0 pg/mL and 315.4 ± 68.0 pg/mL, respectively, versus 45.2 ± 12.4 pg/mL in controls; both values different from control at $p < 0.005$).

In-life observations:

Terminal and necroscopic evaluations: Note: One control and one high-dose female were not pregnant. On day 28 there were 15, 16, 16 and 12 pregnant females in the control, low, mid, and high-dose groups, respectively.

Dams: The mean post-implantation loss was significantly higher in the high-dose group:

Mean post-implantation loss (%):

Vehicle controls 5.2

Low dose 11.4

Mid dose 8.5

High dose 18.4*

* $p \leq 0.05$

No other remarkable differences in "C-section" statistics, including no effect on mean fetal weight.

Offspring: No differences between groups in regard to the incidence of major abnormalities or minor external or visceral abnormalities. The incidence of minor skeletal abnormalities was significantly higher among fetuses from the mid and high-dose groups (18.8% and 22.2%, respectively, versus 12.4% in controls, $p < 0.01$ for both comparisons) due to retarded ossification of the pubic bones. A slightly increased incidence of skeletal variations was also observed in the high-dose group (extra 13th rib, reduced ossification of epiphyses).

Prenatal and postnatal development

2.6.6.6.4 Study title: Calcitriol - Pre- and post-natal development study by the oral route (gavage) in the rat (Segment III)

Key study findings: Calcitriol was evaluated for effects when orally administered to pregnant rats from gestation day 6 through day 20 postpartum at dosages of 0, 0.1, 0.3, and 0.6 $\mu\text{g}/\text{kg}/\text{day}$. No effects were observed on maternal survival, mean duration of gestation, mean litter size, mean numbers of pups born alive or dead, physical development of F1 animals, behavioral development of F1 animals, or reproduction of F1 animals, and no effects on F2 animals were observed during necropsy of F1 dams. Although little evidence of toxicity was observed in this study, mean maternal body weights tended to be lower in all treatment groups throughout the period of treatment, and an apparent trend toward reduced mean maternal body weight gain among treatment groups relative to the vehicle control group was observed over the period of dosing. In a

previous study in female rats that involved the same dosages of calcitriol, serum calcium levels were significantly greater than control values at 0.3 and 0.6 µg/kg/day following 14 days of treatment. These data suggest that the dosages utilized in this study were adequate. 0.6 µg/kg/day was an apparent NOAEL for effects on pre and post-natal development under the conditions of this study.

Study no.: 913/125 (sponsor reference No. RDS.03.SRE.12393)

Volume #, and page #: Mod 4, vol. 58

Conducting laboratory and location: _____

Date of study initiation: 06-AUG-2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Calcitriol, batch Nos. PA2027J0162, 100.2% (assumed to be 100% for dosage calculations)

b(4)

Methods

Doses: 0 (water control), 0 (vehicle control), 0.1, 0.3, and 0.6 µg/kg/day, administered once daily.

Species/strain: Rat/Sprague-Dawley Ico:OFA.SD.(IOPS Caw); females 10-13 weeks (200 g -260 g) at start of treatment.

Number/sex/group: 25 F0 females/group; 20 F1 animals/sex/group

Route, formulation, volume, and infusion rate: Oral (gavage); test article dissolved in Neobee oil M5. Neobee M5 is a medium chain triglyceride.

_____ approximately 2 mL/kg/day (dosing volume adjusted to latest BW)

Route of administration: Oral (gavage) once daily

Satellite groups used for toxicokinetics: No

Study design: F0 females were administered the test articles daily from gestation day 6 (sixth day following confirmed mating) through day 20 postpartum. Each litter was culled on day 4 to yield (if possible) 8 pups per gender (4/sex/group, if possible). 20 F1 animals of each gender (pups of F0 animals) were randomly selected on day 21 postpartum for rearing to sexual maturity. F1 females were paired with a F1 male from the same dose group. All mated F1 females were necropsied on day 13 of gestation.

Parameters and endpoints evaluated: Body weights, food consumption, and clinical signs of all animals were monitored. F0 females were monitored for duration of gestation, litter size, pup viability, and nursing behavior. Gross necropsies were performed. F1 animals were evaluated on (approximately) days 35 and 42 postpartum for performance in a water-filled E maze for overt coordination, swimming ability, learning, and memory. F1 males were monitored for the age of preputial separation and F1 females were monitored for the age of vaginal opening. F1 animals were observed for changes in mating behavior, and were necropsied. F1 females were examined for numbers of corpora lutea, implantation sites, and viable fetuses. F2 fetuses were weighed and examined for

gross external alterations.

Clinical pathology: Blood samples obtained from 10 F0 females per treatment group on day 4 and 9 per group on day 21 post-partum (plus 2 dams in the vehicle control group on day 21), plus 3 pups per dam on days 4 and 21 via decapitation, for measurement of serum calcium and phosphorus. These samples were obtained from animals not used for TK purposes. Urine samples were also obtained from the animals sampled for blood on day 21 (at necropsy) via puncture of the urinary bladder; these samples were analyzed for calcium and phosphorus.

Toxicokinetic data: Blood samples were obtained from 9 F0 females per treatment group (plus 2 dams in the vehicle control group) on days 4 and 20 post-partum, at 2 hours post-dosing, for measurement of drug levels. Blood samples were also obtained from F1 pups (3 pups from each dam that was sampled) on day 4 post-partum following decapitation.

Results

F₀ in-life: At 0.3 µg/kg/day, two F0 females were sacrificed during lactation following total litter loss (days 5 and 7 of lactation). No other deaths prior to scheduled sacrifice. No remarkable clinical signs were observed. Mean maternal body weights tended to be lower in all treatment groups throughout the period of treatment, although the differences did not achieve statistical significance. Mean maternal BW gain was significantly lower than the control value at 0.3 and 0.6 µg/kg/day over some intervals (e.g., days 6-11 of gestation and over days 1-4 post-partum). Statistical analysis of maternal weight gain over the entire period of dosing (day 6 of gestation through day 20 post-partum) was not included in the report, however, the mean body weight changes over this period were lower in the treatment groups than in the vehicle-control group (40.4 g, 39.9 g, 35.4 g, in the low, mid, and high-dose groups, respectively, compared to 48.0 g in the vehicle-treated control group). No differences were observed in pregnancy or delivery data, including the mean duration of gestation, mean litter size, or mean numbers of pups born alive or dead.

F₀ necropsy: No remarkable observations.

F₁ physical development: No remarkable observations, including no treatment-related effects on pup survival to weaning or clinical signs in pups. Mean weights of pups in all treatment groups tended to be slightly lower than pups in the water-treated control group, but were similar to data from the vehicle-treated group. No remarkable effects in physical or functional development were observed, including days of pinna unfolding, incisor eruption, eye opening, vaginal opening, or preputial separation, and there were no differences in performance in reflex tests.

F₁ behavioral evaluation: No remarkable observations, including no effects on performance in water-maze and open-field tests.

F₁ reproduction: No remarkable observations, including no effects on mating performance, pregnancy parameters, pre or post-implantation data, or F1 necropsy data.

F₂ findings: No remarkable observations (during necropsy of F1 dams).

TK data: All values in the control and low-dose groups were below the limit of detection. Approximately half the mid-dose animals and the majority of the high-dose animals had detectable plasma levels of calcitriol at 2 hours post-dosing, although the data were not adequate to permit PK parameters to be calculated.

Clinical pathology: The clinical pathology data (serum and urine calcium and phosphorus) obtained in this study were inconclusive, as adequate data were not obtained from control animals. However, a previous study in female rats that involved the same dosages of calcitriol (study No. 913/124) demonstrated that serum calcium levels were significantly greater than control values at 0.3 and 0.6 µg/kg/day following 14 days of treatment.

2.6.6.7 Local tolerance

2.6.6.7.1 Study title: Primary irritation study of calcitriol vaseline to the eye of male rabbits

Key study findings: The material tested (calcitriol mixed with vaseline, 15 µg/g) was essentially not irritating to the eye under the conditions studied. The material evaluated in this study differed from the clinical formulation of the product (it contained a five-fold greater concentration of calcitriol than does the clinical formulation, and contained no mineral oil or vitamin E). However, in my opinion, the clinical formulation would not be more irritating to the eye than was the formulation tested in this study.

Study no: H.141.407

Volume #, and page #: Mod 4, vol. 61

Conducting laboratory and location: _____

Date of study initiation: 17-JAN-1990

GLP compliance: Yes

QA reports: yes (X) no ():

Drug, lot #, radiolabel, and % purity: Calcitriol, batch No. FLG90A09A (assumed 100% pure)

Formulation/vehicle: Calcitriol mixed with vaseline (15 µg/g)

Methods: 0.1g of material was placed in the conjunctival sac of the left eye of each of three male rabbits. The eyes were examined at 1, 24, 48, and 72 hours post-treatment; fluorescein was used at 24 hours.

Results: At one hour post-treatment observations included slight erythema of the conjunctiva of two of three animals and slight lacrimation of all three animals. These effects had cleared by the time of the 24 hour observation. No effects on the iris or cornea were observed at any time point.

b(4)

2.6.6.8 Special toxicology studies

2.6.6.8.1 Study title: Sensitization study with calcitriol in guinea pigs (maximization test)

Key study findings: Ointment that contained 15 ppm calcitriol was found to be non-sensitizing.

Study no: H.141.405

Volume #, and page #: Mod 4, vol. 61

Conducting laboratory and location: _____

Date of study initiation: 19-DEC-1989

GLP compliance: Yes (stated to have been "essentially in accordance with current OECD GLP principles")

QA reports: yes (X) no ():

Drug, lot #, radiolabel, and % purity: Calcitriol ointment, batch No. FLH 88 J12D (assumed 100% pure)

Formulation/vehicle: Calcitriol (15 ppm) in a vehicle of _____ mineral oil and _____ white petrolatum, with/without _____ and/or _____

b(4)

b(4)

Methods: 0.1 mL of the materials described above were administered through intradermal injection to young adult albino guinea pigs once, followed one week later by topical application of 15 ppm calcitriol ointment to a shaved area of skin (with an occlusive dressing that was left in place for 48 hours). Control animals received vehicle. Two weeks following the topical induction application, a challenge application of 15 ppm calcitriol ointment (or vehicle, for controls) was made with use of a "cup" (presumably, a Hilltop chamber), which remained in place for 24 hours. The application sites were examined 24 and 48 hours after removal of the test article. Note: Historical positive control data, from studies using the same methodology and DNCB, confirmed the validity of the test system.

Results: No skin reactions were observed during the challenge phase in animals that received calcitriol ointment.

2.6.6.9 Discussion and Conclusions

Note: It was not possible to obtain useful toxicokinetic data in most of the nonclinical studies that were conducted with calcitriol, as many of the data points were below the limit of quantitation of the available assays. In the absence of such data, and since the conditions of exposure in the nonclinical studies differed markedly from the clinical conditions of use (e.g., different routes of administration, or different conditions of topical administration), it is impossible to directly compare the daily exposures to

calcitriol in the nonclinical studies to the exposure of a patient that used the proposed new drug product. Therefore, no attempt will be made in this review to quantitatively compare clinical and nonclinical levels of systemic exposure to calcitriol associated with NDA 22-087. However, it should be noted that the pharmacodynamic action of calcitriol on calcium metabolism (e.g., elevated levels of calcium in the serum, increased excretion of calcium, histological evidence of mineralization (calcification) of various tissues) may be viewed as being a "surrogate marker" for elevated systemic exposure to calcitriol. Many of the pivotal nonclinical studies included documentation of such actions on calcium metabolism, suggesting substantial systemic exposure to calcitriol. In comparison, the incidence of hypercalcemia and hypercalciuria in clinical studies conducted under NDA 22-087 suggested no difference between subjects that received the drug product and those that received placebo (according to the "summary of clinical safety" in NDA 22-087; please see the associated clinical reviews for definitive information concerning the clinical studies). These observations suggest that the effective level of systemic exposure to calcitriol achieved in the nonclinical studies substantially exceeded the level that occurs during clinical use of the product.

The drug product being developed under NDA 22-087 contains calcitriol. Calcitriol is the active form of vitamin D₃, and occurs naturally (endogenously) within the human body. The primary sign of toxicity observed in studies that involved repeated exposures to calcitriol was perturbation of calcium homeostasis, including elevated concentrations of calcium and phosphorus in the serum and urine, microscopic evidence of stimulation of bone formation, and mineralization of the kidney. If the exposure is of sufficient magnitude and chronicity, the elevated plasma calcium levels can result in mineralization of tissues throughout the body.

Calcitriol was evaluated in repeat-dose toxicology studies in several species, including both rodents and nonrodents. Studies in mice and rats that involved 13 to 26 weeks of dosing with calcitriol via gavage or topical application to shaved skin indicated that, at sufficient dosage, toxicity representative of the pharmacodynamic effects of calcitriol was observed. These effects included the effects on calcium and phosphorus metabolism, stimulation of bone growth, and mineralization/calcification of various tissues throughout the body that were mentioned above. Severe perturbations of calcium metabolism can be incompatible with life, and in a number of the rodent studies reduced body weight gain and/or reduced survival was observed. It should be noted that rodents, while grooming themselves, lick from the skin and ingest materials that are topically applied. Therefore, systemic toxicity observed in topical rodent studies reflects systemic exposure that occurs via both the transdermal and the oral routes. Also, rodent species tend to be highly sensitive to the pharmacodynamic actions of vitamin D analogs (relative to nonrodents), even when plasma levels of the drug substance are below the limit of quantitation. For these reasons, it is often impossible to meaningfully compare dosages from rodent studies with vitamin D analogs to clinical dosages of topical vitamin D products. Topical nonclinical models that involve nonrodent species (discussed below) are much more reflective of clinically relevant effects.

Repeat-dose toxicology studies conducted with calcitriol in nonrodent species that have been submitted to NDA 22-087 include 13 week studies with minipigs and dogs, and a 9-month study with minipigs. Each of these studies included the proposed clinical formulation (3 ppm calcitriol in the same vehicle proposed for marketing), and involved dosing via the route proposed for clinical use (topical application to the skin). The studies with minipigs also included enriched formulations, that contained a higher concentration of calcitriol than does the proposed clinical formulation.

In a 13-week study with minipigs, formulations that contained 0 (vehicle control), 0.3, 1, 3, or 9 ppm calcitriol were applied daily to shaved skin, 6 hours per day under a non-occlusive dressing, at a rate of 2 mL/kg/day. Parameters monitored included clinical pathology and full histopathology. Essentially no toxicity was observed, although the mean weight of the kidney was significantly increased in animals of both genders in the high-dose group (9 ppm material). Under the conditions of this study ointment that contained 3 ppm calcitriol was a NOAEL, and 9 ppm ointment induced slight toxicity.

A similar study was conducted with beagle dogs that received daily topical applications of formulations that contained 0 (vehicle control), 0.3, 1, or 3 ppm calcitriol, applied to shaved skin for 13 weeks, 6 hours per day under a non-occlusive dressing. All the test materials were applied at a rate of 2 mL/kg/day; material that contained 3 ppm was also applied at a rate of 4 mL/kg/day (as a means of achieving an accelerated level of exposure in lieu of using an enriched formulation). Parameters monitored included clinical pathology and full histopathology. All animals survived to scheduled sacrifice, and there were no effects on general clinical signs or hematology. Treatment-related effects (reflective of the pharmacodynamics of calcitriol) were apparent primarily among animals at the two highest exposure levels (those that received calcitriol 3 ppm ointment at either 2 or 4 mL/kg/day), and included elevated levels of calcium in the serum and urine, slightly increased mean kidney weight (likely a reactive effect of increased excretion of calcium and phosphorus), and minimal to moderate mineralization of various tissues.

In a study in which minipigs received 9 consecutive months of daily topical applications of formulations that contained 0 (vehicle control), 1, 3, 9, or 15 ppm calcitriol, 6 hours per day under a non-occlusive dressing, at a rate of 2 mL/kg/day, the test materials were generally well tolerated. Signs of toxicity (primarily observed only in the highest dosage group) included reduced mean weight gain (females only), slightly reduced erythrocytic parameters (significant in females only), increased mean weight of the kidney, and histological evidence of inflammation of the kidneys (females at 9 ppm and 15 ppm and males at 15 ppm), hypertrophy of the zona glomerulosa of the adrenals (females at 15 ppm only), and hyperostosis of bone (females at 15 ppm only). Under the conditions of this study ointment that contained 3 ppm calcitriol (the same formulation proposed for marketing) was a NOAEL, and 9 ppm ointment induced only slight toxicity.

Calcitriol is an endogenous compound, and as such, extensive evaluation for genetic toxicity was judged to be unnecessary. However, calcitriol was evaluated in the mouse lymphoma TK locus assay and was confirmed to be non-mutagenic.

A two-year carcinogenicity study was conducted in which calcitriol solution was orally administered (via gavage) daily to rats. Dosages of approximately 0.005, 0.03, and 0.1 µg/kg/day were investigated, in addition to control groups. Survival rates did not differ significantly between groups; terminal sacrifice of all groups occurred following 104 weeks of treatment. The incidence of benign pheochromocytomas was significantly increased in female rats (pairwise p-value of 0.0001; trend value of 0.0036). No other tumor incidence data differed according to the Haseman-Lin-Rahman criteria.

A two-year topical carcinogenicity study was conducted in which calcitriol ointment was applied to the skin of mice. Materials that contained calcitriol at concentrations of 0 (vehicle), 0.3, 0.6, and 1.0 ppm were evaluated. The vehicle for the test material was identical to the vehicle of the clinical formulation of Silkis ointment. No statistically significant differences in tumor incidence were observed in this study.

Calcitriol ointment was evaluated for activity as a cocarcinogen with UV light in a 12-month study with hairless mice. The vehicle for the test material was identical to the vehicle of the clinical formulation of Silkis ointment. The median number of weeks on study at which the first tumor (for a given animal) greater than or equal to 1.0 mm in diameter was observed was slightly, but statistically significantly, reduced for both males and females that were treated with the vehicle of the product, relative to untreated animals. Materials that contained calcitriol did not reduce latency to formation of the first tumor greater than or equal to 1.0 mm in diameter relative to vehicle alone. These data suggest that the vehicle of calcitriol ointment slightly enhances UV-induced skin tumor formation (possibly by enhancing UV penetration into the skin), but calcitriol per se does not enhance photo-induced carcinogenesis.

Calcitriol was evaluated for effects upon the fertility of male and female rats, effects upon developmental toxicity of rats and rabbits, and for effects upon pre-natal and perinatal development of rats.

To evaluate calcitriol for effects on fertility, calcitriol was orally administered to male and female rats (males dosed beginning 29 days prior to pairing and beyond and females dosed from 15 days prior to pairing and continuing until day 7 of gestation) at dosages of 0, 0.1, 0.3, and 0.6 µg/kg/day. Male and female reproductive performances were unimpaired in this study, including no effects on the percentages of animals that copulated or became pregnant, latency to mating, pre- or post-implantation losses, numbers of corpora lutea, or numbers of viable embryos. There were no effects on sperm concentration or motility. A dosage of 0.6 µg/kg/day was a NOAEL for reproductive parameters under the conditions of this study, although evidence of mild general toxicity was observed at 0.3 and 0.6 µg/kg/day.

To evaluate calcitriol for effects upon developmental toxicity in rats, calcitriol was orally administered to pregnant female rats on days 6 through 17 of gestation at dosages of 0, 0.1, 0.3, and 0.9 $\mu\text{g}/\text{kg}/\text{day}$. No effects were observed on C-section data, including numbers of live or dead fetuses, post-implantation loss, early or late resorptions, or mean fetal body weight, and there were no effects on the visceral or skeletal malformations or variations of fetuses. A dosage of 0.9 $\mu\text{g}/\text{kg}/\text{day}$ was a NOAEL for reproductive parameters under the conditions of this study, although evidence of mild general toxicity was observed at 0.3 and 0.9 $\mu\text{g}/\text{kg}/\text{day}$.

To evaluate calcitriol for effects upon developmental toxicity in rabbits, calcitriol ointment was topically applied under conditions of occlusion. Exposure was modulated through varying the percentage of the body-surface area that was treated. Evidence of systemic exposure included reduced maternal survival (high-dose group), reduced maternal body weight gain (mid and high-dose groups), and elevated serum calcium and inorganic phosphorus levels in all maternal treatment groups. The mean plasma concentration of calcitriol was significantly elevated in mid and high-dose animals on day 18. Evidence of fetal toxicity included a significantly elevated mean post-implantation loss in the high-dose group, and an increased incidence of minor skeletal abnormalities among fetuses from the mid and high-dose groups due to retarded ossification of the pubic bones. A slightly increased incidence of skeletal variations was also observed in the high-dose group (extra 13th rib, reduced ossification of epiphyses). These effects were likely secondary to maternal toxicity. No effects were observed on the incidence of major fetal abnormalities, or of minor external or visceral abnormalities.

To evaluate calcitriol for effects upon pre-natal and perinatal development in rats, calcitriol was orally administered to pregnant rats from gestation day 6 through day 20 postpartum at dosages of 0, 0.1, 0.3, and 0.6 $\mu\text{g}/\text{kg}/\text{day}$. No effects were observed on maternal survival, mean duration of gestation, mean litter size, mean numbers of pups born alive or dead, physical development of F1 animals, behavioral development of F1 animals, or reproduction of F1 animals, and no effects on F2 animals were observed during necropsy of F1 dams. 0.6 $\mu\text{g}/\text{kg}/\text{day}$ was an apparent NOAEL for effects on pre and post-natal development under the conditions of this study.

Calcitriol ointment was found to be essentially non-irritating to the skin or eyes and to not induce sensitization.

The excipients in the drug product being developed under NDA 22-087 include white petrolatum, mineral oil, and a low level of vitamin E. The proposed use of these materials is acceptable.

The clinical formulation of the drug product and the individual components of the product have been adequately evaluated for safety in nonclinical studies. The database supports the safety of the proposed use of the product.

2.6.6.10 Tables and Figures

See below.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Species	Duration	Route	Exposures	Comments
Mouse	13 Weeks	Topical	1, 2, 3 µg/kg/day	+ Ca ⁺² in serum; mineralization In all treatment groups.
Rat	13 Weeks	Oral	0.01, 0.1, 0.3 µg/kg/day	+ Ca ⁺² in serum in MD & HD groups; + Ca ⁺² in urine all dosage groups; mineralization proportional to dosage.
Minipig	13 Weeks	Topical	0.3, 1, 3, 9 ppm (2 mL/kg/day)	+ mean kidney wt. in HD group; 3 ppm was NOAEL.
Rat	26 Weeks	Topical	0.3, 1, 3, 9 ppm (2 mL/kg/day); theoretical dosages of 0.51, 1.7, 5.1, 15.3 µg/kg/day	100% mortality at 3 and 9 ppm. Reduced BW gain at all dosages. +Ca ⁺² in serum and urine at all dosages.
Minipig	9 Months	Topical	1, 3, 9, 15 ppm (2 mL/kg/day); theoretical dosages of 1.7, 5.1, 15.3, 25.5 µg/kg/day	Reduced BW gain in HD Fem. Incr. mean kidney wt. in HD both genders. 3 ppm was NOAEL; only slight tox. at 9 ppm.
Rat	24 Months	Oral	0.005, 0.03, 0.1 µg/kg/day	Carc. assay; incr. benign pheos. in females.
Mouse	24 Months	Topical	0.3, 0.6, 1 ppm	Carc. assay; no effects on tumor incidence.
Rat	Males 29 days pre-pairing to term.; females 14 days pre-pairing to day G7.	Oral	0.1, 0.3, 0.6 µg/kg/day	No effects on repro. parameters.
Rat	Days G6-G17 (females only)	Oral	0.1, 0.3, 0.9 µg/kg/day	No effects on repro. parameters.
Rabbit	Days G6-G18 (females only)	Topical	0.4, 1.6, 6.4% Of BSA.	Incr. post-implant. loss; minor fetal skeletal abnormalities.
Rat	Days G6-20 post-partum (females only)	Oral	0.1, 0.3, 0.6 µg/kg/day	No effects on repro. parameters.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The product is approvable with respect to nonclinical concerns.

Unresolved toxicology issues (if any): None.

Recommendations: The product is approvable with respect to nonclinical concerns.

Suggested labeling:

Note: Included below are sections 8.1 (Pregnancy) and 13 (NONCLINICAL TOXICOLOGY) of the label of the product, as I recommend they be stated. For comparison, the recommended texts of these sections are contrasted with the texts that were proposed by the sponsor under appendix 1, at the end of this review.

b(4)

b(5)

7

7

b(4)

7

7

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ **Concurrence Yes** ___ **No** ___

3 Page(s) Withheld

 Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

✓ Draft Labeling (b5)

 Deliberative Process (b5)

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

/s/

Norman See
6/30/2008 10:19:22 AM
PHARMACOLOGIST

Barbara Hill
7/2/2008 09:35:43 AM
PHARMACOLOGIST

Appears This Way
On Original

**Division of Dermatologic and Dental
Drug Products (HFD-540)**

**Pharmacology/Toxicology Checklist for
NDA Forward Planning Meeting**

Date: 2/8/08

Reviewer: Norman A. See, Ph.D.

NDA Number: 22-087

Spensor: Galderma Laboratories, L.P.

Product Name: Silkis ointment

Drug Substance(s): Calcitriol

Indication: Psoriasis

Route of Administration: Topical to skin

Expected Date of Draft Review (if filed): 8/1/08

(1) Does the pharmacology/toxicology section of the NDA appear to be organized in a manner that would allow a substantive review to be completed?

Yes.

(2) Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner to enable a timely and substantive review?

Yes.

(3) Is the pharmacology/toxicology section of the NDA sufficiently legible to permit a substantive review to be completed?

Yes.

(4) Based upon a cursory review, does the presentation of data appear to be appropriate (consider tables, graphs, completeness of study reports, inclusion of individual animal data, appropriateness of data analysis, etc.)?

Yes.

(5) Based upon a cursory review, are all necessary nonclinical studies completed and submitted in this NDA?

Yes.

(6) Based upon a cursory review, do the pivotal nonclinical studies appear to have been adequately designed (e.g., appropriate numbers of animals, adequate monitoring

consistent with the proposed clinical use, state-of-the art protocols, etc.)?

Yes.

(7) Based upon a cursory review, were the test materials utilized in the pivotal nonclinical studies identical to the drug product or drug substance proposed for commercial use (including impurity profiles)? If not, or if this matter is unclear, please comment.

Yes.

(8) Has proposed draft labeling been submitted?

Yes.

(9) From a pharmacology/toxicology perspective, should this NDA be filed?

Yes.

Reviewing Pharmacologist

Date Signed

Pharmacology Team Leader

Date Signed

Appears This Way
On Original

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

/s/

Norman See
2/11/2008 09:39:46 AM
PHARMACOLOGIST

Paul Brown
2/12/2008 05:08:30 PM
PHARMACOLOGIST

Appears This Way
On Original

**Division of Dermatologic and Dental
Drug Products (HFD-540)**

**Pharmacology/Toxicology Checklist for
NDA Forward Planning Meeting**

Date: 11/13/06

Reviewer: Norman A. See, Ph.D.

NDA Number: 22-087

Sponsor: Galderma Laboratories, L.P.

Product Name: Silkis ointment

Drug Substance(s): Calcitriol

Indication: Psoriasis

Route of Administration: Topical to skin

Expected Date of Draft Review (if filed): 5/1/07

(1) Does the pharmacology/toxicology section of the NDA appear to be organized in a manner that would allow a substantive review to be completed?

Yes.

(2) Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner to enable a timely and substantive review?

Yes.

(3) Is the pharmacology/toxicology section of the NDA sufficiently legible to permit a substantive review to be completed?

Yes.

(4) Based upon a cursory review, does the presentation of data appear to be appropriate (consider tables, graphs, completeness of study reports, inclusion of individual animal data, appropriateness of data analysis, etc.)?

Yes.

(5) Based upon a cursory review, are all necessary nonclinical studies completed and submitted in this NDA?

Yes.

(6) Based upon a cursory review, do the pivotal nonclinical studies appear to have been adequately designed (e.g., appropriate numbers of animals, adequate monitoring

consistent with the proposed clinical use, state-of-the art protocols, etc.)?

Yes.

(7) Based upon a cursory review, were the test materials utilized in the pivotal nonclinical studies identical to the drug product or drug substance proposed for commercial use (including impurity profiles)? If not, or if this matter is unclear, please comment.

Yes.

(8) Has proposed draft labeling been submitted?

Yes.

(9) From a pharmacology/toxicology perspective, should this NDA be filed?

Yes.

Reviewing Pharmacologist

Date Signed

Pharmacology Team Leader

Date Signed

Appears This Way
On Original

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

/s/

Norman See
11/13/2006 01:57:50 PM
PHARMACOLOGIST

Paul Brown
11/13/2006 03:30:52 PM
PHARMACOLOGIST

Appears This Way
On Original