

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
NDA 22-185

PHARMACOLOGY REVIEW(S)



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:	22-185
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	28-JUN-07
PRODUCT:	Taclonex Scalp Gel
INTENDED CLINICAL POPULATION:	Patients with psoriasis of the scalp
SPONSOR:	LEO Pharmaceutical Products Ltd. A/S
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:	Paul Brown, Ph.D.
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PROJECT MANAGER:	Melinda Bauerlein

Date of review submission to Division File System (DFS): 20-FEB-2008

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	8
2.6.1 INTRODUCTION AND DRUG HISTORY.....	8
2.6.2 PHARMACOLOGY.....	19
2.6.2.1 Brief summary	19
2.6.2.2 Primary pharmacodynamics	19
2.6.2.3 Secondary pharmacodynamics	20
2.6.2.4 Safety pharmacology	20
2.6.2.5 Pharmacodynamic drug interactions.....	20
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	20
2.6.4 PHARMACOKINETICS/TOXICOKINETICS	21
2.6.4.1 Brief summary	21
2.6.4.2 Methods of Analysis	21
2.6.4.3 Absorption	21
2.6.4.4 Distribution.....	21
2.6.4.5 Metabolism.....	21
2.6.4.6 Excretion.....	22
2.6.4.7 Pharmacokinetic drug interactions.....	22
2.6.4.8 Other Pharmacokinetic Studies.....	22
2.6.4.9 Discussion and Conclusions	22
2.6.4.10 Tables and figures to include comparative TK summary	22
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	22
2.6.6 TOXICOLOGY.....	22
2.6.6.1 Overall toxicology summary	22
2.6.6.2 Single-dose toxicity	24
2.6.6.3 Repeat-dose toxicity	24
2.6.6.4 Genetic toxicology.....	51
2.6.6.5 Carcinogenicity.....	60
2.6.6.6 Reproductive and developmental toxicology.....	69
2.6.6.7 Local tolerance	83
2.6.6.8 Special toxicology studies	89
2.6.6.9 Discussion and Conclusions	94
2.6.6.10 Tables and Figures.....	97
2.6.7 TOXICOLOGY TABULATED SUMMARY	97
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	97
APPENDIX/ATTACHMENTS	100

EXECUTIVE SUMMARY

I. Recommendations

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

B. Recommendation for nonclinical studies:

The sponsor has committed to conduct the following nonclinical study post-approval of NDA 22-185:

1. Evaluation of the carcinogenicity of calcipotriene in a two-year oral study in rats.

Note: The sponsor has committed to conduct the following nonclinical studies post-approval of NDA 21-852 (Taclonex ointment), and data from these studies will eventually be used to support labeling of Taclonex gel, as well:

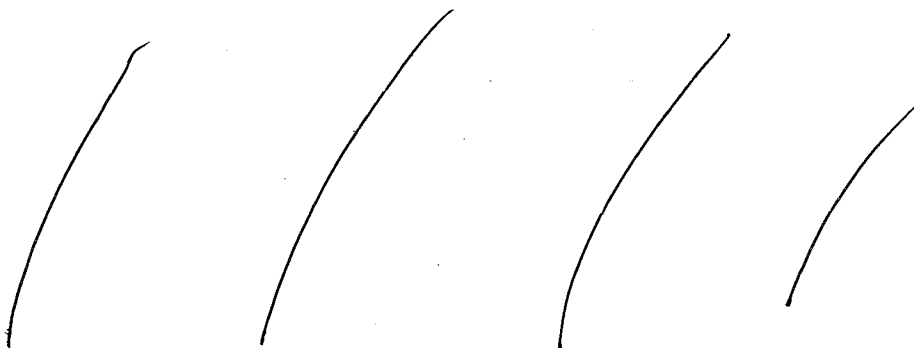
1. Evaluation of the carcinogenicity of betamethasone dipropionate in mice.

2. Evaluation of the carcinogenicity of betamethasone dipropionate in rats.

The sponsor has submitted protocols for these studies, and those protocols were approved by the exec-CAC of CDER (see minutes of exec-CAC meeting dated 20-MAR-2007, NDA 21-852).

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:

Pregnancy:



(4)

1 Page(s) Withheld

 Trade Secret / Confidential (b4)

 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

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b(4)

## II. Summary of nonclinical findings

The product contains both calcipotriene and betamethasone dipropionate.

### Calcipotriene:

The primary sign of toxicity observed in studies that involved application of calcipotriene 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 calcipotriene 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 Taclonex 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.

Calcipotriene was considered negative in the Ames mutagenicity assay, the mouse lymphoma TK locus assay, the human lymphocyte chromosome aberration test, and the mouse micronucleus test.

Calcipotriene was evaluated for activity as a cocarcinogen with UV light in a 12-month study with hairless mice. 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 males that received the greatest exposure to calcipotriene (30 µg/kg/day), while vehicle alone had no effect, suggesting that calcipotriene may enhance the carcinogenic effects of UV light. Calcipotriene was evaluated for activity as a carcinogen in a study in which mice were treated topically for 24 months at dosages of 3, 10 and 30 mcg/kg/day (corresponding to 9, 30 and 90 mcg/m<sup>2</sup>/day). No biologically significant changes in tumor incidence were observed when compared to control. Evaluation of calcipotriene in a standard oral carcinogenicity assay in rats will be accomplished as a post-approval commitment.

Calcipotriene was evaluated for effects upon reproduction. Calcipotriene had no effects on fertility of male or female rats. Teratology studies conducted with calcipotriene in rats and rabbits indicated no effects on the incidence of major malformations, but found that at sufficient levels of systemic exposure calcipotriene can induce minor skeletal

variations, including incomplete ossification of sternebrae, pubic bones, and fore limb phalanges. When assessed for effects on peri-natal or post-natal development, calcipotriene 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.

**Betamethasone dipropionate:**

In a nine-month topical study in which minipigs were treated with Taclonex ointment, little toxicity was observed. Treatment-related findings included slightly reduced mean adrenal weight, minimal to moderate adrenal atrophy, and thinning of the skin. All of those effects were probably secondary to exposure to betamethasone dipropionate. As a glucocorticoid, betamethasone dipropionate is capable of causing reversible adrenal atrophy through negative feedback of the HPA axis. Even with substantial oral doses of betamethasone dipropionate, however, serious toxicity was not observed in rats that were orally dosed for 13 weeks. In that oral rat study, in which rats received up to 0.2 mg/kg/day betamethasone dipropionate, there were no effects on survival, clinical signs, clinical chemistry, or urinalysis, and there was no clear effect on mean body weight, although a trend toward reduced mean body weight with increasing dosage seemed apparent. The mean WBC count decreased in proportion to dosage, as did the mean weights of the spleen and thymus. These are known effects of corticosteroids when systemically administered at sufficient levels. Treatment-related histopathological findings in the oral rat study were limited to the spleen (lymphoid depletion), thymus (cortical atrophy), and lymph nodes (lymphoid depletion or hyperplasia) of high-dose animals of both genders. In all, little toxicity was observed in rats that were orally dosed with betamethasone dipropionate for 13 weeks. Although all plasma samples that were analyzed in that study were below the limit of quantitation for betamethasone dipropionate (75 pg/mL), substantial exposure to the metabolite, betamethasone 17-propionate, was documented.

Betamethasone dipropionate was negative in the Ames assay and in the mouse lymphoma TK locus assay with and without metabolic activation, and in an in vivo micronucleus assay.

The evaluation of betamethasone dipropionate in carcinogenicity assays will be accomplished as a post-approval commitment.

Betamethasone dipropionate was evaluated in a battery of reproductive toxicology studies. No effect on reproductive performance or fertility was observed when betamethasone dipropionate was orally administered to male rats at dosages up to 0.2 mg/kg/day, or in females orally dosed at up to 1.0 mg/kg/day. When administered subcutaneously to pregnant mice on days 7 through 13 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, increased incidence of cleft palate and crooked or short tail, and delayed ossification. A NOAEL was not observed in this study, as fetal toxicity was observed at the lowest exposure that was evaluated (0.156 mg/kg/day). When administered subcutaneously to pregnant rabbits on days 6 through 18 of gestation, betamethasone dipropionate induced

fetal toxicity, including fatality, reduced fetal body weight, external malformations, and skeletal malformations. An exposure of 0.625 µg/kg/day was a NOAEL in this study; fetal toxicity was observed at 2.5 µg/kg/day and above. Betamethasone dipropionate 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 1.0 mg/kg/day. Mean maternal BW was significantly lower at 0.3 and 1.0 mg/kg/day on day 20 of gestation. The mean duration of gestation was slightly but statistically increased at 0.1, 0.3, and 1.0 mg/kg/day. The mean percentage of pups that survived to day 4 was reduced in F1 pups in relation to dosage, although the effects at 0.1 and 0.3 mg/kg/day were minimal. The percentage of pups with a righting-reflex on day 5 of lactation was significantly reduced at 1.0 mg/kg/day. No effects were observed on pup learning ability or reproduction of F1 animals.

Taclonex scalp gel was essentially non-irritating to the skin or eyes.

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ON ORIGINAL



## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-185

Review number: 1

Sequence number/date/type of submission: N-000/28-JUN-2007

Information to sponsor: Yes ( ) No (X)

Sponsor and/or agent: LEO Pharmaceutical Products Ltd. A/S

Manufacturer for drug substance: Leo Pharmaceutical Products, Denmark,  
(calcipotriene) and \_\_\_\_\_ (betamethasone  
dipropionate)

b(4)

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

Division name: Division of Dermatologic and Dental Drug Products

HFD #: 540

Review completion date: 11-FEB-2008

#### Drug:

Trade name: Taclonex Scalp Gel. The Taclonex products are sometimes referred to in older submissions to related INDs and NDAs as "Diavobet", "Daivobet", or "Dovobet".

Generic name: Calcipotriene hydrate and betamethasone dipropionate scalp gel  
(Note: calcipotriene is known as calcipotriol in Europe).

Code name: MC 903 (calcipotriene)/433/M (betamethasone)

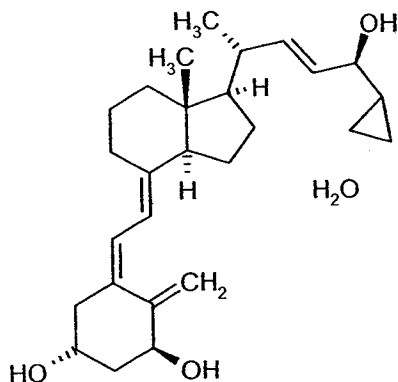
Chemical name: Calcipotriene: (1 $\alpha$ ,3 $\beta$ ,5Z,7E,22E,24S)-24-cyclo-propyl-9, 10-seco-chola-5,7,10(19),22-tetraene-1,3,24-triol. Betamethasone: 9-fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methyl-pregna-1,4-diene-3,20-diene 17,21-dipropionate.

CAS registry number: Calcipotriene: 147657-22-5; Betamethasone: 5593-20-4.

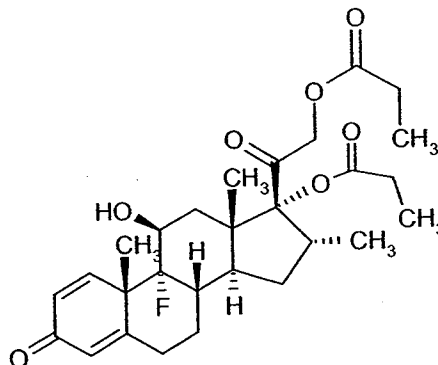
Molecular formula/molecular weight: Calcipotriene hydrate:

C<sub>27</sub>H<sub>40</sub>O<sub>3</sub>•H<sub>2</sub>O/430.6. Betamethasone dipropionate: C<sub>28</sub>H<sub>37</sub>FO<sub>7</sub>/504.59.

## Structures:



Calcipotriene Hydrate



Betamethasone Dipropionate

**Relevant INDs/NDAs/DMFs:** Calcipotriene/betamethasone dipropionate (Taclonex) ointment is marketed under NDA 21-852. Taclonex scalp gel was developed under IND 67,835. Calcipotriene (Dovonex) ointment is marketed under NDA 20-273. The sponsor (Leo Pharmaceutical Products) owns the nonclinical data used to support NDA 20-273. Betamethasone dipropionate ointment was originally approved under NDA 17-691 (Diprosone ointment, Schering Pharmaceuticals). The sponsor of NDA 22-185 does not have the legal right to reference NDA 17-691, although it should be noted that the product is off patent.

**Drug class:** Calcipotriene: Vitamin D analog; betamethasone: corticosteroid

**Intended clinical population:** Patients with psoriasis vulgaris of the scalp

**Clinical formulation (topical ointment):**

| <u>Compound</u>                       | <u>Amount per gram</u> |
|---------------------------------------|------------------------|
| Calcipotriene hydrate*                | 52.2µg                 |
| Betamethasone dipropionate**          | 643µg                  |
| Hydrogenated castor oil.....          | —                      |
| Polyoxypropylene-15-stearyl ether.... | —                      |
| Paraffin, liquid.....                 | —                      |

\*Equivalent to 50µg anhydrous calcipotriene.

\*\*Equivalent to 500µg betamethasone base.

b(4)

**Route of administration:** Topical to the scalp. The proposed use of the product (application to areas of the scalp that are affected by psoriasis) may involve application to up to approximately 10% of the body surface area. The material would be applied once daily for an indefinite period, resulting in chronic exposure to the product.

Approximately 14 g of product may be applied per day to a given patient (this statement is based upon the fact that the label indicates that the maximum weekly dose should not exceed 100 g; 100 g divided by 7 days per week equates to approximately 14 g per day). The actual dosage of a given patient may be less than 14 g per day.

**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. Some of the nonclinical studies were previously reviewed under NDA 21-852; those studies are included here for completeness.

**Pharmacokinetic Studies (briefly summarized in PK section):**

Pharmacokinetic studies with calcipotriene/betamethasone gel:

1. Absorption and excretion of [ $^3\text{H}$ ]-calcipotriene and [ $^3\text{H}$ ]-betamethasone in Dovobet ointment and Dovobet gel in the minipig in vivo, Study No. 806646 (also referred to as study No. LEO Ref. No. AE0504).

**Repeat-Dose Toxicology:**

Repeat-dose toxicology studies with calcipotriene alone:

1. Calcipotriol - Toxicity study by oral gavage administration To Han Wistar rats for 13 weeks, study No. TTOX0410 (also referred to as study No. LOP 052/052427).

Repeat-dose toxicology studies with betamethasone alone:

1. Betamethasone dipropionate toxicity study by dermal administration to CD-1 mice for 13 weeks, Study No. TTOX0409 (also referred to as study No. LOP 051/052449).

Repeat-dose toxicology studies with Polyoxypropylene-15-stearyl ether (an excipient):

1. PPG-15 stearyl ether toxicity study by dermal administration to CD-1 mice for 13 weeks, Study No. TTOX0513 (also referred to as study No. LOP0067/062765).
2. PPG-15 stearyl ether toxicity study by oral gavage administration to Han Wistar rats for 13 weeks, Study No. TTOX0514 (also referred to as study No. LOP0068/062764).

**Carcinogenicity:**

**Carcinogenicity studies with calcipotriene alone:**

1. Dermal carcinogenicity study in mice, study No. 01-2731 (previously reviewed under NDA 20-554).
2. Carcinogenicity Study by Oral Gavage Administration of calcipotriene to Rats for 104 Weeks (Phase IV Commitment to NDA 22-185).

**Reproductive Toxicology:**

**Reproductive toxicology studies with betamethasone alone:**

1. Oral (gavage) fertility and early embryonic development study in the female rat, Study No. RTOX0606 (also referred to as study No. LOP0079).
2. Oral (gavage) pre and post-natal development toxicity study in the rat, study No. RTOX0608 (also referred to as study No. LOP0081).

**Local Tolerance Studies:**

**Local tolerance studies with calcipotriene/betamethasone gel:**

1. Daivobet gel: Acute eye irritation study in rabbits, study No. LTOX0403.
2. Daivobet gel: A 4-week dermal tolerability study in rabbits, study No. LTOX0401.

**Special Toxicology Studies:**

1. Nonclinical photosafety testing to characterize the potential of topically administered calcipotriene solution and calcipotriene/betamethasone gel formulations for four weeks to modify photobiological responses in *skh1-hr* hairless mice, study No. CTOX0502. **b(4)**

**Studies reviewed under NDA 21-852 (and referenced by NDA 22-185, and included in this review for completeness):**

**Pharmacodynamic studies (briefly summarized in PD section):**

**Pharmacodynamic studies with calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):**

1. Effects of MC 903 and  $1,25(\text{OH})_2\text{D}_3$  on cultured keratinocytes from newborn mice, Study No. 35-86/25.

2. Evaluation of MC 903. Binding to the intestinal receptor for rachitic chickens and to rat and human serum, Study No. 35-86/02.
3. Assay of interleukin-1 (IL-1) induced thymocyte proliferation. Effects of MC 903 and  $1,25(\text{OH})_2\text{D}_3$ , Study No. 07-87/01.
4. Effects of  $1,25(\text{OH})_2\text{D}_3$  and its analogues on cell proliferation and differentiation in a human keratinocyte cell line (HaCaT), Study No. 07-92/15.
5. Effects of  $1,25(\text{OH})_2\text{D}_3$ , MC 903, EB 1089 and KH 1060 on the parathyroid hormone related peptide (PTHrP) in human keratinocytes, Study No. 07-93/07.
6. *In vitro* effects of vitamin D analogues on the induction of transglutaminase type I in human keratinocytes, Study No. 07-94/21.
7. Vitamin D receptor activity measured by CAT activity (Transactivation assay), Study No. 07-94/24.
8. EB 1089, KH 1218, KH 1230, KH 1266, MC 903 and  $1\alpha,25(\text{OH})_2\text{D}_3$ : Pharmacological aspects *in vitro* and *in vivo*, Study No. 07-95/09.
9. Evaluation of MC 900 and MC 903. Effects on calcium metabolism *in vivo* and on tumour cell differentiation *in vitro*, Study No. 35-85/17.
10. MC 1046 and MC 1080. Metabolites of MC 903. Effects on cell differentiation, cell proliferation, receptor binding and calcium metabolism, Study No. 07-89/07.
11. MC 900 and MC 902: Effects on cell differentiation, cell proliferation, receptor binding and calcium metabolism, Study No. 07-89/10.
12. EB 1130: Effects on cell proliferation, cell differentiation, receptor binding and calcium metabolism, Study No. 07-89/12.
13. HS 503: Effects on cell differentiation, cell proliferation, receptor binding and calcium metabolism, Study No. 07-93/14.
14. Evaluation of MC 903. Effects on rachitic rats, Study No. 35-86/07.
15. General pharmacological studies on MC 903, Study No. 35-88/02.
16. General pharmacological studies on MC 903, Study No. 35-89/21.
17. Lack of effect of MC 903 on hexobarbital sleeping time in mice, Study No. 35-90/01.
18. Effects of ETH 615 and MC 903 on oxazolone-induced ear inflammation in sensitized mice, a model of contact dermatitis, Study No. 35-92/09.

19. Further evaluation of vitamin D analogues in murine contact dermatitis, Study No. 35-92/14.

20. Effect of MC 903 in spontaneously hypertensive rats on blood pressure, heart rate and diuresis after repeated administration for 28 days, Study No. 35-88/10.

Pharmacodynamic studies with betamethasone alone:

1. Molecular mechanisms of glucocorticosteroid actions; Pulm Pharmacol Ther. 2000;13:115-26.

2. Topical glucocorticoids and the skin-mechanisms of action: an update; Mediators of Inflammation. 1998;7:183-93.

Pharmacodynamic studies with calcipotriene/betamethasone ointment:

1. Combination of calcipotriol and betamethasone dipropionate: Effects on HaCaT cell proliferation, on lymphocyte proliferation and on cytokine production, Study No. 07-01/15.

**Safety Pharmacology studies (briefly summarized in SP section):**

Safety Pharmacology studies with calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):

1. Calcipotriol hydrate: Behavioural Irwin test and effect on body temperature following single oral administration in the rat, Study No. 20040440PGR (LEO Ref. No. SPHA0403).

2. Calcipotriol hydrate: Evaluation of effects on blood pressure, heart rate, electrocardiogram and body temperature after single oral administration to conscious dogs, Study No. 20030645PCC (LEO Ref. No. SPHA0308).

3. Calcipotriol hydrate: Evaluation of effect on respiration in the unrestrained conscious rat following single oral administration, Study No. 20030646PCR (LEO Ref. No. SPHA0307).

Safety Pharmacology studies with betamethasone alone:

1. Betamethasone dipropionate: Behavioural Irwin test and effect on body temperature following single oral administration in the rat, Study No. 20040441PGR (LEO Ref. No. SPHA0402).

2. Betamethasone dipropionate: Evaluation of effects on blood pressure, heart rate, body temperature and electrocardiogram after single oral administration to conscious dogs, Study No. 20030647PCC (LEO Ref. No. SPHA0306).

3. Evaluation of effect on respiration in the unrestrained conscious rat following single oral administration, Study No. 20030648PCR (LEO Ref. No. SPHA0305).

Safety Pharmacology studies with calcipotriene/betamethasone ointment: None.

**Pharmacokinetic Studies (briefly summarized in PK section):**

Pharmacokinetic studies with calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):

1. Calcipotriol. Dermal absorption of  $^3\text{H}$ -calcipotriol in mice, Study No. AE/94/01 (Report No. 36-94/12).
2. Calcipotriol/calcipotriene (BMS-181161). Dermal absorption of  $^3\text{H}$ -calcipotriol in mice without collar protection, Study No. AE/97/01 (Report No. 36-97/15).
3. Absorption of  $^3\text{H}$ -MC 903 following topical application of  $^3\text{H}$ -MC 903 ointment to rats and rabbits, Study No. 890404A3 (File No. 35-89/13).
4. Absorption, distribution, metabolism and excretion of MC 903 in rats dosed with  $^3\text{H}$ -MC 903 i.v. or p.o., Study No. 35-89/12.
5. Dermal absorption of  $^3\text{H}$ -MC 903 in Rabbits, Study No. 35-86/15.
6. Absorption, distribution, metabolism and excretion of MC 903 in mini-pigs dosed with  $^3\text{H}$ -MC 903 i.v. or p.o., Study No. 35-89/15.
7. Whole-body autoradiography of rats after oral or intravenous administration of  $^3\text{H}$ -MC 903, Study No. 35-89/19.
8. MC 903. Metabolism in rats and mini-pigs, Study No. 18-RS 8948.
9. Profiles of  $^3\text{H}$ -calcipotriene and metabolites in minipig, rat and human, Study no. 50922.

Pharmacokinetic studies with betamethasone alone:

1. Studies on absorption, distribution, metabolism and excretion of betamethasone 17, 21-dipropionate (S-3440). Distribution in mice and rabbits, Oyo Yakuri 1981;21:645-55.

Pharmacokinetic studies with calcipotriene/betamethasone ointment:

1. The absorption, tissue distribution and excretion of [ $^3\text{H}$ ]-calcipotriene and [ $^3\text{H}$ ]-betamethasone (Dovobet<sup>®</sup>) in the rat *in vivo*, Study No. 205018.
2. Absorption, excretion and metabolic profiling of  $^3\text{H}$ -Dovobet in minipigs, Study No. AME/03/01.
3. Determination of the *in vitro* metabolic profile of [ $^3\text{H}$ ]-calcipotriol and/or [ $^3\text{H}$ ]-betamethasone dipropionate using the post mitochondrial (S9) liver fractions from mice, rats, rabbits, minipigs and humans, Study No. MET/03/01.

#### Acute Toxicology:

Acute toxicology studies with calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):

1. MC 903. Acute (single dose) toxicity study in mice and rats, Study No. 871124A3.
2. An acute oral toxicity study in rats with 0.005% BMS-181161 ointment, Study No. 09117.
3. A primary skin irritation study in rabbits with 0.005% BMV-30434 ointment and vehicle ointment, Study No. 91-002.
4. BMS-181161. Acute dermal toxicity in rabbits, Study No. 09113.

Acute toxicology studies with betamethasone alone: None

Acute toxicology studies with calcipotriene/betamethasone ointment: None

#### Repeat-Dose Toxicology:

Repeat-dose toxicology studies with calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):

1. Calcipotriol – 4 weeks dermal toxicity study in mice, Study No. 940411T7.
2. BMS-181161. Three-month dermal range-finding study in mice, Study No. 95639.
3. BMS-181161. Three-month dermal toxicity and recovery study in rats, Study No. 91013.



4. MC 903. Oral toxicity in rats. Repeated administration for 4 weeks, Study No. 860210T3.
5. MC 903. 26 week oral toxicity in the rat, Study No. 880212T2.
6. MC 903. Oral toxicity in the beagle dog. Repeated administration for 6 weeks, Study No. 860616T6.
7. A six-month subacute dermal toxicity study of 0.005% BMS-181161 ointment in swine (including a three-month interim evaluation), Study No. 91-001.
8. 52-week dermal toxicity study with BMS-181161 (0.005% ointment) in Hanford minipigs®, Study No. 92613.

Repeat-dose toxicology studies with betamethasone alone:

1. A 13-week oral carcinogenicity range-finding study in rats, Study No. TTOX0301.

Repeat-dose toxicology studies with calcipotriene/betamethasone ointment:

1. Daivobet - A preliminary dermal toxicity study in mice, Study No. TTOX0010.
2. Daivobet ointment. 13-week dermal dose range finding study in the mouse, Study No. LOP0058 (LEO Study No. TTOXO203).
3. Daivobet. A 9-month dermal toxicity study in minipigs, Study No. 48576 (LEO Study No. TTOX0205).

#### **Genetic Toxicology:**

Genetic toxicology studies with calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):

1. MC 903. Bacterial mutagenicity test (Ames test), Study No. 870211N1.
2. An assessment of the mutagenic potential of MC 903 using the mouse lymphoma TK locus assay, Study No. LOP 46 (Study Ref. 871637).
3. MC 903. Metaphase chromosome analysis of human lymphocytes cultured *in vitro*, Study No. LOP 47 (Study Ref. 88113).
4. Mutagenicity testing of MC 903. Micronucleus test in mouse bone marrow, Study No. 870406N1.

Genetic toxicology studies with betamethasone alone:

1. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*, Study No. 339/84 (LEO Study No. GTOX0201).

2. Mutation at the thymidine kinase (*tk*). Locus of mouse lymphoma L5178Y cells (MLA) using the ~~\_\_\_\_\_~~ fluctuation technique, Study No. 339/86 (LEO Study No. GTOX0202). **b(4)**

3. Bone marrow micronucleus test on betamethasone, Study No. 339/85 (LEO Study No. GTOX0203).

Genetic toxicology studies with calcipotriene/betamethasone ointment: None.

Genetic toxicology studies with Polyoxypolyethylene-15-stearyl ether (an excipient):

1. PPG-15 Stearyl Ether: reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*, Study No. 339/116 (LEO Study No. GTOX0302).

2. PPG-15 Stearyl Ether: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the ~~\_\_\_\_\_~~ Fluctuation Technique, Study No. 339/117 (LEO Study No. GTOX0303). **b(4)**

3. PPG-15 Stearyl Ether: Micronucleus Test in Mice, Study No. GTOX0301.

#### **Carcinogenicity:**

Carcinogenicity studies with calcipotriene alone:

1. BMS-181161 solution. 12-month photocarcinogenesis study with ultraviolet radiation in hairless mice, Study No. 1202-031 (LEO Study No. CTOX0102).

Carcinogenicity studies with betamethasone alone:

1. Carcinogenicity Study by Dermal Administration to Mice for 104 Weeks (Phase IV Commitment to NDA 21-852).

2. Betamethasone Dipropionate. Carcinogenicity Study by Oral Gavage Administration to Han Wistar Rats for 104 Weeks (Phase IV Commitment to NDA 21-852).

Carcinogenicity studies with calcipotriene/betamethasone ointment: None.

#### **Reproductive Toxicology:**

Reproductive toxicology studies with calcipotriene alone (some of these studies are discussed in reviews associated with NDA 20-273):

1. MC 903. A study on fertility and general reproductive performance in the rat, Study No. 870727T7.
2. Effect of MC 903 on foetal development in rats, Study No. 870824T8.
3. MC 903. Oral teratology study in the rabbit, Study No. 339/503.
4. MC 903. Peri- and postnatal study in rats, Study No. 880415T3.

Reproductive toxicology studies with betamethasone alone:

1. Effect on the fertility in male rats (oral administration), Study No. RTOX0301.
2. Teratology Studies on betamethasone 17,21-dipropionate, prednisolone and betamethasone 21-disodium phosphate in mice and rats, Oyo Yakuri (Pharmacometrics) 1974;8(6) (Published report).
3. Teratogenicity of betamethasone 17,21-dipropionate (S-3440) in rabbits Kiso to Rinsho (The Clinical Report);11(6), June 1977 (Published report).

Reproductive toxicology studies with calcipotriene/betamethasone ointment: None.

#### **Local Tolerance Studies:**

Local tolerance studies with formulations of calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):

1. Calcipotriol cream. 6 weeks skin irritation test in the rabbit, Study No. 911104I5.
2. Calcipotriol lotion. 6 weeks skin irritation test in the rabbit, Study No. 910612I3.
3. MC 903 ointment. Acute eye irritation study in the rabbit, Study No. 890508I2.

Local tolerance studies of formulations of betamethasone alone: None.

Local tolerance studies with calcipotriene/betamethasone ointment:

1. Calcipotriol betamethasone. Six weeks dermal tolerability study in rabbits, Study No. LTOX/99/02.

#### **Special Toxicology Studies:**

Special toxicology studies with calcipotriene alone (discussed in the Original Summary of NDA 20-273):

1. MC 903 guinea pig maximization test for allergenic potential, Study No. 861111I9.

Studies with betamethasone alone: None.

Studies with calcipotriene/betamethasone ointment: None.

**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

Calcipotriene 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 calcipotriene effects keratinocyte differentiation and proliferation is unclear. Vitamin D receptor agonists are also involved in modulation of calcium metabolism, and induce synthesis of intestinal calcium transport proteins.

Betamethasone is a synthetic corticosteroid, and is therefore an agonist of the glucocorticoid receptor. The betamethasone-receptor complex modulates the activity of certain genes, altering the production and activity of proteins that are involved in the inflammatory response. Such proteins include phospholipase A<sub>2</sub>, cyclooxygenase-2, and NO-synthase. Inhibition of the expression of these enzymes results in reduced production of such inflammatory mediators as prostaglandins, leukotrienes, and nitric oxide. Betamethasone also inhibits keratinocyte proliferation through an unknown mechanism.

### 2.6.2.2 Primary pharmacodynamics

Mechanism of action: Calcipotriene 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 calcipotriene effects keratinocyte differentiation and proliferation is unknown. Betamethasone is a synthetic corticosteroid. The betamethasone-glucocorticoid receptor complex modulates gene expression, indirectly altering the production and activity of such inflammatory mediators as

prostaglandins, leukotrienes, and nitric oxide. Inflammation is a component of the disease known as psoriasis. Betamethasone also inhibits keratinocyte proliferation through an unknown mechanism.

Drug activity related to proposed indication: Calcipotriene inhibits proliferation of keratinocytes within psoriatic lesions, resulting in reduced skin cell turn over. Betamethasone inhibits the inflammatory response that is associated with psoriasis and inhibits proliferation of keratinocytes.

#### **2.6.2.3 Secondary pharmacodynamics**

Vitamin D receptor agonists, such as calcipotriene, 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. Glucocorticoids have numerous effects if systemically administered at sufficient levels, including effects on carbohydrate metabolism and storage in the liver and water and electrolyte metabolism in the kidney.

#### **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 calcipotriene at high systemic levels is capable of impacting 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, although calcipotriene and betamethasone at high systemic levels are capable of impacting kidney function through effects on ion excretion.

Gastrointestinal effects: None known that are relevant to the proposed clinical use, although calcipotriene 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

Systemic absorption of topically applied calcipotriene and betamethasone (in ointment formulation) was approximately 10% of each in rats, while minipigs absorbed approximately 2% of topically applied calcipotriene and 3% of topically applied betamethasone. Both compounds were rapidly and widely distributed in both species. Both compounds are rapidly metabolized to compounds with less activity than the parent compounds. Calcipotriene is primarily excreted in the feces. Approximately one-third of systemically absorbed betamethasone is excreted in the urine, and the remainder in the feces.

To support the NDA for the gel formulation (22-185), data which compared absorption from the ointment and gel formulations were submitted. Absorption (on a percentage basis) of both betamethasone dipropionate and calcipotriene were statistically identical following topical administration of either the gel or ointment formulations that had been spiked with either <sup>3</sup>H-betamethasone dipropionate or <sup>3</sup>H-calcipotriene to minipigs.

### 2.6.4.2 Methods of Analysis

Systemic exposure to topically applied calcipotriene and betamethasone was very low, and was assessed in a few short-term studies through use of <sup>3</sup>H-calcipotriene and <sup>3</sup>H-betamethasone.

### 2.6.4.3 Absorption

When topically applied as components of Dovobet ointment, the percentages of calcipotriene and betamethasone that are systemically absorbed are low (approximately 10% in rats, 3% in minipigs, and less than 1% in humans). The levels of exposure are generally below the limit of detection in most studies, even though the detection limits for both compounds are in the pg per mL range.

### 2.6.4.4 Distribution

Both calcipotriene and betamethasone are rapidly and widely distributed throughout the body in all species that have been studied.

### 2.6.4.5 Metabolism

Calcipotriene is metabolized by rats, minipigs, and humans to MC 1046 (the  $\alpha,\beta$ -unsaturated ketone analog of calcipotriene), which is metabolized further to MC 1080 (a saturated ketone analog). MC 1080 is the major metabolite in plasma. MC 1080 is slowly metabolized to calcitroic acid.

Betamethasone dipropionate is metabolized to betamethasone 17-propionate and betamethasone, including the 6 $\beta$ -hydroxy derivatives of those compounds. Betamethasone 17-propionate is the primary metabolite.

#### **2.6.4.6 Excretion**

Calcipotriene is primarily excreted in the feces. Approximately one-third of systemically absorbed betamethasone is excreted in the urine, and the remainder in the feces.

#### **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 calcipotriene and betamethasone is limited. Calcipotriene is primarily metabolized to MC 1080 (a saturated ketone analog of calcipotriene). Betamethasone dipropionate is primarily metabolized to betamethasone 17-propionate. Calcipotriene is primarily excreted in the feces. Approximately one-third of systemically absorbed betamethasone is excreted in the urine, and the remainder in the feces.

#### **2.6.4.10 Tables and figures to include comparative TK summary**

Not available (due to extremely limited systemic absorption, plasma levels are generally below the limit of detection).

### **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 application of calcipotriene 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 calcipotriene 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 Dovobet 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.

In a nine-month topical study in which minipigs were treated with Dovobet ointment, little toxicity was observed. Treatment-related findings included slightly reduced mean adrenal weight, minimal to moderate adrenal atrophy, and thinning of the skin. All of those effects were probably secondary to exposure to betamethasone dipropionate. As a glucocorticoid, betamethasone dipropionate is capable of causing reversible adrenal atrophy through negative feedback of the HPA axis. Even with substantial oral doses of betamethasone dipropionate, however, serious toxicity was not observed in rats that were orally dosed for 13 weeks. In that oral rat study, in which rats received up to 0.2 mg/kg/day betamethasone dipropionate, there were no effects on survival, clinical signs, clinical chemistry, or urinalysis, and there was no clear effect on mean body weight, although a trend toward reduced mean body weight with increasing dosage seemed apparent. The mean WBC count decreased in proportion to dosage, as did the mean weights of the spleen and thymus. These are known effects of corticosteroids when systemically administered at sufficient levels. Treatment-related histopathological findings in the oral rat study were limited to the spleen (lymphoid depletion), thymus (cortical atrophy), and lymph nodes (lymphoid depletion or hyperplasia) of high-dose animals of both genders. In all, little toxicity was observed in rats that were orally dosed with betamethasone dipropionate for 13 weeks. Although all plasma samples that were analyzed in that study were below the limit of quantitation for betamethasone dipropionate (75 pg/mL), substantial exposure to the metabolite, betamethasone 17-propionate, was documented.

Genetic toxicology: Calcipotriene was considered negative in the Ames mutagenicity assay, the mouse lymphoma TK locus assay, the human lymphocyte chromosome aberration test, and the mouse micronucleus test.

Betamethasone dipropionate was negative in the Ames assay and in the mouse lymphoma TK locus assay with and without metabolic activation, and in an in vivo micronucleus assay.

Carcinogenicity: Calcipotriene was evaluated for activity as a cocarcinogen with UV light in a 12-month study with hairless mice. 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 males that received the greatest exposure to calcipotriene (30 µg/kg/day), while vehicle alone had no effect, suggesting that calcipotriene may enhance the carcinogenic effects of UV light. The evaluation of calcipotriene in a standard carcinogenicity assay will be accomplished as a post-approval commitment.

The evaluation of betamethasone dipropionate in carcinogenicity assays will be accomplished as a post-approval commitment.



Reproductive toxicology: Calcipotriene was evaluated for effects upon reproduction. Calcipotriene had no effects on fertility of male or female rats. Teratology studies conducted with calcipotriene in rats and rabbits indicated no effects on the incidence of major malformations, but found that at sufficient levels of systemic exposure calcipotriene can induce minor skeletal variations, including incomplete ossification of sternebrae, pubic bones, and fore limb phalanges. When assessed for effects on peri-natal or post-natal development, calcipotriene 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.

Betamethasone dipropionate was evaluated in a battery of reproductive toxicology studies. No effect on reproductive performance or fertility was observed when betamethasone dipropionate was orally administered to male rats at exposures up to 0.2 mg/kg/day, or in female rats at oral doses of up to 1.0 mg/kg/day. When administered subcutaneously to pregnant mice on days 7 through 13 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, increased incidence of cleft palate and crooked or short tail, and delayed ossification. A NOAEL was not observed in this study, as fetal toxicity was observed at the lowest exposure that was evaluated (0.156 mg/kg/day). When administered subcutaneously to pregnant rabbits on days 6 through 18 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, external malformations, and skeletal malformations. An exposure of 0.625 µg/kg/day was a NOAEL in this study; fetal toxicity was observed at 2.5 µg/kg/day and above.

Special toxicology: Taclonex scalp gel was essentially non-irritating to the skin or eyes.

#### **2.6.6.2 Single-dose toxicity**

Please see the Original Summary of NDA 20-273 for discussion of the acute toxicology of calcipotriene.

#### **2.6.6.3 Repeat-dose toxicity**

Repeat-dose toxicology studies with calcipotriene alone:

**2.6.6.3.1 Study Title:** Calcipotriol – 4 weeks dermal toxicity study in mice, Study No. 940411T7. Please see Original Summary of NDA 20-611.

**2.6.6.3.2 Study Title:** BMS-181161. Three-month dermal range-finding study in mice, Study No. 95639. In-life 4/95-7/95, report dated 7/9/96, conducted by \_\_\_\_\_ in compliance with Good Laboratory Practice regulations (21 CFR 58). b(4)

**Key study findings:** Toxicity was observed at dosages of 12 µg/kg/day and above; 3 µg/kg/day was a NOAEL. The most notable effects were presumably related to the

pharmacology of the test material (a vitamin D analog), and included altered calcium metabolism and microscopic evidence of stimulation of bone formation and mineralization of the kidney. Excessive toxicity was observed at dosages of 90 µg/kg/day or greater, including dermal irritation at the site of application, increased spleen weight, substantial effects on calcium homeostasis, and microscopic evidence of renal damage and focal mineralization. A dose of 30 µg/kg/day caused small but statistically significant increases in the mean serum calcium concentrations and substantial increases in the rate of excretion of calcium. These effects presumably reflect stimulation of intestinal absorption of calcium with subsequently enhanced excretion to dispose of the excess calcium. A dose of 12 µg/kg/day caused minimal signs of toxicity, including a slight effect on calcium metabolism.

**Methods:** Approximately 7-week old CD-1 (ICR)BR VAF/Plus mice were randomly assigned into treatment groups as indicated below:

b(4)

| Group             | Conc. of Soln. <sup>a</sup> | Amt. per Unit BW <sup>b</sup> | Number of Animals |         |
|-------------------|-----------------------------|-------------------------------|-------------------|---------|
|                   | (µg/mL)                     | (µg/kg)                       | Males             | Females |
| 1 Vehicle Control | 0                           | 0                             | 6                 | 6       |
| 2 Low-dose 1      | 0.75                        | 3                             | 6                 | 6       |
| 3 Low-dose 2      | 3.0                         | 12                            | 6                 | 6       |
| 4 Mid-dose 1      | 7.5                         | 30                            | 6                 | 6       |
| 5 Mid-dose 2      | 22.5                        | 90                            | 6                 | 6       |
| 6 Mid-dose 3      | 30.0                        | 120                           | 6                 | 6       |
| 7 High-dose       | 45.0                        | 180                           | 6                 | 6       |

<sup>a</sup>The products used clinically contain 50 µg of drug per ml or g of material.

<sup>b</sup>Based on an estimated body weight of 25 g.

The animals were dosed once daily by topical application, 7 days per week for 13 weeks. The dose volume for all animals was 100 µl, which was pipetted over a clipped dorsal area at least 3cm x 2cm. The dose was "evenly dispensed over the treatment site by gently stroking with the dosing pipette tip". The treatment sites were not rinsed following dosing, nor were the sites occluded or the mice collared. The vehicle consisted of isopropanol in water (proportions not indicated). Food and water were available *ad libitum*. The parameters that were monitored were mortality and moribundity, abnormal behavior, body weight (measured weekly), signs of dermal irritation (erythema, edema, atonia, desquamation, fissuring, and eschar formation), hematology (including differential cell count), blood chemistry, urinalysis, gross necropsy, organ weights (brain, kidney, liver, ovaries, spleen, and testes), and histopathology (all groups) of the aorta, eyes, femur with bone marrow, kidneys, liver (control and high-dose animals only), skin (both treated and untreated, including the subcutis and underlying muscle layer), spleen, and sternum (with marrow). Note: No toxicokinetic data were obtained.

## Results.

**Survival.** One female at 180 µg/kg/day was sacrificed *in extremis* on day 51 due to a "thin and hunched appearance" and sensitivity to touch. The death was considered to have been related to treatment. No other unscheduled deaths were reported.

**Clinical signs.** The majority of both males and females at 180 µg/kg/day exhibited "rough haircoat". No other remarkable observations were reported.

**Body weight gain.** Both males and females at 180 µg/kg/day exhibited a trend toward reduced body weight gain over weeks 1-14:

#### Summary of Body Weight Gain

##### Males:

| Dosage (µg/kg/day) | Weight Gain (g±S.D.) |
|--------------------|----------------------|
| 0 (Controls)       | 5.7±1.2              |
| 180                | 3.5±5.0              |

##### Females:

| Dosage (µg/kg/day) | Weight Gain (g±S.D.) |
|--------------------|----------------------|
| 0 (Controls)       | 7.1±1.3              |
| 180                | 5.9±4.2              |

The differences were not statistically significant (due to the small group sizes). No other remarkable observations were reported.

**Dermal irritation.** Dose-related erythema, edema, atonia, and desquamation were observed in both males and females that received 90 µg/kg/day or more.

**Hematology.** Slight increases were observed in the eosinophil and monocyte counts in both males and females at 180 µg/kg/day; although apparently related to treatment, the significance of the observation, if any, is unclear. No other remarkable hematological effects were observed.

**Blood chemistry.** As anticipated for calcipotriene (a vitamin D analog), a dose-related increase in serum calcium was observed:

#### Serum Calcium (mg/dL±S.D.)

| Dosage (µg/kg) | 0        | 3        | 12        | 30        | 90         | 120        | 180       |
|----------------|----------|----------|-----------|-----------|------------|------------|-----------|
| Males:         | 8.9±0.26 | 8.8±0.28 | 9.2±0.20* | 9.8±0.44* | 11.2±0.49* | 11.8±0.64* | 13.2±1.1* |
| Females:       | 8.9±0.24 | 9.1±0.31 | 9.1±0.28  | 9.7±0.25* | 11.3±0.95* | 11.7±1.0*  | 12.3±1.3* |

\*Indicates statistically significant at  $p < 0.05$ .

Total protein was slightly (but significantly) elevated in animals that received 120 µg/kg/day or more. No other remarkable effects on blood chemistry were observed.

**Urinalysis.** A dose-related increase in the urinary calcium was observed:

Urinary Calcium (mg/dL±S.D.)

| Dosage   | 0       | 3       | 12       | 30     | 90     | 120    | 180      |
|----------|---------|---------|----------|--------|--------|--------|----------|
| Males:   | 3.2±1.8 | 5.2±8.4 | 9.9±5.6* | 38±27* | 64±26* | 63±39* | 71±25*   |
| Females: | 8.1±4.2 | 12±12   | 25±30    | 34±13* | 35±11* | 64±31* | 116±120* |

\*Indicates statistically significant at  $p < 0.05$ . No other remarkable urological effects were observed.

**Gross necropsy.** Gross observations made in animals at 90 µg/kg/day or greater included thickening and/or crusting of the treated skin, enlargement of the spleen and lymph nodes, and light-colored areas of the diaphragm (mineralization).

**Organ weights.** In both males and females, the absolute and relative (to BW) mean spleen weights increased in proportion to dosage beginning at 90 µg/kg/day. In females, a trend toward increased mean liver weight was observed at 120 µg/kg/day and above.

No other remarkable effects on organ weight were observed in either male or female animals.

**Histopathology.** Treatment-related effects included:

Treated skin. Minimal to moderately severe epidermal hyperplasia in animals given 30 µg/kg/day and above. Minimal to slight subacute inflammation of the dermis of males and females at 90 µg/kg/day or greater.

Kidneys. Minimal to slight tubular degeneration, regeneration, and mineralization in males and females at 90 µg/kg/day or greater; severity was proportional to dosage.

Bone (sternum and femur). A dosage-related hyperostosis (increased amount of bone) was observed in both males and females at 30 µg/kg/day and above.

Lymph nodes. Slight to moderately severe lymphocytic hyperplasia was observed in the axillary, brachial, and inguinal lymph nodes of animals at 30 µg/kg/day and above; severity was proportional to dosage. This finding was considered to be secondary to dermal inflammation.

**2.6.6.3.3 Study Title:** BMS-181161. Three-month dermal toxicity and recovery study in rats, Study No. 91013. Please see Original Summary of NDA 20-273.

**2.6.6.3.4 Study Title:** MC 903. Oral toxicity in rats. Repeated administration for 4 weeks, Study No. 860210T3. Please see Original Summary of NDA 20-273.

**2.6.6.3.5 Study Title:** Calcipotriol - Toxicity study by oral gavage administration To Han Wistar rats for 13 weeks.

**Key study findings:** Calcipotriene was orally administered to rats for 13 weeks at dosages of 0, 3, 15, and 75 µg/kg/day. There were no unscheduled deaths or adverse clinical signs. Mean BW gain over weeks 0-13 was significantly reduced at 75 µg/kg/day in both genders. Mean plasma calcium levels were significantly increased at 15 µg/kg/day and above in both genders, and at 3 µg/kg/day in females. Mean urinary calcium concentration and excretion were significantly increased in all treatment groups in both genders. Histopathological observations were of minor severity, but included minimal to slight mineralization and cortical tubular basophilia in the kidneys in both genders at 15 and 75 µg/kg/day, minimal to slight osteopetrosis of the femur and sternum in both genders at 15 and 75 µg/kg/day, and slight degeneration of the testes, epididymides, and prostate in a minority of males at 75 µg/kg/day.

**Study No:** TTOX0410 (also referred to as study No. LOP 052/052427)

**Amendment #, Vol #, and page #:** NA

**Conducting laboratory and location:** \_\_\_\_\_

**Date study initiated:** 08-DEC-2004

**GLP compliance:** Yes

**QA- Report** Yes (X) No ( )

**Methods:**

**Dosing:**

- species/strain: Rat/Wistar (HsdBrlHan:WIST, outbred)
- #/sex/group or time point: 10; additional 9 per sex per group for toxicokinetic purposes only (6 in control group)
- age: 6 weeks at initiation of dosing
- weight: Males approx 125g, females approx 110 g
- satellite groups used for toxicokinetics or recovery: Yes
- summary of study design:

| Group Number | Dosage of Calcipotriene<br>(µg/kg/day) | No. of Rats |        |
|--------------|----------------------------------------|-------------|--------|
|              |                                        | Male        | Female |
| 1            | 0 (vehicle)                            | 10          | 10     |
| 2            | 3                                      | 10          | 10     |
| 3            | 15                                     | 10          | 10     |
| 4            | 75                                     | 10          | 10     |
| 5*           | 0 (vehicle)                            | 6           | 6      |

|    |    |   |   |
|----|----|---|---|
| 6* | 3  | 9 | 9 |
| 7* | 15 | 9 | 9 |
| 8* | 75 | 9 | 9 |

\*Groups 5-8 used for toxicokinetic purposes only.

- treatment: All main-study animals (groups 1-4) dosed once daily by gavage for 13 weeks.

- route, form, volume: Oral (gavage), solution/suspension, 0.5 mL/kg/day

- drug, lot#, and % purity: Calcipotriene, lot No. 0430517, 94.4% purity

- Formulation/vehicle: Suspended in an 80:20 mixture of propylene glycol and phosphate buffer in water

#### Observations:

- Survival: Yes
- Clinical signs: Yes (twice daily)
- Body weights: Yes (weekly)
- Food consumption: Yes (weekly)
- Ophthalmoscopy: No
- EKG: No
- Hematology: Yes (weeks 4 and 13)
- Clinical chemistry: Yes (weeks 4 and 13)
- Urinalysis: Yes (weeks 4 and 13)
- Organ weights: Yes
- Gross pathology: Yes
- Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes/epididymides, thymus, thyroids, uterus
- Histopathology: Yes, of main study animals in groups 1 (control) and 4 (high dose), plus gross lesions, adrenals, femoral joint, kidneys, mesenteric lymph nodes, prostate, spleen, sternum, testes/epididymides, and thymus from group 2 and 3 animals.
- List of tissues histologically examined: Standard list
- Toxicokinetics: Yes; blood samples obtained from animals in groups 5-8 during week 13 at times of 0 (pre-treatment), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, and 4 hours post-dosing. The samples were analyzed for content of calcipotriene.

#### Results:

- Survival: No unscheduled deaths.
- Clinical signs: No remarkable observations.
- Body weight/Body weight gain: Mean BW gain over weeks 0-13 significantly reduced (approx. 20% less than controls) at 75 µg/kg/day in both genders :

| Group/Gender<br>(µg/kg/day) | Body<br>Weight,<br>Week 13 (g) | Body Weight,<br>Week 13 (% of<br>Controls) | Body Weight<br>Gain,<br>Weeks 0-13 | Body<br>Weight<br>Gain, |
|-----------------------------|--------------------------------|--------------------------------------------|------------------------------------|-------------------------|
|-----------------------------|--------------------------------|--------------------------------------------|------------------------------------|-------------------------|

|                      |        |      | (g)      | Weeks 0-13, (% of Controls) |
|----------------------|--------|------|----------|-----------------------------|
| Group 1/Males (0)    | 388±45 | NA   | 269±44   | NA                          |
| Group 2/Males (3)    | 363±32 | 94%  | 242±30   | 90%                         |
| Group 3/Males (15)   | 375±25 | 97%  | 251±20   | 93%                         |
| Group 4/Males (75)   | 337±35 | 87%  | 216±34** | 80%                         |
| Group 1/Females (0)  | 229±19 | NA   | 122±15   | NA                          |
| Group 2/Females (3)  | 232±26 | 101% | 119±20   | 98%                         |
| Group 3/Females (15) | 233±21 | 102% | 121±18   | 99%                         |
| Group 4/Females (75) | 208±20 | 91%  | 100±18*  | 82%                         |

\*p<0.05; \*\*p<0.01

- Food consumption: No remarkable observations.
- Ophthalmology: NA
- Hematology: No remarkable observations.
- Clinical chemistry: Mean plasma calcium levels were significantly increased at 15 µg/kg/day and above in both genders, and at 3 µg/kg/day in females:

#### Plasma Calcium Levels, week 13

| Group/Gender (µg/kg/day) | Mean Calcium Conc. (mmol/L) | % of Control Value |
|--------------------------|-----------------------------|--------------------|
| Group 1/Males (0)        | 2.82±0.05                   | NA                 |
| Group 2/Males (3)        | 2.83±0.05                   | 100                |
| Group 3/Males (15)       | 2.95±0.11**                 | 105                |
| Group 4/Males (75)       | 3.17±0.09**                 | 112                |
| Group 1/Females (0)      | 2.83±0.09                   | NA                 |
| Group 2/Females (3)      | 2.93±0.08**                 | 104                |
| Group 3/Females (15)     | 3.02±0.07**                 | 107                |

|                         |             |     |
|-------------------------|-------------|-----|
| Group 4/Females<br>(75) | 3.21±0.06** | 115 |
|-------------------------|-------------|-----|

\*\*p<0.01

A few other blood chemistry parameters differed statistically significantly from control values, but did not appear to be biologically significant.

- Urinalysis: Mean urinary calcium concentration and excretion (collected overnight) were significantly increased in all treatment groups in both genders:

#### Urinary Calcium Data, week 13

| Group/Gender<br>(µg/kg/day) | Mean<br>Calcium<br>Conc.<br>(mmol/L) | Volume of<br>Urine<br>Collected<br>(mL) | Total<br>Calcium<br>Excretion<br>(µmol) | % of Control<br>Total<br>Calcium<br>Excretion |
|-----------------------------|--------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------------|
| Group 1/Males<br>(0)        | 5.91±2.1                             | 4.5±1.3                                 | 28±14                                   | NA                                            |
| Group 2/Males<br>(3)        | 12.05±3.5**                          | 3.8±1.9                                 | 48±17**                                 | 171                                           |
| Group 3/Males<br>(15)       | 23.33±4.7**                          | 5.2±2.2                                 | 117±40**                                | 418                                           |
| Group 4/Males<br>(75)       | 36.34±2.6**                          | 4.8±1.3                                 | 172±37**                                | 614                                           |
| Group 1/Females<br>(0)      | 10.53±3.2                            | 4.0±1.0                                 | 41±15                                   | NA                                            |
| Group 2/Females<br>(3)      | 22.42±4.2**                          | 3.2±1.4                                 | 69±25*                                  | 168                                           |
| Group 3/Females<br>(15)     | 24.55±1.7**                          | 3.4±0.95                                | 83±24**                                 | 202                                           |
| Group 4/Females<br>(75)     | 25.18±4.5**                          | 3.6±1.4                                 | 89±29**                                 | 217                                           |

\*p<0.05; \*\*p<0.01

A few other urinalysis parameters differed statistically significantly from control values, but did not appear to be biologically significant.

- Organ Weights: Slightly (5%-15%) increased mean kidney weights (absolute and when adjusted for terminal bodyweight) were seen in all treated groups of females and in males given 75 µg/kg/day in comparison with the control. Statistically significantly higher (approx. 20%) mean adrenal weights (absolute and when adjusted for terminal bodyweight) were noted in males given 15 or 75 µg/kg/day.

- Gross pathology: No remarkable observations.

- Histopathology: Tissues with treatment-related findings were considered to include the adrenals, kidneys, femur, sternum, testes, epididymides, and prostate, but were mild in nature:



Adrenals: Slight "sinusoidal congestion" was observed in a small number of animals at 15 and 75 µg/kg/day. The cause was unclear, and this finding was probably of no toxicological importance.

Kidneys: Minimal to slight mineralization and cortical tubular basophilia and dilation were observed in both genders at 15 and 75 µg/kg/day, increasing somewhat with dosage.

Femur and sternum: Minimal to slight osteopetrosis (increased thickness and density of the bone) was observed in both genders at 15 and 75 µg/kg/day.

Testes, epididymides, and prostate: Approximately 30% of the males at 75 µg/kg/day exhibited minimal to moderate inflammation and degeneration of the seminiferous tubules, reduced or degenerate spermatid cells in the epididymides, and epithelial atrophy in the prostate.

Other tissues: At 75 µg/kg/day, a single male exhibited slight mineralization of the artery at the entrance to the heart, and individual animals exhibited minimal mineralization of the conjunctival ducts and of the cornea.

- Toxicokinetics: All samples analyzed from control, LD, and MD animals were below the limit of quantitation for calcipotriene (60 pg/mL). A few samples from the HD group contained measurable levels of calcipotriene, but the data were not adequate to permit calculation of meaningful pharmacokinetic parameters. A metabolite of calcipotriene, MC1080, was detectable in samples from all treatment groups, but the data were not quantifiable.

**2.6.6.3.6 Study Title:** MC 903. 26 week oral toxicity in the rat, Study No. 880212T2. Please see Original Summary of NDA 20-273.

**2.6.6.3.7 Study Title:** MC 903. Oral toxicity in the beagle dog. Repeated administration for 6 weeks, Study No. 860616T6. Please see Original Summary of NDA 20-273.

**2.6.6.3.8 Study Title:** A six-month subacute dermal toxicity study of 0.005% BMS-181161 ointment in swine (including a three-month interim evaluation), Study No. 91-001. Please see Original Summary of NDA 20-273.

**2.6.6.3.9 Study Title:** 52-week dermal toxicity study with BMS-181161 (0.005% ointment) in Hanford minipigs®, Study No. 92613. Please see Original Summary of NDA 20-611.

Repeat-dose toxicology studies with betamethasone alone:

**2.6.6.3.10 Study Title:** A 13-week oral carcinogenicity range-finding study in rats.

**Key study findings:** Treatment-related findings observed in this study included reduced WBC counts, reduced mean weights for the spleen and thymus, and a trend toward reduced mean body weight. The mean body weight gain was reduced by more than 10% in all female treatment groups, although this observation may have been confounded by the fact that the animals were fasted prior to weighing during week 13. Treatment-related histopathological findings were limited to the spleen (lymphoid depletion), thymus (cortical atrophy), and lymph nodes (lymphoid depletion or hyperplasia) of high-dose animals of both genders.

**Study No:** TTOX0301

**Amendment #, Vol #, and page #:** Mod 4, vol. 26, page 1

**Conducting laboratory and location:** LEO Pharmaceuticals, Denmark

**Date study initiated:** 25-JUN-2003

**Animal phase initiation:** 25-JUN-2003

**Date of final sign-off by study director:** 22-NOV-2004

**GLP compliance:** Yes

**QA- Report** Yes (X) No ( )

**Methods:**

**Dosing:**

- species/strain: Rat/Wistar (HsdBrlHan:WIST, outbred)
- #/sex/group or time point: 10; additional 6 per sex per group for toxicokinetic purposes only
- age: 7-8 weeks at initiation
- weight: Males approx 225g, females approx 170 g
- satellite groups used for toxicokinetics or recovery: Yes
- summary of study design:

| Group Number | Dosage of Betamethasone Dipropionate (mg/kg/day) | No. of Rats |        |
|--------------|--------------------------------------------------|-------------|--------|
|              |                                                  | Male        | Female |
| 1            | 0 (vehicle)                                      | 10          | 10     |
| 2            | 0.02                                             | 10          | 10     |
| 3            | 0.06                                             | 10          | 10     |
| 4            | 0.2                                              | 10          | 10     |
| 5*           | 0 (vehicle)                                      | 2           | 2      |
| 6*           | 0.02                                             | 6           | 6      |
| 7*           | 0.06                                             | 6           | 6      |
| 8*           | 0.2                                              | 6           | 6      |

\*Groups 5-8 used for toxicokinetic purposes only.

- treatment: All main-study animals (groups 1-4) dosed once daily by gavage for 13 weeks.

- route, form, volume: Oral (gavage), solution/suspension, 5 mL/kg/day

- drug, lot#, and % purity: Betamethasone dipropionate, lot No. 0314461, assumed 100% purity

- Formulation/vehicle: Suspended in 1% methylcellulose in water

#### Observations:

- Survival: Yes
- Clinical signs: Yes (daily)
- Body weights: Yes (weekly)
- Food consumption: Yes (weekly)
- Ophthalmoscopy: Yes
- EKG: No
- Hematology: Yes (weeks 6 and 13)
- Clinical chemistry: Yes (weeks 6 and 13)
- Urinalysis: Yes (weeks 6 and 13)
- Organ weights: Yes
- Gross pathology: Yes
- Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes/epididymides, thymus
- Histopathology: Yes, of main study animals in groups 1 (control) and 4 (high dose), plus spleen and thymus from group 2 and 3 animals.
- List of tissues histologically examined: Standard list
- Toxicokinetics: Yes; blood samples obtained from animals in groups 5-8 on day 7 (the eighth day of dosing) at times of 0 (pre-treatment), 1, 2, 3, 5, and 7 hours post-dosing. The samples were analyzed for content of betamethasone dipropionate and the primary metabolite, betamethasone 17-propionate.
- Other: Following 10 weeks of treatment, male main-study animals were paired with untreated females until mating was confirmed (up to 11 days). This was apparently done to generate data to support dosage selection in a separate male fertility study.

#### Results:

- Survival: No remarkable unscheduled deaths.
- Clinical signs: No remarkable observations.
- Body weight/Body weight gain: No statistically significant differences in mean body weight were observed, although there was a suggestion of a trend toward reduced weight with increased dose. Several groups exhibited more than 10% reduction in body weight gain relative to controls over weeks 0-13, including all the female treatment groups:

| Group/Gender<br>(mg/kg/day) | Body<br>Weight,<br>Week 13 (g) | Body Weight,<br>Week 13 (% of<br>Controls) | Body Weight<br>Gain,<br>Weeks 0-13<br>(g) | Body<br>Weight<br>Gain,<br>Weeks 0- |
|-----------------------------|--------------------------------|--------------------------------------------|-------------------------------------------|-------------------------------------|
|-----------------------------|--------------------------------|--------------------------------------------|-------------------------------------------|-------------------------------------|

|                        |        |      |     | 13, (% of Controls) |
|------------------------|--------|------|-----|---------------------|
| Group 1/Males (0)      | 369±37 | NA   | 136 | NA                  |
| Group 2/Males (0.02)   | 367±30 | 100% | 136 | 100%                |
| Group 3/Males (0.06)   | 341±35 | 92%  | 117 | 86%                 |
| Group 4/Males (0.2)    | 349±23 | 95%  | 125 | 92%                 |
| Group 1/Females (0)    | 240±9  | NA   | 66  | NA                  |
| Group 2/Females (0.02) | 226±15 | 94%  | 57  | 86%                 |
| Group 3/Females (0.06) | 223±22 | 93%  | 58  | 88%                 |
| Group 4/Females (0.2)  | 221±12 | 92%  | 52  | 79%                 |

Note: The animals were fasted prior to weighing during week 13, and this undoubtedly reduced the mean body weight gain for all groups. However, since the controls were also fasted, presumably the "body weight gain" statistic, expressed as a percentage, is still relevant.

- Food consumption: No remarkable observations.
- Ophthalmology: No remarkable observations.
- Hematology: The mean WBC count decreased with treatment in a dose-dependent manner. The means for WBCs, lymphocytes, and eosinophil were significantly different from the control value for both genders in the high-dose groups:

Remarkable Hematology Values as a Percentage of Placebo-treated Controls

| Group/Gender (mg/kg/day) | WBC Count, Week 13 (% of Controls) | Lymphocytes, Week 13 (% of Controls) | Eosinophils, Week 13 (% of Controls) |
|--------------------------|------------------------------------|--------------------------------------|--------------------------------------|
| Group 1/Males (0)        | NA                                 | NA                                   | NA                                   |
| Group 2/Males (0.02)     | 95%                                | 100%                                 | 70%                                  |
| Group 3/Males (0.06)     | 93%                                | 91%                                  | 80%                                  |
| Group 4/Males (0.2)      | 70%**                              | 73%**                                | 20%**                                |
| Group 1/Females (0)      | NA                                 | NA                                   | NA                                   |
| Group 2/Females          | 95%                                | 98%                                  | 64%                                  |

|                           |      |      |      |
|---------------------------|------|------|------|
| (0.02)                    |      |      |      |
| Group 3/Females<br>(0.06) | 83%  | 84%  | 36%* |
| Group 4/Females<br>(0.2)  | 72%* | 72%* | 36%* |

\*p<0.05; \*\*p<0.01

- Clinical chemistry: No remarkable observations.
- Urinalysis: No remarkable observations.
- Organ Weights: Mean weights of the spleen and thymus were significantly reduced relative to control values:

#### Absolute Mean Weights of the Spleen and Thymus

| Group/Gender<br>(mg/kg/day) | Mean<br>Weight of<br>Spleen (g) | Mean Weight<br>of Thymus (g) |
|-----------------------------|---------------------------------|------------------------------|
| Group 1/Males<br>(0)        | 0.59±0.05                       | 0.24±0.05                    |
| Group 2/Males<br>(0.02)     | 0.58±0.04                       | 0.21±0.05                    |
| Group 3/Males<br>(0.06)     | 0.54±0.08                       | 0.16±0.07*                   |
| Group 4/Males<br>(0.2)      | 0.47±0.06**                     | 0.13±0.03**                  |
| Group 1/Females<br>(0)      | 0.53±0.7                        | 0.29±0.05                    |
| Group 2/Females<br>(0.02)   | 0.47±0.09                       | 0.19±0.04**                  |
| Group 3/Females<br>(0.06)   | 0.44±0.03**                     | 0.12±0.02**                  |
| Group 4/Females<br>(0.2)    | 0.40±0.05**                     | 0.10±0.02**                  |

\*p<0.05; \*\*p<0.01

The mean organ weight change data were similar when normalized to body weight.

- Gross pathology: Atrophy of the thymus was observed in one male and two females in the high-dose group. No other treatment-related observations.
- Histopathology: Treatment-related findings were limited to the spleen (lymphoid depletion), thymus (cortical atrophy), and lymph nodes (lymphoid depletion or hyperplasia) of high-dose animals of both genders.
- Toxicokinetics: All samples analyzed were below the limit of quantitation for betamethasone dipropionate (75 pg/mL). The concentration of the metabolite, betamethasone 17-propionate, was above the limit of quantitation for this compound (125 pg/mL), and the following parameters were calculated for the

metabolite:

| Dosage                         | Males, Day 7                      |                                    |                             |                          |                          | Females, Day 7                    |                                    |                             |                          |                          |
|--------------------------------|-----------------------------------|------------------------------------|-----------------------------|--------------------------|--------------------------|-----------------------------------|------------------------------------|-----------------------------|--------------------------|--------------------------|
|                                | AUC <sub>0-24</sub><br>(pg•hr/mL) | AUC <sub>0-inf</sub><br>(pg•hr/mL) | C <sub>max</sub><br>(pg/mL) | T <sub>max</sub><br>(hr) | T <sub>1/2</sub><br>(hr) | AUC <sub>0-24</sub><br>(µg•hr/mL) | AUC <sub>0-inf</sub><br>(µg•hr/mL) | C <sub>max</sub><br>(pg/mL) | T <sub>max</sub><br>(hr) | T <sub>1/2</sub><br>(hr) |
| 0.02<br>mg/kg/day<br>(Group 6) | ND                                | ND                                 | 620                         | 1                        | ND                       | 9990                              | 11,410                             | 4438                        | 1                        | 2.3                      |
| 0.06<br>mg/kg/day<br>(Group 7) | ND                                | ND                                 | 2084                        | 1                        | ND                       | 31,985                            | 33,177                             | 14,237                      | 1                        | 1.3                      |
| 0.2<br>mg/kg/day<br>(Group 8)  | 15,700                            | 16,089                             | 7841                        | 1                        | 1.2                      | 103,652                           | 106,122                            | 52,411                      | 1                        | 1.2                      |

ND indicates "not determined", as terminal phase was not adequately defined.

**2.6.6.3.11 Study Title:** Betamethasone dipropionate toxicity study by dermal administration to CD-1 mice for 13 weeks

Key study findings: Little toxicity was observed, with the exception of significantly reduced mean body weight gain at estimated exposures of 33.3 µg/kg/day and above. Non-significant trends toward reduced weight gain may have been apparent in animals (especially females) dosed at 10 µg/kg/day. Small but statistically significant increases in hematocrit, hemoglobin, and RBC count were observed in males at 33.3 µg/kg/day and above, while significantly increased hematocrit was observed in females at 33.3 µg/kg/day, but these effects were of low magnitude and probably do not reflect dose-limiting toxicity. Observed (non-significant) trends in organ-weight changes included, in males, increased weight of the heart and kidneys and decreased weight of the liver and spleen, and in females, decreased mean adjusted weight of the kidneys, spleen, thymus, and uterus. These effects were small, but were probably caused by treatment. No irritation at the treatment site was observed. Histopathological changes considered to be related to treatment were limited to the spleen (decreased cellularity of the red pulp) and thymus (decreased cellularity) of animals at 33.3 µg/kg/day or higher, although these effects were minor and may not indicate dose-limiting toxicity. A dosage of 3.3 µg/kg/day was an apparent NOAEL in females while 10 µg/kg/day was an apparent NOAEL in males (due to reduced mean weight gains at higher exposures).

Study no: LOP 051/052449 (LEO Study No. TTOXO409)

Conducting laboratory and location:

Date of study initiation: 15-DEC-2004

GLP compliance: Yes

b(4)

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: Betamethasone dipropionate ointment (presumably in the vehicle of Taclonex ointment, see formulation at beginning of this review), in concentrations of 0 (vehicle control), 1, 3, 10, and 30 µg/g; batch Nos. 0429717, 0436316, 0429817, 0429916, 0429917, respectively. Note: The stated concentrations refer to betamethasone base; the respective concentrations of betamethasone dipropionate were 0, 1.29, 3.86, 12.9, and 38.6 µg/g. Presumed 100%.

Methods:

Dosing:

**b(4)**

Species/strain: Mice → CD-1 (ICR) BR VAF/Plus

#/sex/group or time point (main study): 10 per sex per group, plus 18 per sex per group in toxicokinetic satellite groups.

Satellite groups used for toxicokinetics or recovery: Yes

Age: Approx. 7 weeks

Weight (at start of treatment): Males 26.5-37.5 g; Females 21.3-29.0 g

Doses in administered units: 0.1 g/day of assigned material (plus an untreated control group).

- summary of study design:

| Group Number<br>(Conc. of Betamethasone<br>Base) | Nominal Dosage of<br>Betamethasone Base<br>(µg/kg/day)* | No. of Mice<br>Per Gender |                        |
|--------------------------------------------------|---------------------------------------------------------|---------------------------|------------------------|
|                                                  |                                                         | Main<br>Study             | Toxicokinetic<br>Group |
| 1<br>(No treatment)                              | 0 (untreated control)                                   | 10                        | None                   |
| 2<br>(0 µg/g)                                    | 0 (vehicle control)                                     | 10                        | 12                     |
| 3<br>(1 µg/g)                                    | 3.3                                                     | 10                        | 27                     |
| 4<br>(3 µg/g)                                    | 10                                                      | 10                        | 27                     |
| 5<br>(10 µg/g)                                   | 33.3                                                    | 10                        | 27                     |
| 6<br>(30 µg/g)                                   | 100                                                     | 10                        | 27                     |

\*Dosage of betamethasone base per day assumes BW of 30 g.

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA) and rubbed in with a gloved finger once daily, 7 days per week, without occlusion, 13 consecutive weeks.

Observations and times:

Clinical signs: Yes, twice daily, including examination of treatment site for edema and erythema, plus a weekly physical exam.

Body weights: Yes, weekly.

Food consumption: Yes, weekly.

Ophthalmoscopy: No

EKG: No

Hematology: Yes, all main-study animals at termination.

Clinical chemistry: Yes, all main-study animals at termination.

Urinalysis: No

Gross pathology: Yes

Organs weighed: Brain, epididymis, heart, kidneys, liver, lungs, ovaries, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, uterus.

Histopathology: Yes (full range of tissues; limited to the untreated and vehicle control groups, plus the high-dose animals, plus the spleen, thymus, adrenals, treatment site, and lachrymal glands from groups 3, 4, and 5).

Toxicokinetics: Yes, samples obtained from satellite animals during week 13 of treatment (3 animals per sex per treatment group) at (control groups skipped at some time points) 0, 0.5, 1, 2, 3, 5, 7, 9, and 12 hours post-treatment. Note: The samples were analyzed for content of the drug substance, betamethasone-17,21-dipropionate, and its metabolite, betamethasone-17-propionate.

Other: None

#### Results:

Mortality: No treatment-related premature deaths. One female in the group 6 satellite group (1/37 HD females evaluated) was found dead during week 2, but in the absence of any other deaths, this was judged to be unrelated to treatment. Clinical signs: No remarkable observations, including no irritation at the treatment site.

Mean Body weight gain: Mean body weight gain was significantly reduced in males and females in groups 5 and 6 relative to the vehicle control group (approximately 36% in males and females treated with 10 µg/g ointment, and approximately 65% in males and 69% in females treated with 30 µg/g material). Non-significant trends toward reduced weight gain may have been apparent in animals (especially females) dosed at 10 µg/kg/day:

#### Body Weight Gain over Weeks 0-13 (g±SD):

| Group Number<br>(Conc. of<br>Betamethasone Base) | Nominal Dosage<br>of Betamethasone<br>Base (µg/kg/day)* | Males     | Females   |
|--------------------------------------------------|---------------------------------------------------------|-----------|-----------|
| 1<br>(No treatment)                              | 0 (untreated<br>control)                                | 8.1±1.96  | 6.6±2.41  |
| 2<br>(0 µg/g)                                    | 0 (vehicle control)                                     | 7.2±2.00  | 5.5±1.96  |
| 3<br>(1 µg/g)                                    | 3.3                                                     | 8.9±1.59  | 5.5±1.75  |
| 4<br>(3 µg/g)                                    | 10                                                      | 6.9±2.27  | 4.1±1.51  |
| 5<br>(10 µg/g)                                   | 33.3                                                    | 4.6±2.89* | 3.5±1.71* |



|                |     |            |            |
|----------------|-----|------------|------------|
| 6<br>(30 µg/g) | 100 | 2.5±2.35** | 1.7±1.06** |
|----------------|-----|------------|------------|

\*p<0.01; \*\*p<0.001 (relative to group 2)

Note: Group 4 females differed significantly from group 1, but not from group 2.

Food consumption: No remarkable observations.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: Small but statistically significant increases in hematocrit, hemoglobin, and RBC count were observed in males in groups 5 and 6, while significantly increased hematocrit was observed in group 5 and 6 females. These effects were of low magnitude and probably do not reflect dose-limiting toxicity.

Clinical chemistry: No remarkable observations.

Urinalysis: NA

Mean organ weights: No significant changes in mean absolute organ weights, although some apparent trends were observed (changes in mean weights in group 5 and/or 6), and some of these attained significance following normalization of the data for changes in mean body weight. Observed trends included: In males: Increased weight of the heart and kidneys, decreased weight of the liver and spleen. In females, decreased mean adjusted weight of the kidneys, spleen, thymus, and uterus. These effects were small, but were probably caused by treatment.

Gross pathology: Reduced size of the spleen in group 6 males and females, and reduced size of the thymus in group 6 females was reported. No other remarkable observations.

Histopathology: Changes considered to be related to treatment were limited to the spleen, thymus, and lachrymal glands, although these effects were minor and may not indicate dose-limiting toxicity:

Spleen: Decreased cellularity of the red pulp was observed in group 6 males (4/10) and females (9/10), and in 2/10 group 5 males, but not at lower exposures or in controls.

Thymus: Decreased cellularity of the thymus was observed in group 6 males and females.

Lachrymal glands: Lymphoid aggregates were observed in all groups of males except group 6. No lymphoid aggregates were observed in any group of females (including controls). The relevance of these data, if any, is unclear, but betamethasone is known to suppress immune cell function, and it is possible the absence of aggregates of lymphocytes in group 6 males may have been related to treatment.

Toxicokinetics: The concentration of betamethasone-17,21-dipropionate in all samples was below the limit of detection (88 pg/mL). The following values were obtained for the metabolite of the dipropionate, betamethasone-17-propionate:

| Group | Males, Week 13 | Females, Week 13 |
|-------|----------------|------------------|
|-------|----------------|------------------|

| Number<br>(Conc. of<br>Ointment) | AUC <sub>0-12</sub><br>(pg•hr/<br>mL) | AUC <sub>0-inf</sub><br>(pg•hr/<br>mL) | C <sub>max</sub><br>(pg/mL) | T <sub>max</sub><br>(hr) | T <sub>1/2</sub><br>(hr) | AUC <sub>0-12</sub><br>(µg•hr/m<br>L) | AUC <sub>0-inf</sub><br>(µg•hr/m<br>L) | C <sub>max</sub><br>(pg/mL) | T <sub>max</sub><br>(hr) | T <sub>1/2</sub><br>(hr) |
|----------------------------------|---------------------------------------|----------------------------------------|-----------------------------|--------------------------|--------------------------|---------------------------------------|----------------------------------------|-----------------------------|--------------------------|--------------------------|
| 3<br>(1 µg/g)                    | 1215                                  | 1264                                   | 338                         | 1                        | 2.3                      | 2944                                  | 3139                                   | 858                         | 1                        | 1.2                      |
| 4<br>(3 µg/g)                    | 2215                                  | ND                                     | 1067                        | 1                        | ND                       | 8147                                  | 8363                                   | 2660                        | 2                        | 2.0                      |
| 5<br>(10 µg/g)                   | 25386                                 | 37208                                  | 8297                        | 3                        | 1.6                      | 32870                                 | 33615                                  | 8403                        | 1                        | 1.7                      |
| 6<br>(30 µg/g)                   | 40336                                 | 40773                                  | 11707                       | 2                        | 1.5                      | 74754                                 | 75734                                  | >21500                      | 1                        | 2.2                      |

ND indicates "not determined", as terminal phase was not adequately defined.

Repeat-dose toxicology studies with calcipotriene/betamethasone ointment:

**2.6.6.3.12 Study Title:** Daivobet - A preliminary dermal toxicity study in mice.

**Key study findings:** Excessive toxicity, likely due to systemic exposure to betamethasone and calcipotriene, was observed, and the study had to be terminated prematurely. It seems likely that the animals ingested some of the applied material, resulting in a high level of systemic exposure to the drug substances. These data are not relevant to the proposed clinical use of the product.

**Study no:** TTOX0010

**Volume #, and page #:** Mod 4, Vol. 27

**Conducting laboratory and location:** Leo Pharmaceutical Products, Ballerup, Denmark

**Date of study initiation:** 01-NOV-2000

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Diavobet ointment, batch No. 993068101, presumed 100%. Vehicle ointment, batch No. 992798302.

**Formulation/vehicle:**

| <u>Compound</u>                 | <u>Amount per gram</u> |
|---------------------------------|------------------------|
| Calcipotriene hydrate.....      | 52.2 µg                |
| Betamethasone dipropionate..... | 643 µg                 |

b(4)

Liquid paraffin.....  
Polyoxypropylene-15-stearyl ether.....

b(4)

**Methods:**

**Dosing:**

Species/strain: Mice/NMRI

#/sex/group or time point (main study): 10 females per group (active ointment and vehicle control). No males in study.

Satellite groups used for toxicokinetics or recovery: No

Age: Not stated

Weight: 25g to 30g initially

Doses in administered units: 0.1 g/day of assigned material. Active treatment animals received approximately 167 µg/kg/day calcipotriene and 1.67mg/kg/day betamethasone (based on BW of 30g).

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA) once daily without occlusion. Remaining ointment was wiped off after six hours. Treatment originally planned to continue daily for six consecutive weeks, but all remaining animals were terminated on study day 11.

**Observations and times:**

Clinical signs: Yes

Body weights: Yes

Food consumption: Yes

Ophthalmoscopy: No

EKG: No

Hematology: Yes

Clinical chemistry: No

Urinalysis: No

Gross pathology: Yes

Organs weighed: Yes (adrenals, kidneys, liver, spleen, thymus)

Histopathology: Yes (limited to gross lesions, adrenals, aortic arch, heart, kidneys, liver, skin, spleen, and thymus)

Toxicokinetics: No

Other: None

**Results:**

Mortality: All mice in treatment group sacrificed prematurely for reasons of humanity; one on day 5, one on day 8, and the remainder on day 11.

Clinical signs: 4/10 mice treated with active material exhibited "slightly irritated/wrinkled/thin skin and small scratch marks" at the treatment site. "Very slight" erythema was observed in all active treatment animals starting on day 9.

No skin reactions were observed in the vehicle control group. The summary does not comment on the presence or absence of non-dermal clinical signs.

Body weights: Animals in the active treatment group "lost weight from day 1 onwards compared to the placebo treated mice".

Food consumption: "Feed intake was increased in the Diavobet treated group".

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: "...marked lymphopenia, eosinophilia and neutrophilia in the Diavobet treated mice".

Clinical chemistry: NA

Urinalysis: NA

Organ weights: In Diavobet-treated mice, decreased absolute and/or relative (to body weight) mean weights of the thymus, spleen, liver, and adrenals were observed, while the relative weight of the kidneys was increased.

Gross pathology: In Diavobet-treated mice, gross observations included atrophy of spleen and thymus, white spots on the kidneys (3/10 treated mice), and (on day of termination) emaciated appearance in all remaining animals.

Histopathology: Changes considered to be related to treatment were observed in the skin (adnexal atrophy and epithelial attenuation), adrenals (atrophy of zona fasciculata), kidneys (cortical tubular regeneration), spleen (atrophy and reduced extramedullary hematopoiesis), and thymus (cortical atrophy). Note that few other tissues were examined, so it is unclear if additional organs exhibited microscopic lesions.

Toxicokinetics: NA (below limit of quantitation).

**2.6.6.3.13 Study Title:** Daivobet ointment. 13-week dermal dose range finding study in the mouse.

Key study findings: Little toxicity was observed, with the exception of reduced body weight gain. This study was of limited value because it did not include clinical chemistry or urinalysis, which are critical to assessment of the toxicology of any vitamin D analog, and because the histopathologic analysis was quite limited.

Study no: LOP0058 (LEO Study No. TTOXO203)

Volume #, and page #: vol. 28

Conducting laboratory and location:

Date of study initiation: 28-JAN-2002

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: Diavobet ointment (see formulation at beginning of this review), in concentrations ( $\mu\text{g/g}$  calcipotriene/ $\mu\text{g/g}$  betamethasone) of 0.125/1.25, 0.5/5, and 2/20; batch Nos. 0134216, 0134416, and 0134417, respectively. Presumed 100%. Vehicle ointment, batch No. 0134216.

Methods:

Dosing:

Species/strain: Mice, — CD-1 (ICR) BR VAF/Plus

b(4)

b(4)

#/sex/group or time point (main study): 10 per sex per group, plus 18 per sex per group in toxicokinetic satellite groups.

Satellite groups used for toxicokinetics or recovery: Yes

Age: Approx. 5 weeks

Weight: Males 20-30 g initially

Doses in administered units: 0.1 g/day of assigned material. Low-dose animals received approximately 0.42 µg/kg/day calcipotriene and 4.2 µg/kg/day betamethasone; mid-dose animals received approximately 1.7 µg/kg/day calcipotriene and 17 µg/kg/day betamethasone; high-dose animals received approximately 6.7 µg/kg/day calcipotriene and 67 µg/kg/day betamethasone (based on BW of 30 g).

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA) once daily, 7 days per week, without occlusion, 13 consecutive weeks.

#### Observations and times:

Clinical signs: Yes, daily, including examination of treatment site for edema and erythema.

Body weights: Yes, twice weekly.

Food consumption: Yes, weekly.

Ophthalmoscopy: No

EKG: No

Hematology: Yes

Clinical chemistry: No

Urinalysis: No

Gross pathology: Yes

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

Histopathology: Yes (control and high-dose animals, but limited to the adrenals, aorta, heart, kidneys, liver, ovaries, pituitary, skin (treated and untreated), spleen, testes, thymus, and gross lesions, plus spleen and thymus from low-dose and mid-dose groups).

Toxicokinetics: Yes, samples obtained from satellite animals during week 4 of treatment (3 animals per sex per treatment group) at 0, 3, 5, 7, 9, and 12 hours post-treatment.

Other: None

#### Results:

Mortality: No treatment-related premature deaths. One low-dose group male was sacrificed on day 47 due to infection resulting from clipper damage. One control male was found dead for no apparent reason on day 92.

Clinical signs: No remarkable observations, although a few animals exhibited "very slight erythema/eschar" at certain time points.

Mean Body weight: Mean body weight was reduced in high-dose males and females on day 92 by 15% and 20%, respectively. The mean weights of the low

and mid-dose females were reduced by over 10%. The differences were apparently not statistically significant.

Mean Body Weight on Day 92 (g±SD):

| Group     | Males    | Females  |
|-----------|----------|----------|
| Control   | 36.0±2.3 | 32.9±2.4 |
| Low-Dose  | 37.1±2.8 | 29.4±2.0 |
| Mid-Dose  | 34.6±2.1 | 29.1±2.0 |
| High-Dose | 30.5±2.8 | 26.3±1.5 |

Mean Body Weight Gain: Mean body weight gain was significantly reduced in mid and high-dose males and in all female treatment groups.

Body Weight Gain over Days 1-92 (g±SD):

| Group     | Males     | Females   |
|-----------|-----------|-----------|
| Control   | 9.6±1.8   | 10.6±2.1  |
| Low-Dose  | 9.8±1.5   | 7.7±2.1** |
| Mid-Dose  | 7.5±1.4*  | 7.0±1.4** |
| High-Dose | 4.2±1.5** | 4.4±1.3** |

\*p<0.01; \*\*p<0.001

Food consumption: No remarkable observations.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: WBC significantly reduced in high-dose animals of both genders, primarily due to reduced lymphocyte levels. No effect on RBC parameters.

Clinical chemistry: NA

Urinalysis: NA

Mean organ weights: Decreased absolute and/or relative (to body weight) mean weights of the thymus and spleen were observed. Mean absolute adrenal weight was reduced in high-dose females. Significant differences were observed in some other organ weight parameters, but the differences were minor and appeared to not be related to treatment.

Gross pathology: No remarkable observations.

Histopathology: Changes considered to be related to treatment were observed in the thymus and in the spleen, but these effects were very minor and probably insignificant. Note that few tissues were examined, so it is unclear if additional organs exhibited microscopic lesions.

Toxicokinetics: Data not submitted (all values probably below LOQ).

#### 2.6.6.3.14 Study Title: Daivobet. A 9-month dermal toxicity study in minipigs.

**Key study findings:** Little toxicity was observed under the conditions of this study. Treatment-related findings included reduced mean adrenal weight, minimal to moderate adrenal atrophy, and thinning of the skin. Systemic exposure to the drug substances was extremely limited in this study, but was apparently comparable to the systemic exposure achieved under clinical conditions.

**Study no:** 48576 (LEO Study No. TTOX0205)

**Volume #, and page #:** Mod 4, vol. 29

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 02-OCT-2002

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Diavobet ointment (see formulation at beginning of this review), in concentrations ( $\mu\text{g/g}$  calcipotriene/ $\mu\text{g/g}$  betamethasone (expressed as betamethasone base, but in form of dipropionate salt)) of 0/0 (vehicle), 2/20, 10/100, and 50/500; batch Nos. 022391601, 022401601, 022411601, and 013248201, respectively. Presumed 100%.

**Methods:**

**Dosing:**

Species/strain: Minipigs/Gottingen SPF

#/sex/group or time point (main study): 5 per sex per group

Satellite groups used for toxicokinetics or recovery: No

Age: Approx. 3 to 4 months

Weight: 5.1 to 8.7 kg initially

Doses in administered units: 0.5 g/kg/day of assigned material. Low-dose animals received approximately 1  $\mu\text{g/kg/day}$  calcipotriene and 10  $\mu\text{g/kg/day}$  betamethasone; mid-dose animals received approximately 5  $\mu\text{g/kg/day}$  calcipotriene and 50  $\mu\text{g/kg/day}$  betamethasone; high-dose animals received approximately 25  $\mu\text{g/kg/day}$  calcipotriene and 250  $\mu\text{g/kg/day}$  betamethasone.

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA) once daily, 7 days per week, for 39 consecutive weeks.

Following application the test materials were covered with gauze. The residual materials were removed after six hours and the site cleaned with soap and water each day. Treatment of 2 male and 2 female high-dose animals had to be skipped for a day or two periodically due to "severe erythema with indication of pain".

**Observations and times:**

Clinical signs: Yes, daily, including examination of treatment site for edema and erythema.

Body weights: Yes, weekly.

Food consumption: Yes, daily.

Ophthalmoscopy: Yes, at baseline and termination

EKG: Yes, at baseline, week 13, and termination

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

Clinical chemistry: Yes, at baseline, weeks 13 and 26, and termination  
Urinalysis: Yes, at baseline, weeks 13 and 26, and termination  
Gross pathology: Yes  
Organs weighed: Yes (adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid, uterus)  
Histopathology: Yes (a full range of tissues from all animals)  
Toxicokinetics: Yes, samples obtained from all animals during week 8 of treatment at 0, 3, 5, 7, 9, and 12 hours post-treatment.  
Other: None

**Results:**

Mortality: No treatment-related premature deaths.  
Clinical signs: No remarkable observations, although a few animals in the mid and high-dose groups exhibited "very slight to well defined erythema" at certain time points.  
Mean Body weight: No remarkable observations.  
Food consumption: No remarkable observations.  
Ophthalmoscopy: No remarkable observations.  
Electrocardiography: No remarkable observations.  
Hematology: No remarkable observations.  
Clinical chemistry: No remarkable observations.  
Urinalysis: Urine volume was increased, and specific gravity reduced, in high-dose males and females (by factors of approximately two). In the absence of histological evidence of renal pathology it is unclear if this finding had any biological significance. In high-dose males, calcium concentration, phosphorus concentration, and calcium/creatinine ratio were increased by approximately two-fold, although no corresponding effect was observed in females. These effects may have resulted from pharmacological actions of calcipotriene.  
Mean organ weights: Mean adrenal weight was reduced in high-dose animals:

Mean Absolute Adrenal Weight, mg $\pm$ SD

| Group     | Males            | Females          |
|-----------|------------------|------------------|
| Control   | 7.15 $\pm$ 1.06  | 7.35 $\pm$ 1.27  |
| Low-Dose  | 6.56 $\pm$ 0.89  | 7.45 $\pm$ 0.40  |
| Mid-Dose  | 6.61 $\pm$ 0.84  | 6.88 $\pm$ 0.79  |
| High-Dose | 5.70 $\pm$ 0.85* | 6.20 $\pm$ 1.21* |

\*p&lt;0.05

Gross pathology: Slight redness of treated areas in mid and high-dose animals. No other remarkable observations.  
Histopathology: Changes considered to be related to treatment were limited to the adrenals and treated skin. In the adrenals, minimal to moderate diffuse cortical atrophy of the zona fasciculata and reticularis was observed in mid and high-dose animals in proportion to exposure (presumably due to the betamethasone). At the treatment site, treatment-related changes included moderate atrophy of the dermis



(all treatment groups) and minimal to moderate epidermal hyperplasia of the epidermis in mid and high-dose animals.

Toxicokinetics: The levels of calcipotriene and betamethasone were at or below the limit of quantitation (LOQ) in nearly all samples (LOQs for calcipotriene and betamethasone were 40 pg/mL and 20 pg/mL, respectively).

Repeat-dose toxicology studies with Polyoxypropylene-15-stearyl ether (an excipient):

**2.6.6.3.15 Study Title:** PPG-15 stearyl ether toxicity study by dermal administration to CD-1 mice for 13 weeks

Key study findings: No toxicity was observed under the conditions of this study.

Study no: LOP0067/062765 (LEO Study No. TTOX0513)

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 09-JAN-2006

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: PPG-15 stearyl ether, in concentrations of 0 (vehicle control, 100% liquid paraffin, batch No. A1294), 10% w/w (90% w/w liquid paraffin), 30% w/w (70% w/w liquid paraffin), and 100%; batch Nos. A1294, 05367310, 05367311, and 051746201, respectively. Presumed 100%.

Methods:

Dosing:

Species/strain: Mice/ —CD-1 (ICR)

#/sex/group or time point (main study): 10 per sex per group

Satellite groups used for toxicokinetics or recovery: No

Age: Approx. 5 weeks

Weight (at start of treatment): Males 25.9-33.8 g; Females 20.8-27.8 g

Doses in administered units: 0.1 mL/day of assigned material. Note: The test materials contained:

Vehicle control = 0.8585 g liquid paraffin per mL

LD test material = PPG-15 stearyl ether 86.7 mg/liquid paraffin 780 mg per mL

MD test material = PPG-15 stearyl ether 265 mg/liquid paraffin 618 mg per mL

HD test material = PPG-15 stearyl ether 948 mg per mL

- summary of study design:

| Group Number | Dosages of PPG-15 Stearyl Ether (mg/kg/day) |
|--------------|---------------------------------------------|
| 1            | 0 (vehicle control)                         |

|   |      |
|---|------|
| 2 | 289  |
| 3 | 883  |
| 4 | 3160 |

\*Dosage per day assumes BW of 30 g.

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA; details of application methodology not indicated) once daily, 7 days per week, without occlusion, 13 consecutive weeks.

Observations and times:

Clinical signs: Yes, twice daily, including examination of treatment site for edema and erythema, plus a weekly physical exam.

Body weights: Yes, weekly.

Food consumption: Yes, weekly.

Ophthalmoscopy: No

EKG: No

Hematology: Yes, all animals at termination.

Clinical chemistry: Yes, all animals at termination.

Urinalysis: No

Gross pathology: Yes

Organs weighed: Brain, epididymis, heart, kidneys, liver, lungs, ovaries, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, uterus.

Histopathology: Yes (full range of tissues; limited to control and HD groups, plus gross lesions from the LD and MD groups).

Toxicokinetics: No

Other: None

Results:

Mortality: No treatment-related premature deaths. One control male and one MD (group 3) female were sacrificed in week 13 for reasons apparently not related to treatment.

Clinical signs: No remarkable observations, including no irritation at the treatment site.

Mean Body weight gain: No remarkable observations.

Food consumption: No remarkable observations.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: No remarkable observations.

Clinical chemistry: No remarkable observations.

Urinalysis: NA

Mean organ weights: No remarkable observations.

Histopathology: No remarkable observations.

Toxicokinetics: NA

**2.6.6.3.16 Study Title:** PPG-15 stearyl ether toxicity study by oral gavage administration to Han Wistar rats for 13 weeks

Key study findings: No toxicity was observed under the conditions of this study.

Study no: LOP0068/062764 (LEO Study No. TTOX0514)

Conducting laboratory and location: \_\_\_\_\_

Date of study initiation: 12-JAN-2006

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: PPG-15 stearyl ether, batch No. 051746202.

Presumed 100%.

b(4)

**Methods:****Dosing:**

Species/strain: Rats/Han Wistar

#/sex/group or time point (main study): 10 per sex per group

Satellite groups used for toxicokinetics or recovery: No

Age: Approx. 5 weeks

Weight (at start of treatment): Males 100-133 g; Females 86-118 g

Doses in administered units: The test material was used undiluted. Dosage was varied by varying the volume administered per day. Controls were sham-treated (no material administered); LD, MD, and HD animals received 0.15, 0.5, and 1.5 mL/kg/day of 100% PPG-15 stearyl ether, respectively (material density was 948 mg/mL).

- summary of study design:

| Group Number | Dosages of PPG-15 Stearyl Ether (mg/kg/day) |
|--------------|---------------------------------------------|
| 1            | 0 (Sham-treated control)                    |
| 2            | 142                                         |
| 3            | 474                                         |
| 4            | 1422                                        |

Route, form, volume, and infusion rate: Oral (gavage) once daily, 7 days per week, 13 consecutive weeks.

Observations and times:

Clinical signs: Yes, twice daily, plus a weekly physical exam.  
Body weights: Yes, weekly.  
Food consumption: Yes, weekly.  
Ophthalmoscopy: No  
EKG: No  
Hematology: Yes, all animals, weeks 6 and 13  
Clinical chemistry: Yes, all animals, weeks 6 and 13  
Urinalysis: Yes, all animals, weeks 6 and 13  
Gross pathology: Yes  
Organs weighed: Adrenals, brain, epididymis, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid, uterus.  
Histopathology: Yes (full range of tissues; limited to control and HD groups, plus gross lesions from the LD and MD groups, plus liver from LD and HD males).  
Toxicokinetics: No  
Other: None

Results:

Mortality: No unscheduled deaths.  
Clinical signs: No remarkable observations.  
Mean Body weight gain: No significant differences. Weight gain over weeks 0-13 in HD females was only 90% of the control value, but in the absence of other signs of toxicity, this appears to have been a spurious observation.  
Food consumption: No remarkable observations.  
Ophthalmoscopy: NA  
Electrocardiography: NA  
Hematology: No remarkable observations.  
Clinical chemistry: No remarkable observations.  
Urinalysis: No remarkable observations.  
Mean organ weights: No remarkable observations.  
Histopathology: No remarkable observations.  
Toxicokinetics: NA

#### 2.6.6.4 Genetic toxicology

Genetic toxicology studies with calcipotriene alone:

**2.6.6.4.1 Study title:** MC 903. Bacterial mutagenicity test (Ames test), Study No. 870211N1. Please see Original Summary of NDA 20-611.

**2.6.6.4.2 Study title:** An assessment of the mutagenic potential of MC 903 using the mouse lymphoma TK locus assay, Study No. LOP 46 (Study Ref. 871637). Please see Original Summary of NDA 20-611.

**2.6.6.4.3 Study title:** MC 903. Metaphase chromosome analysis of human lymphocytes cultured *in vitro*, Study No. LOP 47 (Study Ref. 88113). Please see Original Summary of NDA 20-611.

**2.6.6.4.4 Study title:** Mutagenicity testing of MC 903. Micronucleus test in mouse bone marrow, Study No. 870406N1.

The following statement is excerpted from the Original Summary of NDA 20-611:

Calcipotriene was negative in the Ames mutagenicity assay with and without S-9 activation at 0.01-1.0 mg/plate. Calcipotriene was also negative in the mouse lymphoma TK locus assay (an in vitro mammalian cell mutation assay) with and without metabolic activation at up to 40 µg/plate (>20 µg was toxic). To determine if calcipotriene could induce chromosome aberration in human lymphocytes in vitro, calcipotriene was exposed to cells at up to 11 µg/mL both with and without metabolic activation. Without activation, there was a statistically significant increase (2%) in chromosome aberrations<sup>1</sup>. In the in vivo mouse micronucleus bone marrow assay, no increase in micronucleated polychromatic erythrocytes was noted at 1 mg/kg given I.P.

Genetic toxicology studies with betamethasone alone:

**2.6.6.4.5 Study title:** Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*.

**Key findings:** Betamethasone dipropionate was not mutagenic in an Ames assay.

**Study no:** 339/84 (LEO Study No. GTOX0201)

**Study type** (if not reflected in title): Ames test

**Volume #, and page #:** Mod 4, vol 31

**Conducting laboratory and location:**

**Date of study completion:** 17-SEP-2002

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Betamethasone dipropionate, lot 0105216, 99.9%.

**Formulation/vehicle:** Betamethasone dipropionate dissolved in DMSO

**Methods:**

<sup>1</sup> Although the number of aberrations observed in human lymphocytes at a concentration of 9 µg/mL calcipotriene in the absence of metabolic activation was statistically significantly greater than the number observed in the control culture, this was concluded to be a falsely positive result because: 1) the aberration frequency observed (2%) was within the historical control range for the conducting laboratory (0-5.25%); 2) the control value (0%) was unusually low; and 3) a significant increase was not observed at any other concentration that was studied. This result was therefore disregarded.

b(4)

Strains/species/cell line: Salmonella strains TA98, TA100, TA1535, TA1537; TA102

Dose selection criteria: Compound was not cytotoxic; therefore, 5 mg per plate used as maximum exposure level per ICH S2A document

Basis of dose selection: ICH S2A

Range finding studies: Yes, at exposures up to 5 mg/plate

Test agent stability: NA

Metabolic activation system: Rat liver S9; induced with Aroclor 1254

Controls:

Negative control: vehicle

Positive controls: 2-aminoanthracene (all strains except TA98 in experiments with +S9); benzo[a]pyrene (strain TA98 in experiments with +S9); 2-nitrofluorene (TA98, -S9); sodium azide (TA100 & TA1535, -S9); 9-aminoacridine (TA1537, -S9); glutaraldehyde (TA102, -S9)

Comments: Controls adequate

Exposure conditions:

Incubation and sampling times: 48 to 72 hrs

Doses used in definitive study: 15 to 5000 µg per plate

Study design: Plate method

Analysis:

No. of replicates: 2

Counting method: Automated counter or by hand

Criteria for positive results: A two to three-fold (depending on strain) increase in number of revertants compared to negative control, with dose-response

#### Summary of individual study findings:

Study validity: Acceptable

**Study outcome:** No strain exhibited an increased mutation rate relative to the negative control. Appropriate responses were observed with the positive controls. These data suggest betamethasone dipropionate is not mutagenic.

**2.6.6.4.6 Study title:** Mutation at the thymidine kinase (*tk*). Locus of mouse lymphoma L5178Y cells (MLA) using the — fluctuation technique.

b(4)

**Key findings:** Betamethasone dipropionate was not mutagenic under the conditions of this assay.

**Study No:** 339/86 (LEO Study No. GTOX0202)

**Study Type:** In vitro point mutation assay

**Volume # and Page #:** Mod 4, vol. 31

**Conducting Laboratory:** \_\_\_\_\_

**Date of Study Completion:** 08-AUG-2002

b(4)

**GLP Compliance:** Yes

**QA Reports** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** Betamethasone dipropionate, lot 0105216, 99.9%

**Formulation/vehicle:** Dissolved in DMSO at concentrations up to 100 µg/mL

**Methodology:**

- Strains/Species/Cell line: L5178Y tk<sup>+/+</sup> mouse lymphoma cells, clone 3.7.2C
- Dose Selection Criteria: Cytotoxicity and physical compatibility (precipitate at conc. greater than 50 µg/mL)
- Range finding studies: Examined concentrations from 0 to 100 µg/mL, with and without S9
- Test Agent Stability: Adequate (used within two hours of preparation and protected from light)
- Metabolic Activation System: Aroclor 1254-induced S9 (supernatant of the post-mitochondrial 9000 g fraction from adult male SD rats induced with a single injection of Aroclor-1254)
- 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 and 24 hour exposures without S9; 3 hour exposure with S9
  - Doses used in definitive study: 0-100 µg/mL (studies involving 24 hour exposure in absence of S9 included concentrations up to 200 µg/mL, but useful data were not obtained at the highest concentrations due to cytotoxicity and precipitation)
  - Study design: Following the exposure period, the cells were washed and grown in the presence of TFT (which screens for tk<sup>-/-</sup> mutations)
- Analysis:
  - No. of replicates: Two
  - Criteria for positive results: Considered positive if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of at least 100 mutants per 1,000,000 clonable cells over the background level. Considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 1,000,000 clonable cells over the background level. Considered negative if fewer than 55 mutants per 1,000,000 clonable cells over background.

**Summary of individual study findings:**

- Study Validity: Acceptable

**Study Outcome:** A concentration-response trend was not observed in either presence or absence of S9. Betamethasone dipropionate was not positive (genotoxic) under the criteria established for positive results. Appropriate results were obtained with the controls.

**2.6.6.4.7 Study title:** Bone marrow micronucleus test on betamethasone.

**Key findings:** Betamethasone dipropionate was not clastogenic under the conditions of this assay.

**Study No:** 339/85 (LEO Study No. GTOX0203)

**Study Type:** In vivo clastogenicity assay

**Volume # and Page #:** Mod 4, vol. 32

**Conducting Laboratory:** \_\_\_\_\_

**Date of Study Completion:** 11-NOV-2004

**GLP Compliance:** Yes

**QA Reports** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** Betamethasone dipropionate, lot 0105216, 99.9%

b(4)

**Formulation/vehicle:** Suspended in a solution of 2 mg sodium carboxymethylcellulose, 4 mg polysorbate 80, and 8 mg NaCl per mL (in water).

**Methodology:**

- Strains/Species/Cell line: Rat/Wistar (males only used in definitive assay)
- Dose Selection Criteria: Tolerability in preliminary studies
- Test Agent Stability: Acceptable
- Metabolic Activation System: NA (in vivo assay with endogenous metabolism)
- Controls:
  - Vehicle: See above
  - Negative Control: Vehicle
  - Positive Controls: Cyclophosphamide (20 mg/kg/day)
  - Comments: Controls were adequate
- Exposure Conditions:
  - Doses used in definitive study: 500, 1000, and 2000 mg/kg of betamethasone dipropionate administered once daily by gavage on two consecutive days; cyclophosphamide administered on second day of dosing only.
  - Study design: 6 vehicle control, 6 positive control, 5 LD, 5 MD, and 5 HD mice per sex sacrificed 24 hours post-injection. Following sacrifice marrow from femurs was aspirated, centrifuged, smears produced, fixed, and stained. The slides were examined and 2000 polychromatic erythrocytes were scored for the presence of micronuclei (round, darkly staining nuclear fragments). Blood samples were obtained from vehicle and betamethasone-treated



animals just prior to femur removal; these samples were analyzed for betamethasone content.

- Analysis:
  - Counting method: Microscope
  - Genetic toxicity endpoints: Significantly increased percentage of polychromatic erythrocytes with micronuclei

**Results:**

- Study Validity: Acceptable
- Study Outcome: In main study, no unscheduled deaths or unusual clinical signs occurred. Betamethasone did not increase the incidence of polychromatic erythrocytes with micronuclei. Appropriate results were obtained with the controls.
- Toxicokinetic data: The major metabolite of betamethasone, betamethasone 17-propionate, was present in plasma samples obtained from all animals dosed with betamethasone (but not in samples from vehicle-treated animals). The concentration of the metabolite was roughly proportional to the dose of betamethasone administered.

**Study Outcome:** These data suggest betamethasone dipropionate is not clastogenic.

Genetic toxicology studies with calcipotriene/betamethasone ointment: None.

Genetic toxicology studies with Polyoxypropylene-15-stearyl ether (an excipient):

**2.6.6.4.8 Study title:** PPG-15 Stearyl Ether: reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*.

**Key findings:** PPG-15 stearyl ether was not mutagenic in an Ames assay.

**Study no:** 339/116 (LEO Study No. GTOX0302)

**Study type** (if not reflected in title): Ames test

**Volume #, and page #:** Mod 4, vol 31

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study completion:** 13-JAN-2004

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Polypropylene glycol-15 stearyl ether (PPG-15 stearyl ether), lot 021298101, assumed to be 100% pure.

**Formulation/vehicle:** PPG-15 stearyl ether dissolved in ethanol

**Methods:**

Strains/species/cell line: *Salmonella* strains TA98, TA100, TA1535, TA1537; TA102

Dose selection criteria: Compound was not cytotoxic; therefore, 5 mg per plate used as maximum exposure level per ICH S2A document

b(4)

Basis of dose selection: ICH S2A  
Range finding studies: Yes, at exposures up to 5 mg/plate  
Test agent stability: NA  
Metabolic activation system: Rat liver S9; induced with Aroclor 1254  
Controls:  
    Negative control: vehicle  
    Positive controls: 2-aminoanthracene (all strains except TA98 in experiments with +S9); benzo[a]pyrene (strain TA98 in experiments with +S9); 2-nitrofluorene (TA98, -S9); sodium azide (TA100 & TA1535, -S9); 9-aminoacridine (TA1537, -S9); glutaraldehyde (TA102, -S9)  
    Comments: Controls adequate  
Exposure conditions:  
    Incubation and sampling times: 48 to 72 hrs  
    Doses used in definitive study: 15 to 5000 µg per plate  
    Study design: Plate method  
Analysis:  
    No. of replicates: 2  
    Counting method: Automated counter or by hand  
Criteria for positive results: A two to three-fold (depending on strain) increase in number of revertants compared to negative control, with dose-response

**Summary of individual study findings:**

Study validity: Acceptable

**Study outcome:** No strain exhibited an increased mutation rate relative to the negative control. Appropriate responses were observed with the positive controls. These data suggest PPG-15 stearyl ether is not mutagenic.

b(4)

**2.6.6.4.9 Study title:** PPG-15 Stearyl Ether: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the — Fluctuation Technique.

**Key findings:** PPG-15 stearyl ether was not mutagenic under the conditions of this assay.

**Study No:** 339/117 (LEO Study No. GTOX0303)

**Study Type:** In vitro point mutation assay

**Volume # and Page #:** Mod 4, vol. 31

**Conducting Laboratory:** \_\_\_\_\_

**Date of Study Completion:** 20-FEB-2004

**GLP Compliance:** Yes

**QA Reports** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** PPG-15 stearyl ether, lot 021298101, assumed to be 100% pure.

b(4)

**Formulation/vehicle:** Dissolved in ethanol at concentrations up to 500 µg/mL

**Methodology:**

- Strains/Species/Cell line: L5178Y tk<sup>+</sup> mouse lymphoma cells, clone 3.7.2C
- Dose Selection Criteria: Cytotoxicity and physical compatibility (precipitate at conc. greater than 50 µg/mL)
- Range finding studies: Examined concentrations from 0 to 500 µg/mL, with and without S9
- Test Agent Stability: Adequate (used within two hours of preparation and protected from light)
- Metabolic Activation System: Aroclor 1254-induced S9 (supernatant of the post-mitochondrial 9000 g fraction from adult male SD rats induced with a single injection of Aroclor-1254)
- Controls:
  - Vehicle: Ethanol
  - 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 and 24 hour exposures without S9; 3 hour exposure with S9
  - Doses used in definitive study: 0-500 µg/mL
  - Study design: Following the exposure period, the cells were washed and grown in the presence of TFT (which screens for tk<sup>-</sup> mutations)
- Analysis:
  - No. of replicates: Two
  - Criteria for positive results: Considered positive if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of at least 100 mutants per 1,000,000 clonable cells over the background level. Considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 1,000,000 clonable cells over the background level. Considered negative if fewer than 55 mutants per 1,000,000 clonable cells over background.

**Summary of individual study findings:**

- Study Validity: Acceptable

**Study Outcome:** A concentration-response trend was not observed in either presence or absence of S9. PPG-15 stearyl ether was not positive (genotoxic) under the criteria established for positive results. Appropriate results were obtained with the controls.

**2.6.6.4.10 Study title: PPG-15 Stearyl Ether: Micronucleus Test in Mice.**

**Key findings:** PPG-15 stearyl ether was not clastogenic under the conditions of this assay.

**Study No:** GTOX0301

**Study Type:** In vivo clastogenicity assay

**Volume # and Page #:** Mod 4, vol. 32

b(4)

**Conducting Laboratory:**

**Date of Study Completion:** 21-APR-2004

**GLP Compliance:** Yes

**QA Reports** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** PPG-15 stearyl ether, lot 021298102, assumed to be 100% pure.

**Formulation/vehicle:** Dissolved in sesame oil

**Methodology:**

- Strains/Species/Cell line: Rat/Wistar (males only used in definitive assay)
- Dose Selection Criteria: Tolerability in preliminary studies
- Test Agent Stability: Acceptable
- Metabolic Activation System: NA (in vivo assay with endogenous metabolism)
- Controls:
  - Vehicle: See above
  - Negative Control: Vehicle
  - Positive Controls: Cyclophosphamide (25 mg/kg)
  - Comments: Controls were adequate
- Exposure Conditions:
  - Doses used in definitive study: 500, 1000, and 2000 mg/kg of PPG-15 stearyl ether administered once daily by gavage on two consecutive days; cyclophosphamide administered i.p. on second day of dosing only.
  - Study design: 5 vehicle control, 3 positive control, 5 LD, 5 MD, and 5 HD mice per sex sacrificed 24 hours following the final treatment. Following sacrifice marrow from femurs was aspirated, centrifuged, smears produced, fixed, and stained. The slides were examined and 2000 polychromatic erythrocytes were scored for the presence of micronuclei (round, darkly staining nuclear fragments). Blood samples were obtained from vehicle and betamethasone-treated animals just prior to femur removal; these samples were analyzed for betamethasone content.
- Analysis:
  - Counting method: Microscope
  - Genetic toxicity endpoints: Significantly increased percentage of polychromatic erythrocytes with micronuclei

**Results:**

- Study Validity: Acceptable
- Study Outcome: In main study, no unscheduled deaths or unusual clinical signs occurred. PPG-15 stearyl ether did not increase the incidence of polychromatic erythrocytes with micronuclei. Appropriate results were obtained with the

controls.

**Study Outcome:** These data suggest PPG-15 stearyl ether is not clastogenic.

#### 2.6.6.5 Carcinogenicity

Carcinogenicity studies with calcipotriene alone:

**2.6.6.5.1 Study title:** BMS-181161 solution. 12-month photocarcinogenesis study with ultraviolet radiation 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 significantly reduced for males that received the greatest exposure to calcipotriene (30 µg/kg/day), while vehicle alone had no effect, suggesting that calcipotriene may enhance the carcinogenic effects of UV light. No other statistically significant effects on UV-induced skin tumor formation were observed.

**Study No.:** 1202-031 (LEO Study No. CTOX0102); also referred to as Study No. DN01098. b(4)

**Document #, Volume #, and Page #:** Mod 4, vol. 32-33

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 15-NOV-2001

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Calcipotriol solution vehicle (control), batch 012541601; calcipotriol solution 0.75 µg/mL, batch No. 012541901; calcipotriol solution 2.5 µg/mL, batch No. 012541801; calcipotriol solution 7.5 µg/mL, batch No. 012541701, 100%-102% potency.

**Formulation/vehicle:** Dovonex solution; see reviews of NDA 20-611 for details.

**Methods (unique aspects):**

#### Dosing:

Species/strain: Mouse — SKH1-hrBR (albino hairless) b(4)

#/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

Weight: At start of dosing: males, 22-36 g, females, 20-30 g


Doses in administered units:

| Group | Calcipotriol Exposure (µg/kg/day)* | Calcipotriol Solution Concentration (µg/mL) | Volume of Test Material Applied Per Day (µL/mouse) |
|-------|------------------------------------|---------------------------------------------|----------------------------------------------------|
| 1     | 0                                  | 0                                           | 100                                                |

|   |    |      |     |
|---|----|------|-----|
| 2 | 3  | 0.75 | 100 |
| 3 | 10 | 2.5  | 100 |
| 4 | 30 | 7.5  | 100 |
| 5 | 0  | NA** | 0   |
| 6 | 0  | NA** | 0   |

\*Approximate, based upon assumed BW of 25 g.

\*\*No test material applied to these animals.

The test materials were applied, and the mice irradiated, five days per week (M-F) for 40 weeks. The test materials were applied approximately 70 minutes prior to UVR exposure on Mondays, Wednesdays, and Fridays, and approximately 70 minutes 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 mm  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 following 40 weeks of treatment, with sacrifice during week 52. Mice were sacrificed prematurely if a skin tumor  $\geq 10$  mm diameter was present. All mice in a given dosage/gender group were killed: a) when survival in that group reached 50%; and b) if more than 50% of the surviving mice had tumors  $\geq 4$  mm diameter.

Route, form, volume, and infusion rate: Topical, 100  $\mu$ L/day (see above), once per day M-F for 40 consecutive weeks. The assigned material was applied to the back and sides (approximately 25 cm<sup>2</sup>) 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 (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:

- Survival: No drug-related effects on survival were observed. Increased exposure to UVR resulted in an increased rate of mortality.

## Numbers of Animals Surviving to Scheduled Sacrifice

| Group | Calcipotriol Exposure (µg/kg/day)* | UVR Exposure (RBU/Week) | Number of Males Killed at Scheduled Sacrifice, Week 53 | Number of Females Killed at Scheduled Sacrifice, Week 53 |
|-------|------------------------------------|-------------------------|--------------------------------------------------------|----------------------------------------------------------|
| 1     | 0 (Vehicle)                        | 600                     | 20/36                                                  | 21/36                                                    |
| 2     | 3                                  | 600                     | 16/36                                                  | 18/36                                                    |
| 3     | 10                                 | 600                     | 19/36                                                  | 21/36                                                    |
| 4     | 30                                 | 600                     | 19/36                                                  | 23/36                                                    |
| 5     | 0 (No treatment)                   | 600                     | 19/36                                                  | 15/36                                                    |
| 6     | 0 (No treatment)                   | 1200                    | 0/36                                                   | 0/36                                                     |

\*Approximate, based upon assumed BW of 25 g.

- Clinical signs: All test materials were well tolerated, although some edema was observed, particularly in group 4 animals.
- Body weights: Mean body weights and weight gains tended to be slightly reduced with increased exposure to calcipotriene, particularly in males:

## Body weight gains (mean±SD):

| Group | Calcipotriol Exposure (µg/kg/day)* | UVR Exposure (RBU/Week) | Mean BW Change for Males, Weeks 1-53 | Mean BW Change for Females, Weeks 1-53 |
|-------|------------------------------------|-------------------------|--------------------------------------|----------------------------------------|
| 1     | 0 (Vehicle)                        | 600                     | 10.3±3.1                             | 9.1±2.3                                |
| 2     | 3                                  | 600                     | 8.6±2.6                              | 8.4±2.5                                |
| 3     | 10                                 | 600                     | 7.7±2.3**                            | 8.2±2.7                                |
| 4     | 30                                 | 600                     | 7.6±2.3**                            | 8.6±2.5                                |
| 5     | 0 (No treatment)                   | 600                     | 10.0±2.6                             | 10.9±3.3                               |
| 6     | 0 (No treatment)                   | 1200                    | NA                                   | NA                                     |

\*Approximate, based upon assumed BW of 25 g.

\*\*p<0.01

- 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 males in group 4 (30 µg/kg/day) and for animals of both genders in group 6 (1200 RBU per day).

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

| Group | Calcipotriol Exposure | UVR Exposure (RBU/Week) | Median Week to | Median Week to |
|-------|-----------------------|-------------------------|----------------|----------------|
|-------|-----------------------|-------------------------|----------------|----------------|

|   | ( $\mu\text{g/kg/day}$ )* |      | Tumor<br>$\geq 1$ mm,<br>Males | Tumor<br>$\geq 1$ mm,<br>Females |
|---|---------------------------|------|--------------------------------|----------------------------------|
| 1 | 0 (Vehicle)               | 600  | 42.0                           | 40.0                             |
| 2 | 3                         | 600  | 39.0                           | 36.5                             |
| 3 | 10                        | 600  | 39.5                           | 42.0                             |
| 4 | 30                        | 600  | 37.0**                         | 35.5                             |
| 5 | 0 (No treatment)          | 600  | 43.0                           | 37                               |
| 6 | 0 (No treatment)          | 1200 | 24.0***                        | 23.5***                          |

\*Approximate, based upon assumed BW of 25 g.

\*\* $p < 0.01$  compared to group 1.

\*\*\* $p < 0.001$  compared to group 5.

The median time to first tumor greater than or equal to 1 mm for groups 1 and 5 did not differ statistically. The only difference between groups 1 and 5 was that group 1 animals received vehicle plus 600 RBU per week, while group 5 animals received only 600 RBU per week. These data suggest that the product vehicle did not impact UV-induced tumor formation.

#### 2.6.6.5.2 Study title: Dermal carcinogenicity study in mice

**Key study findings:** Mice were treated with calcipotriene topically for up to 24 months at dosages of 0, 3, 10, and 30  $\mu\text{g/kg/day}$ . No statistically significant differences in tumor incidence were observed in female mice in this study. In male mice the incidence of pooled bronchio/avelolar adenomas and carcinomas was statistically significantly increased, but this finding was regarded as having no biological significance. These data are considered to be consistent with a hypothesis that calcipotriene is not carcinogenic.

Adequacy of the carcinogenicity study and appropriateness of the test model: This study was discussed by the executive carcinogenicity assessment committee on 15-MAY-2007. The committee concluded that the study was acceptable. The committee decided that, under the conditions of this study, the test article had no biologically relevant effects upon tumor incidence in either gender. Although an increased incidence of pooled bronchio/avelolar adenomas and carcinomas was observed in male mice, this observation was considered to spurious and random in nature. This study is adequate to fulfill the sponsor's commitment to submit data from a dermal carcinogenicity study conducted with calcipotriene. The test model (topical application to mouse skin) is considered to be appropriate and to yield data relevant to clinical use of topical products that contain calcipotriene.

Evaluation of tumor findings: No statistically significant differences in tumor incidence were observed in female mice in this study. In male mice the incidence of pooled bronchio/alveolar adenomas and carcinomas was statistically significantly increased (common tumor, test of dose related trends in the vehicle, low, medium, and high dose groups yielded  $p = 0.0053$ ; test of differences between the high dose group and the



vehicle group yielded  $p = 0.0016$ ). However, this observation was considered to random in nature, and to have no biological significance.

**Study No.:** 01-2731 (sponsor study No. DN01097; co-sponsor study No. CTOX0101)

**Document #, Volume #, and Page #:** NA

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 02-NOV-2001

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Calcipotriol solution vehicle (control), batch 012541601, 023731601, and 030801601; calcipotriol solution 0.75 µg/mL, batch No. 012541901, 023761801, and 030801901; calcipotriol solution 2.5 µg/mL, batch No. 012541801, 023761701, and 030801801; calcipotriol solution 7.5 µg/mL, batch No. 012541701, 023761601, and 030801701; approximately 100% potency.

**Formulation/vehicle:** See reviews of NDA 20-273 (Dovonex solution).

**Methods (unique aspects):**

**Dosing:**

Species/strain: Mouse/ ~~CD-1~~ (ICR) BR

#/sex/group or time point (main study): 50/sex/group; housed 1 per cage

Satellite groups used for toxicokinetics or recovery: Yes (10/sex in groups 3, 4, and 5)

Age: Approximately 6 weeks at initiation

Weight: At start of dosing: males, 23-34 g; females, 18-27 g

Study overview/doses administered:

| Group | No. of Animals of Each Gender in Main Study | No. of TK Animals of Each Gender | Calcipotriol Exposure (µg/kg/day)* | Calcipotriol Solution Concentration (µg/mL) | Volume of Test Material Applied Per Day (µL/mouse) |
|-------|---------------------------------------------|----------------------------------|------------------------------------|---------------------------------------------|----------------------------------------------------|
| 1     | 50                                          | 0                                | 0 (untreated)**                    | NA                                          | NA                                                 |
| 2     | 50                                          | 0                                | 0 (vehicle treated)                | 0                                           | 100                                                |
| 3     | 50                                          | 10                               | 3                                  | 0.75                                        | 100                                                |
| 4     | 50                                          | 10                               | 10                                 | 2.5                                         | 100                                                |
| 5     | 50                                          | 10                               | 30                                 | 7.5                                         | 100                                                |

\*Approximate, based upon assumed BW of 25 g.

\*\*No test material applied to these animals.

Route, form, volume, and infusion rate: Applied to clipped surface on back (approx. 10% of BSA) once daily, 7 days per week, without occlusion, for up to 24 months.

Note: The study was in treatment week 78 on 22-MAY-2003, when the sponsor contacted the division with the following mortality data:

No. of surviving animals as of 22-MAY-2003:

| <u>Group</u>           | <u>Males</u> | <u>Females</u> |
|------------------------|--------------|----------------|
| 1 (Untreated controls) | 35           | 37             |
| 2 (Vehicle controls)   | 32           | 30             |
| 3 (3 µg/kg/day)        | 31           | 38             |
| 4 (10 µg/kg/day)       | 18           | 26             |
| 5 (30 µg/kg/day)       | 25           | 41             |

The sponsor requested guidance. The exec-CAC recommended that the sponsor:

1. Cease dosing of group 4 males immediately.
2. Continue dosing of all other groups per the protocol until either (whichever event occurred first):

a) the number of surviving animals of a given gender in either group 1 (untreated control group) or group 5 (high-dose group) reached 15, at which time all animals of that gender in all groups would be sacrificed, or:

b) 104 weeks of dosing was completed.

3. Dosing of animals of a given gender in groups 2, 3, or 4 (other than group 4 males) should cease when the number of surviving animals in that dose/gender group reached 20 (if neither condition listed under point 2, above, had been reached).

Observations and times:

Clinical signs: Yes, daily, plus weekly physical exams. Application site examined for irritation, edema and erythema twice weekly.

Body weights: Yes, weekly through week 13, then every 4 weeks.

Food consumption: Yes, weekly through week 13, then every 4 weeks.

Ophthalmoscopy: No

EKG: No

Hematology: No

Clinical chemistry: No

Urinalysis: No

Gross pathology: Yes

Organs weighed: None

Histopathology: A standard list of tissues from all main-study animals.

Toxicokinetics: After 11 months of dosing, blood samples for toxicokinetic determinations were obtained from satellite ("toxicokinetic") group 5 animals at 1 and 5 hours after dose administration (approximately half of the surviving animals of each gender per time point). At the end of 22 months of dosing, blood samples

for toxicokinetic determinations were obtained from all surviving animals in the satellite groups 3 and 4 at 1 hour after dose administration.

Other: None

#### Results:

Mortality: In males, mortality was significantly higher in the mid-dose (MD) and high-dose (HD) groups. In females, the percent-mortality was similar across groups, in the sense that it was high in all groups. However, comparison of mortality data from control groups in this study to historical control data suggested that survival of control animals in this study was not compromised (see Biostatistics review 1 of IND 67,835). The data are summarized below; more detailed presentation of the mortality data follow:

| Dose ( $\mu\text{g/kg/day}$ ) | Males          |                |    |                 |                 | Females        |                |    |    |    |
|-------------------------------|----------------|----------------|----|-----------------|-----------------|----------------|----------------|----|----|----|
|                               | 0 <sup>b</sup> | 0 <sup>c</sup> | 3  | 10              | 30              | 0 <sup>a</sup> | 0 <sup>b</sup> | 3  | 10 | 30 |
| Total Number                  | 47             | 50             | 49 | 50              | 49              | 49             | 50             | 47 | 47 | 50 |
| No. Preterm Deaths            | 24             | 28             | 29 | 36              | 34              | 32             | 36             | 34 | 35 | 36 |
| No. Survivors                 | 23             | 22             | 20 | 14              | 15              | 17             | 14             | 13 | 12 | 14 |
| Percent Mortality             | 48             | 56             | 58 | 72 <sup>e</sup> | 68 <sup>f</sup> | 64             | 72             | 68 | 70 | 72 |
| Cause of Death:               |                |                |    |                 |                 |                |                |    |    |    |
| Undetermined                  | 7              | 10             | 4  | 6               | 7               | 5              | 3              | 11 | 5  | 7  |
| Obstructive Uropathy          | 5              | 6              | 7  | 15              | 16              | 0              | 1              | 0  | 0  | 1  |
| Lymphoreticular Neoplasm      | 3              | 4              | 7  | 8               | 5               | 8              | 14             | 8  | 17 | 10 |
| Other                         | 9              | 8              | 11 | 7               | 6               | 19             | 18             | 15 | 13 | 18 |

<sup>a</sup>No. of Preterm Deaths excludes accidental deaths; Percent Mortality calculation based on total number of animals at initiation.

<sup>b</sup>Untreated

<sup>c</sup>Vehicle treatment

<sup>e</sup>Significantly different from combined control ( $p \leq 0.0017$ )

<sup>f</sup>Significantly different from combined control ( $p \leq 0.0487$ )

Notes: The table above incorrectly indicates that the second column under females refers to "untreated" animals; for both genders the first column refers to the untreated control group while the second column refers to the vehicle control group. The two major causes of death were considered to be "obstructive uropathy" and "lymphoreticular neoplasia". The "p" values associated with the table above were based upon the sponsor's analysis, which (according to CDER standards) incorrectly involved pooling data from the untreated and the vehicle control groups. However, similar values were generated during analysis by the FDA, in which data from the untreated control groups were excluded (see Biostatistics review 1 of IND 67,835).

## Detailed Mortality Summary for Males:

| Males                  |       |   |   |   |     |     |     | Mortality Summary <sup>a</sup> |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Table 1    |                 |                      |   |   |   |    |    |    |
|------------------------|-------|---|---|---|-----|-----|-----|--------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------------|-----------------|----------------------|---|---|---|----|----|----|
| Initial Number         | Month |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Total Dead | Total Survivors | Percent Survivorship |   |   |   |    |    |    |
|                        | 1     | 2 | 3 | 4 | 5   | 6   | 7   | 8                              | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  |            |                 |                      |   |   |   |    |    |    |
| Group 1 - Untreated    |       |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |                 |                      |   |   |   |    |    |    |
| 50                     | 0     | 0 | 0 | 0 | 1   | 0   | 0   | 1                              | (1) |     |     |     | 0   | 0   | 1   | 1   | 0   | 3   | 2   | 1   | (1) |     | 1   | 2          | 2               | 1                    | 5 | 2 | 1 | 24 | 23 | 52 |
|                        | 0     | 0 | 0 | 0 | 1   | 1   | 1   | 2                              | 2   | 2   | 3   | 4   | 4   | 7   | 9   | 10  | 11  | 13  | 15  | 19  | 21  | 23  | 24  | (3)        |                 |                      |   |   |   |    |    |    |
|                        |       |   |   |   |     |     |     |                                | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (2) | (2) | (3) | (3) | (3) | (3) | (3)        | (3)             |                      |   |   |   |    |    |    |
| Group 2 - 0 ug/kg/day  |       |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |                 |                      |   |   |   |    |    |    |
| 50                     | 0     | 0 | 0 | 1 | 0   | 0   | 0   | 0                              | 2   | 1   | 0   | 1   | 1   | 2   | 3   | 3   | 2   | 2   | 2   | 4   | 2   | 2   | 0   | 28         | 22              | 44                   |   |   |   |    |    |    |
|                        | 0     | 0 | 0 | 1 | 1   | 1   | 1   | 1                              | 3   | 4   | 4   | 5   | 6   | 8   | 12  | 15  | 16  | 18  | 21  | 24  | 26  | 28  | 28  |            |                 |                      |   |   |   |    |    |    |
|                        |       |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |                 |                      |   |   |   |    |    |    |
| Group 3 - 3 ug/kg/day  |       |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |                 |                      |   |   |   |    |    |    |
| 50                     | 0     | 0 | 0 | 0 | 0   | 1   | 0   | 1                              | 0   | 0   | 1   | 1   | 4   | 0   | 2   | 2   | 3   | 3   | 1   | 3   | 4   | 2   | 0   | 28         | 21              | 42                   |   |   |   |    |    |    |
|                        | 0     | 0 | 0 | 0 | 0   | 1   | 1   | 2                              | 2   | 2   | 3   | 4   | 8   | 8   | 10  | 12  | 15  | 18  | 19  | 22  | 26  | 28  | 28  | (1)        |                 |                      |   |   |   |    |    |    |
|                        |       |   |   |   |     |     |     |                                |     |     |     |     | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) |            |                 |                      |   |   |   |    |    |    |
| Group 4 - 10 ug/kg/day |       |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |                 |                      |   |   |   |    |    |    |
| 50                     | 0     | 0 | 2 | 0 | 2   | 1   | 1   | 0                              | 1   | 1   | 0   | 3   | 3   | 5   | 5   | 3   | 2   | 3   | 1   | 2   | 1   | 0   | 0   | 36         | 14              | 28                   |   |   |   |    |    |    |
|                        | 0     | 0 | 2 | 2 | 4   | 5   | 6   | 6                              | 7   | 8   | 8   | 11  | 14  | 19  | 24  | 27  | 29  | 32  | 33  | 35  | 36  | 36  | 36  |            |                 |                      |   |   |   |    |    |    |
|                        |       |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |                 |                      |   |   |   |    |    |    |
| Group 5 - 30 ug/kg/day |       |   |   |   |     |     |     |                                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |                 |                      |   |   |   |    |    |    |
| 50                     | 0     | 0 | 1 | 0 | 2   | 0   | 0   | 0                              | 0   | 2   | 3   | 1   | 2   | 3   | 1   | 0   | 5   | 4   | 2   | 1   | 3   | 4   | 0   | 34         | 15              | 32                   |   |   |   |    |    |    |
|                        | 0     | 0 | 1 | 1 | 3   | 3   | 3   | 3                              | 3   | 5   | 8   | 9   | 11  | 14  | 15  | 15  | 20  | 24  | 26  | 27  | 30  | 34  | 34  | (1)        |                 |                      |   |   |   |    |    |    |
|                        |       |   |   |   | (1) | (1) | (1) | (1)                            | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) | (1) |            |                 |                      |   |   |   |    |    |    |

<sup>a</sup>The numbers above the line represent mortality occurring monthly and the numbers below the line represent cumulative mortality. Accidental deaths are presented in parentheses at the time of occurrence, but were treated as scheduled sacrifices for survival and mortality analyses.

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## Detailed Mortality Summary for Females:

| Females                |       |   |   |   |     |     | Mortality Summary* |   |   |     |    |    |    |     |    |    |    |    |    |    |    |    |    |     |               |                    |                         |    |    |    | Table 1 |  |  |
|------------------------|-------|---|---|---|-----|-----|--------------------|---|---|-----|----|----|----|-----|----|----|----|----|----|----|----|----|----|-----|---------------|--------------------|-------------------------|----|----|----|---------|--|--|
| Initial<br>Number      | Month |   |   |   |     |     |                    |   |   |     |    |    |    |     |    |    |    |    |    |    |    |    |    |     | Total<br>Dead | Total<br>Survivors | Percent<br>Survivorship |    |    |    |         |  |  |
|                        | 1     | 2 | 3 | 4 | 5   | 6   | 7                  | 8 | 9 | 10  | 11 | 12 | 13 | 14  | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24  |               |                    |                         |    |    |    |         |  |  |
| Group 1 - Untreated    |       |   |   |   |     |     |                    |   |   |     |    |    |    |     |    |    |    |    |    |    |    |    |    |     |               |                    |                         |    |    |    |         |  |  |
| 50                     | 0     | 0 | 0 | 0 | 0   | 0   | 0                  | 0 | 0 | (1) | 0  | 2  | 1  | 2   | 2  | 2  | 1  | 1  | 2  | 6  | 4  | 2  | 3  | 4   | 0             | 32                 | 17                      | 36 |    |    |         |  |  |
|                        | 0     | 0 | 0 | 0 | 0   | 0   | 0                  | 0 | 0 | 0   | 2  | 3  | 5  | 7   | 9  | 10 | 11 | 13 | 19 | 23 | 25 | 28 | 32 | 32  | (1)           |                    |                         |    |    |    |         |  |  |
| Group 2 - 0 ug/kg/day  |       |   |   |   |     |     |                    |   |   |     |    |    |    |     |    |    |    |    |    |    |    |    |    |     |               |                    |                         |    |    |    |         |  |  |
| 50                     | 0     | 0 | 0 | 0 | 0   | 0   | 1                  | 0 | 2 | 0   | 0  | 0  | 3  | 3   | 2  | 3  | 4  | 3  | 2  | 5  | 1  | 3  | 2  | 1   | 36            | 14                 | 28                      |    |    |    |         |  |  |
|                        | 0     | 0 | 0 | 0 | 0   | 0   | 1                  | 1 | 3 | 3   | 3  | 3  | 6  | 9   | 11 | 14 | 17 | 20 | 24 | 27 | 29 | 32 | 35 | 36  |               |                    |                         |    |    |    |         |  |  |
| Group 3 - 3 ug/kg/day  |       |   |   |   |     |     |                    |   |   |     |    |    |    |     |    |    |    |    |    |    |    |    |    |     |               |                    |                         |    |    |    |         |  |  |
| 50                     | 0     | 0 | 1 | 0 | 0   | (1) | 1                  | 0 | 0 | 0   | 0  | 0  | 3  | 1   | 0  | 0  | 3  | 6  | 5  | 5  | 6  | 0  | 3  | 34  | 16            | 38                 |                         |    |    |    |         |  |  |
|                        | 0     | 0 | 1 | 1 | 1   | 1   | 2                  | 2 | 2 | 2   | 2  | 2  | 5  | 6   | 6  | 6  | 9  | 15 | 20 | 25 | 31 | 31 | 34 | (3) |               |                    |                         |    |    |    |         |  |  |
| Group 4 - 10 ug/kg/day |       |   |   |   |     |     |                    |   |   |     |    |    |    |     |    |    |    |    |    |    |    |    |    |     |               |                    |                         |    |    |    |         |  |  |
| 50                     | 0     | 0 | 0 | 0 | (1) | (1) | 1                  | 0 | 3 | 1   | 0  | 2  | 0  | (1) | 2  | 1  | 2  | 0  | 1  | 3  | 6  | 1  | 1  | 6   | 2             | 4                  | 0                       | 35 | 12 | 30 |         |  |  |
|                        | 0     | 0 | 0 | 0 | 1   | 1   | 4                  | 5 | 5 | 7   | 7  | 8  | 9  | 11  | 11 | 12 | 15 | 21 | 22 | 23 | 29 | 31 | 35 | 35  | (3)           |                    |                         |    |    |    |         |  |  |
| Group 5 - 30 ug/kg/day |       |   |   |   |     |     |                    |   |   |     |    |    |    |     |    |    |    |    |    |    |    |    |    |     |               |                    |                         |    |    |    |         |  |  |
| 50                     | 0     | 0 | 0 | 0 | 0   | 0   | 0                  | 0 | 0 | 0   | 0  | 2  | 2  | 1   | 1  | 0  | 2  | 4  | 3  | 7  | 5  | 2  | 4  | 3   | 36            | 17                 | 34                      |    |    |    |         |  |  |
|                        | 0     | 0 | 0 | 0 | 0   | 0   | 0                  | 0 | 0 | 0   | 0  | 2  | 4  | 5   | 6  | 6  | 8  | 12 | 15 | 22 | 27 | 29 | 33 | 36  |               |                    |                         |    |    |    |         |  |  |

\*The numbers above the line represent mortality occurring monthly and the numbers below the line represent cumulative mortality. Accidental deaths are presented in parentheses at the time of occurrence, but were treated as scheduled sacrifices for survival and mortality analyses.

Clinical signs: No remarkable observations, including no dermal irritation at the treatment site.

Mean Body weight: No treatment-related effects on mean body weight were observed.

Mean Body Weight Gain: No treatment-related effects on mean body weight gain were observed.

Body Weight Gain over Weeks 0-93 ( $g \pm SD$ ):

| Group     | Males                  | Females                |
|-----------|------------------------|------------------------|
| Control   | 13.6 $\pm$ 3.21 (n=49) | 12.8 $\pm$ 3.01 (n=45) |
| Low-Dose  | 13.2 $\pm$ 2.49 (n=22) | 11.8 $\pm$ 2.86 (n=20) |
| Mid-Dose  | 11.2 $\pm$ 2.47 (n=14) | 12.3 $\pm$ 4.23 (n=18) |
| High-Dose | 14.2 $\pm$ 3.84 (n=19) | 11.6 $\pm$ 3.07 (n=23) |

None significant. "Control" values represent pooled data from the untreated and vehicle-treated groups.

Food consumption: No remarkable observations.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: NA

Clinical chemistry: NA

Urinalysis: NA

Mean organ weights: NA

Gross pathology: Distension of the urinary bladder and dilation of the renal pelvis were observed slightly more frequently in treated animals than in controls (males only). No other remarkable observations.

Histopathology:

Non-neoplastic: In males administered 10 and 30 µg/kg/day, the incidence of minimal mineralization of the renal cortex and minimal to moderate mineralization of the myocardium of the heart was slightly increased. Increased incidence of minimal to moderate "basophilic cortical tubules" was observed in MD and HD males, possibly suggesting degeneration/regeneration of the epithelium. Females administered 30 µg/kg/day exhibited an increased incidence of minimally to moderately dilated proximal tubules.

Neoplastic: (See Biostatistics review 1 of IND 67,835, dated 02-APR-2007, for complete information): No statistically significant differences in tumor incidence were observed in female mice in this study. In male mice the incidence of pooled bronchio/alveolar adenomas and carcinomas was statistically significantly increased (common tumor, test of dose related trends in the vehicle, low, medium, and high dose groups yielded  $p = 0.0053$ ; test of differences between the high dose group and the vehicle group yielded  $p = 0.0016$ ). However, this observation was considered to random in nature, and to have no biological significance.

Toxicokinetics: All values were below the LOQ (60 pg/mL).

**2.6.6.5.3 Study title:** Carcinogenicity Study by Oral Gavage Administration of calcipotriene to Rats for 104 Weeks. By agreement between the sponsor and the Division, this study will be regarded as being a Phase 4 commitment to NDA 22-185.

Carcinogenicity studies with betamethasone alone:

**2.6.6.5.4 Study title:** Carcinogenicity Study by Dermal Administration to Mice for 104 Weeks. By agreement between the sponsor and the Division, this study is regarded as being a Phase 4 commitment to NDA 21-852.

**2.6.6.5.5 Study title:** Betamethasone Dipropionate. Carcinogenicity Study by Oral Gavage Administration to Han Wistar Rats for 104 Weeks. By agreement between the sponsor and the Division, this study is regarded as being a Phase 4 commitment to NDA 21-852.

Carcinogenicity studies with calcipotriene/betamethasone ointment: None.

#### **2.6.6.6 Reproductive and developmental toxicology**

## **Fertility and early embryonic development**

Fertility and early embryonic development study conducted with calcipotriene alone:

**2.6.6.6.1 Study title:** MC 903. A study on fertility and general reproductive performance in the rat, Study No. 870727T7. Please see Original Summary of NDA 20-273 for details of this study. Briefly, F0 males were treated for 63 days prior to and throughout pairing with F0 females, which were treated beginning 14 days prior to pairing with males and continuing until day 28 post-partum. Exposures of 0, 6, 18, and 54 µg/kg/day were studied. F1 and F2 animals were not dosed. No effects on mortality or clinical signs were observed. Body weight gain was significantly reduced in high-dose F0 animals prior to mating. Mating performance and pregnancy rate were not affected by treatment. Body weight gain of F0 females during gestation was reduced in proportion to dosage. Mean litter data from F0 females sacrificed on day 20 were comparable across groups, and no major malformations were observed. Increased incidence of delayed ossification of the skull, ribs, and hyoid bones was observed in all treatment groups, but the magnitude of the increase did not appear to increase in proportion to dosage. No effects on the incidence of minor visceral anomalies or effects on development or behavior of F1 or F2 animals were noted.

Fertility and early embryonic development study conducted with betamethasone dipropionate alone:

**2.6.6.6.2 Study title:** Effect on the fertility in male rats (oral administration).

**Key study findings:** Betamethasone, orally administered to males only, had no effects on the reproductive parameters that were monitored in this study, including time to mating, fertility index, numbers of fetuses, or implantation losses. A dose of 0.2 mg/kg/day (the highest dose studied) was considered to be a NOEL under the conditions of this study.

**Study no.:** RTOX0301

**Volume #, and page #:** Mod 4, vol. 34

**Conducting laboratory and location:** LEO Pharmaceutical Products, Ballerup, Denmark

**Date of study initiation:** 05-SEP-2003

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, and % purity:** Betamethasone dipropionate; lot No. 0314461; assumed 100% pure. Methyl cellulose 1% w/v (vehicle); lot No. 0319716.

### **Methods**

Doses: 0 (vehicle), 0.02, 0.06, and 0.2 mg/kg/day

Species/strain: Rat/Wistar (HsdBrHn:WIST)

Number/sex/group: 10 (Note: Only males were treated)

Route, formulation, volume, and infusion rate: Oral (gavage); betamethasone suspended in 1% methyl cellulose (4, 12, and 40 µg/mL); 5 mL/kg/day

Satellite groups used for toxicokinetics: Yes (2 controls and six test animals per group)  
Study design: Main study animals: Males dosed for 10 weeks prior to pairing with untreated females (paired until either a positive vaginal smear was obtained or else until 11 days passed without mating). Dams killed on day 14 of pregnancy and numbers of corpora lutea, implantations, resorptions, and fetuses recorded. Toxicokinetic animals: Blood samples drawn following 8 administrations at 0, 1, 2, 3, 5, and 7 hours post-dosing.  
Parameters and endpoints evaluated: Clinical signs, body weight, and maternal/fetal parameters (see above).

## Results

Note: The males used in this study were part of a 13 week oral study to select dosages to be used in a carcinogenicity study ("A 13-week oral carcinogenicity range-finding study in rats", Study No. TTOX0301, reviewed above). Please see the review of that study for information concerning mortality, clinical signs, body weight, food consumption, and toxicokinetics.

Mortality: See review of study TTOX0301, in the repeat-dose toxicology portion of this summary.

Clinical signs: See review of study TTOX0301, in the repeat-dose toxicology portion of this summary.

Body weight: See review of study TTOX0301, in the repeat-dose toxicology portion of this summary.

Food consumption: See review of study TTOX0301, in the repeat-dose toxicology portion of this summary.

Toxicokinetics: See review of study TTOX0301, in the repeat-dose toxicology portion of this summary.

Necropsy: See review of study TTOX0301, in the repeat-dose toxicology portion of this summary.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): No remarkable observations on any of the reproductive parameters, including time to mating, fertility index, numbers of fetuses, or implantation losses.

**2.6.6.6.3 Study title:** Oral (gavage) fertility and early embryonic development study in the female rat.



**Key study findings:** When administered to female rats for 15 days prior to mating and continuing until gestation day 6, betamethasone had no effects on mating or fertility.

**Study no.:** RTOX0606 (also referred to as study No. LOP0079)

**Volume #, and page #:** Submitted to amendment 4F to NDA 21-852

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** 18-SEP-2006

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Betamethasone dipropionate, lot No. 053386101, 99.5%

**Formulation/vehicle:** The test materials were prepared as suspensions in 1.0% methylcellulose in purified water.

**Methods:**

Species/strain: Rat/ — CD(SD)IGS BR VAF/Plus

b(4)

Doses employed: 0 (control), 0.1, 0.3, and 1.0 mg/kg/day

Route of administration: Oral (gavage) once daily

Study design: Females only were dosed. The animals were treated beginning 14 days prior to pairing with untreated males, during pairing, and continuing until day 6 of gestation (day 0 was the day a positive sperm smear was observed).

Dams were killed on day 13 of gestation.

Number/group: 20

Parameters and endpoints evaluated: Maternal survival, clinical signs, body weight, food consumption, estrous cycling (smear), mating behavior, and gross necropsy. Numbers of live, dead, and resorbed fetuses were determined. The thymus and spleen of the F0 female animals were weighed.

Toxicokinetic data: Blood samples were obtained from 3 F0 females per group on day 10 of gestation at 0.5, 1, and 2 hours after dosing for measurement of drug levels.

**Results:**

In-life (maternal) observations:

Maternal Mortality: None

Clinical signs: None

Maternal body weight: Mean body weight gain tended to be slightly lower in treatment groups during the period of dosing, which differences achieving statistical significance over some time intervals, but the differences were small and this may have merely reflected the fact that the mean weight of the control animals on day 1 of treatment was less than in the treatment groups.

Food consumption: No remarkable observations.

Latency to mate: No remarkable observations.

Fertility index (% pregnant): No remarkable observations.

Mean weight of F0 female organs: The mean weights of the thymus and spleen were significantly reduced in all treatment groups.

Terminal and necroscopic evaluations (offspring):

No. of corpora lutea: No remarkable observations.

Mean implantations: No remarkable observations.

No. of Live fetuses at C-section (expressed as percentage of implantations): No remarkable observations.

No. of early resorptions: No remarkable observations.

No. of late resorptions: No remarkable observations.

TK data:

Mean plasma levels in F0 females, day 10 of gestation:

| Group             | Betamethasone<br>Dipropionate (pg/mL) | Betamethasone<br>17-Propionate (pg/mL) |
|-------------------|---------------------------------------|----------------------------------------|
| 1 (Control)       | BLQ                                   | BLQ                                    |
| 2 (0.1 mg/kg/day) | BLQ                                   | 25,900                                 |
| 3 (0.3 mg/kg/day) | BLQ                                   | 62,933                                 |
| 4 (1.0 mg/kg/day) | 304                                   | ALQ                                    |

Data shown are from 1 hour post-dosing; levels at 0.5 and 2 hours were similar.

BLQ = Below limit of quantitation (75 pg/mL for betamethasone dipropionate; 125 pg/mL for betamethasone 17-propionate)

ALQ = Above limit of quantitation (100,000 pg/mL for betamethasone 17-propionate)

## Embryofetal development

Embryofetal development studies with calcipotriene alone:

**2.6.6.6.4 Study title:** Effect of MC 903 on foetal development in rats, Study No. 870824T8. Please see Original Summary of NDA 20-273 for details of this study. Briefly, pregnant Wistar rats were dosed daily with calcipotriene at exposures of 0, 6, 18,

or 54 µg/kg/day on days 6-15 of gestation. There were no remarkable effects on survival, behavior, body weight, litter parameters, or the incidence of major malformations. A slight trend toward an increase in the incidence of minor skeletal variations, including "coma" shaped extra ribs, was apparent, but this is unlikely to be relevant to the levels of systemic exposure to calcipotriene that would result from use of the Dovobet ointment.

**2.6.6.6.5 Study title:** MC 903. Oral teratology study in the rabbit, Study No. 339/503. Please see Original Summary of NDA 20-273 for details of this study. Briefly, pregnant New Zealand rabbits were dosed daily with calcipotriene at exposures of 0, 4, 12, or 36 µg/kg/day on days 6-18 of gestation. Mortality was increased in the high-dose group (7 F0 females died or were killed following abortion, compared to 0 in the control group and 2 unscheduled deaths in both the low and mid groups). Body weight gain was reduced in mid and high-dose animals. The post-implantation loss was increased in the high-dose group, while the mean fetal weight was reduced. There were no remarkable effects on the incidence of major malformations. An increase in the incidence of minor skeletal variations, including incomplete ossification of sternebrae, pubic bones, and fore limb phalanges was observed in the high-dose group.

Embryofetal development studies with betamethasone alone:

**2.6.6.6.6 Study title:** Teratology Studies on betamethasone 17,21-dipropionate, prednisolone and betamethasone 21-disodium phosphate in mice and rats, Oyo Yakuri (Pharmacometrics) 1974;8(6) (Published report).

Note: This is a review of an article published in Japanese in a Japanese journal (Hasegawa Y. et al., Oyo Yakuri (Pharmacometrics), 8(6), 1974). The version of the article that was submitted was apparently translated by the sponsor. This review is limited to the portion of the data that concerned betamethasone dipropionate, but includes both a mouse study and a rat study.

Mouse study:

**Key study findings:** When administered subcutaneously to pregnant mice on days 7 through 13 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, increased incidence of cleft palate and crooked or short tail, and delayed ossification. A NOAEL was not observed in this study, as fetal toxicity was observed at the lowest exposure that was evaluated (0.156 mg/kg/day).

**Study no.:** Not stated

**Volume #, and page #:** Mod 4, vol. 35

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Not stated

**GLP compliance:** No

**QA reports:** yes ( ) no (X)

**Drug, lot #, radiolabel, and % purity:** Not stated

**Formulation/vehicle:** 0.5% gum arabic in water

b(4)

**Methods:**

Species/strain: Mouse/ICR-JCL

Doses employed: 0 (control), 0.156, 0.625, and 2.5mg/kg/day

Route of administration: Subcutaneous injection into dorsal side of neck (once daily).

Study design: Virgin females were paired with males; day on which mating was confirmed (plug or sperm in vagina) was designated day 0. Animals were dosed for six days starting on day 7 of gestation. Dams were killed on day 18 and C-sectioned.

Number/sex/group: 23 in control and low-dose groups, 22 in mid and high-dose groups.

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.

**Results:**

In-life (maternal) observations:

Maternal mortality: Apparently none

Clinical signs: Not mentioned

Maternal body weight: No effect at low dose, but "markedly reduced" in the mid and high-dose groups (quantitative data not submitted).

Food consumption: NA

Toxicokinetics: NA

Terminal and necroscopic evaluations (offspring):

Body weight of live fetuses (male/female combined; grams, mean $\pm$ SD):

Significantly reduced in all treatment groups (1.33 $\pm$ 0.12, 1.26 $\pm$ 0.14, 1.13 $\pm$ 0.14, and 0.95 $\pm$ 0.15 at 0, 0.156, 0.625, and 2.5mg/kg/day, respectively).

No. of Live fetuses at C-section (expressed as percentage of implantations):

Significantly reduced in mid and high-dose groups (90%, 87%, 75%, and 18% at 0, 0.156, 0.625, and 2.5mg/kg/day, respectively).

No. of resorbed fetuses (early/late resorptions not specified; expressed as percentage of implantations): Significantly increased in mid and high-dose groups (10%, 10%, 15%, and 71% at 0, 0.156, 0.625, and 2.5mg/kg/day, respectively).

No. of "macerated" fetuses (late resorptions?; expressed as percentage of implantations): Significantly increased in mid and high-dose groups (0%, 2%, 6%, and 8% at 0, 0.156, 0.625, and 2.5mg/kg/day, respectively).

No. of dead fetuses (expressed as percentage of implantations): Significantly increased in mid and high-dose groups (0%, 1%, 4%, and 4% at 0, 0.156, 0.625, and 2.5mg/kg/day, respectively).

Percentage of live fetuses exhibiting selected non-skeletal anomalies:

Cleft palate: Significantly increased in all treatment groups (2%, 12%, 45%, and 96% at 0, 0.156, 0.625, and 2.5mg/kg/day, respectively).

Crooked or short tail: Significantly increased in high-dose group only (0%, 1%, 1%, and 20% at 0, 0.156, 0.625, and 2.5mg/kg/day, respectively).

Skeletal anomalies: Skeletal effects were apparently limited to delayed ossification of the cervical vertebra, sternbrae, and occipital squama.

Rat study:

**Key study findings:** When administered subcutaneously to pregnant rats on days 9 through 15 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, and adrenal hypertrophy and hemorrhage.

**Study no.:** Not stated

**Volume #, and page #:** Mod 4, vol. 35

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Not stated

**GLP compliance:** No

**QA reports:** yes ( ) no (X)

**Drug, lot #, radiolabel, and % purity:** Not stated

**Formulation/vehicle:** 0.5% gum arabic in water

b(4)

#### Methods:

Species/strain: Rat/SD-JCL

Doses employed: 0 (control), 20, 80, and 320mg/kg/day

Route of administration: Subcutaneous injection into dorsal side of neck (once daily).

Study design: Virgin females were paired with males; day on which mating was confirmed (plug or sperm in vagina) was designated day 0. Animals were dosed for six days starting on day 9 of gestation. Dams were killed on day 21 and C-sectioned.

Number/sex/group: 23 in control and mid-dose groups, 24 in low-dose group, and 22 in high-dose group.

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.

#### Results:

In-life (maternal) observations:

Maternal Mortality: Apparently none  
Clinical signs: Not mentioned  
Maternal body weight: No effect at any dose  
Food consumption: NA  
Toxicokinetics: NA

Terminal and necroscopic evaluations (offspring):

Body weight of live fetuses (male/female combined; grams, mean $\pm$ SD):  
Significantly reduced in all treatment groups ( $4.90\pm0.49$ ,  $4.56\pm0.40$ ,  $4.50\pm0.42$ ,  
and  $4.49\pm0.41$  at 0, 20, 80, and 320mg/kg/day, respectively).

No. of Live fetuses at C-section (expressed as percentage of implantations):  
Significantly reduced in high-dose group only (94%, 93%, 95%, and 89% at 0, 20,  
80, and 320 mg/kg/day, respectively).

No. of resorbed fetuses (early/late resorptions not specified; expressed as  
percentage of implantations): Significantly increased in high-dose group only  
(6%, 7%, 5%, and 11% at 0, 20, 80, and 320mg/kg/day, respectively).

No. of "macerated" fetuses (late resorptions?): None

No. of dead fetuses: No remarkable observations

Gross non-skeletal anomalies: The only reported non-skeletal gross anomalies  
were "adrenal hypertrophy and hemorrhage", observed in 100% of fetuses from  
all three treatment groups, but no control fetuses, and "hydronephrosis with  
hydroureter", the incidence of which was significantly increased in the mid and  
high-dose groups (1%, 2%, 8, and 8% at 0, 20, 80, and 320mg/kg/day,  
respectively).

Skeletal anomalies: No remarkable observations

**2.6.6.6.7 Study title:** Teratogenicity of betamethasone 17,21-dipropionate (S-3440) in  
rabbits, Kiso to Rinsho (The Clinical Report);11(6), June 1977 (Published report).

Note: This is a review of an article that was apparently published in Japanese in a  
Japanese journal (Hasegawa Y. et al., Kiso to Rinsho (The Clinical Report), 11(6), 1977).  
The version of the article that was submitted was apparently translated by the sponsor.

**Key study findings:** When administered subcutaneously to pregnant rabbits on days 6 through 18 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, external malformations, and skeletal malformations. An exposure of 0.625 µg/kg/day was a NOAEL in this study; fetal toxicity was observed at 2.5 µg/kg/day and above.

**Study no.:** Not stated

**Volume #, and page #:** Mod 4, vol. 35

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** Not stated

**GLP compliance:** No

**QA reports:** yes ( ) no (X)

**Drug, lot #, radiolabel, and % purity:** Not stated

**Formulation/vehicle:** 1% gum arabic in water

**Methods:**

Species/strain: Rabbit/New Zealand white

Doses employed: 0 (control), 0.625, 2.5, and 10 µg/kg/day

Route of administration: Subcutaneous injection into dorsal skin (once daily)

Study design: Virgin females were paired with males; day on which mating was confirmed (plug or sperm in vagina) was designated day 0. Animals were dosed for 13 days on days 6 to 18 of gestation. Dams were killed on day 28 and C-sectioned.

Number/sex/group: 12 in control and low-dose groups, 14 in mid-dose group, and 15 in high-dose group.

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.

**Results:**

In-life (maternal) observations:

Maternal mortality: Apparently none

Clinical signs: Not mentioned

Maternal body weight: Maternal weight in low-dose animals appeared to become somewhat suppressed (judging by a graphical presentation of the data) beginning about day 14, but recovered by day 28. Maternal weight was suppressed in a dose-dependent manner in mid and high-dose groups; high-dose animals weighed approximately the same on day 28 as on day 6 (quantitative data not submitted).

Food consumption: NA

Toxicokinetics: NA

Terminal and necroscopic evaluations (offspring):

Body weight of live fetuses (male/female combined; grams, mean $\pm$ SD): Significantly reduced in mid and high-dose groups (33.6 $\pm$ 4.7, 33.0 $\pm$ 4.8, 30.7 $\pm$ 6.0, and 25.6 $\pm$ 5.0 at 0, 0.625, 2.5, and 10  $\mu$ g/kg/day, respectively).

No. of Live fetuses at C-section (expressed as percentage of implantations): Significantly reduced in high-dose group only (96%, 97%, 93%, and 30% at 0, 0.625, 2.5 and 10  $\mu$ g/kg/day, respectively).

No. of resorbed fetuses (early/late resorptions not specified; expressed as percentage of implantations): Significantly increased in mid and high-dose groups (3%, 1%, 6%, and 64% at 0, 0.625, 2.5, and 10  $\mu$ g/kg/day, respectively).

No. of "macerated" fetuses (late resorptions?; expressed as percentage of implantations): No significant differences

Percentage of live fetuses exhibiting selected non-skeletal anomalies: None in control or low-dose groups. External anomalies were observed in 9% of the live fetuses in the mid-dose group, including isolated instances of exencephaly, kinked tail, gastroschisis, and umbilical hernia. External anomalies were observed in 55% of the live fetuses in the high-dose group, including auricular dysplasia, cleft palate, meningocele, gastroschisis, umbilical hernia, kinked tail, club foot, and club hand.

Skeletal anomalies: Remarkable effects on skeletal development were limited to the mid and high-dose groups. In the mid-dose group, 12% of the fetuses examined exhibited an absence of phalanges of the first digit. In the high-dose group, 50% of the fetuses examined exhibited skeletal malformations, including absence of phalanges of the first digit, cranial dysplasia, and club hand.

#### **Summary of individual study findings:**

Note: The teratology studies conducted with betamethasone dipropionate reviewed above are old, non-GLP studies, the reports of which are available only as translations of Japanese journal articles. Original animal data, as well as many details of the conduct and results of the studies, are not available. It is unclear why rats were apparently much less sensitive to betamethasone than were mice and rabbits (high-doses of 2.5 mg/kg/day and 10  $\mu$ g/kg/day were used in mice and rabbits, respectively, while high-dose rats received 320 mg/kg/day, apparently with similar levels of toxicity). Therefore, I discount the portion of the study that involved rats, and will base evaluation of the teratology of betamethasone on the portions of the study that involved mice and rabbits, as they appear to be the more sensitive species. If the data from these studies had been negative I probably would not consider the studies to be of sufficient quality to be of regulatory use. However, in view of the fact that the submitted data are positive, and considering that topical betamethasone products have been approved and used extensively for many years, I consider these data to be adequate for the current regulatory purpose.



Mouse teratology study: When administered subcutaneously to pregnant mice on days 7 through 13 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, increased incidence of cleft palate and crooked or short tail, and delayed ossification. Fetal toxicity generally increased in incidence or severity with increased exposure to the test material, and some fetal toxicity was observed even in the low-dose group, which did not exhibit maternal toxicity. A NOAEL was not observed in this study, as fetal toxicity was observed at the lowest exposure that was evaluated (0.156 mg/kg/day).

Rabbit teratology study: When administered subcutaneously to pregnant rabbits on days 6 through 18 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, external malformations, and skeletal malformations. An exposure of 0.625 µg/kg/day was a NOAEL in this study; fetal toxicity was observed at 2.5 µg/kg/day and above.

Embryofetal development studies with calcipotriene/betamethasone ointment: None.

### **Prenatal and postnatal development**

Prenatal and postnatal development studies of calcipotriene alone:

**2.6.6.6.8 Study title:** MC 903. Peri- and postnatal study in rats, Study No. 880415T3. Please see Original Summary of NDA 20-273 for details of this study. Briefly, pregnant Wistar rats were dosed daily with calcipotriene at exposures of 0, 6, 18, or 54 µg/kg/day from day 15 of gestation through day 20 post-partum. There were no remarkable effects on any parameter, including survival, behavior, body weight, litter parameters, or the ability to nurse or rear pups.

Prenatal and postnatal development studies of betamethasone alone:

**2.6.6.6.9 Study title:** Oral (gavage) pre and post-natal development toxicity study in the rat.

**Key study findings:** Betamethasone dipropionate 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 1.0 mg/kg/day. Mean maternal BW was significantly lower at 0.3 and 1.0 mg/kg/day on day 20 of gestation. The mean duration of gestation was slightly but statistically increased at 0.1, 0.3, and 1.0 mg/kg/day. The mean percentage of pups that survived to day 4 was reduced in F1 pups in relation to dosage, although the effects at 0.1 and 0.3 mg/kg/day were minimal. The percentage of pups with a righting-reflex on day 5 of lactation was significantly reduced at 1.0 mg/kg/day. No effects were observed on pup learning ability or reproduction of F1 animals.

**Study no.:** RTOX0608 (also referred to as study No. LOP0081)

**Volume #, and page #:** NA

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** 30-AUG-2006

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Betamethasone dipropionate, Lot #053386101.

**Formulation/vehicle:** The test materials were prepared as suspensions. 1% methylcellulose and 0.1% citric acid in purified water was used as the vehicle (pH 4.0).

## Methods:

Species/strain: Rat/ CD(SD)IGS BR VAF/Plus

Number/sex/group: 22

Doses employed: 0 (control), 0.1, 0.3, and 1.0 mg/kg/day

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) 4 pups per gender. 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.

Toxicokinetic data: Blood samples were obtained from 3 F0 females per group on day 10 of gestation for measurement of drug levels.

## Results

**F<sub>0</sub> in-life:** At 1.0 mg/kg/day, two F0 females were sacrificed during lactation following total litter loss (days 1 and 3 of lactation). One animal at 0.3 mg/kg/day was sacrificed on day 23 of gestation (parturition had commenced, but littering could not be completed). This was judged to be incidental (not related to treatment), as no other F0 females, including those in the 1.0 mg/kg/day group, exhibited these signs. Mean maternal BW was slightly, but significantly lower at 0.3 and 1.0 mg/kg/day on day 20 of gestation:

Mean BW on day 20-21 of gestation:

| Group       | Mean BW (g) |
|-------------|-------------|
| 1 (control) | 389±23      |
| 2 (0.1)     | 387±26      |
| 3 (0.3)     | 360±17***   |
| 4 (1.0)     | 368±24***   |

\*\*\*p<0.001

At 0.3 and 1.0 mg/kg/day, group mean BW values remained lower than control values throughout lactation.

The mean duration of gestation was slightly but statistically increased at 0.1, 0.3, and 1.0 mg/kg/day:

| Group       | Mean Gestation Period (Days) |
|-------------|------------------------------|
| 1 (control) | 22.2±0.4                     |
| 2 (0.1)     | 22.6±0.5*                    |
| 3 (0.3)     | 22.8±0.5***                  |
| 4 (1.0)     | 23.2±0.4***                  |

\*p<0.05; \*\*\*p<0.001

No significant differences in the mean numbers of pups born alive or dead.

F<sub>0</sub> necropsy: The mean weights of the spleen and thymus were reduced in relation to dosage. No other remarkable observations.

F<sub>1</sub> physical development: The mean cumulative survival index (% of pups surviving to day 4) was reduced in F<sub>1</sub> pups in relation to dosage:

| Group       | Mean F <sub>1</sub> Pup Survival Index |
|-------------|----------------------------------------|
| 1 (control) | 98.7                                   |
| 2 (0.1)     | 93.1*                                  |
| 3 (0.3)     | 91.2*                                  |
| 4 (1.0)     | 78.2**                                 |

\*p<0.05; \*\*p<0.01

The cause of the reduced pup survival was presumably due to reduced maternal ability to nurse, as well as cannibalism, as dosage-related increases in the numbers of missing pups and pups with little or no milk in the stomach were observed. Two litters at 1.0 mg/kg/day were completely lost (days 1 and 3 postpartum). Pup survival following culling on lactation day 4 was also slightly lower in treatment groups, but the difference

was not significant. Mean pup weight was slightly reduced at 0.3 and 1.0 mg/kg/day, although the values were similar to historical control values.

F<sub>1</sub> behavioral evaluation: The percentage of pups with a righting-reflex on day 5 of lactation was significantly reduced at 1.0 mg/kg/day (86.5%, compared to 96.4% of controls). No other remarkable observations.

F<sub>1</sub> reproduction: No remarkable observations.

F<sub>2</sub> findings: No remarkable observations (during necropsy of F1 dams).

TK data:

Mean plasma levels in F0 females, day 10 of gestation:

| Group             | Betamethasone<br>Dipropionate (pg/mL) | Betamethasone<br>17-Propionate (pg/mL) |
|-------------------|---------------------------------------|----------------------------------------|
| 1 (Control)       | BLQ                                   | BLQ                                    |
| 2 (0.1 mg/kg/day) | BLQ                                   | 24,471                                 |
| 3 (0.3 mg/kg/day) | 101                                   | 83,794                                 |
| 4 (1.0 mg/kg/day) | 458                                   | ALQ                                    |

BLQ = Below limit of quantitation (75 pg/mL for betamethasone dipropionate;  
125 pg/mL for betamethasone 17-propionate)

ALQ = Above limit of quantitation (100,000 pg/mL for betamethasone 17-  
propionate)

#### 2.6.6.7 Local tolerance

Local tolerance studies with formulations of calcipotriene alone (some of these studies are discussed in the Original Summary of NDA 20-273):

**2.6.6.7.1 Study title:** Calcipotriol cream. 6 weeks skin irritation test in the rabbit, Study No. 911104I5. Please see Original Summary of NDA 20-273.

**2.6.6.7.2 Study title:** Calcipotriol lotion. 6 weeks skin irritation test in the rabbit, Study No. 910612I3. Please see Original Summary of NDA 20-273.

**2.6.6.7.3 Study title:** MC 903 ointment. Acute eye irritation study in the rabbit, Study No. 890508I2. Please see Original Summary of NDA 20-273.

Local tolerance studies of formulations of betamethasone alone: None.

Local tolerance studies with calcipotriene/betamethasone ointment:

**2.6.6.7.4 Study title:** Calcipotriol betamethasone. Six weeks dermal tolerability study in rabbits.

**Key study findings:** Local effects included occasionally observed very slight erythema, apparently caused by either the vehicle or the application procedure, and, histologically, slight to moderate pilosebaceous metaplasia and minimal to moderate keratin cysts. Body weight loss and decreased skin-fold thickness indicate loss of body fat, presumably due to systemic effects of betamethasone and/or calcipotriene. Overall, these data suggest Dovobet ointment was reasonably well tolerated under the conditions of this study.

**Study no:** LTOX/99/02

**Volume #, and page #:** Mod 4, Vol. 39

**Conducting laboratory and location:** Leo Pharmaceutical Products, Ballerup, Denmark

**Date of study initiation:** 21-MAY-1999

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Taclonex ointment, batch No. 9838381, presumed 100%. Vehicle ointment, batch No. 983638101.

**Formulation/vehicle:**

| <u>Compound</u>                        | <u>Amount per gram</u> |
|----------------------------------------|------------------------|
| Calcipotriene hydrate.....             | 52.2µg                 |
| Betamethasone dipropionate.....        | 643µg                  |
| <hr/>                                  |                        |
| Liquid paraffin.....                   | /                      |
| Polyoxypropylene-15-stearyl ether..... | /                      |

b(4)

**Methods:**

**Dosing:**

Species/strain: Rabbits/New Zealand White

#/sex/group or time point (main study): 6 males were used, each of which received both active and placebo ointment. No females were studied.

Satellite groups used for toxicokinetics or recovery: No

Age: Not stated

Weight: 2.0 kg to 2.8 kg initially

Doses in administered units: Four areas of shaved skin on the back of each animal, each area approximately 3cm x 4cm, were selected. 0.1 g of active ointment was applied once daily to one site, while 0.1 g of vehicle was applied to a second site; these materials were gently spread on the skin using a latex glove. Two sites remained untreated, but were massaged with a latex glove. No dressing was applied. The animals were placed in restraining boxes for four hours following treatment to prevent access to the application sites. At the end of the four hour treatment period the sites were wiped with gauze to remove any

remaining material. Treatment continued daily for six weeks. Each animal received approximately 2 µg/kg/day calcipotriene and 20 µg/kg/day betamethasone base (based on BW of 2.5 kg).

Route, form, volume, and infusion rate: See above

**Observations and times:**

Clinical signs: Yes

Body weights: Yes

Food consumption: Yes

Ophthalmoscopy: No

EKG: No

Hematology: No

Clinical chemistry: No

Urinalysis: No

Gross pathology: Yes

Organs weighed: Yes (adrenals only)

Histopathology: Yes (limited to skin from the four selected areas)

Toxicokinetics: No

Other: Skin erythema and thickness

**Results:** Note: The study did not include a true control group (all animals received active ointment), so there was nothing against which to compare systemic parameters.

Mortality: No unscheduled deaths

Clinical signs: Animals appeared thin beginning week 2.

Body weights: The mean body weight decreased by approximately 100 g during the six week treatment period.

Food consumption: The animals apparently ate all available food.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: NA

Clinical chemistry: NA

Urinalysis: NA

Organ weights: NA

Gross pathology: Observations thought to be related to treatment included livers that were enlarged, light colored, soft textured, and rounded, and adrenals that were considered smaller than usual.

Histopathology: Changes considered to be related to treatment were observed in areas of skin treated with both active and vehicle ointment (but not in untreated skin), and included slight to moderate pilosebaceous metaplasia and minimal to moderate keratin cysts. The severity of the cysts tended to be higher in the areas that received active ointment. Note that only skin was examined histologically.

Other: Very slight erythema was occasionally observed in the areas treated with either active or placebo ointment. The skin-fold thickness of all skin areas (including untreated areas) decreased during the study, apparently due to fat loss.

Toxicokinetics: NA

Local tolerance studies with calcipotriene/betamethasone gel:

**2.6.6.7.5 Study title:** Daivobet gel: Acute eye irritation study in rabbits

**Key study findings:** Taclonex gel did not induce irritation or other observed toxicity when placed within the conjunctival sac on one occasion.

**Study no:** LTOX0403

**Volume #, and page #:** NA

**Conducting laboratory and location:** Leo Pharmaceutical Products, Ballerup, Denmark

**Date of study initiation:** 30-MAR-2004

**GLP compliance:** Yes

**QA report:** yes ( ) no (X)

**Drug, lot #, radiolabel, and % purity:** Taclonex gel, batch No. 033586101, presumed 100%.

**Formulation/vehicle:** See above.

**Methods:**

**Dosing:**

Species/strain: Rabbits/New Zealand White

#/sex/group or time point: 5 males. No females were studied.

Satellite groups used for toxicokinetics or recovery: No

Age: Not stated

Weight: 2.0 kg to 2.5 kg initially

Doses in administered units: Two drops (approximately 100 mg total) of gel were placed in the right eye (conjunctival sac) of each animal on a single occasion.

The left eyes remained untreated.

Route, form, volume, and infusion rate: See above

**Observations and times:** The animals were examined daily for three days. Ocular irritation was assessed pre-treatment and at 1, 6, 24, 48, and 72 hours post-treatment. Ophthalmoscopy was performed prior to treatment and at termination.

**Results:** No remarkable effects were observed, including no ocular irritation.

**2.6.6.7.6 Study title:** Daivobet gel: A 4-week dermal tolerability study in rabbits

**Key study findings:** Local effects included very slight to well-defined erythema; this was caused by vehicle as well as by the drug product, although it tended to be more severe and appear more quickly at sites treated with product than at vehicle treated sites. Initial body weight loss (reversed when food availability was increased) and decreased skin-fold thickness indicate loss of body fat, presumably due to systemic effects of

betamethasone and/or calcipotriene. Overall, these data suggest Dovobet gel was reasonably well tolerated under the conditions of this study.

**Study no:** LTOX0401

**Volume #, and page #:** NA

**Conducting laboratory and location:** Leo Pharmaceutical Products, Ballerup, Denmark

**Date of study initiation:** 12-FEB-2004

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Taclonex gel, batch No. 033586101, presumed 100%. Placebo (vehicle), batch No. 040591601.

**Formulation/vehicle:** See above.

**Methods:**

**Dosing:**

Species/strain: Rabbits/New Zealand White

#/sex/group or time point (main study): 6 males were used, each of which received both active and placebo ointment. No females were studied.

Satellite groups used for toxicokinetics or recovery: No

Age: Not stated

Weight: 2.2 kg to 2.5 kg initially

Doses in administered units: Three areas of shaved skin on the back of each animal, each area approximately 3 cm x 4 cm, were selected. 0.1 g of drug product was applied once daily to one site, while 0.1 g of vehicle was applied to a second site; these materials were gently spread on the skin using a latex glove.

The third site remained untreated, but was massaged with a latex glove. No dressing was applied. The animals were placed in restraining boxes for four to six hours following treatment to prevent access to the application sites. At the end of the four to six hour treatment period the sites were wiped with gauze to remove any remaining material. Treatment continued daily for four weeks. Each animal received approximately 2 µg/kg/day calcipotriene and 20 µg/kg/day betamethasone base (based on BW of 2.5kg).

Route, form, volume, and infusion rate: See above.

**Observations and times:**

Clinical signs: Yes, daily

Body weights: Yes, weekly

Food consumption: Yes

Ophthalmoscopy: No

EKG: No

Hematology: No

Clinical chemistry: No

Urinalysis: No

Gross pathology: Yes

Organs weighed: Yes (adrenals only)



Histopathology: Yes (limited to skin from the selected areas)

Toxicokinetics: No

Other: Skin erythema and thickness

**Results:** Note: The adrenals were weighed because it was noticed that some animals lost weight during the first week of treatment, and it was suspected that the betamethasone might be inducing systemic effects. The study did not include a true control group (all animals received active ointment), so animals from the eye-irritation study, reviewed above, were used as controls with respect to adrenal weight. It was assumed that the single, ocular exposure to Dovobet gel in that study would not affect adrenal weight, and I agree that this was a reasonable assumption.

Mortality: No unscheduled deaths

Clinical signs: Very-slight to well-defined erythema was observed in nearly all animals at application sites for both drug product and vehicle; however, the drug product tended to induce slightly more severe erythema than did vehicle. The drug product induced erythema within 2 days, while vehicle did not induce erythema until day 14. Skin-fold thickness increased during the first week of treatment in both the product and the vehicle application sites, but diminished thereafter, and by the end of the study was slightly below the starting point. The fold thickness at the untreated site also diminished throughout the study (presumably due to fat loss).

Body weights: The mean body weight decreased slightly during the first week of the study. The amount of food offered to the animals was then increased, which offset the weight loss. The weight loss was ascribed to systemic effects of betamethasone.

Food consumption: The animals apparently ate all available food.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: NA

Clinical chemistry: NA

Urinalysis: NA

Organ weights: The mean weight of the adrenals was significantly reduced ( $0.115 \pm 0.017$  g compared to  $0.253 \pm 0.088$  g in treated and control animals respectively. Note: The animals from which the "control" adrenals were obtained weighed approximately 20% more than did the "test" animals, and this may be responsible for some of the difference in mean adrenal weight).

Gross pathology: The adrenals appeared smaller than usual.

Histopathology: Slight to moderate acanthosis/hyperkeratosis, minimal infiltration of the dermis by inflammatory cells, and slight follicular epithelial hyperplasia were observed at the application sites for both product and vehicle, but not at the sham operated site.

Other: NA

Toxicokinetics: NA

#### 2.6.6.8 Special toxicology studies

Special toxicology studies with calcipotriene alone (discussed in the Original Summary of NDA 20-273):

**2.6.6.8.1 Study title:** MC 903 guinea pig maximization test for allergenic potential, Study No. 861111I9. Please see Original Summary of NDA 20-273.

Studies with betamethasone alone: None.

Studies with calcipotriene/betamethasone ointment: None.

Studies with multiple formulations:

**2.6.6.8.2 Study title:** Nonclinical photosafety testing to characterize the potential of topically administered calcipotriene solution and calcipotriene/betamethasone gel formulations for four weeks to modify photobiological responses in - .skh1-*hr* hairless mice **b(4)**

**Key study findings:** This study attempted to compare the effects of topically applied calcipotriene solution to the effects of topical calcipotriene/betamethasone gel, with respect to effects upon UVR-induced skin damage. Hairless mice were treated topically with the assigned material for 28 days, followed by a single exposure to UVR. The study assessed such parameters as the gross appearance of the treated skin, the thickness of a skinfold, and the histopathology of the skin. Relative abundance or activity of cell turnover, thymidine dimer formation, p53 activity, myeloperoxidase activity, and apoptotic cells were used in an attempt to assess relative levels of UVR-induced DNA damage. No clear trends were apparent in the parameters that were used to assess DNA damage or cell proliferation.

Note: Data from the 12-month photocarcinogenesis study that was conducted with calcipotriene solution, which indicated that topical application of calcipotriene preparations enhances UVR-induced carcinogenesis, should be regarded as being more definitive than the data obtained in study CTOX0502. **b(4)**

**Study no:** CTOX0502

**Volume #, and page #:** NA

**Conducting laboratory and location**

**Date of study initiation:** 13-JUL-2006

**GLP compliance:** Yes

**QA report:** yes ( ) no (X) **b(4)**

**Drug, lot #, radiolabel, and % purity:** Calcipotriene solution, lot Nos. 06 194 113, 05 365 150, and 05 365 151. Calcipotriene/betamethasone dipropionate gel, lot Nos. 06 194 112, 05 352 153, and 05 352 152.

**Formulation/vehicle:** The vehicles of the materials used are not clearly identified, but it is presumed that the vehicles of Dovonex solution and Taclonex gel were used.

**Methods:**

**Dosing:**

Species/strain: Mouse/Albino hairless → SKH1-hr

#/sex/group or time point: 24 females per group. No males were studied.

Satellite groups used for toxicokinetics or recovery: No

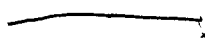
Age: 7 weeks

Weight: Approx. 25 g at initiation of treatment.

The design of the study is summarized below:

**Experimental Design Table**

| Group | Formulation (Concentration)                          | Formulation Administration Volume (mcL/mouse/day) | Number of Mice Per Group <sup>c</sup> | UVR Exposure MEDi <sup>a</sup> | Assigned Mouse Numbers                   |
|-------|------------------------------------------------------|---------------------------------------------------|---------------------------------------|--------------------------------|------------------------------------------|
| 1     | Untreated                                            | NA <sup>b</sup>                                   | 24                                    | 0                              | 1001-1024                                |
| 2     | Untreated                                            | NA <sup>b</sup>                                   | 24                                    | 1                              | 1025-1048                                |
| 3     | Untreated                                            | NA <sup>b</sup>                                   | 24                                    | 2                              | 1049-1051, 7080 <sup>d</sup> , 1053-1072 |
| 4     | Calcipotriene Solution Vehicle (0) mcg/mL            | 100                                               | 24                                    | 1                              | 1073-1096                                |
| 5     | Calcipotriene Solution (1 mcg/mL)                    | 100                                               | 24                                    | 1                              | 1097-1120 <sup>e</sup>                   |
| 6     | Calcipotriene Solution (3 mcg/mL)                    | 100                                               | 24                                    | 1                              | 1121-1144                                |
| 7     | Calcipotriene Solution (10 mcg/mL)                   | 100                                               | 24                                    | 1                              | 1145-1168                                |
| 8     | Calcipotriene/Betamethasone Gel Vehicle (0/0 mcg/mL) | 100                                               | 24                                    | 1                              | 1169-1192                                |
| 9     | Calcipotriene/Betamethasone Gel (1/10 mcg/g)         | 100                                               | 24                                    | 1                              | 1193-1216 <sup>f</sup>                   |
| 10    | Calcipotriene/Betamethasone Gel (3/30 mcg/g)         | 100                                               | 24                                    | 1                              | 1217-1240 <sup>f</sup>                   |
| 11    | Calcipotriene/Betamethasone Gel (10/100 mcg/g)       | 100                                               | 24                                    | 1                              | 1241-1264                                |
| 12    | Triamcinolone (5000 mcg/g)                           | 100                                               | 24                                    | 1                              | 1265-1288                                |

Groups 1-3 received no treatment (except exposure to UVR for groups 2 and 3, as described below). The other groups received application of either calcipotriene solution, calcipotriene/betamethasone gel, or triamcinolone in a gel vehicle, 100 µL/mouse/day for 28 consecutive days. The UVR source was a 6.5 kilowatt xenon long arc, water cooled lamp horizontally suspended within a metal frame holding one optical filter (15 cm by 15 cm, 1 mm thick:  glass). During exposure, the mice were located approximately 1.2 meters from the UVR source.

UVR exposure was monitored by a customized detector that recorded both intensity and UVR dose in Robertson-Berger Units (RBU). Mice in group 1 were not exposed to UVR. Mice in groups 2 and 4 through 12 were exposed to an UVR dose of approximately 1 instrumental minimal erythema dose for free-ranging mice (MED<sub>if</sub>, 800 RBU) over a period of 2 hours  $\pm$  10 minutes. Mice in group 3 were exposed to an UVR dose of approximately 2 MED<sub>if</sub> (1600 RBU) over a period of 4 hours  $\pm$  15 minutes. 12 mice per group were used for evaluation for DNA strand breaks, thymine dimer formation, myeloperoxidase alterations, inflammatory cell infiltration, epidermal thickness, epidermal cellularity, and other histomorphologic changes. At 1 hour after UVR exposure, these mice were sacrificed and the dorsal skin was removed and fixed. 12 mice in group 1 were similarly processed. 12 mice per group were assigned to evaluate sun burn cell formation, DNA synthesis, p53 alterations, inflammatory cell infiltration, and dermal histomorphologic changes, and at 6 hours after UVR exposure these mice were injected with bromodeoxyuridine (BrdU, 200 mg/kg) via the intraperitoneal route (BrdU is a thymidine analog that, through use of immunohistochemistry, can be used to identify cells that were synthesizing DNA immediately prior to sacrifice of the animal). Two hours post-injection these mice were sacrificed and the dorsal skin was removed and fixed. Sections were stained for presence of BrdU, thymidine dimers, p53, and myeloperoxidase (markers of DNA damage and cellular damage), and were subjected to a TUNEL assay (terminal dUTP nick-end labeling) to identify apoptotic cells.

### Results:

Skin reactions: No remarkable observations in control groups (groups 1-3). In groups treated with calcipotriene solution (groups 4-7) the incidence of "wrinkling" increased with concentration of calcipotriene, while increased thickness of the skin was observed in group 7 only:

**Text Table 1. Skin Reactions: Calcipotriene Solutions**

| Group | Concentration (mcg/mL) | Wrinkling <sup>a</sup> | Thickening <sup>a</sup> |
|-------|------------------------|------------------------|-------------------------|
| 1     | Untreated              | 0/24                   | 0/24                    |
| 4     | 0 (Vehicle)            | 4/24                   | 0/24                    |
| 5     | 1                      | 8/24                   | 0/24                    |
| 6     | 3                      | 13/24**                | 0/24                    |
| 7     | 10                     | 24/24***               | 10/24***                |

a. Number of affected mice/total number of mice in the group

\*\* Significantly different from the Groups 1 value ( $p \leq 0.01$ ). analysis restricted to Groups 1 through 12.

\*\*\* Significantly different from the Group 4 value ( $p \leq 0.01$ ). analysis restricted to Groups 4 through 7.

Skin reactions observed in mice treated with calcipotriene/betamethasone gel are summarized below:

**Text Table 2. Skin Reactions: Calcipotriene/Betamethasone Gel**

| Group | Concentration (mcg/g) | Erythema Grade 1 <sup>a</sup> | Edema Grade 1 <sup>a</sup> | Flaking Grade 1 <sup>a</sup> | Flaking Grade 2 <sup>a</sup> | Wrinkling <sup>a</sup> | Thinning <sup>a</sup> |
|-------|-----------------------|-------------------------------|----------------------------|------------------------------|------------------------------|------------------------|-----------------------|
| 1     | Untreated             | 0/24                          | 0/24                       | 0/24                         | 0/24                         | 0/24                   | 0/24                  |
| 8     | 0 (Vehicle)           | 0/24                          | 0/24                       | 12/24**                      | 0/24                         | 21/24**                | 0/24                  |
| 9     | 1/10                  | 0/24                          | 0/24                       | 11/24**                      | 0/24                         | 21/24**                | 0/24                  |
| 10    | 3/30                  | 0/24                          | 0/24                       | 11/24**                      | 0/24                         | 23/24**                | 24/24**&&             |
| 11    | 10/100                | 2/24**                        | 2/24**                     | 23/24**&&                    | 3/24**&&                     | 23/24**                | 24/24**&&             |

a. Number of affected mice/total number of mice in the group

\*\* Significantly different from the Groups 1 value ( $p \leq 0.01$ ), analysis restricted to Groups 1 through 12.

&& Significantly different from Group 8 value ( $p \leq 0.01$ ), analysis restricted to Groups 8 through 11.

Skinfold thickness on day 28: No meaningful differences were observed in thickness of the skinfold between groups treated with calcipotriene solution (groups 4-7), but this parameter tended to reduce somewhat with increasing exposure to corticosteroids (groups 8-12):

| Group | Skin Thickness (mm) |
|-------|---------------------|
| 1     | 0.63±0.04           |
| 2     | 0.64±0.05           |
| 3     | 0.67±0.06           |
| 4     | 0.61±0.05           |
| 5     | 0.64±0.06           |
| 6     | 0.62±0.04           |
| 7     | 0.68±0.05           |
| 8     | 0.69±0.06           |
| 9     | 0.56±0.05           |
| 10    | 0.47±0.04           |
| 11    | 0.43±0.03           |
| 12    | 0.46±0.04           |

Histological indicators of photo-induced skin damage: As expected, increased exposure to UVR induced increased signs of DNA damage (comparing groups 1-3, see tables 1 and 2, below). Data concerning the DNA-damage parameters that were assessed in skin samples from mice treated with calcipotriene or calcipotriene/betamethasone were difficult to interpret, as often a dose-response relationship was lacking. However, when compared to their respective vehicle control groups, some general trends may have been apparent. Calcipotriene solution slightly increased epidermal hyperplasia, while calcipotriene/betamethasone gel slightly reduced epidermal hyperplasia. Skin treated with calcipotriene solution appeared to exhibit slightly increased p53 expression, while skin from mice treated with calcipotriene/betamethasone gel exhibited slightly reduced p53 expression. Calcipotriene solution appeared to have little effect on UVR-induced thymine dimer formation, while calcipotriene/betamethasone gel seemed to slightly reduce dimer formation.

Both preparations seemed to slightly reduce the formation of "sunburn cells", and both appeared to slightly increase cell proliferation, as indicated by BrdU labeling. It is unclear whether these differences were genuine, as some type 1 errors (falsely positive comparisons) may have resulted from the multiple statistical comparisons.

Table 1  
Group Summary Biomarker Data – 1<sup>st</sup> Set of Mice – Sacrifice 1 Hour Post UVR

| Group | Formulation (Concentration)                    | Formulation Administration Volume (mL/mouse/day) | UVR Exposure MED <sup>1</sup> | Epidermal Cellularity <sup>2</sup> |     | Epidermal Thickness <sup>3</sup> |     | TUNEL Labeling Index <sup>4</sup> |      | Thymine Dimer Labeling Index <sup>5</sup> |      | Myeloperoxidase Labeling Index <sup>6</sup> |      |
|-------|------------------------------------------------|--------------------------------------------------|-------------------------------|------------------------------------|-----|----------------------------------|-----|-----------------------------------|------|-------------------------------------------|------|---------------------------------------------|------|
|       |                                                |                                                  |                               | Mean                               | SD  | Mean                             | SD  | Mean                              | SD   | Mean                                      | SD   | Mean                                        | SD   |
| 1     | Untreated                                      | NA <sup>7</sup>                                  | 0                             | 18.7                               | 1.7 | 18.7                             | 3.8 | 0.04                              | 0.07 | 0.0                                       | 0.0  | 0.00                                        | 0.00 |
| 2     | Untreated                                      | NA <sup>7</sup>                                  | 1                             | 18.6                               | 1.9 | 22.3 <sup>1</sup>                | 2.9 | 0.37                              | 0.62 | 177.1 <sup>1</sup>                        | 12.1 | 0.00                                        | 0.00 |
| 3     | Untreated                                      | NA <sup>7</sup>                                  | 2                             | 18.7                               | 1.4 | 23.5 <sup>1</sup>                | 3.0 | 0.51 <sup>1</sup>                 | 0.50 | 189.7 <sup>1</sup>                        | 20.3 | 0.00                                        | 0.00 |
| 4     | Calcipotriene Solution Vehicle (0)             | 100                                              | 1                             | 18.6                               | 1.5 | 21.3                             | 2.4 | 0.22                              | 0.38 | 173.7                                     | 18.0 | 0.00                                        | 0.00 |
| 5     | Calcipotriene Solution (1 mcg/mL)              | 100                                              | 1                             | 20.9 <sup>2</sup>                  | 1.4 | 23.5                             | 3.2 | 1.27                              | 2.26 | 165.7                                     | 19.9 | 0.00                                        | 0.00 |
| 6     | Calcipotriene Solution (3 mcg/mL)              | 100                                              | 1                             | 22.6 <sup>2</sup>                  | 2.8 | 24.4 <sup>2</sup>                | 3.7 | 0.36                              | 0.40 | 165.3                                     | 17.3 | 0.00                                        | 0.00 |
| 7     | Calcipotriene Solution (15 mcg/mL)             | 100 <sup>1</sup>                                 | 1                             | 24.1 <sup>2</sup>                  | 2.5 | 30.6 <sup>2</sup>                | 4.2 | 0.77 <sup>2</sup>                 | 0.76 | 165.3                                     | 24.9 | 0.00                                        | 0.00 |
| 8     | Calcipotriene/Betamethasone Gel Vehicle (0/0)  | 100                                              | 1                             | 21.0 <sup>2</sup>                  | 3.3 | 29.2 <sup>2</sup>                | 5.3 | 0.30                              | 0.32 | 172.7                                     | 10.1 | 0.00                                        | 0.00 |
| 9     | Calcipotriene/Betamethasone Gel (1/10 mcg/g)   | 100                                              | 1                             | 20.5                               | 1.6 | 27.0                             | 2.5 | 0.71                              | 1.99 | 161.0                                     | 14.5 | 0.00                                        | 0.00 |
| 10    | Calcipotriene/Betamethasone Gel (3/30 mcg/g)   | 100                                              | 1                             | 18.2 <sup>3</sup>                  | 2.2 | 21.6 <sup>3</sup>                | 2.1 | 0.20                              | 0.30 | 148.0 <sup>3</sup>                        | 11.1 | 0.00                                        | 0.00 |
| 11    | Calcipotriene/Betamethasone Gel (10/100 mcg/g) | 100                                              | 1                             | 17.0 <sup>4</sup>                  | 1.9 | 25.4                             | 3.1 | 1.99                              | 3.33 | 160.4                                     | 20.1 | 0.00                                        | 0.00 |
| 12    | Triamcinolone (5000 mcg/g)                     | 100                                              | 1                             | 17.0 <sup>4</sup>                  | 1.4 | 21.5 <sup>3</sup>                | 3.8 | 1.93                              | 6.26 | 139.6 <sup>5</sup>                        | 12.2 | 0.00                                        | 0.00 |

1. Instrumental MED for free-ranging mice (MED<sup>1</sup>): MED<sup>1</sup> refers to a UVR dose adequate to elicit a barely perceptible response in skin based on an instrument used to measure UVR. A MED<sup>1</sup> is approximately equivalent to 2 standard erythema doses (2 SED).
2. Not applicable

<sup>3</sup>Group mean  $\pm$  standard deviation (SD) for nucleated epidermal cells per 100  $\mu$ m segment of basement membrane; see Table legend for details

<sup>4</sup>Group mean  $\pm$  standard deviation (SD) for epidermal thickness (um); see Table legend for details

<sup>5</sup>Group mean  $\pm$  standard deviation (SD) for TUNEL-positive epidermal cells per mm of basement membrane; see Table legend for details

<sup>6</sup>Group mean  $\pm$  standard deviation (SD) for thymine dimer positive epidermal cells per mm of basement membrane; see Table legend for details

<sup>7</sup>Group mean  $\pm$  standard deviation (SD) for epidermal cells expressing myeloperoxidase per mm of basement membrane; see Table legend for details

<sup>1</sup>Statistically significant compared to Group 1 Untreated Control;  $P \leq 0.05$

<sup>2</sup>Statistically significant compared to Group 2 Untreated UVR 1 MED<sup>1</sup>;  $P \leq 0.05$

<sup>3</sup>Statistically significant compared to Group 4 Calcipotriene Solution Vehicle;  $P \leq 0.05$

<sup>4</sup>Statistically significant compared to Group 9 Calcipotriene/Betamethasone Gel Vehicle;  $P \leq 0.05$

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Table 2  
Group Summary Biomarker Data – 2<sup>nd</sup> Set of Mice – Sacrifice 8 Hours Post UVR

| Group | Formulation (Concentration)                    | Formulation Administration Volume (mL/mouse/day) | UVR Exposure MED <sup>1</sup> | Epidermal Cellularity <sup>2</sup> |     | Epidermal Thickness <sup>3</sup> |     | Sunburn Cell Index <sup>4</sup> |     | BrdU Labeling Index <sup>5</sup> |     | p53 Labeling Index <sup>6</sup> |      |
|-------|------------------------------------------------|--------------------------------------------------|-------------------------------|------------------------------------|-----|----------------------------------|-----|---------------------------------|-----|----------------------------------|-----|---------------------------------|------|
|       |                                                |                                                  |                               | Mean                               | SD  | Mean                             | SD  | Mean                            | SD  | Mean                             | SD  | Mean                            | SD   |
| 1     | Untreated                                      | NA <sup>7</sup>                                  | 0                             | 20.3                               | 1.5 | 22.7                             | 8.0 | 0.0                             | 0.0 | 10.3                             | 3.3 | 0.0                             | 0.0  |
| 2     | Untreated                                      | NA <sup>7</sup>                                  | 1                             | 18.8 <sup>a</sup>                  | 1.0 | 21.7                             | 2.8 | 3.9 <sup>a</sup>                | 1.2 | 9.2 <sup>a</sup>                 | 0.4 | 99.0 <sup>a</sup>               | 17.9 |
| 3     | Untreated                                      | NA <sup>7</sup>                                  | 2                             | 17.4 <sup>a</sup>                  | 1.3 | 21.1                             | 2.0 | 2.8 <sup>a</sup>                | 1.1 | 6.0 <sup>a</sup>                 | 0.0 | 86.5 <sup>a</sup>               | 12.9 |
| 4     | Calcipotriene Solution Vehicle (0)             | 100                                              | 1                             | 19.1                               | 1.2 | 22.1                             | 2.8 | 2.8 <sup>a</sup>                | 0.8 | 0.1                              | 0.1 | 99.0                            | 15.1 |
| 5     | Calcipotriene Solution (1 mcg/mL)              | 100                                              | 1                             | 21.5 <sup>a</sup>                  | 1.7 | 23.0                             | 2.2 | 2.8                             | 0.9 | 0.1                              | 0.1 | 107.7 <sup>a</sup>              | 16.8 |
| 6     | Calcipotriene Solution (3 mcg/mL)              | 100                                              | 1                             | 23.0 <sup>a</sup>                  | 1.8 | 26.5 <sup>a</sup>                | 2.9 | 2.8                             | 1.0 | 0.8 <sup>a</sup>                 | 0.6 | 122.6 <sup>a</sup>              | 12.4 |
| 7     | Calcipotriene Solution (10 mcg/mL)             | 100                                              | 1                             | 28.5 <sup>a</sup>                  | 2.3 | 30.1 <sup>a</sup>                | 2.6 | 1.6 <sup>a</sup>                | 0.6 | 1.8 <sup>a</sup>                 | 1.1 | 126.7 <sup>a</sup>              | 21.3 |
| 8     | Calcipotriene/Betamethasone Gel Vehicle (0/0)  | 100                                              | 1                             | 23.0 <sup>a</sup>                  | 1.6 | 33.1 <sup>a</sup>                | 0.1 | 4.7                             | 3.1 | 0.1                              | 0.2 | 90.0                            | 18.0 |
| 9     | Calcipotriene/Betamethasone Gel (1/10 mcg/g)   | 100                                              | 1                             | 19.9 <sup>a</sup>                  | 1.2 | 25.4 <sup>a</sup>                | 2.8 | 3.3                             | 1.1 | 0.7 <sup>a</sup>                 | 0.8 | 86.0                            | 15.1 |
| 10    | Calcipotriene/Betamethasone Gel (3/30 mcg/g)   | 100                                              | 1                             | 17.9 <sup>b</sup>                  | 1.4 | 23.3 <sup>b</sup>                | 2.5 | 2.2 <sup>a</sup>                | 1.2 | 1.1 <sup>a</sup>                 | 0.8 | 76.3 <sup>a</sup>               | 22.7 |
| 11    | Calcipotriene/Betamethasone Gel (10/100 mcg/g) | 100                                              | 1                             | 19.5 <sup>a</sup>                  | 2.1 | 24.8 <sup>a</sup>                | 3.5 | 2.5 <sup>b</sup>                | 0.8 | 4.5 <sup>b</sup>                 | 4.1 | 80.3 <sup>b</sup>               | 9.5  |
| 12    | Triamcinolone (5000 mcg/g)                     | 100                                              | 1                             | 18.2 <sup>a</sup>                  | 1.3 | 21.2 <sup>a</sup>                | 2.4 | 3.3                             | 1.2 | 1.0 <sup>b</sup>                 | 1.0 | 97.7 <sup>b</sup>               | 14.8 |

1. Instrumental MED (MED<sub>i</sub>): MED<sub>i</sub> refers to a UVR dose adequate to elicit a barely perceptible response in skin based on an instrument used to measure UVR. A MED<sub>i</sub> is approximately equivalent to 2 standard erythema doses (2 SED).

2. Not applicable

<sup>a</sup>Group mean ± standard deviation (SD) for nucleated epidermal cells per 100 µm segment of basement membrane; see Table legend for details

<sup>b</sup>Group mean ± standard deviation (SD) for epidermal thickness (µm); see Table legend for details

<sup>c</sup>Group mean ± standard deviation (SD) for sunburn cells per mm of basement membrane; see Table legend for details

<sup>d</sup>Group mean ± standard deviation (SD) for BrdU positive epidermal cells per mm of basement membrane; see Table legend for details

<sup>e</sup>Group mean ± standard deviation (SD) for epidermal cells expressing p53 per mm of basement membrane; see Table legend for details

<sup>f</sup>Statistically significant compared to Group 1 Untreated Control; P ≤ 0.05

<sup>g</sup>Statistically significant compared to Group 2 Untreated UVR 1 MED<sub>i</sub>; P ≤ 0.05

<sup>h</sup>Statistically significant compared to Group 4 Calcipotriene Solution Vehicle; P ≤ 0.05

<sup>i</sup>Statistically significant compared to Group 8 Calcipotriene/Betamethasone Gel Vehicle; P ≤ 0.05

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## 2.6.6.9 Discussion and Conclusions

The product contains both calcipotriene and betamethasone dipropionate. The safety database submitted in support of NDA 22-185 includes nonclinical studies conducted with both the individual active ingredients as well as studies conducted with test materials that contained both active ingredients. The primary sign of toxicity observed in studies that involved application of calcipotriene was perturbation of calcium homeostasis. Calcipotriene is an analog of vitamin D, and, at sufficient levels of systemic exposure, induces elevations in the levels of calcium in the plasma and urine. If the exposure is of sufficient magnitude and chronicity, the elevated plasma calcium levels can result in mineralization of tissues throughout the body. In a three-month topical study in which material that contained calcipotriene (but not betamethasone) was applied to mice at exposures ranging from 0 to 180 µg/kg/day, toxicity was observed at dosages of 12 µg/kg/day and above; 3 µg/kg/day was a NOAEL. The most notable effects included significantly 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 calcipotriene 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 Taclonex 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. Treatment-related findings included slightly reduced mean adrenal weight, minimal to moderate adrenal atrophy, and thinning of the skin. All of those effects were probably secondary to exposure to betamethasone dipropionate. As a glucocorticoid, betamethasone dipropionate is capable of causing reversible adrenal atrophy through negative feedback of the HPA axis. Even with substantial oral doses of betamethasone dipropionate, however, serious toxicity was not observed in rats that were orally dosed for 13 weeks. In that oral rat study, in which rats received up to 0.2 mg/kg/day betamethasone dipropionate, there were no effects on survival, clinical signs, clinical chemistry, or urinalysis, and there was no clear effect on mean body weight, although a trend toward reduced mean body weight with increasing dosage seemed apparent. The mean WBC count decreased in proportion to dosage, as did the mean weights of the spleen and thymus. These are known effects of corticosteroids when systemically administered at sufficient levels. Treatment-related histopathological findings in the oral rat study were limited to the spleen (lymphoid depletion), thymus (cortical atrophy), and lymph nodes (lymphoid depletion or hyperplasia) of high-dose animals of both genders. In all, little toxicity was observed in rats that were orally dosed with betamethasone dipropionate for 13 weeks. Although all plasma samples that were analyzed in that study were below the limit of quantitation for betamethasone dipropionate (75 pg/mL), substantial exposure to the metabolite, betamethasone 17-propionate, was documented.

Calcipotriene was considered negative in the Ames mutagenicity assay, the mouse lymphoma TK locus assay, the human lymphocyte chromosome aberration test, and the mouse micronucleus test.

Betamethasone dipropionate was negative in the Ames assay and in the mouse lymphoma TK locus assay with and without metabolic activation, and in an in vivo micronucleus assay.

Calcipotriene was evaluated for activity as a cocarcinogen with UV light in a 12-month study with hairless mice. 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 males that received the greatest exposure to calcipotriene (30 µg/kg/day), while vehicle alone had no effect, suggesting that calcipotriene may enhance the carcinogenic effects of UV light. Calcipotriene was evaluated for activity as a carcinogen in a study in which mice were treated topically for 24 months at dosages of 3, 10 and 30 mcg/kg/day (corresponding to 9, 30 and 90 mcg/m<sup>2</sup>/day). No biologically significant changes in tumor incidence were observed when compared to control. Evaluation of calcipotriene in a standard oral carcinogenicity assay in rats will be accomplished as a post-approval commitment.

The evaluation of betamethasone dipropionate in carcinogenicity assays will be accomplished as a post-approval commitment.

Calcipotriene was evaluated for effects upon reproductive function:



In a study for effects on fertility and reproductive success, in which F0 males were treated for 63 days prior to and throughout pairing with F0 females, and the females were treated beginning 14 days prior to pairing with males and continuing until day 28 post-partum, at exposures of 0, 6, 18, and 54 µg/kg/day, calcipotriene did not induce major malformations or affect the reproductive performance of either males or females. Body weight gain was significantly reduced in high-dose F0 animals. Increased incidence of delayed ossification of the skull, ribs, and hyoid bones was observed in all treatment groups, but the magnitude of the increase did not appear to increase in proportion to dosage. No effects on the incidence of minor visceral anomalies or effects on development or behavior of F1 or F2 animals were noted.

In an assessment of the effects of calcipotriene on embryofetal development, pregnant Wistar rats were dosed daily with calcipotriene at exposures of 0, 6, 18, or 54 µg/kg/day on days 6-15 of gestation. There were no remarkable effects on survival, behavior, body weight, litter parameters, or the incidence of major malformations. A slight trend toward an increase in the incidence of minor skeletal variations, including "coma" shaped extra ribs, was apparent, but this is unlikely to be relevant to the levels of systemic exposure to calcipotriene that would result from use of the Dovobet ointment. In a similar study conducted in rabbits, pregnant New Zealand rabbits were dosed daily with calcipotriene at exposures of 0, 4, 12, or 36 µg/kg/day on days 6-18 of gestation. Mortality was increased in the high-dose group (7 F0 females died or were killed following abortion, compared to 0 in the control group and 2 unscheduled deaths in both the low and mid groups). Body weight gain was reduced in mid and high-dose animals. The post-implantation loss was increased in the high-dose group, while the mean fetal weight was reduced. There were no remarkable effects on the incidence of major malformations. An increase in the incidence of minor skeletal variations, including incomplete ossification of sternbrae, pubic bones, and fore limb phalanges was observed in the high-dose group.

Calcipotriene was assessed for effects on peri-natal or post-natal development. Pregnant Wistar rats were dosed daily with calcipotriene at exposures of 0, 6, 18, or 54 µg/kg/day from day 15 of gestation through day 20 post-partum. There were no remarkable effects on any parameter, including survival, behavior, body weight, litter parameters, or the ability to nurse or rear pups.

Betamethasone dipropionate was evaluated in a battery of reproductive toxicology studies. No effect on reproductive performance or fertility was observed when betamethasone dipropionate was orally administered to male rats at dosages up to 0.2 mg/kg/day, or in females orally dosed at up to 1.0 mg/kg/day. When administered subcutaneously to pregnant mice on days 7 through 13 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, increased incidence of cleft palate and crooked or short tail, and delayed ossification. A NOAEL was not observed in this study, as fetal toxicity was observed at the lowest exposure that was evaluated (0.156 mg/kg/day). When administered subcutaneously to pregnant rabbits on days 6 through 18 of gestation, betamethasone dipropionate induced fetal toxicity, including fatality, reduced fetal body weight, external malformations, and

skeletal malformations. An exposure of 0.625 µg/kg/day was a NOAEL in this study; fetal toxicity was observed at 2.5 µg/kg/day and above. Betamethasone dipropionate 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 1.0 mg/kg/day. Mean maternal BW was significantly lower at 0.3 and 1.0 mg/kg/day on day 20 of gestation. The mean duration of gestation was slightly but statistically increased at 0.1, 0.3, and 1.0 mg/kg/day. The mean percentage of pups that survived to day 4 was reduced in F1 pups in relation to dosage, although the effects at 0.1 and 0.3 mg/kg/day were minimal. The percentage of pups with a righting-reflex on day 5 of lactation was significantly reduced at 1.0 mg/kg/day. No effects were observed on pup learning ability or reproduction of F1 animals.

Taclonex scalp gel was essentially non-irritating to the skin or eyes.

The only excipient in the product that was of potential toxicological concern is polyoxypropylene-15-stearyl ether (which is a synonym for polypropylene glycol-15-stearyl ether; PPG-15 SE). This excipient was included in the formulations of Taclonex ointment and gel that have been evaluated in a battery of nonclinical and clinical studies, and was therefore evaluated for safety in those studies. PPG-15 SE was evaluated in 13-week studies in mice and rats via the topical dermal and oral routes, respectively. No toxicity was observed in those studies. PPG-15 SE was evaluated in a battery of genetic toxicology studies, including an Ames test, a mouse lymphoma assay, and a micronucleus study. PPG-15 SE is not genotoxic. PPG-15 SE is an excipient in several topical products that are approved for chronic use. The proposed use of this excipient is acceptable.

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

#### **2.6.6.10 Tables and Figures**

Not applicable.

#### **2.6.7 TOXICOLOGY TABULATED SUMMARY**

Not applicable.

#### **OVERALL CONCLUSIONS AND RECOMMENDATIONS**

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

Unresolved toxicology issues (if any): The sponsor has committed to conduct the following nonclinical study post-approval of NDA 22-185:

1. Evaluation of the carcinogenicity of calcipotriene in a two-year oral study in rats.

Note: The sponsor has committed to conduct the following nonclinical studies post-approval of NDA 21-852 (Taclonex ointment), and data from these studies will eventually be used to support labeling of Taclonex gel, as well:

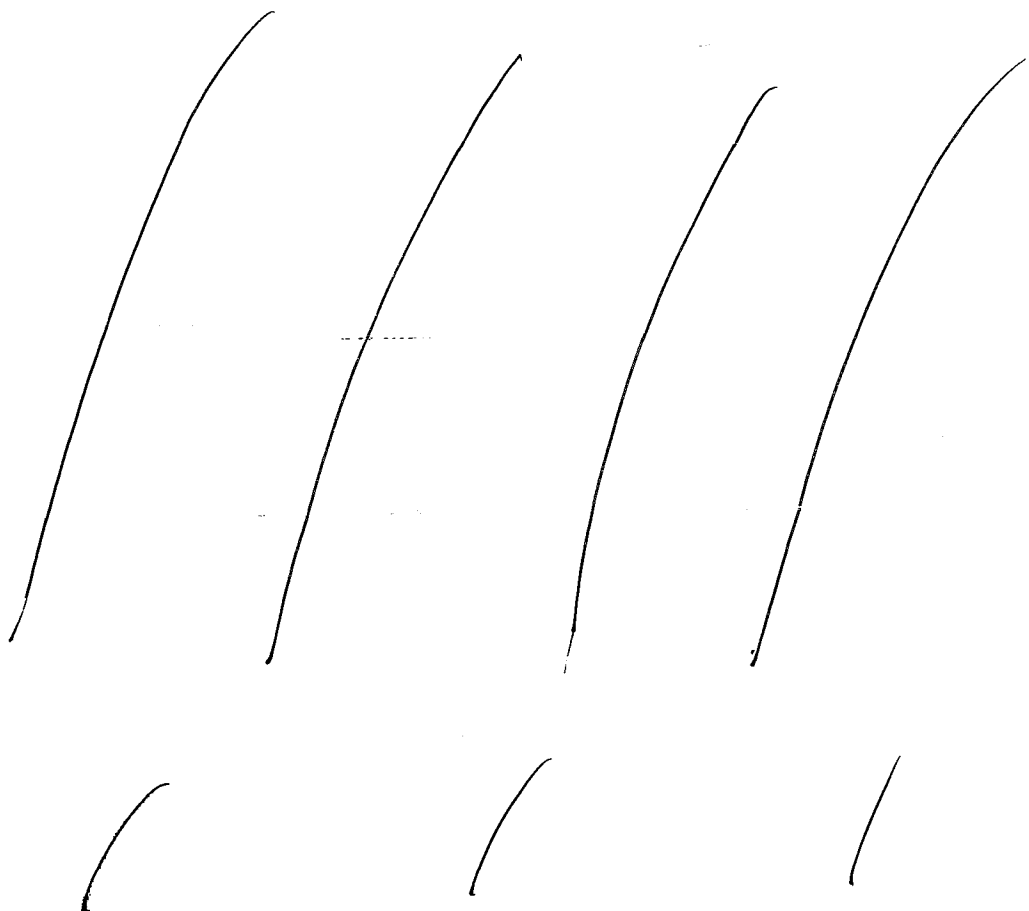
1. Evaluation of the carcinogenicity of betamethasone dipropionate in mice.
2. Evaluation of the carcinogenicity of betamethasone dipropionate in rats.

The sponsor has submitted protocols for these studies, and those protocols were approved by the exec-CAC of CDER (see minutes of exec-CAC meeting dated 20-MAR-2007, NDA 21-852).

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

Suggested labeling:

**b(4)**



1 Page(s) Withheld

☒ Trade Secret / Confidential (b4)

☐ Draft Labeling (b4)

☐ Draft Labeling (b5)

☐ Deliberative Process (b5)

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Reviewer: Norman A. See, Ph.D.

NDA No. 22-185

Supervisor Signature _____ Concurrence Yes ____ No ____

APPENDIX/ATTACHMENTS

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Norman See
2/20/2008 01:22:27 PM
PHARMACOLOGIST

Paul Brown
2/20/2008 04:45:13 PM
PHARMACOLOGIST

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

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

Date: 8/15/07

Reviewer: Norman A. See, Ph.D.

NDA Number: 22-185

Sponsor: Parexel International

Product Name: Taclonex gel

Drug Substance(s): Calcipotriene/betamethasone

Indication: Psoriasis

Route of Administration: Topical

Date CDER Received: 6/19/07

Expected Date of Draft Review (if filed): 2/1/08

(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) Are all necessary nonclinical studies completed and submitted in this NDA?

Yes.

**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
8/15/2007 01:39:17 PM
PHARMACOLOGIST