CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-852

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:	**21-852**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**09-MAR-05**
PRODUCT:	**Dovobet ointment**
INTENDED CLINICAL POPULATION:	**Patients with psoriasis vulgaris**
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.**
DIVISION DIRECTOR (ACTING):	**Stanka Kukich, M.D.**
PROJECT MANAGER:	**Felicia Curtis**

Date of review submission to Division File System (DFS): 1/4/06

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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 studies post-approval of the NDA:

1. Evaluation of the carcinogenicity of calcipotriene (this matter is currently being evaluated by the sponsor as a post-approval commitment to NDA 20-273).

2. Evaluation of the carcinogenicity of betamethasone dipropionate in mice. The sponsor should submit a protocol for this study with appropriate supporting documents for evaluation by the executive carcinogenicity assessment committee of CDER following approval of NDA 21-852.

3. Evaluation of the carcinogenicity of betamethasone dipropionate in rats. The sponsor should submit a protocol for this study with appropriate supporting documents for evaluation by the executive carcinogenicity assessment committee of CDER following approval of NDA 21-852.

4. Evaluation of betamethasone dipropionate for effects upon female fertility, including prenatal and postnatal function.

C. Recommendations on labeling: It is recommended that the "Carcinogenesis" and "Pregnancy" sections of the label be modified to the statements indicated below:

Carcinogenesis, mutagenesis, impairment of fertility:

Long-term animal studies have not been performed to evaluate the carcinogenic potential of Tradename® ointment or any of the active constituents.

In a study in which albino hairless mice were exposed to both ultra-violet radiation (UVR) and topically applied calcipotriene, a reduction in the time required for UVR to induce the formation of skin tumors was observed (statistically significant in males only), suggesting that calcipotriene may enhance the effect of UVR to induce skin tumors. Patients who apply Tradename® ointment to exposed portions of the body should avoid excessive exposure to either natural or artificial sunlight (including tanning booths, sun lamps, etc.). Physicians may wish to limit or avoid use of phototherapy in patients that use Tradename® ointment.

Calcipotriene did not elicit any genotoxic effects in the Ames mutagenicity assay, the mouse lymphoma TK locus assay, the human lymphocyte chromosome aberration test, or the mouse micronucleus test.

Betamethasone dipropionate did not elicit any genotoxic effects in the Ames mutagenicity assay, the mouse lymphoma TK locus assay, or in the rat micronucleus test.

Studies in rats at oral doses of up to 54 mcg/kg/day (324 mcg/m^2/day) of calcipotriene indicated no impairment of fertility or general reproductive performance.

Studies in rats at oral doses of up to 0.2 mg/kg/day (1,200 mcg/m^2/day) of betamethasone dipropionate indicated no impairment of male fertility.

Pregnancy:

Teratogenic Effects: Pregnancy Category C
Animal reproduction studies have not been conducted with Tradename® ointment. Tradename® ointment contains calcipotriene that has been shown to be fetotoxic and betamethasone dipropionate that has been shown to be teratogenic in animals when given systemically. There are no adequate and well-controlled studies in pregnant women. Tradename® ointment should be used during pregnancy only if the potential benefit to the patient justifies the potential risk to the fetus.

Teratogenicity studies with calcipotriene were performed by the oral route in rats and rabbits. In rabbits, increased maternal and fetal toxicity were noted at a dosage of 12 mcg/kg/day (144 mcg/m^2/day); a dosage of 36 mcg/kg/day (432 mcg/m^2/day) resulted in a significant increase in the incidence of incomplete ossification of the pubic bones and forelimb phalanges of fetuses. In a rat study, a dosage of 54 mcg/kg/day (324 mcg/m^2/day) resulted in a significantly increased incidence of skeletal abnormalities (enlarged fontanelles and extra ribs). The enlarged fontanelles are most likely due to calcipotriene's effect upon calcium metabolism. The estimated maternal and fetal no-effect levels in the rat (108 mcg/m^2/day) and rabbit (48 mcg/m^2/day) studies are lower than the estimated maximum topical dose in man (approximately 460 mcg/m^2/ day).
Corticosteroids are generally teratogenic in laboratory animals when administered systemically at relatively low dosage levels. Betamethasone dipropionate has been shown to be teratogenic in mice and rabbits when given by the subcutaneous route at doses of 156 mcg/kg/day (468 mcg/m^2/day) and 2.5 mcg/kg/day (30 mcg/m^2/day) respectively. Those dose levels are lower than the estimated maximum topical dose in man (5,948 mcg/m^2/ day). The abnormalities observed included umbilical hernia, exencephaly and cleft palates.

Pregnant women were excluded from the clinical trials conducted with Tradename® ointment.

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 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.

Calcipotriene was negative in the Ames mutagenicity assay and in the mouse lymphoma TK locus assay with and without metabolic activation, and in the in vivo mouse micronucleus bone marrow assay. Calcipotriene was positive (induced chromosome aberrations) in an in vitro assay in human lymphocytes in the absence of metabolic activation.

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.

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 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.

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 exposures up to 0.2 mg/kg/day. Evaluation of betamethasone dipropionate for effects upon female fertility, including prenatal and postnatal function, will be regarded as being a Phase 4 commitment to NDA 21-852. 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.

Dovonex or Dovobet ointment were essentially non-irritating to the skin or eyes and were nonsensitizing.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

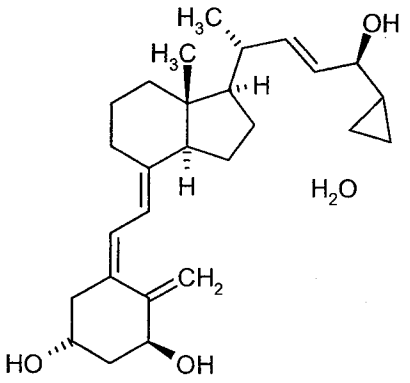
NDA number: 21-852
Review number: 1
Sequence number/date/type of submission: N-000/09-MAR-2005
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: LEO Pharmaceutical Products Ltd. A/S
Manufacturer for drug substance: Leo Pharmaceutical Products, Denmark, (calcipotriene) and [redacted] betamethasone dipropionate)

Reviewer name: Norman A. See, Ph.D.
Division name: Division of Dermatologic and Dental Drug Products
HFD #: 540
Review completion date: 17-OCT-2005

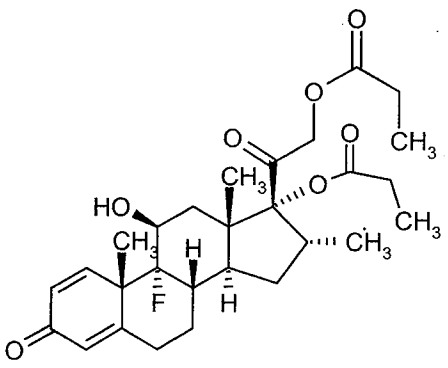
Drug:

Trade name: Dovobet ointment
Generic name: Calcipotriene hydrate and betamethasone dipropionate ointment (Note: calcipotriene is known as calcipotriol in Europe).
Code name: MC 903 (calcipotriene)/433/M (betamethasone)
Chemical name: Calcipotriene: (1α,3β,5Z,7E,22E,24S)-24-cyclo-propyl-9, 10-seco-chola-5,7,10(19),22-tetraene-1,3,24-triol. Betamethasone: 9-fluoro-11β,17,21-trihydroxy-16β-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: $C_{27}H_{40}O_3 \cdot H_2O$/430.6. Betamethasone dipropionate: $C_{28}H_{37}FO_7$/504.59.

Structures:



Calcipotriene Hydrate



Betamethasone Dipropionate

Relevant INDs/NDAs/DMFs: 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 21-852 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

Clinical formulation (topical ointment):

Compound	Amount per gram
Calcipotriene hydrate*.....	
Betamethasone dipropionate**.....	
α-tocopherol.....	
.....	
Polyoxypropylene-15-stearyl ether...	
.....	

*Equivalent to

**Equivalent t

Route of administration: Topical to the skin. The proposed use of the product (application to areas of the skin that are affected by psoriasis) may involve application to up to approximately of the body surface area. The material would be applied once daily for an indefinite period, resulting in chronic exposure to the product. Approximately of product may be applied per day to a given patient (the

exact amount varies greatly, depending on the severity of the disease and the percentage of the skin that is affected).

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

Studies reviewed within this submission: Note: This product was developed under IND 62,993, which was originally submitted 27-JUL-2001. 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.

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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$, Study No. 07-87/01.

4. Effects of $1,25(OH)_2D_3$ and its analogues on cell proliferation and differentiation in a human keratinocyte cell line (HaCaT), Study No. 07-92/15.

5. Effects of $1,25(OH)_2D_3$, 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 $1\alpha,25(OH)_2D_3$: 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}H-calcipotriol in mice, Study No. AE/94/01 (Report No. 36-94/12).

2. Calcipotriol/calcipotriene (BMS-181161). Dermal absorption of ^{3}H-calcipotriol in mice without collar protection, Study No. AE/97/01 (Report No. 36-97/15).

3. Absorption of ^{3}H-MC 903 following topical application of ^{3}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}H-MC 903 i.v. or p.o., Study No. 35-89/12.

5. Dermal absorption of ^{3}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}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}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}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}H]-calcipotriene and [^{3}H]-betamethasone (Dovobet®) in the rat *in vivo*, Study No. 205018.

2. Absorption, excretion and metabolic profiling of ^{3}H-Dovobet in minipigs, Study No. AME/03/01.

3. Determination of the *in vitro* metabolic profile of [^{3}H]-calcipotriol and/or [^{3}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% BMY-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 microtitre® fluctuation technique, Study No. 339/86 (LEO Study No. GTOX0202).

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 Polyoxypropylene-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 Microtitre® Fluctuation Technique, Study No. 339/117 (LEO Study No. GTOX0303).

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).

2. BMS-181161. Dermal carcinogenicity study in mice, Protocol No. 01-2731 (currently underway as a Phase 4 commitment to NDA 20-273).

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. Female fertility, Prenatal and Postnatal study conducted as a phase IV commitment to NDA 21-852.

3. 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).

4. 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 <u>not</u> 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 A2, 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 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.

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 ^{3}H-calcipotriene and ^{3}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 α,β-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 6β-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 negative in the Ames mutagenicity assay and in the mouse lymphoma TK locus assay with and without metabolic activation, and in the in vivo mouse micronucleus bone marrow assay. Calcipotriene was positive (induced chromosome aberrations) in an in vitro assay in human lymphocytes in the absence of metabolic activation.

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. Evaluation of betamethasone dipropionate for effects upon female fertility, including prenatal and postnatal function, will be regarded as being a Phase 4 commitment to NDA 21-852. 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: Dovonex or Dovobet ointment were essentially non-irritating to the skin or eyes and were nonsensitizing.

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 [redacted] in compliance with Good Laboratory Practice regulations (21 CFR 58).

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 Crl:CD-1 (ICR)BR VAF/Plus mice were randomly assigned into treatment groups as indicated below:

Group	Conc. of Soln.[a] (µg/mL)	Amt. per Unit BW[b] (µg/kg)	Number of Animals 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

[a]The products used clinically contain 50 µg of drug per ml or g of material.
[b]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 $p \leq 0.05$.

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 $p \leq 0.05$. 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: MC 903. 26 week oral toxicity in the rat, Study No. 880212T2. Please see Original Summary of NDA 20-273.

2.6.6.3.6 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.

2.6.6.3.7 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.

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

Repeat-dose toxicology studies with betamethasone alone:

2.6.6.3.9 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 (mg/kg/day)	Body Weight, Week 13 (g)	Body Weight, Week 13 (% of Controls)	Body Weight Gain, Weeks 0-13 (g)	Body Weight Gain, 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 (0.06)	83%	84%	36%*
Group 4/Females (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 (mg/kg/day)	Mean Weight of Spleen (g)	Mean Weight of Thymus (g)
Group 1/Males (0)	0.59±0.05	0.24±0.05
Group 2/Males (0.02)	0.58±0.04	0.21±0.05
Group 3/Males (0.06)	0.54±0.08	0.16±0.07*
Group 4/Males (0.2)	0.47±0.06**	0.13±0.03**
Group 1/Females (0)	0.53±0.7	0.29±0.05
Group 2/Females (0.02)	0.47±0.09	0.19±0.04**
Group 3/Females (0.06)	0.44±0.03**	0.12±0.02**
Group 4/Females (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-proprionate, 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_{0-24} (pg•hr/mL)	AUC_{0-inf} (pg•hr/mL)	C_{max} (pg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{0-24} (µg•hr/mL)	AUC_{0-inf} (µg•hr/mL)	C_{max} (pg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)
0.02 mg/kg/day (Group 6)	ND	ND	620	1	ND	9990	11,410	4438	1	2.3
0.06 mg/kg/day (Group 7)	ND	ND	2084	1	ND	31,985	33,177	14,237	1	1.3
0.2 mg/kg/day (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.

Repeat-dose toxicology studies with calcipotriene/betamethasone ointment:

2.6.6.3.10 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:

Compound	Amount per gram
Calcipotriene hydrate.....	
Betamethasone dipropionate.....	
α-tocopherol.....	

Polyoxypropylene-15-stearyl ether.....

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 fasiculata), 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).

2.6.6.3.11 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: [redacted].
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 (µg/g calcipotriene/µg/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/Crl: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. 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).

2.6.6.3.12 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: [redacted]
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 (μg/g calcipotriene/μg/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/ [redacted]
#/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 μg/kg/day calcipotriene and 10 μg/kg/day betamethasone; mid-dose animals received approximately 5 μg/kg/day calcipotriene and 50 μg/kg/day betamethasone; high-dose animals received approximately 25 μg/kg/day calcipotriene and 250 μg/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±SD

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

*$p<0.05$

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 fasiculata 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).

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[1]. In the in vivo mouse micronucleus bone marrow assay, no increase in micronucleated polychromatic erythrocytes was noted at 1 mg/kg given I.P.

[1] 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.

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: [redacted]
Date of study completion: 17-SEP-2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Betamethasone dipropionate, lot 0105216, [redacted]

Formulation/vehicle: Betamethasone dipropionate dissolved in DMSO

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
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 [redacted]® fluctuation technique.

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: [redacted]
Date of Study Completion: 08-AUG-2002
GLP Compliance: Yes
QA Reports Yes (X) No ( )
Drug, lot #, radiolabel, and % purity: Betamethasone dipropionate, lot 0105216, [redacted]

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

Methodology:

- Strains/Species/Cell line: L5178Y $tk^{+/-}$ mouse lymphoma cells, clone 3.7.2C
- Dose Selection Criteria: Cytotoxicity and physical compatibility (precipitate at conc. greater that 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 $tk^{-/-}$ 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: [redacted]
Date of Study Completion: 11-NOV-2004
GLP Compliance: Yes
QA Reports Yes (X) No ( )
Drug, lot #, radiolabel, and % purity: Betamethasone dipropionate, lot 0105216,

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: [redacted]
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
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.

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 [redacted] 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: [redacted]
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.

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

Methodology:

- Strains/Species/Cell line: L5178Y $tk^{+/-}$ mouse lymphoma cells, clone 3.7.2C
- Dose Selection Criteria: Cytotoxicity and physical compatibility (precipitate at conc. greater that 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 $tk^{-/-}$ 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
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.
Document #, Volume #, and Page #: Mod 4, vol. 32-33
Conducting laboratory and location: [redacted]
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% potency.
Formulation/vehicle: Dovonex solution; see reviews of NDA 20-611 for details.

Methods (unique aspects):

Dosing:

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

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 ≥ 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 ≥ 4 mm diameter.

Route, form, volume, and infusion rate: Topical, 100 µL/day (see above), once per day M-F for 40 consecutive weeks. The assigned material was applied to the back and sides (approximately 25 cm^2) of the mice.

Observations and times:

Clinical signs: Animals observed twice daily for viability and weekly for general appearance. Clinical signs and local skin reactions (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	UVR Exposure	Mean BW	Mean BW

	Exposure (μg/kg/day)*	(RBU/Week)	Change for Males, Weeks 1-53	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 (μg/kg/day)*	UVR Exposure (RBU/Week)	Median Week to Tumor ≥ 1 mm, Males	Median Week to Tumor ≥ 1 mm, 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: BMS-181161. Dermal carcinogenicity study in mice, Protocol No. 01-2731; a 104-week topical mouse bioassay with calcipotriene that is currently ongoing in relation to NDA 20-273, NDA 20-554, and NDA 20-611 (topical calcipotriene (Dovonex) for psoriasis) as a Phase 4 commitment. The protocol was discussed by the

exec-CAC on 4/2/96 and on 3/4/97. The study is expected to be completed in 2005, and will be reviewed upon submission.

Carcinogenicity studies with betamethasone alone:

2.6.6.5.3 Study title: Carcinogenicity Study by [redacted] By agreement between the sponsor and the Division, this study will be regarded as being a Phase 4 commitment to NDA 21-852. The sponsor has committed to submission of a protocol for the study and supporting data within four months of approval of NDA 21-852.

2.6.6.5.4 Study title: Betamethasone Dipropionate. Carcinogenicity Study by [redacted] agreement between the sponsor and the Division, this study will be regarded as being a Phase 4 commitment to NDA 21-852. The submission contained a draft protocol for the study, which will be presented to the executive carcinogenicity assessment committee of CDER if NDA 21-852 is approved (the sponsor will only conduct the carcinogenicity study if the NDA is approved).

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 (HsdBrlHan: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: Female fertility, Prenatal and Postnatal study. By agreement between the sponsor and the Division, this study will be regarded as being a Phase 4 commitment to NDA 21-852.

Fertility studies with calcipotriene/betamethasone ointment: None.

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: [redacted]
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 [redacted] in water

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±SD): Significantly reduced in all treatment groups (1.33±0.12, 1.26±0.14, 1.13±0.14, and 0.95±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, sternebrae, 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: [redacted]
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 [redacted] in water

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±SD): Significantly reduced in all treatment groups (4.90±0.49, 4.56±0.40, 4.50±0.42, and 4.49±0.41 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: [redacted]
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 — 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±SD): Significantly reduced in mid and high-dose groups (33.6±4.7, 33.0±4.8, 30.7±6.0, and 25.6±5.0 at 0, 0.625, 2.5, and 10 μg/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 μg/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 μg/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 µg/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: As noted above under section 2.6.6.6.3, evaluation of effects of betamethasone dipropionate on female fertility, prenatal and postnatal development will be regarded as being a Phase 4 commitment to NDA 21-852.

Prenatal and postnatal development studies with calcipotriene/betamethasone ointment: None.

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: Diavobet ointment, batch No. 9838381, presumed 100%. Vehicle ointment, batch No. 983638101.

Formulation/vehicle:

Compound	Amount per gram
Calcipotriene hydrate.....	
Betamethasone dipropionate.....	
α-tocopherol.....	
.....	
Polyoxypropylene-15-stearyl ether.....	
.....	

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

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.

2.6.6.9 Discussion and Conclusions

The product contains both calcipotriene and betamethasone dipropionate. The safety database submitted in support of NDA 21-852 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 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. 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 negative in the Ames mutagenicity assay and in the mouse lymphoma TK locus assay with and without metabolic activation, and in the in vivo mouse micronucleus bone marrow assay. Calcipotriene was positive (induced chromosome aberrations) in an in vitro assay in human lymphocytes in the absence of metabolic activation.

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. The evaluation of calcipotriene and betamethasone dipropionate in standard carcinogenicity assays will be accomplished as post-approval commitments.

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 sternebrae, 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 exposures up to 0.2 mg/kg/day. By agreement between the sponsor and the Division, evaluation of betamethasone dipropionate for effects upon female fertility, including prenatal and postnatal function, will be regarded as being a Phase 4 commitment to NDA 21-852. 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.

Dovonex or Dovobet ointment were essentially non-irritating to the skin or eyes and were nonsensitizing.

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 Dovobet ointment 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 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 studies post-approval of the NDA:

1. Evaluation of the carcinogenicity of calcipotriene (this matter is currently being evaluated by the sponsor as a post-approval commitment to NDA 20-273).

2. Evaluation of the carcinogenicity of betamethasone dipropionate in mice. The sponsor should submit a protocol for this study with appropriate supporting documents for evaluation by the executive carcinogenicity assessment committee of CDER following approval of NDA 21-852.

3. Evaluation of the carcinogenicity of betamethasone dipropionate in rats. The sponsor should submit a protocol for this study with appropriate supporting documents for evaluation by the executive carcinogenicity assessment committee of CDER following approval of NDA 21-852.

4. Evaluation of betamethasone dipropionate for effects upon female fertility, including prenatal and postnatal function.

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

Suggested labeling:

Carcinogenesis, mutagenesis, impairment of fertility:

Long-term animal studies have not been performed to evaluate the carcinogenic potential of Tradename® ointment or any of the active constituents.

In a study in which albino hairless mice were exposed to both ultra-violet radiation (UVR) and topically applied calcipotriene, a reduction in the time required for UVR to induce the formation of skin tumors was observed (statistically significant in males only), suggesting that calcipotriene may enhance the effect of UVR to induce skin tumors. Patients who apply Tradename® ointment to exposed portions of the body should avoid excessive exposure to either natural or artificial sunlight (including tanning booths, sun lamps, etc.). Physicians may wish to limit or avoid use of phototherapy in patients that use Tradename® ointment.

Calcipotriene did not elicit any genotoxic effects in the Ames mutagenicity assay, the mouse lymphoma TK locus assay, the human lymphocyte chromosome aberration test, or the mouse micronucleus test.

Betamethasone dipropionate did not elicit any genotoxic effects in the Ames mutagenicity assay, the mouse lymphoma TK locus assay, or in the rat micronucleus test.

Studies in rats at oral doses of up to 54 mcg/kg/day (324 mcg/m^2/day) of calcipotriene indicated no impairment of fertility or general reproductive performance.

Studies in rats at oral doses of up to 0.2 mg/kg/day (1,200 mcg/m^2/day) of betamethasone dipropionate indicated no impairment of male fertility.

Pregnancy:

Teratogenic Effects: Pregnancy Category C
Animal reproduction studies have not been conducted with Tradename® ointment. Tradename® ointment contains calcipotriene that has been shown to be fetotoxic and betamethasone dipropionate that has been shown to be teratogenic in animals when given systemically. There are no adequate and well-controlled studies in pregnant women. Tradename® ointment should be used during pregnancy only if the potential benefit to the patient justifies the potential risk to the fetus.

Teratogenicity studies with calcipotriene were performed by the oral route in rats and rabbits. In rabbits, increased maternal and fetal toxicity were noted at a dosage of 12 mcg/kg/day (144 mcg/m^2/day); a dosage of 36 mcg/kg/day (432 mcg/m^2/day) resulted in a significant increase in the incidence of incomplete ossification of the pubic bones and forelimb phalanges of fetuses. In a rat study, a dosage of 54 mcg/kg/day (324 mcg/m^2/day) resulted in a significantly increased incidence of skeletal abnormalities (enlarged fontanelles and extra ribs). The

enlarged fontanelles are most likely due to calcipotriene's effect upon calcium metabolism. The estimated maternal and fetal no-effect levels in the rat (108 mcg/m^2/day) and rabbit (48 mcg/m^2/day) studies are lower than the estimated maximum topical dose in man (approximately 460 mcg/m^2/ day). Corticosteroids are generally teratogenic in laboratory animals when administered systemically at relatively low dosage levels. Betamethasone dipropionate has been shown to be teratogenic in mice and rabbits when given by the subcutaneous route at doses of 156 mcg/kg/day (468 mcg/m^2/day) and 2.5 mcg/kg/day (30 mcg/m^2/day) respectively. Those dose levels are lower than the estimated maximum topical dose in man (5,948 mcg/m^2/ day). The abnormalities observed included umbilical hernia, exencephaly and cleft palates.

Pregnant women were excluded from the clinical trials conducted with Tradename® ointment.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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
1/4/2006 02:12:10 PM
PHARMACOLOGIST

Paul Brown
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