

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-835

PHARMACOLOGY REVIEW



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-835
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 12/22/04
PRODUCT: CLOBEX Spray 0.05%
INTENDED CLINICAL POPULATION: patients with psoriasis
SPONSOR: Dow Pharmaceutical Sciences
DOCUMENTS REVIEWED: electronic submission
REVIEW DIVISION: Division of Dermatological and Dental Drug
Products (HFD-540)
PHARM/TOX REVIEWER: Jill C Merrill
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DIVISION DIRECTOR: Jonathan Wilkin
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TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	5
2.6.1 INTRODUCTION AND DRUG HISTORY	5
2.6.2 PHARMACOLOGY	8
2.6.2.1 Brief summary	8
2.6.2.2 Primary pharmacodynamics	8
2.6.2.3 Secondary pharmacodynamics	9
2.6.2.4 Safety pharmacology	9
2.6.2.5 Pharmacodynamic drug interactions	9
2.6.3 PHARMACOLOGY TABULATED SUMMARY	10
2.6.4 PHARMACOKINETICS/TOXICOKINETICS	10
2.6.4.1 Brief summary	10
2.6.4.2 Methods of Analysis	10
2.6.4.3 Absorption	10
2.6.4.4 Distribution	10
2.6.4.5 Metabolism	11
2.6.4.6 Excretion	11
2.6.4.7 Pharmacokinetic drug interactions	11
2.6.4.8 Other Pharmacokinetic Studies	11
2.6.4.9 Discussion and Conclusions	12
2.6.4.10 Tables and figures to include comparative TK summary	12
2.6.5 PHARMACOKINETICS TABULATED SUMMARY	12
2.6.6 TOXICOLOGY	12
2.6.6.1 Overall toxicology summary	12
2.6.6.2 Single-dose toxicity	18
2.6.6.3 Repeat-dose toxicity	18
2.6.6.4 Genetic toxicology	36
2.6.6.5 Carcinogenicity	36
2.6.6.6 Reproductive and developmental toxicology	36
2.6.6.7 Local tolerance	50
2.6.6.8 Special toxicology studies	50
2.6.6.9 Discussion and Conclusions	51
2.6.6.10 Tables and Figures	51
2.6.7 TOXICOLOGY TABULATED SUMMARY	51
OVERALL CONCLUSIONS AND RECOMMENDATIONS	51
APPENDIX/ATTACHMENTS	55

EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability – CLOBEX™ (clobetasol propionate) Spray 0.05% for the treatment of psoriasis is approvable from a pharmacological/toxicological perspective
- B. Recommendation for nonclinical studies – no additional nonclinical studies are recommended for CLOBEX™ (clobetasol propionate) Spray 0.05% at this time. However, the sponsor has committed to performing carcinogenicity studies as a phase 4 commitment
- C. Recommendations on labeling - minor changes were recommended to the sponsor's proposed labeling. These changes are detailed in the Overall Conclusions and Recommendations

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The sponsor conducted a 90 day dermal toxicity study of 0.05% clobetasol propionate spray in micro-pigs. Local effects in the skin included irritation, breakdown of connective tissue, hyperkeratosis, increase in basophilic material and epidermal inflammation. The systemic effects were typical of corticosteroids, such as thymic and adrenal atrophy, which were observed grossly and microscopically. Systemic effects of the clobetasol propionate were observed even though the toxicokinetic measurements seldomly detected quantifiable levels of drug. This phenomenon is also observed in human studies of potent corticosteroids. A NOEL was not identified in this study. Adrenal atrophy, white blood cell changes and skin effects were noted even at the low dose of 150 mg/kg (3900 mg/m²). This dose was equal to approximately 1.6 mg/cm² at the site of application. A dosage level of less than 12.5 µg/kg/day was considered to be the NOEL for maternal toxicity and a dosage level of 12.5 µg/kg/day was considered the NOAEL for viability and growth in the offspring after subcutaneous administration to rats on gestation day 7 through lactation day 25. According to CPSC-FSHA guidelines, clobetasol propionate (0.05%) was considered to be an ocular irritant in both rinsed and nonrinsed eyes when tested in rabbits. However, it was classed as nonirritating to rabbit skin. Clobetasol propionate was not sensitizer when tested in the guinea pig maximization test.

- B. Pharmacologic activity - Clobetasol propionate is a fluorinated corticosteroid with anti-inflammatory and anti-proliferative activity.
- C. Nonclinical safety issues relevant to clinical use - Systemic absorption of topical corticosteroids can produce reversible hypothalamic pituitary axis suppression with the potential for glucocorticoid insufficiency after withdrawal from treatment. A warning about this adverse effect is included in the labels of currently approved formulations of clobetasol propionate and will

be included in the CLOBEX™ (clobetasol propionate) Spray 0.05% label. Clobetasol propionate, like other corticosteroids, is teratogenic in multiple species when administered at sufficient doses and at the vulnerable gestational periods. Topical application of clobetasol propionate appears to be less likely to result in teratogenic effects probably due to lower exposure to clobetasol propionate by the topical route than by systemic exposure.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-835

Review number: 0000

Sequence number/date/type of submission: 0000/12-22-04/ NDA submission

Information to sponsor: Yes (x)

Sponsor and/or agent: Dow Pharmaceuticals

Manufacturer for drug substance: ██████████

Reviewer name: Jill C. Merrill

Division name: Dermatological and Dental Drug Products

HFD #: 540

Review completion date:

Drug:

Trade name: CLOBEX™ (clobetasol propionate) Spray 0.05%

Generic name: Clobetasol propionate spray, 0.05%

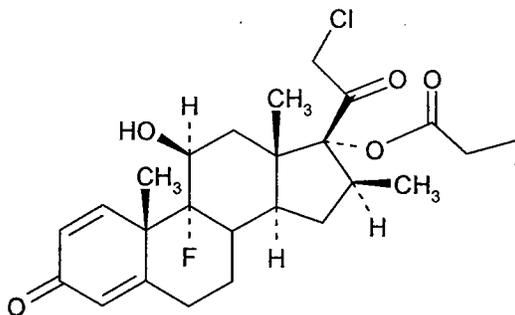
Code name: not provided

Chemical name: 21-chloro-9-fluoro-11 β ,17-dihydroxy-16 β -methylpregna-1,4-diene-3,20-dione 17 propionate

CAS registry number: 25122-46-7

Molecular formula/molecular weight: C₂₅H₃₂ClFO₅/466.7

Structure:



Relevant INDs/NDAs/DMFs: NDA 19-322, IND 62,543

Drug class: fluorinated corticosteroid

Intended clinical population: patients with psoriasis

Clinical formulation:

Clobetasol propionate spray 0.05% (nonaerosol pump spray):

Component	% w/w
Clobetasol Propionate, USP	0.05
Alcohol, USP (190 proof)	*
Isopropyl Myristate, NF	*
Sodium Lauryl Sulfate, NF	
Undecylenic acid, USP	

*

Route of administration: topical

Introduction: This NDA was submitted under section 505(b)(1) of the FD&C Act as discussed at the pre-NDA meeting (October 5, 2004). It is supported with pharmacology/toxicology information obtained from studies performed by the sponsor as well as studies for which they obtained the right-of-reference.

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

Studies reviewed within this submission:

A 90-day dermal toxicity study of clobetasol propionate 0.05% spray in micro-pigs (0215-S2.R-07-01)

A 9-month dermal toxicity study with 1-month recovery in Hanford minipigs with clobetasol propionate spray 0.05% (0215-S2.R-17-03)

A three-month dermal toxicity study in Sprague Dawley rats with a 4-week recovery with clobetasol propionate spray (0215-S2.R-22-04)

Subcutaneous fertility and general reproduction toxicity study of clobetasol propionate in rats (0215-S2.R-19-03)

Subcutaneous developmental and perinatal/postnatal reproduction toxicity study of clobetasol propionate in rats, including a postnatal behavioral/functional evaluation (0215-S2.R-18-03)

A dermal developmental toxicity (segment II) study in Sprague Dawley rats with isopropyl myristate (0215-S2.R-08-02)

A dermal developmental toxicity (segment II) study in New Zealand White rabbits with isopropyl myristate (0215-S2.R-13-02)

A dermal developmental toxicity (segment II) study in New Zealand White rabbits with isopropyl myristate (0215-S2.R-09-02)

1 Page(s) Withheld

✓ Trade Secret / Confidential

 Draft Labeling

 Deliberative Process

Withheld Track Number: Pharm/Tox-1

A preliminary (non-GLP) dermal tolerance study of various dilutions of isopropyl myristate in female New Zealand White rabbits. Study no: 3351.20, also referred to as 0215-S2.R-10-02. This study was previously submitted/reviewed under IND 62,543 (SN019) by Dr. Paul Brown.

Topical developmental and perinatal/postnatal reproduction toxicity study of isopropyl myristate in rats, including a postnatal behavioral/functional evaluation. Study No: 2304-004, also referred to as 0215-S2.R-20-03. This study was previously submitted/reviewed under IND 62,543 (SN0050).

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Corticosteroids have anti-inflammatory and anti-proliferative properties and as such are widely used in the treatment of psoriasis. They are divided into two categories based on their specific activities; glucocorticoids being associated with glucose metabolism and anti-inflammatory effects, and mineralcorticoids being associated with Na⁺ retention. Topical corticosteroids, including clobetasol propionate, fall into the glucocorticoid category.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: The mechanism of anti-inflammatory activity of topical steroids in general is unclear. However, corticosteroids are thought to act by induction of phospholipase A2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their precursor, arachidonic acid. Arachidonic acid is released from membrane phospholipids by phospholipase A₂.

Glucocorticoids bind to glucocorticoid receptors (GR) in the cell cytoplasm, activating and translocating them to the nucleus where they bind with specific glucocorticoid response elements (GRE) on the DNA, resulting in the induction of gene transcription and the production of specific cellular proteins. Glucocorticoids upregulate lipocortins, inhibiting phospholipase A2 and thereby preventing the release of arachidonic acid from membrane phospholipids. Glucocorticoids also affect the release of multiple inflammatory factors from various cell types including intracellular adhesion molecules that are critical for leukocyte localization, acute phase reactants which include the third component of complement, and histamine. As a result, glucocorticoid treatment results in decreased release of vasoactive and chemoattractive factors, diminished secretion of proteolytic and lipolytic enzymes, decreased extravasation of leukocytes to areas of injury, and ultimately, decreased fibrosis, resulting in a diminished inflammatory response.

Drug activity related to proposed indication: Clobetasol propionate has anti-inflammatory activity and anti-proliferative activity that are both important for the efficacious treatment of psoriasis.

2.6.2.3 Secondary pharmacodynamics

No data available.

2.6.2.4 Safety pharmacology

The safety pharmacology section is based on studies performed by Irie *et al.*, (1975) involving various physiological systems in mice, rats, guinea pigs and rabbits. This paper was published in Japanese and the sponsor has provided a summary of the data in the NDA. The doses used were considered much larger than the expected maximum clinical doses. No effects were observed at doses <20 mg/kg (200x the effective dose of 0.1 mg/kg).

Neurological effects: Irie *et al.*, dosed mice with intraperitoneal administration of 300 and 500 mg/kg clobetasol propionate and 500, 1000 and 2000 mg/kg betamethasone valerate. After 1 hour, a dose of 500 mg/kg clobetasol 17- propionate resulted in decreased alertness, reactivity, touch response and pain response, as well as limb tone. In addition, within 20 minutes of administration there was inhibition of spontaneous locomotor activity. These effects were not observed at 300 mg/kg clobetasol 17- propionate or with betamethasone valerate. An ocular dose of 100 mg/mL clobetasol propionate and betamethasone valerate in guinea pigs did not produce local anesthesia or local irritation.

Cardiovascular effects: Several studies were performed to study the effects of clobetasol propionate on the cardiovascular system in rabbits. No effects were observed on the cardiovascular system.

Pulmonary effects: No studies to address pulmonary effects were submitted.

Renal effects: A diuretic effect was observed in mice following subcutaneous administration of 50 or 200 mg/kg.

Gastrointestinal effects: No studies to address gastrointestinal effects were submitted.

Abuse liability: No studies to address abuse liability were submitted.

Other: No studies to address other aspects of safety pharmacology were submitted.

2.6.2.5 Pharmacodynamic drug interactions

No data available.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

This section is not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The pharmacokinetic data presented below are derived from *in vitro* percutaneous penetration studies, dermal application of creams and ointments, as well as dermally and subcutaneously applied radioactive drug. No pharmacokinetic studies were performed with CLOBEX™ (clobetasol propionate) Spray, 0.05%.

2.6.4.2 Methods of Analysis

This section is not applicable.

2.6.4.3 Absorption

Clobetasol propionate is absorbed through the skin according to its known polarity relative to other corticosteroids. Ponec and Polano (1979) compared the relative rates of absorption of various percutaneously used corticosteroids through the epidermis. The amount penetrating in 0.1% ethanolic solutions was consistent with the known polarities of the different corticosteroids. Clobetasol propionate, having intermediate polarity, penetrated the epidermis in amounts that were also intermediate relative to hydrocortisone, the most polar, and clobetasol butyrate, the least polar.

Occlusion significantly improved the absorption of the drug into plasma regardless of the vehicle used. Tritiated clobetasol 17-propionate was dermally administered to rats in either a cream or ointment formulation, under occlusion or non-occluded (Study report # WBP 84/018). The occluded treatments provided greater plasma concentrations of radioactivity than the non-occluded treatments (2.55 and 1.45 ng equiv./mL, respectively). There were no significant differences in plasma concentrations of radioactivity resulting from cream or ointment treatments (1.8 vs 2.06 ng equiv./mL, respectively). Peak plasma concentrations of radioactivity were attained at 48 hours after dosing and were significantly higher in the occluded vs. the non-occluded groups (3.97 and 2.25 ng equiv./mL, respectively).

2.6.4.4 Distribution

Tissue distribution of radioactivity, determined only after subcutaneous administration in Wistar rats, showed high levels in the liver, GI tract, kidneys, adrenals, pituitary, thyroid, prostate and fat. The radioactivity in these organs (or tissues) in many cases, showed a trend of change with time similar to that found with plasma radioactivity (Tokiwa *et al.*).

2.6.4.5 Metabolism

The metabolism of clobetasol propionate has never been fully characterized or quantified; however it is assumed that its metabolism follows that of systemically administered adrenocortical steroids. The metabolism of steroid hormones involves sequential addition of oxygen or hydrogen atoms followed by conjugation to form water-soluble derivatives. The double bond at the 4,5 position is reduced both in the liver and extrahepatically to produce inactive compounds. Reduction of the 3-ketone group to a 3-hydroxyl group occurs only in the liver. Most of these reduced compounds are subsequently conjugated with glucuronide or sulfate in the liver, and to a lesser extent in the kidney. These sulfate esters and glucuronides form water-soluble derivatives that are excreted in the urine (Schimmer, 1996).

Dermal application of clobetasol propionate was found to maximally induce the drug metabolizing enzyme ethoxycoumarin-O-dealkylase (ECOD) in the skin 6-fold at a concentration of 0.05%, the concentration most often used clinically. Activity increased for the first 16 hours after treatment, reaching a maximum at between 18-24 hours, and then declining (Finnen *et al.* (1984).

2.6.4.6 Excretion

The primary excretion route of clobetasol propionate after dermal dosing in Wistar rats was via feces. The totals excreted via feces and urine up to the 96th hour after administration was 9.20%, 1.22%, and 8.86% of the administered radioactivity for cream, ointment and solution, respectively. The remaining amounts in the body (excluding site of application) were 0.92, 0.42%, and 2.85% of the administered amounts, respectively. These results indicated that when applied dermally, the absorption was greater in the cases of cream and solution than with the ointment. It was also revealed that when the drug was administered in the form of cream or solution (dermally), a greater plasma concentration of the drug could be maintained for a long period of time, even after a single administration (Tokiwa *et al.*, and Study Report # WBP 84/018).

Seventy-two hours after subcutaneous administration of [³H] 0.05% clobetasol propionate solution in Wistar rats, 16.2% and 70.8% of the radioactivity of the administered dose had been excreted via urine and feces, respectively. The amount remaining in the body was 4.62% of the administered dose. 54% of the dose was excreted into the bile by the 45th hour after administration, and there was very little enterohepatic circulation. Exhalation of radioactivity was less than 1% of the dose in 48 hours after dosing (Tokiwa *et al.*, and Study Report # WBP 84/018).

2.6.4.7 Pharmacokinetic drug interactions

No data available.

2.6.4.8 Other Pharmacokinetic Studies

No data available.

2.6.4.9 Discussion and Conclusions

As measured *in vitro* using human epidermis, clobetasol propionate is absorbed according to its known polarity relative to other corticosteroids. In rats occlusion significantly improved the absorption of the drug into plasma regardless of the vehicle used. Peak radioactive drug concentration in plasma was reached in 4 hours after subcutaneous application rather than 8 hours for dermally applied drug; however, concentrations remained high for 96 hours following dermal applications and decreased much more rapidly ($T_{1/2} = 9$ hours) following subcutaneous application. Tissue distribution of subcutaneous administered radioactive clobetasol propionate showed high levels in liver, GI tract, kidneys, adrenals, pituitary, thyroid, prostate and fat, which decreased over time similar to levels in the plasma. The metabolism of clobetasol propionate is assumed to follow that of other corticosteroids, namely conjugation of hepatically and non-hepatically reduced inactive compounds with glucuronide or sulfate to produce water-soluble compounds that are excreted in the urine. Dermal application of clobetasol propionate was found to maximally induce the drug metabolizing enzyme ethoxycoumarin-O-dealkylase in the skin 6-fold at a concentration of 0.5%, the concentration most often used clinically. Clobetasol propionate is primarily excreted in the feces; approximately 71% of a subcutaneous administered dose is excreted in the feces by 72 hours. Dermally applied clobetasol propionate is also primarily excreted in the feces with a longer plasma half-life.

2.6.4.10 Tables and figures to include comparative TK summary

This section is not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

This section is not applicable.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The sponsor conducted a 90 day dermal toxicity study of 0.05% clobetasol propionate spray in micro-pigs. Local effects in the skin included irritation, breakdown of connective tissue, hyperkeratosis, increase in basophilic material and epidermal inflammation. The systemic effects were typical of corticosteroids, such as thymic and adrenal atrophy, which were observed grossly and microscopically. Systemic effects of the clobetasol propionate were observed even though the toxicokinetic measurements seldomly detected quantifiable levels of drug. This phenomenon is also observed in human studies of potent corticosteroids. A NOEL was not identified in this study. Adrenal atrophy, white blood

cell changes and skin effects were noted even at the low dose of 150 mg/kg (3900 mg/m²). This dose was equal to approximately 1.6 mg/cm² at the site of application.

The sponsor also conducted a 9-month dermal toxicity study with a 1-month recovery of 0.05% clobetasol propionate spray in minipigs. Dermal observations were limited to very slight to moderate erythema and very slight to slight edema, focal pinpoint to 10% eschar formation, desquamation, fissuring and blanching. Decreases in mean body weight were observed in the mid- and high-dose males and all treated female groups during the treatment period. There were no toxicologically meaningful differences in hematology, coagulation, clinical chemistry or ophthalmology data. Absolute adrenal weights were statistically decreased in both males and females in all treatment groups at the end of the treatment phase. Concentrations of clobetasol propionate in minipig plasma were generally not detectable or below the lower limit of quantitation of . However, occasional measurable low levels of clobetasol propionate suggest that the animals receiving dermal doses of clobetasol propionate were indeed exposed systemically. Treatment-related histopathology findings were observed in all treatment groups and included differences in the adrenals, thymus, spleen, treated and untreated skin, mammary gland, bone, testes and epididymides, ovaries, large intestine and liver. Based on the results of this study, a NOEL for local irritation would be 60 mg/kg/day, however, a NOAEL for systemic toxicity could not be established following the dermal administration of CLOBEX™ (clobetasol propionate) Spray, 0.05% for 9 months in Hanford minipigs.

Genetic toxicology:

Clobetasol Propionate

The sponsor has conducted two genetic toxicology studies with clobetasol propionate. Both of these studies were submitted to the IND and previously reviewed by Dr. Paul Brown (IND 62,543, SN0019). Summaries of these studies appear below:

Study Title: *In vitro* mammalian chromosome aberration assay (0215-S2.R-04-01)
Clobetasol propionate was evaluated for its clastogenic potential in an *in vitro* mammalian chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the absence and presence of an Aroclor-induced S9 activation system. Clobetasol propionate was soluble in DMSO at a concentration of 50 mg/mL, the maximum concentration tested. The cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9 activated test system, and all cells were harvested at 20 hours after treatment initiation. In the absence of at least 50% toxicity at any dose level, the highest dose level evaluated was 4670 µg/mL (10 mM) in the non-activated and S9 activated 4 hour exposure groups. The presence of precipitate at every dose level selected for analysis in the non-activated and S9 activated 4 hour exposure groups did not interfere with the microscopic evaluation. Selection of doses for microscopic analysis was based on toxicity (the lowest dose with at least 50% reduction in mitotic index, relative to the solvent control) in the non-activated 20 hour exposure group. Two lower doses were also evaluated in each harvest. It was concluded that treatment of CHO *in*

vitro with clobetasol propionate at doses up to 4670 µg/mL did not produce an increase in the percentage of cells with structural or numerical chromosome aberrations.

Study Title: Mammalian erythrocyte micronucleus test (0215-S2.R-06-02)

A mammalian erythrocyte micronucleus study was conducted to evaluate the potential for clobetasol propionate to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of ICR mice. The ratio of polychromatic erythrocytes to total erythrocytes decreased 6 to 35% in animals treated with clobetasol propionate at doses up to 2000 mg/kg. These reductions suggest bioavailability of the test article to the bone marrow. The number of micronucleated polychromatic erythrocytes was not increased by clobetasol propionate treatment at any dose or timepoint. Clobetasol propionate was concluded to be negative in the mouse micronucleus assay at doses up to 2000 mg/kg.

Mutagenicity studies have also been performed with other clobetasol propionate formulations and are described in the labeling of those products. Clobetasol was negative for mutagenicity in the Ames test, the yeast gene conversion assay and the bacterial DNA fluctuation test. However, since the sponsor has not built a clinical bridge to those products and is not referring to the Agency's findings of safety, these studies can not be used to support NDA 21-835 for CLOBEX™ (clobetasol propionate) Spray 0.05%.

Isopropyl Myristate

Blevins and Taylor (1982) determined that isopropyl myristate was not mutagenic using the Salmonella/microsome test. Isopropyl myristate was spot tested (50 µg) with and without S9 mixture (50 µL/plate). Isopropyl myristate was not mutagenic in any of the strains tested (TA1538, TA1537, TA1535, TA100, and TA98).

Carcinogenicity:

Clobetasol Propionate

No carcinogenicity studies were included in this NDA submission. However, the sponsor has committed to conducting carcinogenicity studies as a phase 4 commitment.

Isopropyl Myristate

The Cosmetic Ingredient Review (CIR) monograph on isopropyl myristate (CIR Compendium, 1995), notes that isopropyl myristate is not carcinogenic in a limited study when applied to the skin of mice.

Reproductive toxicology:

Clobetasol Propionate

The sponsor has obtained the right of reference to two dermal reproductive and developmental toxicity studies conducted in rats with Clobetasol Propionate Lotion that were submitted to IND 54,230 and reviewed under SN008 by Dr. Paul Brown. Summaries of these previously reviewed studies appear below:

Study Title: Preliminary study of embryo-fetal toxicity in the CD rat by dermal administration (1.CG.03.SRE.12055)

The primary objective of this preliminary embryo-fetal toxicity study was to assess the influence of dermal administration of clobetasol propionate lotion at concentrations of 0.01, 0.025 and 0.05% w/w, at a volume-dosage of 2 mL/kg bodyweight during the organogenesis phase of gestation, on the progress and outcome of pregnancy in the rat. In addition the study was performed to assist in the choice of dosages for the main embryo-fetal toxicity study (1.CG.03.SRE.12081). Dermal administration of 2mL/kg bodyweight clobetasol propionate lotion at 0.01%, 0.025%, and 0.05% to CD rats from Day 6 to 19 of gestation was associated with marked dose-related maternal and fetal toxicity. The report recommends that exposures in the main study be conducted for 6 hours with occlusion to reduce possible oral exposure. In addition, the report recommends that dose volumes be reduced to 1 mL/kg and that the lowest concentration be reduced to 0.005% and that the highest concentration remain at 0.05%.

Study Title: Study of embryo-fetal toxicity in the CD rat by dermal administration (1.CG.03.SRE.12081)

Clobetasol propionate lotion was administered to 22 pregnant rats by dermal administration at 1 mL/kg, at concentrations of 0.005, 0.015, or 0.05% (equivalent to 0.05, 0.15, or 0.5 mg/kg/day) or placebo lotion, from days 6 to 17 of gestation inclusive. Dermal sites were occluded for 6 hours each day. Topically applied clobetasol propionate caused maternal toxicity at all doses used in the study. Fetal immaturity was observed at all dose levels. Fetal survival was reduced for the 0.015 and 0.05% doses. Dose-related abnormalities were observed in the fetuses. There was an effect on fetal growth with treated groups showing low fetal weights, reduced skeletal ossification and umbilical herniation. The dose related reduction in ano-genital distance and displaced testes observed in male fetuses may be due to an effect of the clobetasol propionate on the androgen-dependent nature of these parameters. Some of the abnormalities, such as cleft palate, are commonly observed in teratogenicity studies with corticosteroids.

The study confirms that topically applied corticosteroids can be absorbed in sufficient amounts to have systemic effects such as teratogenicity.

Isopropyl Myristate

The sponsor has conducted a dermal developmental reproductive toxicity study of isopropyl myristate in rats. This study was submitted to IND 62,543 and reviewed under SN0050. A summary of this study appears below:

Study Title: Topical developmental and perinatal/postnatal reproduction toxicity study of isopropyl myristate in rats, including a postnatal behavioral/functional evaluation (0215-S2.R-20-03)

The primary objective of this study was to detect adverse effects of isopropyl myristate treatment at dosages of 0, 200, and 600 mg/kg/day in female rats from implantation through lactation and weaning on gestation, parturition, lactation and maternal behavior in female rats and on the development of the offspring of the treated female rats. There were no adverse effects on reproduction in the F0 generation or development in the F1 generation as evaluated in this study. On the basis of these data, the maternal NOEL for isopropyl myristate was 200 mg/kg/day. The 600 mg/kg/day dosage caused increased adverse skin reactions, including grade 1 erythema and grade 1 and 2 flaking. The reproductive NOEL in the dams was greater than 600 mg/kg/day and the NOEL for viability and growth in the offspring was also greater than 600 mg/kg/day.

The sponsor also conducted dermal developmental toxicity studies with isopropyl myristate in rats and rabbits. In rats, twice daily dermal dosing with 0, 50, 150 and 500 mg/kg/dose isopropyl myristate resulted in very slight dermal irritation for females in the 50 and 150 mg/kg/dose groups and included incidences of very slight, barely perceptible erythema and desquamation. Mild to moderate dermal irritation was observed for females in the 500 mg/kg/dose group and included very slight to moderate erythema, very slight to slight edema, focal and/or pinpoint irritation and desquamation. No statistically significant or toxicologically meaningful differences in fetal malformations or developmental variations were observed in the test article-treated groups. Results from this study determined the NOAEL for isopropyl myristate on maternal toxicity was 150 mg/kg/dose. The NOAEL for isopropyl myristate for developmental toxicity in rats from gestation day 6 to 17 was 500 mg/kg/dose.

In rabbits, twice daily dermal dosing with 0, 50, 150, and 300 mg/kg/dose resulted in dermal findings for females in the control and each test article-treated group. The severity of the findings appeared to be slightly greater in the 300 mg/kg/dose group when compared to the control, 50 and 150 mg/kg/dose groups. No statistically significant or toxicologically meaningful differences in fetal external, visceral and skeletal malformations or variations were observed in the test article-treated groups as compared to controls. The maternal toxicity NOAEL was 150 mg/kg/dose. The developmental toxicity NOAEL was 300 mg/kg/dose.

Special toxicology:

Clobetasol Propionate

The sponsor has conducted three special toxicology studies with clobetasol propionate spray, 0.05%. All of these studies were previously submitted to the IND and reviewed by Dr. Paul Brown (IND 62,543, SN0000). Summaries of these studies appear below:

Study Title: A primary skin irritation study in rabbits with 0.05% clobetasol propionate (0215-S2.R-01-01)

This study was performed to assess the potential irritant and/or corrosive effects of the test article in New Zealand White rabbits when administered by single dermal doses to one intact and one abraded site and left either occluded or non-occluded. The report concludes that the test articles appear to produce some irritation especially when occluded. Much if the irritation appears to be due to the vehicle and may actually be decreased when the corticosteroid is present. The calculated primary irritation indices were 1.25 and 0.42 for clobetasol propionate spray, 0.05% occluded and non-occluded, respectively and 3.42 for placebo occluded. The report classified the test article as nonirritating since the primary irritation indices were all below 5.

Study Title: A primary eye irritation study in rabbits with 0.05% clobetasol propionate (0215-S2.R-02-01)

This study was performed to assess the potential irritant or corrosive effects of clobetasol propionate spray, 0.05% to the eyes of New Zealand White rabbits. Results were similar in rinsed and unrinsed eyes. Corneal opacity was observed in all animals by 24 hours. The corneal opacity resolved by 7 days. Iritis and conjunctivitis were also observed in all animals. In some cases the conjunctivitis was characterized by marked erythema and obvious swelling of the eyelids with partial eversion. The iritis was resolved by 49 hours in all animals and the conjunctivitis was resolved by day 7. Clobetasol propionate formulation (0.05%) was considered to be a moderate irritant to ocular tissue according to the Kay and Calandra Ocular Evaluation. According to the CPSC-FSHA guidelines clobetasol propionate is considered an irritant for both rinsed and non-rinsed groups.

Study Title: A dermal sensitization study in guinea pigs with 0.05% clobetasol propionate – maximization design (0215-S2.R-03-01)

The purpose of this study was to determine if clobetasol propionate spray, 0.05% was a dermal sensitizer. The 0.05% clobetasol propionate formulation was not tested at full strength in this assay since it was too irritating. A 5% dilution of the formulation in acetone did not produce a contact sensitization response in the guinea pig. Concurrent positive control animals were not included in this study. The report states that the assay had responded properly to a positive control agent within the six months preceding the test.

Isopropyl Myristate

The sponsor has conducted three special toxicology studies with isopropyl myristate. All of these studies were previously submitted to the IND and reviewed by Dr. Paul Brown (IND 62,543, SN0019). Summaries of these studies appear below:

Study Title: A preliminary (non-GLP) dermal tolerance study of isopropyl myristate in female Sprague-Dawley rats (0215-S2.R-05-01).

The purpose of this study was to evaluate the potential dermal tolerance of isopropyl myristate when administered to female rats twice daily, at approximately 4 hours apart, for 10 consecutive days. This information was then used to plan and design a subsequent definitive Segment II study in rats (0215-S2.R-08-02). Isopropyl myristate, either neat or diluted to 50% with ethanol, produced adverse clinical signs, severe dermal irritation and marked body weight losses when applied twice daily. Although this study was planned for 10 days, it was terminated after 7 days because of the severe irritation. A follow-up range-finding study was conducted to identify one or more concentrations of isopropyl myristate which can be tolerated in rats following repeated dermal application (0215-S2.R-12-02).

Study Title: A preliminary (non-GLP) dermal tolerance study of various dilutions of isopropyl myristate in female Sprague-Dawley rats (0215-S2.R-12-02).

The purpose of this study was to assess the dermal tolerance of the test article in female rats when administered at various dilutions, twice daily, at approximately 4 hours apart, for 12 consecutive days. The results from this study were then used to plan and design the subsequent, definitive Segment II study in rats (0215-S2.R-08-02). Twice daily dermal administration of isopropyl myristate at dose levels ranging from 25 to 500 mg/kg/dose (concentrations ranging from 2.5% to 50% w/v in mineral oil) was generally well tolerated by female rats. Minor clinical signs, moderate dermal irritation and slight weight loss were observed at the high-dose level of 500 mg/kg/dose (i.e., 1000 mg/kg/day). The report concludes that this dose would still be appropriate as a high dose in a Segment II reproductive toxicity study in rats.

Study Title: A preliminary (non-GLP) dermal tolerance study of various dilutions of isopropyl myristate in female New Zealand White rabbits (0215-S2.R-10-02).

The purpose of this study was to assess the dermal tolerance of isopropyl myristate in non-pregnant female rabbits when administered at various dilutions, twice daily, at approximately 4 hours apart, for 13 consecutive days. The results from this study were then used in planning and designing a subsequent, definitive developmental toxicity study in rabbits (0215-S2.R-09-02). Twice daily dermal administration of isopropyl myristate diluted in mineral oil at dose levels ranging from 25 to 500 mg/kg/dose for 13 days was well tolerated by non-pregnant rabbits. There were some minor clinical signs, moderate dermal irritation, and net decreases in mean body weight, especially in the higher dose groups (250 and 500 mg/kg/dose). The report concludes that this dose would be appropriate as a high dose in a reproductive toxicity study in rabbits.

2.6.6.2 Single-dose toxicity

No single-dose toxicity studies were included in this NDA submission.

2.6.6.3 Repeat-dose toxicity

Study title: A 90-day dermal toxicity study of clobetasol propionate 0.05% spray in micro-pigs

Key study findings: Based on the results of this study, the NOEL for local changes at the administration site following dermal treatment of clobetasol propionate 0.05% spray to micro-pigs for 13 weeks was less than 150 mg/kg/day or less than ~ 1.6 mg/cm² at the site of application. The NOAEL for systemic changes was also less than 150 mg/kg/day based on the findings of minimal to mild adrenal cortical atrophy in all test article-treated animals.

Laboratory Study no.: -429002

Sponsor Study no.: 0215-S2.R-07-01

Volume #, and page #: electronic document

Conducting laboratory and location: _____

Date of study initiation: July 26, 2001

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Clobetasol Propionate 0.05% Spray, batch # LB-062,
Vehicle Spray for Clobetasol Propionate, batch # LB-061,
negative for clobetasol propionate

Methods

Doses: 0, 150, 450, 750 mg/kg/day

Species/strain: micro-pigs/Yucatan

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: test article was applied to clipped intact dorsal skin (~15% of total BSA) twice daily ~ 5 hours apart

Satellite groups used for toxicokinetics or recovery: NA

Age: ~ 3-5 months

Weight: males: 11.2 – 19.2 kg; females: 12.1 – 16.2 kg

Group #	Test Article	Dosage Level ^a (mg/kg/day)	Dosage Level ^b (mg/m ² /day)	Dosage Volume (mL/kg/day) ^c	Number of Animals	
					Males	Females
1	Placebo	0	0	0.904	4	4
2	Clobetasol Propionate	150	3900	0.181	4	4
3	Clobetasol Propionate	450	12000	0.542	4	4
4	Clobetasol Propionate	750	19000	0.904	4	4

^aThe test article formulation and placebo were applied undiluted based upon the mg/kg/day dose levels.

^bThe dosage expressed as mg/m²/day approximated the mg/kg/day dosage, assuming a 12-18 kg body weight range over the dosing phase of the study.

^cThe dose volumes (mL/kg/day) were calculated based on the specific gravity (0.83 g/mL) of the test article formulation.

Observations and times:

Mortality: observed twice daily for mortality and moribundity

Clinical signs: performed on all animals prior to each daily dosing and ~1 hour following each dose.

Dermal observations: application sites were examined weekly for erythema, edema and other findings prior to placebo or test article application. Erythema and edema were evaluated in accordance with the method of Draize, based on a 4-step grading system of very slight, slight, moderate, and severe.

Body weights: recorded weekly beginning 2 weeks prior to test article administration, on study day -1 and just prior to scheduled necropsy

Feed consumption: not performed

Ophthalmoscopy: conducted on all animals prior to initiation of dosing (study week -1) and near the end of the dosing period (study week 12)

EKG: not performed

Hematology: blood samples were collected from all pigs prior to initiation of dosing (week -2) and prior to scheduled necropsy (study week 12). The following parameters were evaluated: total leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, prothrombin time, activated partial thromboplastin time, differential leukocyte count, red cell morphology

Clinical chemistry: blood samples were collected from all pigs prior to initiation of dosing (week -2) and prior to scheduled necropsy (study week 12). The following parameters were evaluated: albumin, total protein, globulin, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, glucose, total cholesterol, calcium, phosphorus, potassium, sodium, triglycerides.

Urinalysis: urine samples were collected from all pigs prior to initiation of dosing (week -2) and prior to scheduled necropsy (study week 12). The following parameters were evaluated: specific gravity, pH, urobilinogen, total volume, sodium, chloride, potassium, color, appearance, protein, glucose, occult blood, ketones, bilirubin, leukocytes, nitrites, microscopy of sediment

Toxicokinetics: blood samples were collected from 4 pigs/sex/group on study day 0 and 84 at 0 (pre-dose), 0.5, 1, 2, 6, 8, and 24 hours after dosing. Samples were processed to plasma and stored at -70°C until analyzed.

Gross pathology: necropsy included examination of external surfaces, all orifices, and the cranial, thoracic, abdominal and pelvic cavities including viscera.

Organ weights: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thyroid.

Histopathology: the following organs were collected and placed in 10% neutral buffered formalin (except as noted): adrenal glands, aorta, bone with marrow (femur, sternum), bone marrow smear (not placed in formalin), brain (forebrain, midbrain, hindbrain), epididymides (placed in Bouin's), eyes with optic nerve (placed in Davidson's), esophagus, stomach (esophageal, cardiac, fundic, pyloric), duodenum, jejunum, ileum,

cecum, colon, rectum, heart, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), mammary gland, ovaries, pancreas, peripheral nerve (sciatic), pituitary, prostate, salivary glands (mandibular), seminal vesicles, skeletal muscle, spinal cord (cervical, midthoracic, lumbar), spleen, testes (placed in Bouin's), thymus, thyroid, tongue, trachea, urinary bladder, uterus with cervix, vagina, gross lesions, treated application site, untreated skin.

Adequate Battery: yes (x)

Results

Mortality: all animals survived to scheduled necropsy

Clinical signs: there were no test article-related clinical observations.

Dermal observations: Erythema was noted in all groups, including controls, but was noted with an increased incidence in the 450 and 750 mg/kg/day group males and 150, 450, and 750 mg/kg/day females. The erythema was generally very slight to slight in these groups; however, severe erythema was noted for one male and two females in the 750 mg/kg/day group. Test article-related focal eschar formation was observed for all animals in the 150, 450, and 750 mg/kg/day groups. The incidence of eschar formation occurred in a dose-related manner with the greatest incidence noted in the 750 mg/kg/day group. Desquamation was noted in all groups, including the control group and was not indicative of test article irritation or considered adverse. Encrustation, which was noted for one 150 mg/kg/day group female and one control group female, was also not considered adverse or indicative of test article treatment.

Body weights: decreased mean body weight gains were consistently observed throughout the dosing period resulting in mean body weights that were 16% and 10% lower than the control group for the 750 mg/kg/day group males and females, respectively, and 13% lower for both males and females in the 450 mg/kg/day group at the end of the study.

Ophthalmoscopy: there were test article-related ocular findings

Hematology: there was a test article-related trend for increased neutrophils and decreased lymphocytes observed in the treated groups compared to the controls. The changes were statistically significant ($p < 0.05$ or $p < 0.01$) for mean absolute lymphocyte counts in the 450 and 750 mg/kg/day group females. However, these changes are consistent with the pharmacological activity of the test article, which is indicative of hematological effects induced by exogenously administered steroids.

Clinical chemistry: test article-related changes were limited to lower mean alkaline phosphatase levels in the 450 and 750 mg/kg/day groups. The changes were statistically significant ($p < 0.05$ or $p < 0.01$) in the 450 mg/kg/day group females and the 750 mg/kg/day group males and females. The decreases observed in alkaline phosphatase levels for this study were not considered adverse or of significant toxicological relevance since the biological relevance of lower ALP levels is uncertain and there were no correlating microscopic findings.

Urinalysis: urinalysis parameters were unaffected by test article administration

Gross pathology: scabbing and dry skin at the application site for the 450 and 750 mg/kg/day. These macroscopic findings correlated with the minimal to mild superficial inflammation and minimal hyperkeratosis observed microscopically. A small thymus was noted in a 450 mg/kg/day female and a small adrenal gland was noted in a 750 mg/kg/day group male.

Organ weights: decreases in mean adrenal gland weights were noted in all test article-treated groups when compared to the control groups. The decreases were statistically significant ($p < 0.01$) for both mean absolute and mean relative (to final body weight and to brain weight) for both sexes in the 150, 450, and 750 mg/kg/day groups.

Histopathology: Minimal to mild degeneration of dermal collagen and/or minimal increases in basophilic ground substance were observed in one or more treated skin sites for males and females in the 150, 450, and 750 mg/kg/day groups. In addition, similar changes were noted in the untreated skin of 3 males in the 450 and 750 mg/kg/day groups and 3 females in the 750 mg/kg/day group, suggesting that collagen degeneration and increased ground substance were systemic effects of the test article. The treated skin sites were often observed with minimal increases in superficial keratin (hyperkeratosis) and minimal to moderate focal accumulations of leukocytes (epidermitis) in the superficial crust. Although regarded as test article-related, hyperkeratosis and epidermitis were generally minimal to mild in severity and were consistent with the repeated topical application of a mild irritant.

Bilateral, minimal to mild atrophy of the adrenal cortex was noted in the males and females in all test article-treated groups. These microscopic changes correlated with the decreased mean adrenal gland weights observed for these groups and as such are consistent with the known pharmacologic activity of the test article, which can lead to suppression of the hypothalamic pituitary axis (HPA) resulting in adrenal atrophy.

Thymic atrophy was present in 1/4 and 3/4 males in the 450 and 750 mg/kg/day groups, respectively, and in 2/4 and 2/4 females in the 450 and 750 mg/kg/day groups, respectively. Atrophy was minimal to mild in most animals; however, it was regarded as moderate in one 750 mg/kg/day group female. Thymic atrophy correlated with the test article-related effect observed for reducing circulating lymphocytes and was consistent with both age-related physiologic involution and with the expected pharmacologic activity of the test article.

Reviewer's comments: Although the report considers the adrenal cortical atrophy to not be an adverse effect because it is minimal to mild in severity and likely to resolve on discontinuation of the test article, this effect will be regarded as adverse and as such prevent a determination of a NOAEL.

Toxicokinetics: Concentrations of clobetasol in micro-pig plasma were generally not detectable or were below the lower limit of quantitation of — The lack of data

precluded determination of most toxicokinetic parameters. However, the occasional measurable doses of clobetasol suggest that animals receiving dermal doses of clobetasol were exposed systemically.

Other: Based on the results of this study, the NOEL for local changes at the administration site following dermal treatment of clobetasol propionate 0.05% spray to micro-pigs for 13 weeks was less than 150 mg/kg/day or less than ~ 1.6 mg/cm² at the site of application. The NOAEL for systemic changes was also less than 150 mg/kg/day based on the findings of minimal to mild adrenal cortical atrophy in all test article-treated animals.

Study title: A three-month dermal toxicity study in Sprague Dawley rats with a 4-week recovery with clobetasol propionate spray

Key study findings: Based on the results of this study, a NOAEL for dermal administration of clobetasol propionate spray was the 0.001% concentration following administration of 0.001%, 0.005%, 0.015% and 0.05%.

Laboratory Study no.: 3551.26

Sponsor Study no.: 0215.-S2.R-22-04

Volume #, and page #: electronic document

Conducting laboratory and location: _____

Date of study initiation: February 13, 2004

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity:

clobetasol propionate spray 0.001%, lot #LB-04003, # LB-04017,	% purity
clobetasol propionate spray 0.005%, lot #LB-04004, LB-04018,	purity
clobetasol propionate spray 0.015%, lot # LB-04005, LB-04019	% purity
clobetasol propionate spray 0.05%, lot # LB-04006, LB-04020,	purity

Vehicle Control (w/o IPM), lot #LB-04001, negative for clobetasol propionate

Vehicle Control, LB-04002, negative for clobetasol propionate

Methods

Doses:

Species/strain: rats/Sprague Dawley _____:CD@(SD)IGS BR

Number/sex/group or time point (main study): see study design

Route, formulation, volume, and infusion rate: topical to clipped dorsal surface (~15% BSA), twice daily, ~ 4 hours apart. Animals wore collars designed to prevent oral ingestion for the daily 8-hour dosing period.

Satellite groups used for toxicokinetics or recovery:

Age: ~8 weeks of age

Weight: males: 208 - 272 g; females: 159 - 209 g

Sampling times:

Unique study design or methodology (if any):

Group	No. of Animals ^b		Dosage Material	Formulation Dose Level (mg/cm ²)	Area of Exposure (cm x cm) ^a	Formulation Dosage Volume (mL/kg/dose)
	Male	Female				
1	15/5	15/5	Vehicle Control (w/o IPM)	1	20	0.16
2	15/5	15/5	Vehicle Control	1	20	0.16
3	10(6)	10(6)	Clobetasol Propionate Spray (0.001%)	1	20	0.16
4	10(6)	10(6)	Clobetasol Propionate Spray (0.005%)	1	20	0.16
5	10(6)	10(6)	Clobetasol Propionate Spray (0.015%)	1	20	0.16
6	15/5(6)	15/5(6)	Clobetasol Propionate Spray (0.05%)	1	20	0.16

^aRotation between 2 ~ 20 cm² areas was required.

^bAnimals listed to the right of the "/" were retained for the 4-week drug-free recovery period. Animals designated within the parentheses were utilized for the toxicokinetic phase.

Observations and times:

Mortality: mortality and moribundity checks were conducted twice daily

Clinical signs: detailed clinical observations were performed weekly and cage-side observations were performed daily 0.5-2 hours after first dosing.

Dermal observations: dermal scoring was performed at least once weekly during the dosing phase. Application sites were examined for erythema, edema, desquamation and other dermal changes. During recovery dermal observations were performed weekly.

Body weights: prior to initiation of treatment and weekly thereafter

Feed consumption: individual feed consumption was measured on the same days as the body weights.

Ophthalmoscopy: performed once prior to in-life treatment, and just prior to the end of the dosing phase. Because there were no treatment-related ocular findings, no exam was performed on recovery animals.

EKG: not performed

Hematology: evaluated on the scheduled day of termination. Blood samples were collected from the orbital sinus of overnight fasted animals while under light isoflurane anesthesia. The following parameters were evaluated: erythrocyte count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, platelet count, RBC morphology, reticulocyte count, total and differential leukocyte counts, activated partial thromboplastin time, prothrombin time.

Clinical chemistry: evaluated on the scheduled day of termination. Blood samples were collected from the orbital sinus of overnight fasted animals while under light isoflurane anesthesia. The following parameters were evaluated: A/G ratio, alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, calcium, cholesterol, creatinine, electrolyte balance (sodium, potassium and chloride), globulin, glucose, phosphorus, total bilirubin, total serum protein, urea nitrogen.

Toxicokinetics: blood samples were collected from a total of six rats per sex per test article group at ~ 1, 2, and 4 hours after the first dose on days 0 and 89. Rats were lightly anesthetized with isoflurane and ~ 1.0 mL of blood was collected from the retro-orbital plexus into tubes containing K₃EDTA. Samples were centrifuged and the plasma was stored at -70°C. Samples were shipped to _____ for analysis.

Urinalysis: urine samples were collected overnight from all animals on the day of scheduled termination. The following parameters were evaluated: bilirubin, blood, color and gross appearance, glucose, ketones, leukocytes, microscopic examination of spun deposits, nitrites, pH, protein, specific gravity, urobilinogen, volume.

Gross pathology: all animals were subjected to a complete gross necropsy at time of death or scheduled euthanasia (days 91 and 92 for the main phase and day 119 for the recovery phase) which included examination of all external surfaces and all viscera. Rats were fasted overnight and euthanized by carbon dioxide inhalation.

Organ weights : adrenal glands, brain, heart, kidneys, liver, ovaries, thyroid/parathyroid, and testes. Paired organs were weighed together.

Histopathology: the following organs and tissues were preserved in 10% neutral buffered formalin: accessory genital organs (epididymides, seminal vesicles, prostate, or uterus and vagina), adrenals, all gross lesions, aorta, brain (including sections of the medulla/pons, cerebellar cortex, and cerebral cortex), cecum, colon, duodenum, esophagus, exorbital lachrymal glands, eyes, femur (including articular surface and bone marrow), heart, ileum, jejunum, kidneys, liver (three sections collected), lungs (with bronchi), mammary gland, mandibular lymph node, mediastinal lymph node, mesenteric lymph node, pancreas, peripheral nerve (sciatic), pituitary, rectum, skeletal muscle (thigh), skin- treated (dorsal back), skin- untreated (hip region), spinal cord at three levels (cervical, midthoracic, lumbar), spleen, sternum with bone marrow, stomach (glandular, nonglandular), submaxillary salivary gland, testes/ovaries, thymus, thyroid/parathyroid, trachea, urinary bladder. Tissues were trimmed, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Adequate Battery: yes (x)

Results

Mortality: One group 2 male and one group 6 female were found dead on days 82 and 85, respectively, and one group 6 female was euthanized moribund on day 89. Neither clinical or histopathological indications of the cause of death were evident in the male rat. Microscopic evidence of immune suppression expressed by lymphoid atrophy was observed for the group 6 female found dead. The euthanized female had a septic condition as indicated by sites of acute inflammation within the brain, kidney, cecum and heart. This animal also displayed lymphoid atrophy within the spleen and thymus suggesting that immune suppression may have contributed to this condition. All other males and females survived to scheduled euthanasia at the end of the treatment or recovery period.

Clinical signs: No overt clinical signs of toxicity were noted during the study. The most remarkable clinical sign observed during the treatment period was thin appearance in all group 6 females. No remarkable clinical signs were observed for males or females during the recovery period.

Dermal observations: dermal findings were noted for males and females in the IPM vehicle control (group 2) and test article-treated groups (groups 3 to 6) during the treatment period. The most common dermal findings in groups 2 through 6 included grade 1 erythema (very slight, barely perceptible) and desquamation. Additional dermal findings in groups 2 through 6 included grade 2 erythema (well-defined), grade 1 edema (very slight, barely perceptible), grade 1 eschar (focal and/or pinpoint areas up to 10% of the test site), and eschar exfoliation. The severity of the dermal findings appeared to be greater in the IPM vehicle control group (group 2), and group 6. No positive dermal findings were noted for males or females in the vehicle control group (group 1). During the recovery phase, the most remarkable dermal observation was a low incidence of grade 1 erythema in group 6.

Body weights: A dose-related decrease in mean body weights was noted for males in groups 3, 4, 5, and 6 and females in groups 4, 5, and 6 during the treatment period. At the end of the treatment period, mean body weights were ~ 13%, 23%, 34% and 50% lower than group 1 for males in groups 3, 4, 5, and 6 and ~ 20%, 34%, and 45% lower than group 1 for females in groups 4, 5, and 6, respectively. Mean body weights of recovery animals in group 6 remained lower than controls during the recovery period. At the end of the recovery period, mean body weight of males in group 6 was ~ 38% lower than group 1, while the mean body weight of females in group 6 was ~ 25% lower than group 1. Mean body weights of males in group 2 were also lower than controls at the end of the treatment period (~ 8%) and remained lower than controls during the recovery period. At study termination (day 118), mean body weight for males in group 2 was ~ 15% lower than controls. Mean body weights for females in group 2 were comparable to controls throughout the study.

Feed consumption: Mean feed consumption was statistically decreased for males and females in groups 4, 5, and 6 during the treatment period compared to group 1. Mean feed consumption remained statistically decreased for recovery males in group 6 during the first part of the recovery period (days 91-98).

Ophthalmoscopy: no test article-related ocular abnormalities were noted during the treatment period.

Hematology: At the end of the treatment period (day 91), statistically significant differences in hematology and coagulation parameters for males included a lower mean segmented neutrophil value in group 2; lower monocyte values in groups 2, 3, and 6; lower mean leukocyte values in groups 2 and 6; a lower mean platelet count in group 5; lower mean lymphocyte values in groups 5 and 6; a higher mean eosinophil value in group 5; a higher mean MCV value in group 6; and higher mean APTT values in groups 5 and 6 compared to group 1. In females statistically significant differences in hematology and coagulation parameters included lower mean leukocyte and lymphocyte values in groups 4, 5, and 6; lower mean erythrocyte and prothrombin time in group 6; and higher mean MCV, MCH and segmented neutrophil values in group 6 compared to group 1. At the end of the recovery period, statistically significant differences in hematology and coagulation parameters were limited to a lower mean monocyte value for males in group 2; a lower mean lymphocyte value for males in group 6; a higher mean reticulocyte value for males in group 6; lower mean leukocyte values for females in groups 2 and 6; and a lower mean lymphocyte value for females in group 6.

Clinical chemistry: At the end of the treatment period (day 91), statistically significant differences in clinical chemistry parameters for males included a lower mean glucose value in group 3; a lower mean creatinine value in group 4; lower total protein and globulin values in group 6; higher mean ALT, A/G ratio and phosphorus levels in groups 5 and 6; and higher mean AST, BUN and cholesterol values in group 6 compared to group 1. In females, statistically significant differences in clinical chemistry parameters included lower mean creatinine levels in groups 4, 5, and 6; lower mean total protein and globulin values in group 6; higher mean potassium values on groups 2 and 6; a higher mean calcium value in group 4; higher mean phosphorus values in groups 4, 5, and 6; higher mean AST, ALT, BUN and sodium values in groups 5 and 6; and a higher mean A/G ratio and cholesterol value in group 6 compared to group 1. At the end of the recovery period, statistically significant differences in clinical chemistry parameters included higher mean alkaline phosphatase and phosphorus values in group 6 males, lower mean total protein, albumin and globulin values in the group 6 females, and higher mean alkaline phosphatase, glucose and phosphorus values in group 6 females.

Urinalysis: statistically significant parameters were limited to a lower mean total urine volume for group 5 and 6 females on Day 92 and a higher mean specific gravity for group 6 females on day 92 compared to group 1. There were no statistically significant urinalysis parameters noted at the end of the recovery period.

Ophthalmology: no test article-related ocular findings were noted in any animals at the end of the treatment period.

Gross pathology:

Subcutaneous hemorrhage of the treated skin, dark red lung and abnormal content in the small intestine were noted for the group 2 male (#6922) found dead on day 82.

Dehydration, body fat depletion, abnormal content of the stomach, duodenum and cecum, distended ileum and jejunum, small thymus, dark red lung, foci on the lung, small spleen, linear striations on the stomach and reddened stomach were noted for the group 6 female (#85) found dead on day 85.

Flaking of the treated skin, dehydration, body fat depletion, small thymus, foci on the brain, foci on the lung and tan areas on the kidney were noted for the group 6 female (#91) euthanized moribund on day 89.

Remarkable necropsy findings at the end of the treatment period included small thymus for males in group 5, small adrenals, small thymus and stomach foci for males in group 6; small adrenals, dehydration, small thymus and stomach foci for females in group 5; small adrenals, dehydration, body fat depletion, abnormal contents of the cecum, small thymus and stomach foci for females in group 6. Flaking of the treated skin was also observed for males and females in groups 2 through 6 at the end of the treatment period.

Organ weights: lower mean adrenal gland weights and adrenal gland to body weight ratios of males and females in group 6 correlated with microscopic changes observed in the adrenal glands. All other differences in absolute and relative organ weights were attributed to the lower mean body weights noted for males and females in groups 4, 5, and 6 at the end of the treatment period. There were no statistically significant differences in the absolute or relative adrenal weights of males or females in group 6 at the end of the recovery period.

Histopathology: No histopathological indications of the cause of death were noted for the group 2 male found dead. Microscopic evidence of immune suppression expressed by lymphoid atrophy in various lymph nodes and splenic white pulp was observed for one group 6 female found dead. The group 6 female euthanized moribund had a septic condition as indicated by sites of acute inflammation within the brain, kidney, cecum, and heart. This animal also displayed lymphoid atrophy within the spleen and thymus suggesting that immune suppression may have contributed to this condition.

Treatment-related changes were observed in the adrenal gland, liver, lung, lymph nodes, mammary gland, spleen, stomach, thymus and treated skin at the end of the treatment period.

Adrenal: minimal to mild atrophy of the adrenal glands was noted in 12 male and 12 female rats from group 6. Histologically there was a decrease in the width of the zona fasciculata of the adrenal cortex. The incidence and severity of adrenal atrophy was reduced at lower doses of the test article with only minimal atrophy in one group 4 male and five group 5 females.

Liver: minimal to mild accumulations of macrophages within hepatic sinusoids of 13 group 6 females. These cells were variably filled with an olive pigment. Minimal

numbers of pigment laden, sinusoidal macrophages were observed in five group 5 females; none were present in group 3 and 4 females.

Lung: minimal to moderate multifocal aggregates of alveolar macrophages (alveolar histiocytosis) were evident in 14 male and 13 female rats in group 6. Only minimal alveolar histiocytosis was observed in the control and lower dose test article groups.

Lymph nodes: occasional minimal to moderate lymphoid atrophy within a variety of lymph nodes (mesenteric, mandibular, and mediastinal lymph nodes) from group 6 males and females. Most of the observations (19/24) were minimal in severity.

Mammary gland: minimal to moderate physiologic hyperplasia within the mammary gland of 10 group 6 females. Only minimal hyperplasia was observed in groups 3, 4, and 5 females, a severity level similar to the group 2 vehicle controls. Although the severity was similar, the incidence of findings increased in a dose-response manner in groups 3, 4, and 5 (3, 4, and 7 observations, respectively).

Spleen: minimal to severe lymphoid atrophy was observed in splenic white pulp in 11 male and 13 female rats from group 6. Only minimal to mild splenic, lymphoid atrophy was observed in one male each in groups 3 and 4 and two males in group 5. One minimal and one mild observation of splenic, lymphoid atrophy were observed in group 5 females.

Stomach: increased incidence of minimal to mild, focal, necrosis within the glandular mucosa of male and female rats from group 6. Several gastric lesions noted at necropsy in two group 5 females also displayed necrotic foci.

Thymus: minimal to severe thymic atrophy was noted in 13 group 6 males and 13 group 6 females. Minimal thymic atrophy was evident in one group 4 male and four group 5 males. Five group 5 females displayed minimal thymic atrophy and one group 5 female displayed moderate atrophy.

Treated skin: sites of dermal exposure displayed minimal to mild treatment-related changes. Dermal sites were assessed for the following changes: inflammation, epidermal hyperplasia, hyperkeratosis, and parakeratosis. Male and female rats in group 2 treated with the vehicle control containing isopropyl myristate (IPM) and groups 3 through 6 treated with the vehicle control with IPM and the test article displayed various combinations and roughly similar incidences and severities of the above changes. Male and female rats in group 1 treated with the vehicle control without IPM displayed only minimal changes. This relationship suggests a slight effect of the vehicle with IPM.

At the end of the recovery period, the above mentioned changes were markedly reduced in incidence and severity. Adrenal gland, lymph node, stomach and thymus tissues from males and females in groups 1, 2, and 6 appeared normal at the end of the recovery period. Minimal splenic white pulp atrophy was limited to one group 6 male, and minimal mammary gland hyperplasia was observed in four group 6 females. The minimal presence of pigment laden macrophages within the hepatic sinusoids was noted

in one group 6 female. Within the lung, alveolar histiocytosis was reduced to minimal to mild severity in four group 6 males and five group 6 females. Dermal test sites demonstrated reductions in incidence and severity of all parameters assessed.

Toxicokinetics: The systemic absorption of clobetasol 17-propionate following topical application of clobetasol propionate spray formulation ranging in concentration from 0.001% to 0.05% clobetasol 17-propionate was low. Blood levels ranged from less than 0.1 ng/mL (limit of quantitation) at the lower dose levels to a maximum of 3.17 ng/mL at the highest dose applied for 90 days. There was no apparent difference in systemic absorption between males and females.

Summary of clobetasol 17-propionate Cmax results following topical application of clobetasol propionate spray:

Dose Level (Group)	Day	Males Cmax (ng/mL)	Females Cmax (ng/mL)
0.001% (Group 7)	0	BLQ	BLQ
	89	0.173	0.108
0.005% (Group 8)	0	BLQ	0.169
	89	0.492	0.550
0.015% (Group 9)	0	0.123	0.444
	89	0.735	2.02
0.05% (Group 10)	0	0.571	0.762
	89	3.17	2.59

Other: Based on the results of this study, the NOAEL for dermal administration of Clobetasol Propionate Spray was considered to be the 0.001% following administration for 91 consecutive days. The effects noted during or at the end of the treatment period resolved almost completely by the end of the recovery period.

Study title: A 9-month dermal toxicity study with 1-month recovery in Hanford minipigs with clobetasol propionate spray 0.05%

Key study findings: Based on the results of this study, a NOEL for local irritation would be 60 mg/kg/day. However, a NOAEL for systemic toxicity could not be established.

Laboratory Study no.: 3551.27

Sponsor Study no.: 0215.-S2.R-17-03

Volume #, and page #: electronic document

Conducting laboratory and location: _____

Date of study initiation: May 22, 2003

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: clobetasol propionate spray 0.05%, lot # 669, _____ purity
Clobetasol propionate spray, placebo, lot # 672, negative for clobetasol propionate

Methods

Doses: see study design

Species/strain: minipigs/Hanford

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

Route, formulation, volume, and infusion rate: topical to clipped dorsal surface (~15% BSA), twice daily, ~ 5 hours apart. Each test site was covered by an 8-ply gauze dressing.

Satellite groups used for toxicokinetics or recovery:

Age: ~6-12 weeks of age

Weight: males: 4.12 – 19.08 kg; females: 4.3 – 15.77 kg

Sampling times:

Unique study design or methodology (if any):

Group	# of Animals		Test Material	Multiple ^a Human/Animal Dose (mg/kg/day)	Formulation Dosage Level ^{b,c,d}		Formulation Dose Volume ^e	
	Males	Females			mg/kg/day	mg/m ² /day ^f	mL/kg/dose	mL/kg/day
1	5(2)	5(2)	Placebo	0	0	0	0.144	0.288
2	5(2)	5(2)	Clobetasol 0.05%	0.5	60	1440	0.036	0.072
3	5(2)	5(2)	Clobetasol 0.05%	1.0	120	2880	0.072	0.144
4	5(1)	5(2)	Clobetasol 0.05%	2.0	240	5760	0.144	0.288

Note: Animals designated in parentheses “()” were maintained for an ~ 28-day drug-free recovery period. Due to early non-treatment related deaths, the number of group 4 males designated for the recovery phase was adjusted from 2 to 1.

^aAnticipated human dose was 120 mg formulation/kg/day (*b.i.d.*).

^bThe test article formulation and placebo were applied undiluted based on the mg/kg/day dose levels.

^cThe test article formulation and placebo were applied twice daily (*b.i.d.*), ~ 5 hours apart.

^dEstimated from the conversion multiple of 24 for a 10 to 20 kg animal (Freireich et al., 1966).

^eThe dose volumes (mL/kg/day) were calculated based on the specific gravity (0.83 g/mL) of the test article formulation.

^fThe intended target area was ~ 15% of the total body surface area.

Observations and times:

Mortality: mortality and moribundity checks were conducted twice daily

Clinical signs: detailed clinical observations were performed weekly and cage-side observations were performed daily 0.5-2 hours after dosing.

Dermal observations: dermal scoring was performed at least once weekly during the dosing and recovery phases. Application sites were examined for erythema, edema, and other derma findings. Erythema and edema were evaluated in accordance with the Draize method.

Body weights: prior to initiation of treatment and weekly thereafter

Feed consumption: not performed due to feeding habits of this species

Ophthalmoscopy: performed once prior to in-life treatment, once during the third month and ninth month of dosing and just prior to the end of the recovery phase

EKG: not performed

Hematology: evaluated once prior to in-life initiation, during the third month and ninth month of dosing, and near the conclusion of the recovery phase. Blood samples were collected from the anterior vena cava from overnight fasted animals. The following parameters were evaluated: erythrocyte count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelet count, RBC morphology, reticulocyte count, total and differential leukocyte counts, activated partial thromboplastin time, prothrombin time.

Clinical chemistry: evaluated once prior to in-life initiation, during the third month and ninth month of dosing, and near the conclusion of the recovery phase. Blood samples were collected from the anterior vena cava from overnight fasted animals. The following parameters were evaluated: A/G ratio, alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, calcium, cholesterol, creatinine, creatine phosphokinase, electrolyte balance (sodium, potassium and chloride), globulin, glucose, phosphorus, total bilirubin, total serum protein, urea nitrogen.

Toxicokinetics: blood samples were collected from 5 pigs/sex/group on days 0 and 97 and from 4 pigs/sex/group on day 270 at the following time points: 0 hour (pre-dose) and 1, 4, 8, and 24 hours post-dose. Samples were collected from the anterior vena cava into glass tubes containing sodium heparin as the anticoagulant. The target sample size was 5 mL whole blood (to yield 2.5 mL plasma) per sample. Samples were centrifuged and the plasma was stored at -70°C. Samples were shipped to _____ for analysis.

Urinalysis: not performed

Gross pathology: all animals were subjected to a complete gross necropsy at time of death or scheduled euthanasia. Minipigs were fasted overnight and euthanized by *i.v.* overdose of sodium pentobarbital followed by exsanguination.

Organ weights : adrenal glands, brain, heart, kidneys, liver, ovaries, thyroid, pituitary, spleen, testes. Paired organs were weighed together.

Histopathology: with the exception of the bone marrow smear, the following organs were preserved in 10% neutral buffered formalin: accessory genital organs (epididymides, seminal vesicles, prostate, or uterus and vagina), adrenals, all gross lesions, aorta, brain (including sections of the medulla/pons, cerebellar cortex, and cerebral cortex), cecum, colon, duodenum, ear tag, esophagus, eyes (including optic nerve), femur (including articular surface and bone marrow), bone marrow smear (rib), gallbladder, heart, ileum, jejunum, kidneys, liver (three sections collected), lungs (with bronchi), mammary gland, mandibular lymph node, mediastinal lymph node, mesenteric lymph node, pancreas, peripheral nerve (sciatic), pituitary, rectum, skeletal muscle (thigh), skin- treated (dorsal back), skin- untreated (hip region), spinal cord at three levels (cervical, midthoracic, lumbar), spleen, sternum with bone marrow, stomach, submandibular salivary gland, testes/ovaries (including oviducts), thymus/parathyroid, thyroid, trachea, urinary bladder. Tissues were trimmed, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Adequate Battery: yes (x)

Results

Mortality: No test article-related mortality or overt clinical signs of toxicity occurred during the treatment or recovery period. Seven minipigs were either found dead or euthanized for humane reasons during the treatment period. Three of these deaths were ruled accidental or were associated with the trauma of the blood collection procedure. Three additional animals were euthanized for humane reasons due to the clinical observation of prolapsed rectum and one female died as a result of a collapsed lung. All other animals survived to scheduled euthanasia at the end of the treatment or recovery periods.

Clinical signs: The most notable clinical sign during the study was impaired mobility. On occasion, this observation is associated with the age of the animal and the flooring system utilized in the home cage. No overt clinical signs of toxicity were noted in any animals during the treatment or recovery period.

Dermal observations: Due to technical error, the test material was administered to a much smaller test area than what was intended on days 0-49. This resulted in an increase in concentrated drug over a decreased test area. The result of this error produced pronounced dermal observations including eschar, eschar exfoliation, fissuring, blanching, desquamation, very slight to moderate erythema and very slight to severe edema. The actual range of surface area (cm²) and % surface area for days 0-49 were as follows:

Group/Sex	Actual Range on Days 0-49 (cm ²)	% Surface Area on Days 0-49
Group 2 Males	24-67	0.98-1
Group 3 Males	26-64	1-0.99
Group 4 Males	27-71	1-1.6
Group 2 Females	25-67	0.99-1
Group 3 Females	25-62	0.99
Group 4 Females	27-67	1.0

The error was detected on day 49 and an additional test site was used for the rest of the treatment period. However, the dosing site was not expanded to the corrected calculated exposure area until study day 56. Dermal findings observed on the fresh test site included very slight to moderate erythema with very slight to slight edema, focal pinpoint to 1% eschar formation, desquamation, fissuring and blanching on up to 25% of the test site. The actual range of surface area (cm²) and % surface area for days 56-266 were as follows:

Group/Sex	Actual Range on Days 0-49 (cm ²)	% Surface Area on Days 0-49
Group 2 Males	100-957	2.2-8.5
Group 3 Males	195-1131	4-10.8
Group 4 Males	192-1080	4-11.1
Group 2 Females	80-851	1.9-8.7
Group 3 Females	156-1080	3.6-11.5
Group 4 Females	182-1218	3.9-11.8

Dermal observations for the corrected test area were much less pronounced than the original test area. The dermal irritation in test article-treated groups diminished during the recovery period.

Body weights: Decreases in mean body weight were observed in the 120 and 240 mg/kg/day males and all treated female groups during the treatment period. These decreased body weights remained lower than controls throughout the recovery period.

Ophthalmoscopy: no test article-related ocular abnormalities were noted during the treatment or recovery period.

Hematology: All differences noted for hematology and coagulation parameters were within the historical control range and are not considered to be toxicologically meaningful.

Clinical chemistry: Creatinine kinase was decreased in low and high-dose males and high-dose females on Day 270. Alkaline phosphatase was decreased in all female treated groups on Day 88 and in the high-dose females only on Day 270. All other parameters were within the range of their respective historical controls.

Gross pathology: The most notable necropsy findings pertained to the treated skin site and included edema, fissuring, flaking, thickening, subcutaneous hemorrhage, subcutaneous foci, reddened and dark red areas at the end of the treatment period. At the end of the recovery period, gross observations at the treated skin site were limited to a low incidence of thickening and flaky skin.

Organ weights: Absolute adrenal weights were decreased in both males and females in all treated groups at scheduled euthanasia.

Histopathology: treatment-related histopathology findings were observed in all treatment groups and included differences in the adrenals, thymus, spleen, treated and untreated skin, mammary gland, bone, testes and epididymides, ovaries, large intestine and liver.

Adrenal: Minimal to moderate cortical atrophy, minimal or mild cortical vacuolation, minimally or mildly increased prominence of the zona glomerulosa, and minimal or moderate nodular hyperplasia occurred in all treated groups.

Thymus and spleen: Increased incidence and/or severity of thymic lymphocytic depletion occurred in all treated groups. Additionally, minimally decreased lymphocytic prominence was associated with the splenic white pulp of one or two minipigs in some of the treated groups.

Treated skin: Minimal or mild hyperkeratosis, minimal epidermal attenuation (thinning), minimal to moderate inflammation associated with the epidermis/hair follicles, minimal to moderate decreased dermal collagen with minimally to moderately increased prominence of adipocytes, and minimal or mild adnexal atrophy occurred in all of the treated groups.

Untreated skin: Minimal or mild hyperkeratosis, minimal epidermal attenuation (thinning), minimal or mild inflammation associated with the epidermis/hair follicles, minimal to moderate decreased dermal collagen with minimally to moderately increased prominence of adipocytes, minimal or mild adnexal atrophy, and minimal epidermal hyperplasia occurred in the treated groups.

Mammary gland: Mildly to moderately decreased prominence of the mammary gland of females was of increased incidence and severity in all treated groups.

Bone: Minimal or mild trabecular attenuation occurred in all treated groups in association with the femur and in all treated male groups in association with the sternum.

Testes and epididymides: Minimal to moderate tubular degeneration occurred in the testes of all treated groups, and minimal to mild luminal cellular debris and minimal or mild decreased prominence of sperm occurred in the epididymides of all treated groups.

Ovaries: The incidence and/or severity of follicular atresia were increased in all treated groups relative to controls.

Large intestine: Minimally or mildly increased prominence of adipocytes occurred in all treated groups.

Liver: Minimally enlarged hepatocytes having more abundant often finely granular eosinophilic cytoplasm were noted in all treated groups.

At the end of the recovery period, microscopic changes interpreted to be test article-related occurred in all treated groups and were associated with the adrenals, treated skin, untreated skin, mammary gland, bones, testes and epididymides, ovaries and large intestine. Test article-related effects observed in the thymus, spleen, and liver at the end of treatment were not present at the end of the recovery.

Toxicokinetics: Concentrations of clobetasol in minipig plasma were generally not detectable or below the lower limit of quantitation (LLOQ) of 0.1 ng/mL. The lack of data precluded determination of most toxicokinetic parameters. However occasional measurable low levels of clobetasol suggest that animals receiving dermal doses of clobetasol were systemically exposed.

Reviewer's comment: The final report mistakenly reports the LLOQ as 0.1 mg/mL. However, review of the toxicokinetic report from [REDACTED] clearly states the LLOQ is [REDACTED].

Other:

Based on the results of this study, a NOEL for local irritation would be 60 mg/kg/day. However, a NOAEL for systemic toxicity could not be established.

2.6.6.4 Genetic toxicology

The genetic toxicology studies included in this submission, *In vitro* mammalian chromosome aberration assay (0215-S2.R-04-01) and Mammalian erythrocyte micronucleus test (0215-S2.R-06-02), were previously reviewed under IND 62,543 (SN019). No new genetic toxicology studies were included in this NDA submission.

2.6.6.5 Carcinogenicity

No carcinogenicity studies were included in this NDA submission.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Subcutaneous fertility and general reproduction toxicity study of clobetasol propionate in rats.

Key study findings: Based on these data the paternal and maternal NOEL for general toxicity of clobetasol propionate was less than 12.5 µg/kg/day. The male reproductive NOEL was also less than 12.5 µg/kg/day, based on increased weights in the seminal vesicles at the 12.5, 25, and 50 µg/kg/day dosages. The female reproductive NOEL was 12.5 µg/kg/day.

Study no.: 0215-S2.R-19-03

Volume #, and page #: electronic submission

Conducting laboratory and location: _____

19044-1241

Date of study initiation: December 30, 2003

GLP compliance: Yes

QA reports: yes (x)

Drug, lot #, and % purity: clobetasol propionate, lot # 7817/MI, _____ purity

Methods

Doses: 0, 12.5, 25, 50 µg/kg/day

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

Number/sex/group: 25/sex/group

Route, formulation: subcutaneous, *b.i.d.*

Vehicle: 0.04% Tween in saline

Satellite groups used for toxicokinetics: no

Group	Dosage ^a (µg/kg/day)	Dosage ^a (µg/kg/dose)	Concentration (µg/mL)	Dosage Volume (mL/kg/dose)	Number of Rats per sex
1	0 (Vehicle)	0 (Vehicle)	0	2	25
2	12.5	6.25	3.125	2	25
3	25	12.5	6.25	2	25
4	50	25	12.5	2	25

^a Dosage calculations were adjusted for the  purity of the test article.

Male rats were administered the test article and/or vehicle twice daily (at least 6 hours apart) beginning 70 days before cohabitation (maximum 14 days) and continuing through the day before sacrifice. Female rats were administered the test article and/or vehicle twice daily (at least 6 hours apart) beginning 15 days before cohabitation and continuing through DG 7. Rats were injected at approximately the same time each day.

Within each dosage group, one male rat was mated with one female rat. The cohabitation period consisted of a maximum of 14 days. Female rats with a copulatory plug were considered to be DG 0 (day 0 of gestation).

Parameters and endpoints evaluated:

Mortality: twice daily

Clinical observations: weekly during acclimation period, twice daily during dosage administration, once daily (females only) during postdosage period

Body weights: weekly during acclimation period, daily during dosage and postdosage (females only) administration

Estrous cycle: evaluated by examination of vaginal cytology for 14 days before dosing and then until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed in situ during the cohabitation period.

Gross pathology: Males: after cohabitation period thoracic, abdominal and pelvic viscera were examined and sperm concentration and motility were assessed. Females: DG 13 animals were caesarean-sectioned and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The number of corpora lutea was recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites, and live and dead embryos.

Organ weights: Males: testis, epididymis, seminal vesicle, prostate

Results

F0 generation male rats

Mortality: no deaths were caused by dosages of clobetasol propionate as high as 50 µg/kg/day. One rat in the 12.5 µg/kg/day dosage group was sacrificed as the result of an injury to the palate.

Clinical signs: urine-stained fur occurred in a slightly increased number of rats in the 50 µg/kg/day dosage group, but the incidence was not statistically significant. All other clinical observations were considered unrelated to clobetasol propionate.

Body weight: significant body weight loss ($p \leq 0.01$) or significantly reduced body weight gains ($p \leq 0.05$ or $p \leq 0.01$) occurred in the 12.5, 25, and 50 µg/kg/day dosage groups at every tabulated weekly interval during the study, with the exception of DSs 57 to 64 in the 12.5 µg/kg/day dosage group, and for the entire precohabitation period (DS 1 to 70) and the entire dosage period (calculated as DSs 1 to 92 and DSs 1 to termination). Average body weights were significantly reduced ($p \leq 0.01$) in all three treated groups beginning on DS 8 and continuing weekly until sacrifice.

Feed consumption: after initial reductions in relative feed consumption values during the first two weeks of dosage, relative feed consumption values were increased in all three treated groups beginning in the fourth week of dosage, resulting in significant increases in the 50 µg/kg/day dosage group for the entire precohabitation period and in the 25 and 50 µg/kg/day dosage groups for the entire dosage period.

Necropsy: There were no test article-related necropsy observations.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): All mating and fertility parameters [number of days in cohabitation, rats that mated, the fertility index (number of pregnancies per number of rats that mated), rats with confirmed mating dates during the first and second week of cohabitation and number of pregnancies per number of rats in cohabitation] were unaffected by dosages of clobetasol propionate as high as 50 µg/kg/day.

F0 generation female rats

Mortality: no deaths were caused by dosages of clobetasol propionate as high as 50 µg/kg/day.

Clinical signs: All clinical observations were considered unrelated to clobetasol propionate.

Body weight: significant body weight loss occurred for the entire precohabitation period (DSs 1 to 15) in the 12.5, 25, and 50 µg/kg/day dosage groups. Body weights were significantly reduced in the three treated groups beginning on DS 3 and continuing through DS 15.

Feed consumption: absolute and relative feed consumption values were significantly reduced in the 12.5, 25, and 50 µg/kg/day dosage groups for the entire precohabitation period. Gestation body weight gains were significantly reduced for the gestation dosage period (calculated as DGs 0 to 8) and significantly increased during the postdosage period (DGs 8 to 13) in all three treated groups. Gestation body weights were

significantly reduced in all three treated groups on DGs 0 through 13. Absolute feed consumption values were significantly reduced in the 12.5, 25, and 50 µg/kg/day dosage groups for the gestation period. Relative feed consumption values were significantly increased in the 50 µg/kg/day dosage group for the entire gestation period.

Necropsy: There were no test article-related necropsy observations

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): The number of estrous stages per 14 days was comparable among the four dosage groups before the start of administration. During the precohabitation dosage period, the number of estrous stages was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 25 and 50 µg/kg/day dosage groups. Additionally there were 1, 1, 2 and 3 females rats in the four respective groups with six or more consecutive days of diestrous during this period.

All mating and fertility parameters [number of days in cohabitation, rats that mated, the fertility index (number of pregnancies per number of rats that mated), rats with confirmed mating dates during the first and second week of cohabitation and number of pregnancies per number of rats in cohabitation] were unaffected by dosages of clobetasol propionate as high as 50 µg/kg/day.

Caesarean-sectioning observations were based on 24 (96.0%), 22 (88.0%), 24 (96.0%) and 23 (92.0%) pregnant rats in groups 1 through 4, respectively. The average number of nonviable embryos was significantly increased ($p \leq 0.05$) in the 50 µg/kg/day dosage group. Additionally the percentage of nonviable embryos per litter was increased in this dosage group. The litter averages for corpora lutea and implantations were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in the 25 and 50 µg/kg/day dosage groups; however, these changes were not considered toxicologically important because: 1) the values were within the ranges observed historically at the testing facility; and 2) an increase in the numbers of corpora lutea and implantations are not considered an adverse event. Despite these changes, the litter averages for viable embryos were comparable among the four dosage groups and did not significantly differ. No dam had a litter consisting of only nonviable embryos.

Conclusions: Based on these data the paternal and maternal NOEL for general toxicity of clobetasol propionate was less than 12.5 µg/kg/day. The male reproductive NOEL was also less than 12.5 µg/kg/day, based on increased weights in the seminal vesicles at the 12.5, 25, and 50 µg/kg/day dosages. The female reproductive NOEL was 12.5 µg/kg/day.

Embryofetal development

Study title: A dermal developmental toxicity (segment II) study in Sprague Dawley rats with isopropyl myristate.

Key study findings: Based on the results of this study, a dosage level of 150 mg/kg/dose was considered to be the NOAEL for maternal toxicity and a dosage level of 500 mg/kg/dose was considered to be the NOAEL for developmental toxicity in this study following twice daily dermal administration of isopropyl myristate from gestation day 6 to gestation day 17. Therefore on a daily basis the NOAEL for maternal toxicity and the NOAEL for developmental toxicity were determined to be 300 mg/kg/day and 1000 mg/kg/day, respectively.

Laboratory Study no.: 3551.14

Sponsor Study no.: 0215-S2.R-08-03

Volume #, and page: electronic document

Conducting laboratory and location: _____

45887

Date of study initiation: March 26, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: isopropyl myristate (IPM), Lot # QD0529, purity

Vehicle, lot #: Mineral oil, lot # 011518

Methods

Doses: 0, 50, 150, 500 mg/kg, *b.i.d.*

Species/strain: rats/ CD® (SD)IGS BR

Number/sex/group: 25 females/dosage group

Route, formulation, volume, and infusion rate: topical, *b.i.d.*, IPM in mineral oil, 1.0 mL/kg/dose

Satellite groups used for toxicokinetics: all animals were used for TK

Study design:

Group	Number of Females	Dosage Material	Dose Level (mg/m ² /dose)	Dose Level (mg/kg/dose)	Dosage Conc. (mg/mL)	Dosage Volume (mL/kg/dose)
1	25	Mineral Oil, USP	0	0	0	1.0
2	25	IPM ^a	300 ^b	50	50	1.0
3	25	IPM ^a	900 ^b	150	150	1.0
4	25	IPM ^a	3000 ^b	500	500	1.0

^aIPM = isopropyl myristate diluted in mineral oil, USP

^bEstimated dose level per square meter, based on a conversion factor of 6 for rats

Time-mated female rats received twice daily topical applications (~4 hours apart) of the vehicle or test article to a clipped dorsal surface on gestation day 6 (DG 6) through gestation day 17 (DG 17). The clipped area was at least 5 cm x 6 cm (30 cm²). A plastic Elizabethan collar was placed on each female rat before the first daily dose and removed

following completion of the second daily (4-hour) exposure period. At that time, test sites were washed with saline and rinsed with RO water and dried with gauze pads.

Blood samples were collected from all animals on the last day of dosing (gestation day 17) following the second daily dose as shown in the following table:

Group	Dosage Level (mg/kg/dose)	0.5hr*	1 hr*	2 hr*	4 hr*	24 hr*	Number of Samples
1	0	5 dams	25				
2	50	5 dams	25				
3	150	5 dams	25				
4	500	5 dams	25				

*hours post-dose

Blood was collected from each animal via the orbital plexus while under light isoflurane anesthesia. The blood samples were collected sequentially from 5 animals/group for each timepoint until all animals were sampled. No animal was sampled more than once. Plasma was obtained from each sample and stored frozen (~-70°C) at  for possible future toxicokinetic analysis.

Parameters and endpoints evaluated:

Mortality: twice daily

Clinical observations: once daily during study (after second dose application on days of dosing)

Dermal observations: females examined for dermal reactions on gestation days 7, 12, and 17 according to scale for Evaluating Skin Reactions presented in Appendix A

Body weights: recorded on gestation days 0 (provided by supplier), 5, 6, 9, 12, 15, 17, and 20. Body weight changes were calculated for the following gestation intervals: 0-5, 5-6, 6-9, 9-12, 12-15, 15-17, 17-20, 6-17, and 0-20. Also corrected maternal body weight change (body weight change for gestation days 0-20 minus gravid uterine weight) was calculated

Feed consumption: recorded on gestation days 6, 9, 12, 15, 17, and 20

Scheduled euthanasia and cesarean: females euthanized on gestation day 20 (CO₂ inhalation) and subjected to morphological examination. The thoracic, abdominal and pelvic cavities were opened and the viscera examined. Abnormalities were recorded and internal gross lesions were retained in 10% neutral buffered formalin for possible microscopic examination. The uterus was weighed (gravid weight only), examined externally and opened for internal examination. The number of viable and nonviable fetuses and early and late resorptions were recorded as were the number of corpora lutea. Fetal morphological examinations: fetuses were examined for external and internal (visceral) or skeletal abnormalities. Findings were classified as malformations or developmental variations based on the severity of the anatomical changes and their potential for interference with normal organ and/or body functions. Viable fetuses were

sexed, weighed, and euthanized with sodium pentobarbital (*i.p.*). Approximately one-half of the fetuses were fixed in Bouin's solution for subsequent visceral examination using Wilson's technique. Approximately one-half were fixed in 95% isopropyl alcohol, macerated in a 1 to 2% potassium hydroxide solution, stained with Alizarin Red S for skeletal examination.

Results

Maternal survival and pregnancy status: all females survived to scheduled cesarean section on gestation day 20. The pregnancy rate was 92% in the control and 50 mg/kg/dose groups, and 96% in the 150 and 500 mg/kg/dose groups.

Clinical observations: incidence of reddish vaginal discharge was higher for females in the 150 and 500 mg/kg/dose groups and the overall incidence of urine stain, dark material around the eyes and ocular discharge was higher for females in the 500 mg/kg/dose group compared to controls. Type and incidence of clinical signs in the 50 mg/kg/dose group were comparable to controls.

Dermal observations: in the 50 and 150 mg/kg/dose groups incidences of grade 1 (very slight, barely perceptible) erythema and desquamation were noted. Mild to moderate dermal irritation was observed for females in the 500 mg/kg/dose group and consisted of grade 1 to 3 erythema (very slight to moderate to severe), grade 1 to 2 edema (very slight to slight), grade 1 eschar (focal and/or pinpoint areas on up to 10% of the test site) and desquamation. No signs of dermal irritation were observed in the vehicle control group.

Body weights, weight changes, gravid uterine weight/net body weight change: mean body weights of females in the 500 mg/kg/dose group were statistically lower (~7-9%) than controls on gestation days 12, 15, 17, and 20. Mean body weights of females in the 50 and 150 mg/kg/dose groups were comparable to controls. Mean body weight change of females in the 500 mg/kg/dose group was statistically lower than controls during the early part of the treatment period (gestation days 6-9 and 9-12) and for the overall treatment (gestation days 6-17) and study (gestation days 0-20) periods. Mean maternal body weight change (gestation day 20 body weight minus gestation day 0 body weight) and mean corrected maternal body weight change (maternal body weight change minus gravid uterine weight) of females in the 500 mg/kg/dose group were statistically lower than controls. However, mean gravid uterine weight of females in the 500 mg/kg/dose group was comparable to controls.

Feed consumption: mean feed consumption (grams/animal/day) of females in the 500 mg/kg/dose group was statistically lower than controls during the gestation days 9-12 and 12-15.

Maternal necropsy findings: enlarged axillary lymph nodes for 1 female in the 150 mg/kg/dose group and 12 females in the 500 mg/kg/dose group and enlarged iliac lymph nodes for 2 females in the 500 mg/kg/dose group were the only remarkable findings in test article-treated animals.

Cesarean section findings: all parameters evaluated (mean number of corpora lutea, implantation sites, live fetuses, postimplantation loss, early and late resorptions and mean fetal body weight) were comparable between the controls and test article-treated groups.

Fetal morphological evaluations: no statistically significant or toxicologically meaningful differences in fetal malformations or developmental variations were observed in the test

article-treated groups. A low incidence of malformations and variations known to occur spontaneously was observed sporadically throughout the groups, including the controls.

Conclusion: Based on the results of this study, a dosage level of 150 mg/kg/dose was considered to be the NOAEL for maternal toxicity and a dosage level of 500 mg/kg/dose was considered to be the NOAEL for developmental toxicity in this study following twice daily dermal administration of isopropyl myristate from gestation day 6 to gestation day 17. Therefore on a daily basis the NOAEL for maternal toxicity and the NOAEL for developmental toxicity were determined to be 300 mg/kg/day and 1000 mg/kg/day, respectively.

Study title: A dermal developmental toxicity (segment II) study in New Zealand White rabbits with isopropyl myristate.

Key study findings: During the course of this study, a greater than normal rate of maternal late term abortions and litters with fetuses that had domed heads were observed. Since about 35% of the control females were affected with these findings, it was determined that the study was invalid. The rate of occurrence for these findings was far outside that recorded in the contract laboratory's historical control database. An exhaustive search to determine the possible cause for these unexpected findings revealed no definitive answer.

Laboratory Study no.: 3551.15

Sponsor Study no.: 0215-S2.R-09-02

Volume #, and page: electronic document

Conducting laboratory and location: _____

45887

Date of study initiation: July 15, 2002

GLP compliance: Yes

QA reports: no (x)

Reviewer's comments: The protocol was amended so as not to require a QA audit of the data because the study was determined to be invalid. In addition the sponsor amended the protocol to require only an abbreviated letter report. The letter report includes the following information in letter format: the purpose of the study, test article receipt date, in-life initiation and completion dates, as well as the results, a summary and conclusion to address the reasons for considering this study to be invalid. It does not include any raw data.

Drug, lot #, and % purity: isopropyl myristate (IPM), Lot # QD0529, purity _____

Vehicle, lot #: Mineral oil, lot # not provided

Methods:

Twenty time-mated female rabbits per group were dosed twice daily by the dermal route from gestation day 6 to 18 with increasing dosage levels of isopropyl myristate (50, 150, 300 mg/kg/dose) in the vehicle control material consisting of mineral oil. Clinical

observations, body weights and feed consumption were monitored throughout the study period.

Results:

There were 2, 1, 5, and 6 females that aborted prior to scheduled cesarean section in the control, 50, 150, and 500 mg/kg/dose groups, respectively. In addition, at scheduled cesarean section there were 5, 1, 5, and 2 litters with fetuses that had domed heads in the control, 50, 150, and 500 mg/kg/dose groups, respectively. The rate of occurrence for these findings was far outside the [REDACTED]. At the sponsor's request, no fetal skeletal examinations were performed. However, the specimens will be retained at [REDACTED] and stored in formol (solution containing a 50/50 mixture of 10% neutral buffered formalin in isopropanol) at specified storage conditions for possible future examination.

Conclusion:

Based on these unexpected findings, specifically in the control group animals, this dermal developmental toxicity study in rabbits with isopropyl myristate was determined to be invalid. All data and specimens collected during the study period will be retained and archived at [REDACTED] for the time period specified in the protocol.

Reviewer's comments: The following study (0215-S2.R-13-02) was conducted at the same facility with the same test article under the same conditions, except the doses were 0, 50, 150 and 300 mg/kg/dose, b.i.d.

Study title: A dermal developmental toxicity (segment II) study in New Zealand White rabbits with isopropyl myristate.

Key study findings: Based on the results of this study, a dosage level of 150 mg/kg/dose was considered to be the NOAEL for maternal toxicity and a dosage level of 300 mg/kg/dose was considered to be the NOAEL for developmental toxicity in this study following twice daily dermal administration of isopropyl myristate from gestation day 6 to gestation day 18.

Laboratory Study no.: 3551.24

Sponsor Study no.: 0215-S2.R-13-02

Volume #, and page: electronic document

Conducting laboratory and location: [REDACTED]

45887

Date of study initiation: September 19, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: isopropyl myristate (IPM), Lot # QD0529, purity

Vehicle, lot #: Olive oil, lot # QO0059

Methods

Doses: 0, 50, 150, 300 mg/kg, b.i.d.

Species/strain: rabbits/New Zealand White

Number/sex/group: 20 females/dosage group

Route, formulation, volume, and infusion rate: topical, *b.i.d.*, IPM in olive oil, 1.0 mL/kg/dose

Satellite groups used for toxicokinetics: all animals were used for TK

Study design:

Group	Number of Females	Dosage Material	Dose Level (mg/m ² /dose)	Dose Level (mg/kg/dose)	Dosage Conc. (mg/mL)	Dosage Volume (mL/kg/dose)
1	20	Olive Oil, NF	0	0	0	1.0
2	20	IPM ^a	500 ^b	50	50	1.0
3	20	IPM ^a	1500 ^b	150	150	1.0
4	20	IPM ^a	3000 ^b	300	300	1.0

^aIPM = isopropyl myristate diluted in olive oil, NF

^bEstimated dose level per square meter, based on a conversion factor of 10 for rabbits

Reviewer's comments: The conversion factor listed in the FDA guidance document 'Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers' lists the conversion factor for rabbits as 12, not 10.

Time-mated female rabbits received twice daily topical applications (~4 hours apart) of the vehicle or test article to a clipped dorsal surface on gestation day 6 (DG 6) through gestation day 18 (DG 18). The clipped area was at least 10 cm x 12 cm (120 cm²). Following the first daily dose, the test site was covered with an 8-ply gauze dressing which was secured with double-sided tape and a stockinette sleeve attached to a foam collar was fitted over the torso of the animal to reduce unwanted oral exposure. The binder and gauze were removed off the test site for the second daily dose and then reapplied. The wrapping material and gauze were changed daily and the test site was thoroughly cleaned with gauze soaked in 5% v/v XXXXXXXXXX body wash in RO deionized (RODI) water, then rinsed with gauze soaked in RODI water only and dried with gauze pads.

Blood samples were collected from all animals on the last day of dosing (gestation day 18) following the second daily dose as shown in the following table:

Group	Dosage Level (mg/kg/dose)	0.5hr*	1 hr*	2 hr*	4 hr*	24 hr*	Number of Samples
1	0	4 dams	4 dams	5 dams	4 dams	4 dams	20
2	50	4 dams	4 dams	5 dams	3 dams	3 dams	18
3	150	4 dams	4 dams	5 dams	4 dams	4 dams	20
4	300	4 dams	4 dams	5 dams	4 dams	4 dams	20

*hours post-dose following the second daily dose

Note: 2 dams in the 50 mg/kg/dose group were found dead prior to gestation day 18.

Blood was collected from each animal via the marginal ear vein. The blood samples were collected sequentially from 4 animals/group for each timepoint until all animals were sampled. No animal was sampled more than once. Plasma was obtained from each sample and stored frozen (~-70°C) at ■ for possible future toxicokinetic analysis.

Parameters and endpoints evaluated:

Mortality: twice daily

Clinical observations: once daily during study (after second dose application on days of dosing)

Dermal observations: females examined for dermal reactions on gestation days 7, 12, and 18 according to scale for Evaluating Skin Reactions presented in Appendix A

Body weights: recorded on gestation days 0 (provided by supplier), 5, 6, 9, 12, 15, 18, 24, 27 and 29. Body weight changes were calculated for the following gestation intervals: 0-5, 5-6, 6-9, 9-12, 12-15, 15-18, 18-24, 24-27, 27-29, 24-29, 6-18, and 0-29. Also corrected maternal body weight change (body weight change for gestation days 0-29 minus gravid uterine weight) was calculated

Feed consumption: recorded daily on gestation days 6 to 29

Unscheduled death and euthanasia: animals that aborted, delivered prematurely, were found dead or euthanized for humane reasons during the study (*i.v.*, injection of sodium pentobarbital via the marginal ear vein) were subjected to a gross necropsy examination.

Scheduled euthanasia and cesarean: females euthanized on gestation day 29 (*i.v.*, injection of sodium pentobarbital via the marginal ear vein) and subjected to morphological examination. The thoracic, abdominal and pelvic cavities were opened and the viscera examined. Abnormalities were recorded and internal gross lesions were retained in 10% neutral buffered formalin for possible microscopic examination. The uterus was weighed (gravid weight only), examined externally and opened for internal examination. The number of viable and nonviable fetuses and early and late resorptions were recorded as were the number of corpora lutea.

Fetal morphological examinations: fetuses were examined for external and internal (visceral) or skeletal abnormalities. Findings were classified as malformations or developmental variations based on the severity of the anatomical changes and their potential for interference with normal organ and/or body functions. Viable fetuses were sexed, weighed, and euthanized by intrathoracic injection with sodium pentobarbital.

Each fetus was dissected, eviscerated, skinned, and fixed 95% isopropyl alcohol. Following fixation, the fetuses were macerated in a 1 to 2% potassium hydroxide solution, and stained with Alizarin Red S for skeletal examination.

Results

Maternal survival and pregnancy status: not all females survived to scheduled cesarean section. There were six 50 mg/kg/dose group females that were either euthanized for humane reasons, found dead or euthanized aborted; three 150 mg/kg/dose group females were either euthanized aborted or found dead; and three 300 mg/kg/dose group females were either euthanized due to premature delivery or euthanized aborted. Of these unscheduled events, all but the two 50 mg/kg/dose females found dead occurred after completion of dosing on gestation day 18. The pregnancy rate for the females that survived to scheduled euthanasia was as follows: 100% in the control, 50 and 150 mg/kg/dose groups, and 94.1% in the 300 mg/kg/dose groups. At cesarean section, there were 20 control females, 14 group 2 (50 mg/kg/dose) females, 17 group 3 (150 mg/kg/dose) females and 17 group 4 (300 mg/kg/dose) females available for examination. In spite of the number of deaths and abortions that were seen in the treatment groups (2-4), there were enough litters available to provide for adequate evaluation.

Clinical observations: ocular discharge, reddened eyelids, scabs, hairloss, swelling, urine stain, fecal stain, few feces, feces small in size, no feces, reddish colored fluid in cage/tray, soft stools, reddish vaginal discharge and diarrhea were observed in the control and all treatment groups.

Dermal observations: dermal findings were observed for females in the control and each test article-treated group. The severity of findings appeared to be slightly greater in the 300 mg/kg/dose group when compared to the control, 50 and 150 mg/kg/dose groups. Dermal findings throughout the groups included grade 1 erythema (very slight, barely perceptible), to maximized grade 4 erythema (notable dermal lesions); grade 1 edema (very slight, barely perceptible) to grade 3 edema (moderate, raised approximately 1 mm); grade 1 eschar (focal and/or pinpoint areas up to 10% of the test site), to grade 4 eschar (>50% of the test site) and desquamation.

Body weights, weight changes, gravid uterine weight/net body weight change: There were no statistically significant differences in mean body weights or body weight changes noted during the study for the test article-treated groups as compared to the controls. Mean maternal body weight change (gestation day 29 body weight minus gestation day 0 body weight) and mean gravid uterine weight of females in the test article-treated groups were higher compared to controls. Mean corrected maternal body weight change (maternal body weight change minus gravid uterine weight) was decreased in the controls as compared to the test article-treated animals. However, these changes were not statistically significant.

Feed consumption: there were no statistically significant changes noted in mean feed consumption (grams/animal/day) between the test article-treated and control animals during the course of the study.

Maternal necropsy findings: maternal necropsy revealed one 300 mg/kg/dose female to be nongravid. Otherwise there were no toxicologically meaningful findings in the test article-treated females.

Cesarean section findings: there were 19 control females, 14 group 2 (50 mg/kg/dose) females, 17 group 3 (150 mg/kg/dose) females and 16 group 4 (300 mg/kg/dose) females with viable fetuses at scheduled cesarean section. Only one control female was found with no viable fetuses. All cesarean section parameters evaluated for the surviving females, including the mean number of corpora lutea, implantation sites, preimplantation loss, live fetuses, postimplantation loss, early or late resorptions and mean fetal body weights, were comparable between the control and test article-treated groups.

Fetal morphological evaluations: No statistically significant or toxicologically meaningful differences in fetal external, visceral and skeletal malformations or variations were noted in the test article-treated groups as compared to the controls. A low incidence of malformations and variations known to occur spontaneously was observed throughout the groups, including controls and was within the historical control range. Fetal malformations only noted in the test article-treated litters included multiple anomalies (one 300 mg/kg/dose fetus), gastroschisis (one 300 mg/kg/dose fetus), flexed paw (two 300 mg/kg/dose fetuses in two litters), hydrocephaly (one 50 mg/kg/dose fetus), interventricular septal defect (one 300 mg/kg/dose fetus), heart and great vessel anomaly (one 300 mg/kg/dose fetus), frontal bone fusion (one 300 mg/kg/dose fetus) vertebral anomaly with/without rib anomaly (one 50 mg/kg/dose fetus and one 150 mg/kg/dose fetus), centrum anomaly (one 50 mg/kg/dose fetus), spina bifida occulta (one 300 mg/kg/dose fetus) and caudal vertebra(e) malaligned severe (one 300 mg/kg/dose).

Conclusion: Based on the results of this study, a dosage level of 150 mg/kg/dose was considered to be the NOAEL for maternal toxicity and a dosage level of 300 mg/kg/dose was considered to be the NOAEL for developmental toxicity in this study following twice daily dermal administration of isopropyl myristate from gestation day 6 to gestation day 18.

Prenatal and postnatal development

Study title: Subcutaneous developmental and perinatal/postnatal reproduction toxicity study of clobetasol propionate in rats, including a postnatal behavioral/functional evaluation

Key study findings: On the basis of these data, the maternal NOEL for clobetasol propionate was less than 12.5 µg/kg/day. The 12.5, 25, and 50 µg/kg/day dosages caused reduced body weight gain and feed consumption during the gestation period, with reduced body weights at all levels and reduced feed consumption at 50 µg/kg/day continuing through the lactation period. The reproductive NOEL in the dams was 25 µg/kg/day; one dam in the 50 µg/kg/day dosage group had a prolonged, uncompleted delivery. The NOAEL for viability and growth in the offspring was 12.5 µg/kg/day.

Laboratory Study no.: 2304-002

Sponsor Study no.: 0215-S2.R-18-03

Volume #, and page #: electronic document

Conducting laboratory and location: _____**Date of study initiation:** November 25, 2003**GLP compliance:** yes**QA reports:** yes (x) no ()**Drug, lot #, and % purity:** clobetasol propionate, lot # 7817/MI, purity _____**Methods**

Doses: 0, 12.5, 25, 50 µg/kg/day

Species/strain: rat _____ CD®(SD)IGS BR VAF/Plus® (female)

Number/sex/group: 25 females/group

Route, formulation, volume, and infusion rate: subcutaneous, 0.04% Tween® 80 in saline, 2 mL/kg/dose, twice daily

Satellite groups used for toxicokinetics:

Study design:

Female rats received a twice daily subcutaneous dose of vehicle or test article on gestation day 7 (DG 7) through lactation day 25 (DL 25) or DG 24 (rats that did not deliver a litter). Each dam and delivered litter were housed in a common nesting box until DL 25. Each litter was housed in a common nesting box on DLs 26 through 28. After day 28 postpartum, F1 generation rats were individually housed and at ~ 90 days they were assigned to a 21-day cohabitation. Male rats were sacrificed after cohabitation and female rats were sacrificed on DG 21.

F₀ Generation Rats

Dosage Group	Dosage ^a (µg/kg/day)	Dosage (µg/kg/dose)	Concentration (µg/mL)	Dosage Volume (mL/kg/dose)	Number of Rats
1	0 (Vehicle)	0 (Vehicle)	0	2	25
2	12.5	6.25	3.125	2	25
3	25	12.5	6.25	2	25
4	50	25	12.5	2	25

^aDosage calculations were adjusted for the _____ purity of the test article.**F₁ Generation Rats**

Dosage Group	Maternal Dosage (µg/kg/day)	Number of Rats per Sex
1	0 (Vehicle)	25
2	12.5	25
3	25	25
4	50	25

Results

F₀ in-life: No drug-related deaths occurred. One mid-dose dam was found dead on DL 22. This death was not considered drug-related because it was not dosage dependent and probably related to kidney lesions which are commonly seen in this species/strain of rats. No drug-related clinical observations were noted. Body weight gains were significantly

reduced in the 12.5, 25, and 50 µg/kg/day dosage groups for the entire gestation dosage period (DGs 7 to 20). Body weights were significantly reduced in all three treated groups on DGs 8 through 20. Maternal body weights continued to be significantly reduced in the three treated groups throughout the lactation period (DLs 1 through 25); however body weight gains were significantly reduced in the 25 and 50 µg/kg/day dosage groups only on DLs 7 to 10. One pregnant dam in the 50 µg/kg/day dosage group began to deliver a litter on DG 23 but did not complete delivery by DG 28 and was sacrificed. The dystocia in this dam was considered a possible effect of the test article at the highest dosage. Stillbirths were significantly increased in the 25 and 50 µg/kg/day dosage groups. Pup mortality was significantly increased in the 50 µg/kg/day dosage group on DLs 1 through 7. One dam in the 50 µg/kg/day dosage group had all pups die by DL 7.

F₀ necropsy: All necropsy observations were considered unrelated to clobetasol propionate.

F₁ physical development: Pup body weights were significantly reduced in the 12.5, 25, and 50 µg/kg/day dosage groups on DL 1, in the 25 and 50 µg/kg/day dosage groups on DL 7 and in the 50 µg/kg/day dosage group on DLs 14 and 28. Umbilical hernia occurred in 1, 4, and 12 pups in 1, 3, and 3 litters in the 12.5, 25, and 50 µg/kg/day dosage groups. All F₁ generation male and female rats appeared normal at necropsy.

F₁ behavioral evaluation: There were no statistically significant or biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F₁ generation male and female rats, as evaluated by performance in a passive avoidance paradigm or watermaze.

F₁ reproduction: There were no statistically or biologically important effects on the mating and fertility parameters evaluated in the F₁ generation male and female rats.

Conclusions:

On the basis of these data, the maternal NOEL for clobetasol propionate was less than 12.5 µg/kg/day. The 12.5, 25, and 50 µg/kg/day dosages caused reduced body weight gain and feed consumption during the gestation period, with reduced body weights at all levels and reduced feed consumption at 50 µg/kg/day continuing through the lactation period. The reproductive NOEL in the dams was 25 µg/kg/day; one dam in the 50 µg/kg/day dosage group had a prolonged, uncompleted delivery. The NOAEL for viability and growth in the offspring was 12.5 µg/kg/day.

2.6.6.7 Local tolerance

No local tolerance studies were included in this NDA submission.

2.6.6.8 Special toxicology studies

No special toxicology studies were included in this NDA submission.

2.6.6.9 Discussion and Conclusions

In SN049 to IND 62,543 the sponsor indicates that the following studies have been initiated:

RDS.03.SPR.12428: A 90-day dermal (skin painting) toxicity study in rats with clobetasol lotion and Clobetasol shampoo vehicle.

RDS.03.SPR.12465: A 90-day dermal (skin painting) toxicity study in rats with clobetasol lotion and Clobetasol spray.

RDS.03.SPR.12427: A 13-week topical range-finding study of clobetasol propionate lotion and clobetasol propionate spray placebo in hairless mice, with simulated sunlight.

Final reports are expected in September 2005, October 2005, and August 2005 for study # 12428, 12465, and 12427, respectively.

2.6.6.10 Tables and Figures

This section is not applicable.

2.6.7 TOXICOLOGY TABULATED SUMMARY

This section is not applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Based on the nonclinical data available for clobetasol propionate NDA 21-835 is approvable from a pharmacological/toxicological perspective.

Unresolved toxicology issues (if any): There are no unresolved toxicology issues for NDA 21-835, at this time.

Recommendations: The sponsor-proposed labeling has been revised and the recommended nonclinical portions are provided in the next section. Although the data for isopropyl myristate are important for establishing safety, data related to excipients do not generally appear in the label and so the isopropyl myristate data have been removed from the suggested labeling.

Suggested labeling:

3 Page(s) Withheld

 Trade Secret / Confidential

✓ Draft Labeling

 Deliberative Process

Withheld Track Number: Pharm/Tox-

1



Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

References

Blevins RD and Taylor DE. Mutagenicity screening of twenty-five cosmetic ingredients with the Salmonella/microsome test. *J. Environ. Sci. Health* (1982) A17(2):217-239.

Irie D, *et al.* General pharmacology of clobetasol 17-propionate (SN-201) a new synthetic corticosteroid. *J. Med. Soc. Toho, Japan* (1975) 22 (3-4):318-331.

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/s/

Jill Merrill
9/8/2005 11:00:44 AM
PHARMACOLOGIST

corrected as per your comments

Paul Brown
9/8/2005 02:13:09 PM
PHARMACOLOGIST

Memorandum

To: NDA 21-835

From: Jill C Merrill, Ph.D.

Re:

Drug: CLOBEX Spray 0.05%

Indication: patients with psoriasis

Sponsor: Dow Pharmaceutical Sciences

Review date: October 24, 2005

Comments to be conveyed to the sponsor:

We remind you of your postmarketing study commitments in your submission dated March 11, 2005 (SN0049 to IND 62,543). These commitments with a recommended timeline are listed below.

1. The applicant commits to conduct a dermal carcinogenicity study with CLOBEX (clobetasol propionate) Spray 0.05%.

90-day dose range-finding study report:	December 16, 2005
Study protocol submission:	March 16, 2006
Study start date:	November 16, 2006
Final report submission:	November 16, 2009

2. The applicant commits to conducting a study to determine the photoco-carcinogenic potential of CLOBEX (clobetasol propionate) Spray 0.05%.

90-day dose range-finding study report:	December 16, 2005
Study protocol submission:	March 16, 2006
Study start date:	November 16, 2006
Final report submission:	November 16, 2009

Jill C. Merrill
Reviewing Toxicologist

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/s/

Jill Merrill
10/24/2005 10:09:00 AM
PHARMACOLOGIST

phase 4 commitments and recommended timeline

Paul Brown
10/24/2005 10:22:45 AM
PHARMACOLOGIST