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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

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65,369)
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Product: VELTIN Gel
Indication: Acne vulgaris
Applicant: Stiefel Laboratories Inc.

Reviewer's note: The original IND was filed by Connetics Corporation (Connetics). Connetics was acquired by Stiefel on December 28, 2006. The names Connetics and Stiefel are considered synonymous in this document.

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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	3
1.1	RECOMMENDATIONS	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	6
2	DRUG INFORMATION	7
3	STUDIES SUBMITTED.....	10
4	PHARMACOLOGY	12
4.1	PRIMARY PHARMACOLOGY	12
4.2	SECONDARY PHARMACOLOGY	13
4.3	SAFETY PHARMACOLOGY	13
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	14
5.1	PK/ADME.....	14
5.2	TOXICOKINETICS	15
6	GENERAL TOXICOLOGY.....	15
6.1	SINGLE-DOSE TOXICITY	15
6.2	REPEAT-DOSE TOXICITY	15
7	GENETIC TOXICOLOGY	25
8	CARCINOGENICITY	26
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	36
10	SPECIAL TOXICOLOGY STUDIES.....	37
11	INTEGRATED SUMMARY AND SAFETY EVALUATION.....	38
12	APPENDIX/ATTACHMENTS.....	40

1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

Veltin Gel for the treatment of acne vulgaris is approvable from a pharmacological/toxicological perspective.

1.1.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended at this time.

1.1.3 Labeling

A number of changes were recommended to nonclinical portions of the sponsor's proposed labeling. These changes are provided below and detailed in the Appendix (Attachment #6).

8.1 Pregnancy

Pregnancy Category C.

There are no well-controlled studies in pregnant women treated with VELTIN Gel. VELTIN Gel should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

A limit teratology study performed in Sprague Dawley rats treated topically with VELTIN Gel or 0.025% tretinoin gel at a dose of 2 mL/kg during gestation days 6 to 15 did not result in teratogenic effects. Although no systemic levels of tretinoin were detected, craniofacial and heart abnormalities were described in drug-treated groups. These abnormalities are consistent with retinoid effects and occurred at 16 times the recommended clinical dose assuming 100% absorption and based on body surface area comparison. For purposes of comparison of the animal exposure to human exposure, the recommended clinical dose is defined as 1 g of VELTIN Gel applied daily to a 50 kg person.

Clindamycin

Reproductive developmental toxicity studies performed in rats and mice using oral doses of clindamycin up to 600 mg/kg/day (480 and 240 times the recommended clinical dose based on body surface area comparison, respectively) or subcutaneous doses of clindamycin up to 180 mg/kg/day (140 and 70 times the recommended clinical dose based on body surface area comparison, respectively) revealed no evidence of teratogenicity.

Tretinoin

Oral tretinoin has been shown to be teratogenic in mice, rats, hamsters, rabbits, and primates. It was teratogenic and fetotoxic in Wistar rats when given orally at doses greater than 1 mg/kg/day (32 times the recommended clinical dose based on body surface area comparison). However, variations in teratogenic doses among various strains of rats have been reported. In the cynomolgous monkey, a species in which tretinoin metabolism is closer to humans than in other species examined, fetal malformations were reported at oral doses of 10 mg/kg/day or greater, but none were observed at 5 mg/kg/day (324 times the recommended clinical dose based on body surface area comparison), although increased skeletal variations were observed at all doses. Dose-related teratogenic effects and increased abortion rates were reported in pigtail macaques.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Clindamycin

[See Microbiology (12.4).]

Tretinoin

Although the exact mode of action of tretinoin is unknown, current evidence suggests that topical tretinoin decreases cohesiveness of follicular epithelial cells with decreased microcomedone formation. Additionally, tretinoin stimulates mitotic activity and increased turnover of follicular epithelial cells causing extrusion of the comedones.

12.4 Microbiology

Clindamycin binds to the 50S ribosomal subunit of susceptible bacteria and prevents elongation of peptide chains by interfering with peptidyl transfer, thereby suppressing protein synthesis. Clindamycin has been shown to have *in vitro* activity against *Propionibacterium acnes* (*P. acnes*), an organism that has been associated with acne vulgaris; however, the clinical significance of this activity against *P. acnes* was not examined in clinical studies with VELTIN Gel. *P. acnes* resistance to clindamycin has been documented. Resistance to clindamycin is often associated with resistance to erythromycin.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of VELTIN Gel or the effect of VELTIN Gel on fertility.

VELTIN Gel was negative for mutagenic potential when evaluated in an *in vitro* Ames *Salmonella* reversion assay. VELTIN Gel was equivocal for clastogenic

potential in the absence of metabolic activation when tested in an *in vitro* chromosomal aberration assay.

Clindamycin

Once daily dermal administration of 1% clindamycin as clindamycin phosphate in the VELTIN Gel vehicle (32 mg/kg/day, 13 times the recommended clinical dose based on body surface area comparison) to mice for up to 2 years did not produce evidence of tumorigenicity.

Fertility studies in rats treated orally with up to 300 mg/kg/day of clindamycin (240 times the recommended clinical dose based on body surface area comparison) revealed no effects on fertility or mating ability.

Tretinoin

In two independent mouse studies where tretinoin was administered topically (0.025% or 0.1%) three times per week for up to two years no carcinogenicity was observed, with maximum effects of dermal amyloidosis. However, in a dermal carcinogenicity study in mice, tretinoin applied at a dose of 5.1 µg (1.4 times the recommended clinical dose based on body surface area comparison) three times per week for 20 weeks acted as a weak promoter of skin tumor formation following a single application of dimethylbenz[*a*]anthracene (DMBA).

In a study in female SENCAR mice, papillomas were induced by topical exposure to DMBA followed by promotion with 12-O-tetradecanoyl-phorbol 13-acetate or mezerein for up to 20 weeks. Topical application of tretinoin prior to each application of promoting agent resulted in a reduction in the number of papillomas per mouse. However, papillomas resistant to topical tretinoin suppression were at higher risk for pre-malignant progression.

Tretinoin has been shown to enhance photoco-carcinogenicity in properly performed specific studies, employing concurrent or intercurrent exposure to tretinoin and UV radiation. The photoco-carcinogenic potential of the clindamycin tretinoin combination is unknown. Although the significance of these studies to humans is not clear, patients should avoid exposure to sun.

The genotoxic potential of tretinoin was evaluated in an *in vitro* Ames *Salmonella* reversion test and an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells. Both tests were negative.

In oral fertility studies in rats treated with tretinoin, the no-observed-effect-level was 2 mg/kg/day (64 times the recommended clinical dose based on body surface area comparison).

1.2 Brief Discussion of Nonclinical Findings

The sponsor has conducted subchronic toxicity tests in both a rodent and nonrodent model on the previous formulation, Velac Gel (1% clindamycin, 0.025% tretinoin). Velac Gel was determined to be nonmutagenic in an *in vitro* Ames *Salmonella* reversion test and was tested without effect in a limit teratology test in rodents. However, Velac Gel was determined to have equivocal clastogenic activity in a chromosome aberration assay and when tested in a 26-week dermal carcinogenicity study in Tg.AC mice, the vehicle alone caused a statistically significant increased incidence of skin papillomas compared to the untreated controls. Clindamycin in the Velac Gel vehicle caused further significant dose-related increases in papillomas relative to the vehicle controls and untreated animals. These nonclinical findings formed the basis of an action letter (June 10, 2005) in which the drug was not approved. The sponsor has subsequently reformulated Velac Gel, (b) (4) and (b) (4) of polyoxyethylene 4 monolauryl ether (POE 4) (b) (4) was necessary (b) (4). The reformulated clindamycin 1% - tretinoin 0.025% gel (CT Gel) contains the same concentration of active ingredients as found in the previous formulation and, with the exception of the aforementioned (b) (4) (b) (4) POE 4, is identical to Velac Gel.

Given the similarities between Velac Gel and CT Gel, the pharmacology, pharmacokinetics and toxicology are expected to be similar and the supportive nonclinical information previously reviewed for Velac Gel (chronic toxicology, genetic toxicology, reproductive and developmental toxicology) stands in support of the reformulated CT Gel. The nonclinical deficiencies noted in the action letter, specifically the positive carcinogenicity signal in the Tg.AC mouse dermal carcinogenicity model, have been addressed by reformulation. Clindamycin at the clinical concentration (1%) in the reformulated vehicle has been tested in a 2-year dermal carcinogenicity study (NPB00012; reviewed within) with adequate results. Consistent with the Agency's guidance (FDA Minutes of the Post-Action Meeting, September 15, 2005), the sponsor has obtained the right of reference to a 27-week dermal carcinogenicity study of clindamycin phosphate (0, 0.5, 1, and 2 %) in a different gel-formulation to provide evidence that clindamycin does not display carcinogenic potential across a range of concentrations bracketing the clinical concentration of the CT Gel product. Although the study did not indicate a carcinogenic concern for clindamycin, technical inadequacies precluded further evaluation of this study and the results will not appear in the label (see Section 3.2 Studies Not Reviewed). The testing of the clinical clindamycin concentration in the vehicle and the long history of safe use of clindamycin are considered to be sufficient to support its use in the CT Gel. The sponsor was previously informed that literature information describing both the carcinogenic potential of tretinoin and the limitations of this information could appear in the drug product label (IND 65,369, 2-6-03).

Since the sponsor has relied on literature data to provide pivotal toxicology information to support the safety of clindamycin and tretinoin (refer to Section 2.5, Regulatory Background) this NDA is a 505(b)(2) regulatory submission.

2 Drug Information

2.1 Drug

Veltin Gel (proposed name; clindamycin phosphate/tretinoin, 1.2% / 0.025%)

2.1.1 CAS Registry Number

Clindamycin phosphate: 24729-96-2

Tretinoin: 302-79-4

2.1.2 Generic Name

Clindamycin phosphate and tretinoin (all *trans*-retinoic acid) gel

2.1.3 Code Name

Not provided.

2.1.4 Chemical Name

Clindamycin phosphate: methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo*- α -D-*galacto*-octopyranoside 2-(dihydrogen phosphate)

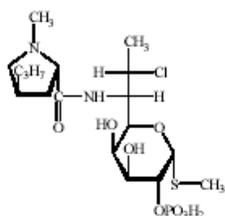
Tretinoin: *all-E* 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid

2.1.5 Molecular Formula/Molecular Weight

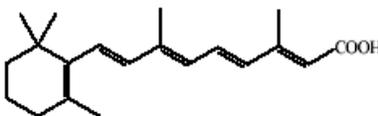
Clindamycin phosphate: $C_{18}H_{34}ClN_2O_8PS$ / MW = 504.97

Tretinoin: $C_{20}H_{28}O_2$ / MW = 300.44

2.1.6 Structure



Clindamycin phosphate



Tretinoin

2.1.7 Pharmacologic class

Clindamycin – lincosamide antibiotic
Tretinoin - retinoid

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 65,369, Velac Gel, Connetics

(b) (4)

2.3 Clinical Formulation

2.3.1 Drug Formulation

CT Gel is what was used for the 2-year dermal mouse carcinogenicity study.
Composition of Veltin™, Velac Gel and CT Gel (minus tretinoin)

Formulations	Veltin™	Velac Gel	CT Gel
Active ingredients	Amount (% w/w)		
Clindamycin phosphate, USP	1.00	1.00	1.00
Tretinoin	0.025	0.025	0.00
Inactive ingredients			
Butylated hydroxytoluene, NF	(b) (4)		
Carbomer 940 (b) (4), NF			
Citric acid, USP			
Edetate disodium, USP			
Methylparaben, NF			
Polyoxyethylene 4 Monolaurate Ether (POE 4) (b) (4)			
Propylene Glycol, USP			
Tromethamine, USP			
Purified water, USP			
Total:			

Note: Although the clinical formulation also contains tretinoin (0.025%), only clindamycin phosphate was assessed in the 2-year mouse dermal carcinogenicity study. The sponsor will rely on literature data to address the carcinogenic potential of tretinoin.

2.3.2 Comments on Novel Excipients

(b) (4) polyoxyethylene 4 monolaurate ether (POE 4; (b) (4)) were both components of the prototype formulation, Velac Gel. As such these excipients were present in the nonclinical studies conducted

with Velac Gel (90-Day dermal toxicity study in rats with clindamycin, 3600.7; A 13-week dermal toxicity study in Hanford minipigs with Velac Gel, 3600.10; Limit teratology study in the rat by the dermal route, 94/BSM004/0576; 26-Week dermal carcinogenicity study in Tg.AC mice, AA81EW.7D8T.BTL; numerous *in vitro* genetic toxicology studies). They were both present in the vehicle which caused an increased incidence of skin papillomas compared to the untreated control in the mouse dermal carcinogenicity study which formed the basis for the nonapprovable letter for the original NDA submission (6-10-05). The sponsor has since reformulated the drug product (b) (4)

(b) (4) POE 4 (b) (4) as a component of CT Gel, has been tested in a 2-year mouse dermal carcinogenicity study (reviewed in the appropriate section below) with adequate results.

POE 4 is listed in the CTFA International Cosmetic Ingredient Dictionary as a synonym for laureth-4 (CAS # 5274-68-0). Laureth -4 is not listed in the FDA Inactive Ingredient Database (FDA IID). However, laureth-4 is a surfactant and emulsifying agent produced by reacting ethylene oxide with lauryl alcohol and as such belongs to a generic class of polyoxyethers of lauryl alcohol (CAS # 9002-92-0). Included in this class are other laureths, including laureth-23 (which is listed in the FDA IID). The numerical designation refers to the average number of repeating ethylene oxide units in the molecule. The Cosmetic Ingredient Review expert panel has conducted research on two of the many laureths (laureth-4 and laureth-23) and made an inclusive approval of all laureths for the use in cosmetics at up to 6% (originally reviewed in Journal of American College of Toxicology 2(7):1-15, 1983; confirmed in International Journal of Toxicology 24(S1):59-63, 2005). Given its relationship to the generic class of polyoxyethers of lauryl alcohol and the nonclinical testing conducted as a component of CT Gel in the 2-year dermal carcinogenicity study, no further testing is recommended.

2.3.3 Comments on Impurities/Degradants of Concern

Clindamycin is a synthetic derivative of the natural antibiotic lincomycin. The sponsor has set a specification for the maximum amount of (b) (4) the drug product. (b) (4) is a tretinoin-related substance that is a drug substance impurity, a process impurity and a CT Gel drug product degradation product. Use of (b) (4) (b) (4) is associated with exposure to (b) (4) a (b) (4) degradation product. The sponsor proposes setting a specification limit for (b) (4) the (b) (4) concentration. Pharmacology/toxicology has informed the Chemistry reviewer, Dr. Shulin Ding, that these levels are acceptable in memoranda which are appended to this review.

2.4 Proposed Clinical Population and Dosing Regimen

1 g Veltin Gel to be applied once daily by patients ≥12 years of age with acne vulgaris. A 12-week treatment duration was evaluated in Phase 3 clinical studies.

2.5 Regulatory Background

This is a 505(b)(2) regulatory submission. It is supported by studies owned by the sponsor and by referring to the published literature (for fertility and peri-/postnatal development information for clindamycin and tretinoin and for tretinoin carcinogenicity information) for which the sponsor does not have the rights to the underlying data. An NDA for an earlier formulation received a non-approvable letter based on a positive response to the vehicle alone in a Tg.AC mouse dermal carcinogenicity study. The sponsor subsequently modified the vehicle by (b) (4) polyoxyethylene 4 monolaurate ether.

3 Studies Submitted

3.1 Studies Reviewed

CT Gel – W0265: *In vitro* human skin penetration (Study No. 2006-171-JL)

A 35-day toxicity study of clindamycin administered by the dermal (skin painting) route to rats (Study No. NPB00013)

A 90-day toxicity study of clindamycin administered by the dermal (skin painting) route to rats with a 21-day interim sacrifice interval (Study No. NPB00019)

A two-year carcinogenicity study of clindamycin administered dermally (skin painting) to mice (NPB00012)

3.2 Studies Not Reviewed

27-Week Dermal Carcinogenicity Study in Tg.AC Mice ((b) (4) Study No. AA56ZZ.7D8T.BTL)

The sponsor has the right to reference this Tg.AC mouse study which evaluated the tumorigenic potential of clindamycin (0.5%, 1.0%, 2.0%) in a topical gel containing 17.3% acrylate copolymer. The incidence of mice that developed skin tumors (latent or actual papillomas) at the site of application (SOA) was 1/20, 1/25, 19/19, 0/22, 1/25, and 2/23 males; and 1/20, 1/23, 19/20, 1/22, 0/23, and 1/21 females (shelf control, vehicle control, 50 µg TPA in 17.3% acrylate copolymer, 25 mg/kg, 50 mg/kg, 100 mg/kg clindamycin phosphate, respectively). The incidence of animals that developed skin tumors at a site other than the application site (NSOA) included 1/20, 1/25, 0/19, 0/22, 4/25, and 2/23 male mice; and 0/20, 3/23, 0/20, 6/22, 7/23, and 4/21 female mice from the same respective groups. Therefore, it is concluded that daily administration of clindamycin in a topical gel containing 17.3% acrylate copolymer for 27 weeks did not

increase the incidence of dermal tumors at the site of application in hemizygous Tg.AC transgenic mice. However, the study was confounded by the reallocation of previously randomized females with more severe dermal abrasions to the positive control group. It is noteworthy that the positive control in this study, 50 µg TPA in acrylate vehicle is 40-fold the amount of TPA required in the same model when acetone is used as the vehicle. Randomization of the animals assigned to experimental groups is necessary to ensure that underlying variables do not skew the data for each of the groups and is a fundamental aspect of experimental design. As such the study will not be further reviewed here.

3.3 Previous Reviews Referenced

Each of the following reviews contains studies used to support the approval of the current drug product. In addition, pivotal studies are listed individually under the document in which they are reviewed.

IND 65,369, Velac Gel; review # 1; reviewed by Dr. Paul Brown, 02-06-03.

An investigation in the rabbit of the comedolytic activity of a gel containing tretinoin 0.025% and clindamycin phosphate 1.2% (Study no. 92014).

Evaluation of the mutagenic activity of clindamycin-tretinoin gel in the Ames *salmonella*/microsome test (with independent repeat) (Study No. 094793)

Evaluation of the mutagenic activity of tretinoin gel in the Ames *salmonella*/microsome test (with independent repeat) (Study No. 094771)

Evaluation of the mutagenic activity of vehiculum clindamycin-tretinoin gel in the Ames *salmonella*/microsome test (with independent repeat) (Study No. 094782)

In vitro assessment of the clastogenic activity of clindamycin tretinoin gel in cultured human lymphocytes (Study No. 94/BSM004/0576)

Evaluation of the ability of tretinoin to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) (Study No. 187537)

Evaluation of the ability of clindamycin phosphate to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) (Study No. 187548)

Salmonella- Escherichia coli/mammalian-microsome reverse mutation assay with polyoxyethylene 4 monolauryl ether (Study No. 24043-0-409OECD)

Chromosome aberrations in Chinese hamster ovary (CHO) cells with polyoxyethylene 4 monolauryl ether (Study No. 24043-0-437OECD)

Limit teratology study in the rat by the dermal route (Study No. 93/BSM001/1174)

IND 65,369, Velac Gel; review # 2; reviewed by Dr. Paul Brown, 03-11-03.

IND 65,369, Velac Gel; review # 3; reviewed by Dr. Paul Brown, 01-16-04.

Evaluation of the mutagenic activity of clindamycin phosphate in the *salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (Study No. 187559)

IND 65,369, Velac Gel; review # 4; reviewed by Dr. Paul Brown, 06-24-04.

IND 65,369, Velac Gel; review # 5; reviewed by Dr. Jill Merrill, 09-19-07

Report on the maximum feasible volume of study drug that can be applied dermally to young CD-1 mice (CNCT 001)

A 90-day toxicity study of clindamycin administered by the dermal (skin painting) route to mice with a 21-day interim sacrifice interval (NPB00018)

NDA 50-803, Velac Gel (clindamycin 1%, tretinoin 0.025%), reviewed by Dr. Jill Merrill, 05-16-05.

A 90-day dermal toxicity study in rats with clindamycin (Study No. 3600.7)

A 13-week dermal toxicity study in Hanford minipigs with Velac® Gel (Study No. 3600.10)

26-Week dermal carcinogenicity study in Tg.AC mice (Study No. AA81EW.7D8T.BTL)

4 Pharmacology

4.1 Primary Pharmacology

Clindamycin phosphate:

Clindamycin binds to the 50S subunit of bacterial ribosomes and thereby interferes with bacterial protein synthesis. Clindamycin is primarily bacteriostatic. Clindamycin is active against gram positive cocci and most anaerobic gram negative organisms. Its activity against the anaerobe *Propionibacterium acnes* may account for its effectiveness in the treatment of acne vulgaris. Clindamycin decreases the percentage of skin surface free fatty acids, and decreases the number of *Propionibacterium acnes* in comedones.

Tretinoin:

Tretinoin, also known as all- trans- retinoic acid, is a member of the retinoid family of

compounds and is an endogenous metabolite of vitamin A. Tretinoin, like other retinoids, regulates gene transcription through interaction with intracellular retinoic acid receptors. Retinoids impact a variety of cellular and physiologic processes. Although the exact mechanism by which retinoids are beneficial in acne is unknown, current evidence suggests that topical tretinoin decreases cohesiveness of follicular epithelial cells and decreases microcomedone formation. Additionally, tretinoin stimulates mitotic activity and increases turnover of follicular epithelial cells causing the stratum corneum to become thinner and extrude comedones. Tretinoin has also been shown to suppress sebum gland activity, most probably by virtue of its antiproliferative effect on sebum producing cells.

The combination of clindamycin and tretinoin in Veltin Gel potentially offers comedolytic, anti-bacterial, and anti-inflammatory pharmacological properties that could be effective in the treatment of acne.

The sponsor has submitted a study entitled "An investigation in the rabbit of the comedolytic activity of a gel containing tretinoin 0.025% and clindamycin phosphate 1.2%" (Study no. 92014). This study was previously reviewed (IND 65,369 2-06-2003) by Dr. Paul Brown. A summary of this previously reviewed study appears below:

This study addresses the comedolytic activity of three gels. One is a gel containing tretinoin (0.025%) and clindamycin phosphate (1.2%) in a base that is slightly different than the proposed product. The other two gels contain 0.025% tretinoin only; one in the same base as the clindamycin/tretinoin gel and the other is a reference product. In this study rabbits were treated with coal tar on both ears for two weeks to induce comedones. Then only the left ear was treated with one of the gels. The animals were then sacrificed and the epidermis and dermis of the ears were separated and comedones were counted. The clindamycin/tretinoin gel caused a 53% decrease; tretinoin in the same base caused a 42% decrease and the reference tretinoin gel a 14% decrease in comedones compared to the corresponding right ears. The submitted study suggests that a gel containing clindamycin and tretinoin is comedolytic. The study also suggests that the combination of clindamycin and tretinoin may be more comedolytic than tretinoin alone.

4.2 Secondary Pharmacology

No studies to address secondary pharmacology were included in this submission.

4.3 Safety Pharmacology

Although the sponsor has not submitted nonclinical safety pharmacology studies, transient neuromuscular blockade is a recognized side effect of clinical use of antibiotics, including clindamycin. Extensive analysis of the blockade has led to the conclusion that clindamycin exerts its main effect post-synaptically at the neuromuscular junction, with a minor component of the inhibition also occurring pre-synaptically. The basis for these effects has been determined to be the lipophilic nature of the structure of

clindamycin, which allows the molecule to compete with calcium for entry into nerve terminals, resulting in interference with nerve transmission. The effect of clindamycin on neuromuscular transmission has potential relevance to gastrointestinal smooth muscle function and the development of enterocolitis. However, because systemic exposure following topical application of clindamycin is low, it is not anticipated that patients receiving treatment with Veltin Gel will be affected.

Pharmacodynamic Drug Interactions

No studies to address pharmacodynamic drug interactions were included in this submission. However, VELTIN Gel should not be used in combination with photosensitizing drugs because of the possibility of tretinoin-augmented phototoxicity. Additionally patients should not expose VELTIN Gel-treated skin to certain compounds because of a possible interaction with tretinoin. These include concomitant topical medications, soaps/cosmetics with a drying effect, products with a high concentration of alcohol, astringents, spices, or lime. VELTIN Gel should be used with caution in patients receiving neuromuscular blocking agents because clindamycin has been shown to enhance their action.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The pharmacokinetic characteristics of both tretinoin and clindamycin by multiple routes of administration have been investigated for over three decades and are well characterized. ADME information available in the public domain indicates that systemic exposure to topical tretinoin or clindamycin is limited when compared to systemic levels observed following parenteral administration. It is also recognized that the presence of cutaneous lesions and the possibility of topical drug ingestion by study animals could produce greater systemic exposure than would be seen via strictly percutaneous routes. Despite these factors, systemic exposure following topical application was significantly lower in the majority of nonclinical studies when compared to other routes of administration.

In a limit teratology study conducted in rats no tretinoin was detected in the plasma of animals treated during days 6-15 of gestation with 2 mL/kg of the clindamycin/tretinoin gel (20 mg/kg tretinoin). The limit of detection was 2 ng/mL.

Please refer to the original NDA 50-803 review (05-16-05) for a more detailed discussion of the pharmacokinetic characteristics of clindamycin and tretinoin.

The sponsor has conducted a non-GLP *in vitro* study to compare the skin penetration profiles of tretinoin and clindamycin phosphate in Velac Gel, CT Gel, and CT Gel enriched with 5% clindamycin only (2006-171-JL). Test articles were applied on split-thickness skin sections from three different healthy human donors to capture *inter-*

individual variation in a temperature- and humidity-controlled diffusion chamber, designed to mimic *in vivo* conditions. Test articles were applied to four or five replicates per donor to capture *intra*-individual variation within each skin donor. The receptor fluid was collected hourly from 0 to 6 hours post application into scintillation vials, for subsequent analysis. At the end of the treatment period, the skin surface was washed and the skin was split into epidermis and dermis. All samples were analyzed for tretinoin and clindamycin phosphate using LC/MS/MS. There were no significant differences between Velac Gel or CT Gel in the delivery of clindamycin or tretinoin in the skin (epidermis and dermis) and through the skin over 6 hours. The CT Gel containing 5% clindamycin, resulted in a 2-3 fold increase in clindamycin in the epidermis and a trend for higher delivery of clindamycin through the skin over 6 hours compared to Velac Gel and CT Gel.

Reviewer's comment: Although the clindamycin concentration is the same in both the Velac Gel and the CT Gel (1%), the sponsor's clinical testing indicates an increased in vivo exposure to clindamycin from the CT Gel compared to the Velac Gel. However, the nonclinical testing that was done in support of CT gel was performed at sufficient excess to cover this increase in bioavailability (refer to memorandum #3, Appendix).

5.2 Toxicokinetics

Toxicokinetics were considered in the individual nonclinical studies.

6 General Toxicology

6.1 Single-Dose Toxicity

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

6.2 Repeat-Dose Toxicity

The sponsor has conducted a 90-day dermal toxicity study in rats (Study No. 3600.7, reviewed NDA 50-803, 5-16-2005) with clindamycin only in the Velac gel vehicle. Dermal administration of 1% and 5% clindamycin gel (0, 1, 3, and 5 mg/kg/day, 10 rats/sex/group) did not cause any remarkable dermal irritation or signs of systemic toxicity. Therefore, the NOAEL for males and females in this study was 5 mg/kg/day clindamycin. A 13-week dermal toxicity study in minipigs (Study No. 3600.10, reviewed NDA 50-803, 5-16-2005) was conducted with Velac Gel (0.025% tretinoin, 1% clindamycin), a 3-fold (0.075% tretinoin, 3% clindamycin) and a 5-fold (0.125% tretinoin, 5% clindamycin) enriched Velac gel formulation and an arm with 0.125% tretinoin only in Velac Gel vehicle (see study design table below). The 3x and 5x Velac Gels increased the local exposure to the clindamycin/tretinoin combination, whereas systemic exposure was increased by increasing the area of skin treated by 5x (the maximum feasible area of exposure, ~20% BSA). The study design provided below is taken from the original review:

Group	Total Velac Gel exposure	Test Material Formulation	Exposure Area	Clindamycin Dosage Level (mg/kg/day)	Tretinoin Dosage Level (mg/kg/day)	Formulation Dosage Level (mg/kg/day) ^b	Final Formulation Dose Volume (mL/kg/day)
1	0	0	5x	0.0	0.0	0	0.1
2	0	5x	5x	0.0	0.125	500	0.1
3	1x	1x	1x	0.2	0.005	20	0.02
4	15x	3x	5x	3.0 ^a	0.075	300	0.1
5	25x	5x	5x	5.0 ^a	0.125	500	0.1

^aAlthough the final study report describes the clindamycin dosage level for group 4 and 5 as 1.0 and 3.0 mg/kg/day, respectively, given that they represent 15x and 25x Velac Gel exposure then these values must be 3.0 (0.2 x 15 = 3.0) and 5.0 (0.2 x 25 = 5.0) mg/kg/day. Therefore the above table depicting the study design has been modified.

^bThis value depicts a theoretical formulation dosage level and as such represents a multiple of the exposure area times the formulation strength.

No evidence of systemic toxicity was noted at any dose level. Microscopic changes in the treated skin were observed at a similar incidence in the 500 mg/kg/day tretinoin group and the 20, 300 and 500 mg/kg/day Velac Gel groups. These changes were attributed to tretinoin and not clindamycin. Based on these data, the systemic NOEL was 500 mg/kg/day Velac Gel and the local/dermal NOEL could not be established.

Study title: A 35-day toxicity study of clindamycin administered by the dermal (skin painting) route to rats

Key study findings: Once daily dermal administration of CTG Vehicle and 1% Clindamycin in CTG Vehicle at 5, 10 and 15 mg/kg/day produced mild dermal irritation with focal eschar/ulceration during the first week of dosing. There was no notable difference in the dermal response between the vehicle and test article-treated groups. Tolerance to the administered dose appeared to occur during the next four weeks of dosing as dermal responses diminished (but did not completely resolve) by Day 36 of the study. The dermal response to 1% Clindamycin in Velac Gel (Group 5, comparative control) administered at 5 mg/kg/day was typically less severe relative to the CTG Vehicle and 1% Clindamycin in CTG Vehicle treated animals.

Study no.: NPB00013

Volume #, and page #: SDN47: vol 1, page 3 (IND 65,369)

Conducting laboratory and location:  (b) (4)

Date of study initiation: January 9, 2006

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

1.0% Clindamycin in CTG vehicle, Lot # 451-53, 98.5% of LC

1.0% Clindamycin in Velac gel vehicle, Lot # SIAB-C, 99.75% of LC

CTG vehicle, Lot #451-25, purity not provided but certificate states 'clindamycin absent'

Methods

Doses: 0, 5, 10, 15, 5 mg/kg

Species/strain: rats/Sprague Dawley Crl:CD(SD)

Number/sex/group: 5/sex/group

Route, formulation, volume: topical to clipped dorsal skin, uncovered test site

Satellite groups used for early sacrifice: one male and one female from each of Groups 1-4 were dosed on days 1-7 and euthanized on Day 8.

Age: ~ 8 weeks of age

Weight: males: 255 to 273 g; females: 178 to 205 g

Sampling times:

Unique study design or methodology: Due to the development of notable dermal lesions in Groups 1-4 during the first week of dosing, test article application was discontinued on Day 7 for one male and one female from each of Groups 1-4 (those that appeared to have the most severe dermal changes within each group/sex). The selected animals were euthanized on Day 8 and dermal tissue was collected from each test site for histopathologic evaluation. This early termination was conducted to further define the nature of the dermal lesions observed grossly. Dosing in the remaining Groups 1-4 animals was continued through Day 35 to determine if the dermal findings would progress and/or if an adaptive response would occur at the test site. A fifth group (1% Clindamycin in Velac Gel) was added to serve as a comparative control for the 1% Clindamycin in CTG Vehicle test article. Dosing for Group 5 started on Day 8 and continued to termination.

Reviewer's comment: It would have been a better study design to have tested the comparative control (1% clindamycin in Velac Gel) for the same treatment period as the experimental (1% clindamycin in CTG Vehicle).

Group No.	No. of Animals		Dose Material	Clindamycin Dose Level (mg/kg)	Formulation Dose Volume (mL/kg)	Clindamycin Dose Concentration (mg/mL)	BSA Treatment Area (%)	Necropsy Day
	Males	Females						
1	5	5	CTG Vehicle (vehicle control)	0	1.5	0	15	8 ^a /36 ^b
2	5	5	1% Clindamycin in CTG Vehicle	5	0.5	10	5	8 ^a /36 ^b
3	5	5	1% Clindamycin in CTG Vehicle	10	1.0	10	10	8 ^a /36 ^b
4	5	5	1% Clindamycin in CTG Vehicle	15	1.5	10	15	8 ^a /36 ^b
5	5	5	1% Clindamycin in Velac Gel Vehicle (comparative control)	5	0.5	10	5	36 ^c

^aOne male and one female in Groups 1-4 were dosed on Days 1-7 and euthanized on Day 8.

^bFour males and four females in Groups 1-4 were dosed on Days 1-35 and euthanized on Day 36.

^cThe Group 5 animals were added to the study and dosed on Days 8-35 and euthanized on Day 36.

Reviewer's note: The dorsal skin of each rat was clipped with an electric clipper on the day prior to dose initiation and approximately weekly throughout the remainder of the study.

Observation and Times:

Clinical signs: general health/mortality and moribundity checks were performed twice daily. Detailed clinical observations were performed prior to the first day dosing and prior to dosing on Days 1, 8, 15, 22, and 29 and on Day 36. Cage-side observations were performed daily (Days 1-35) ~ 0.5 to 2 hours following dosing.

Dermal observations: Detailed clinical observations were performed prior to the first day dosing and prior to dosing on Days 1, 8, 15, 22, and 29 and on Day 36. The dermal grading system was based on Draize.

Body weights: recorded at randomization and on Days 1, 8, 15, 22, 29 and 36.

Feed consumption: recorded on Days 1, 8, 15, 22, 29, and 36.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: all surviving animals were euthanized by exsanguination while under carbon dioxide induced anesthesia. The necropsy examination included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surface of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Organ weights: not performed.

Histopathology: Treated skin, untreated skin, and gross abnormalities were collected from Groups 1-4 and preserved in 10% neutral buffered formalin. No tissues were collected from Group 5 animals.

Adequate Battery: Minimally, but usually a complete set of tissues is examined microscopically during a toxicity study

Peer review: no

Results:

Mortality: no treatment-related mortalities were observed during this study. One Group 2 female (#4334) was euthanized moribund on Day 33 and was noted to have dark material around the facial area, decreased activity, thin appearance, decreased defecation, hunched posture, and ocular discharge during the two days prior to

euthanasia. Gross necropsy revealed reddened mandibular lymph nodes and thickened glandular stomach. A definitive cause of death could not be established, this mortality was not considered to be test article-related since it occurred in the low-dose group and there were no comparable clinical or gross necropsy abnormalities noted in the remaining animals.

Clinical signs: no toxicologically meaningful clinical abnormalities noted. Signs noted (hair loss, scabs) were generally typical of the species and route of administration.

Dermal observations: During the first 3 weeks of treatment, Groups 1, 3, and 4 males and females exhibited slight erythema and pinpoint eschar. Minor irritation was observed in Group 2 and 5 males and females with no dermal response noted in group 6 animals. During the remaining 4 weeks of study (Days 9-36), the dermal irritation in Groups 1-4 gradually diminished with no eschar present by study termination. Dermal response in Group 5 animals during days 9-36 was typically less severe relative to Groups 1-4.

The following 2 tables give the group total dermal irritation score calculated by multiplying the dermal grade by the number of animals affected (i.e., 1 animal with a grade of erythema-1 and 2 animals with a grade erythema-2 = total score of 5) for each interval.

Group total irritation scores for males:

Group no.	Study Interval				
	Days 1-8	Days 9-15	Days 16-22	Days 23-29	Days 30-36
1	7	4	1	3	3
2	2	2	2	1	1
3	7	2	1	1	1
4	9	4	2	2	2
5	-	3	0	0	1

Group total irritation scores for females:

Group no.	Study Interval				
	Days 1-8	Days 9-15	Days 16-22	Days 23-29	Days 30-36
1	2	1	1	1	1
2	4	1	2	1	0
3	5	2	1	2	2
4	7	4	5	2	1
5	-	1	0	0	0

Body weights: some sporadic differences, but no clear test article-related effects on body weight in the males or females during this study.

Feed consumption: no statistically significant or toxicologically meaningful changes in feed consumption were noted.

Gross pathology: no treatment-related internal gross abnormalities observed in any animal.

Histopathology: Microscopic examination of the vehicle and test article-treated skin sites revealed an overall comparable dermal response among Groups 1-4 with a more pronounced reaction on Day 8 relative to the sites examined from day 36. On Day 8, this response generally consisted of mild to moderate epidermal hyperplasia, hyperkeratosis, serocellular crusting, multifocal epidermal necrosis with ulceration, and minimal to moderate mixed mononuclear inflammatory cell infiltrates. On Day 36 the lesions were diminished relative to the Day 8 response, but generally consisted of minimal to mild diffuse epidermal hyperplasia, minimal to mild hyperkeratosis, and minimal mixed mononuclear inflammatory cell infiltrates. Only two rats were noted to have serocellular crusting and focal epidermal necrosis. No meaningful lesions were noted on the untreated skin sites collected from animals on day 8 or day 36.

Conclusion: Once daily dermal administration of CTG Vehicle and 1% Clindamycin in CTG Vehicle at 5, 10 and 15 mg/kg/day produced mild dermal irritation with focal eschar/ulceration during the first week of dosing. There was no notable difference in the dermal response between the vehicle and test article-treated groups. Tolerance to the administered dose appeared to occur during the next four weeks of dosing as dermal responses diminished (but did not completely resolve) by Day 36 of the study. The dermal response to 1% Clindamycin in Velac Gel (Group 5, comparative control) administered at 5 mg/kg/day was typically less severe relative to the CTG Vehicle and 1% Clindamycin in CTG Vehicle treated animals.

Reviewer's comment: Although 1% clindamycin in Velac Gel (Group 5) was designed as a comparative control, the difference in timing and the length of the exposure period (28 days versus 35 days) makes a direct comparison difficult.

Study title: A 90-day toxicity study of clindamycin administered by the dermal (skin painting) route to rats with a 21-day interim sacrifice interval

Key study findings: Based on study results, dermal application of 1% Clindamycin in CTG Vehicle or Velac Gel vehicle for up to 90 days was well tolerated systemically as there were no test article-related mortalities or evidence of systemic toxicity. However, dermal responses at the site of application produced both macroscopic and microscopic lesions indicative of dermal irritation more in the CTG vehicle (Group 1) and 1% Clindamycin in CTG vehicle (Groups 3 and 4) treated groups through the first 21 days of dosing. Clipping the animals either 1 or 3 days prior to the first dermal dose did not appear to impact the irritation potential as the dermal reactions of Groups 3 and 4 animals were similar. A less notable dermal response was observed in the Velac Gel Vehicle (Group 2) and 1% Clindamycin in Velac Gel Vehicle (Group 5) treated animals during this period. Continuation of dosing in the CTG Vehicle (Group 1) and 1% Clindamycin in CTG Vehicle (Groups 3 and 4) animals through 90 days resulted in the

development of tolerance to the dermal dose as the macroscopic and microscopic lesions were generally reduced in severity.

Study no.: NPB00019

Volume #, and page #: SDN47: vol 3, page 1 (IND 65,369)

Conducting laboratory and location:

(b) (4)

Date of study initiation: February 22, 2006

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

1.0% Clindamycin in CTG vehicle, Lot # XBA-C, purity not provided

1.0% Clindamycin in Velac gel vehicle, Lot # 451-62, purity not provided

CTG vehicle, Lot # WMB-C, purity not provided

Velac gel vehicle, Lot # 451-71, purity not provided

Methods

Doses: 0, 0, 10, 10, 10 mg/kg

Species/strain: rats/Sprague Dawley Crl:CD(SD)

Number/sex/group : 3/sex/group

Route, formulation, volume: topical to clipped dorsal skin, uncovered test site

Satellite groups used for early sacrifice:

5/sex/group for Groups 1, 3-5, 3/sex/group for Group 6

Age: ~ 8 weeks of age

Weight: males: 245 to 279 g; females: 205 to 235 g

Sampling times:

Unique study design or methodology (if any): the dorsal skin of each rat was clipped prior to dose initiation on Day -1 for Groups 1, 2, 4, and 5 and Day -3 for Group 3. In either case, the clipped area represented ~ 15% of BSA.

Reviewer's note: Group 3 and 4 were designed to determine the impact of the pretest dermal clipping procedure on the resulting dermal response.

Group No.	No. of Animals		Dose Material	Clindamycin Dose Level (mg/kg)	Formulation Dose Volume (mL/kg)	Clindamycin Dose Concentration (mg/mL)	Necropsy Day
	Males	Females					
1	8	8	CTG Vehicle	0	1	0	22 ^a /92 ^b
2	5	5	Velac Gel Vehicle	0	1	0	22
3	8	8	1% Clindamycin in CTG Vehicle	10	1	10	22 ^a /92 ^b
4	8	8	1% Clindamycin in CTG Vehicle	10	1	10	22 ^a /92 ^b
5	5	5	1% Clindamycin in Velac Gel vehicle	10	1	10	22
6 ^c	3	3	Sham control	0	0	0	22

^aFive males and 5 females in Groups 1, 3, 4 were dosed on days 1-21 and euthanized on Day 22.

^bThree males and 3 females in Groups 1, 3, 4 were dosed on Days 1-91 and euthanized on Day 92.

^cAnimals in Group 6 were sham treated on Days 8-21 and euthanized on Day 22.

Observation and Times:

Clinical signs: general health/mortality and moribundity checks were performed twice daily. Detailed clinical observations were performed once prior to treatment and weekly during the treatment period. Cage-side observations were performed daily ~ 0.5 to 2 hours following dosing.

Dermal observations: performed once prior to treatment and weekly during the treatment period (Days -3, 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 91). The dermal grading system was based on Draize.

Body weights: recorded at randomization and weekly throughout the treatment period (Days -3, 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 91).

Feed consumption: weekly throughout treatment period (Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 91).

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: not performed.

Clinical chemistry: not performed.

Urinalysis: not performed.

Gross pathology: all surviving animals were euthanized by exsanguination while under carbon dioxide induced anesthesia. The necropsy examination included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial

cavity and external surface of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Organ weights: not performed.

Histopathology: Treated skin, untreated skin, and gross abnormalities were collected from all animals and preserved in 10% neutral buffered formalin.

Adequate Battery: minimally, but usually a complete set of tissues is examined microscopically during a toxicity study

Peer review: no

Results:

Mortality: no mortality or morbidity observed during this study.

Clinical signs: no toxicologically meaningful clinical abnormalities noted. Signs noted (hair loss, scabs) were generally typical of the species and route of administration.

Dermal observations: During the first 3 weeks of treatment, Groups 1, 3, and 4 males and females exhibited notable erythema and eschar. Minor irritation was observed in Group 2 and 5 males and females with no dermal response noted in group 6 animals. During the last 10 study weeks, irritation was reduced in Groups 1, 3, and 4 males and Groups 3 and 4 females. In Group 1 females the dermal response noted during the last 10 weeks was similar to that noted during the first three weeks.

Male and female dermal irritation ranges

Sex	Study Days	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Male	1-22	1-4 Ery 1-3 Esch	1-2 Ery	1-4 Ery 1-3 Esch	1-4 Ery 1-2 Esch	1 Ery	0 Ery
	23-91	1-2 Ery 1 Esch	NA	1-2 Ery 1 Esch	1-2 Ery	NA	NA
Female	1-22	1-2 Ery 1 Esch	1 Ery	1-2 Ery 1 Esch	1-4 Ery 1-3 Esch	1-2 Ery	0 Ery
	23-91	1-2 Ery 1 Esch	NA	1-2 Ery	1-2 Ery	NA	NA

Body weights: some sporadic differences, but no clear test article-related effects on body weight in the males or females during this study.

Feed consumption: no statistically significant or toxicologically meaningful changes in feed consumption were noted.

Gross pathology: no treatment-related internal gross abnormalities observed in any animal.

Histopathology: Microscopic changes indicating dermal irritation were observed in all vehicle (Groups 1 and 2) and test article-treated groups (Groups 3, 4, and 5) on Day 22 and in Groups 1, 3, and 4 on Day 91.

Day 22 individual histopathology scores in the treated skin

Sex	Male						Female					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Number examined	5	5	5	5	5	3	5	5	5	5	5	3
Epidermal hyperplasia	(5)	(5)	(5)	(5)	(5)	(3)	(5)	(5)	(5)	(5)	(5)	(0)
-minimal	-	-	-	-	2	2	2	4	-	-	2	-
-mild	-	4	-	-	3	1	1	1	3	1	2	-
-moderate	4	1	5	5	-	-	2	-	2	4	1	-
-marked	1	-	-	-	-	-	-	-	-	-	-	-
Hyperkeratosis	(5)	(5)	(5)	(5)	(5)	(3)	(5)	(0)	(5)	(5)	(2)	(0)
-minimal	-	4	-	-	3	3	3	-	2	-	1	-
-mild	1	1	2	2	2	-	2	-	3	3	1	-
-moderate	4	-	3	3	-	-	-	-	-	2	-	-
Adnexal hyperplasia	(4)	(5)	(5)	(5)	(5)	(0)	(2)	(1)	(5)	(5)	(3)	(0)
-minimal	1	2	-	-	2	-	-	-	3	1	2	-
-mild	1	3	4	2	3	-	2	1	2	3	1	-
-moderate	2	-	1	2	-	-	-	-	-	1	-	-
-marked	-	-	-	1	-	-	-	-	-	-	-	-

Note : () = number affected. – = no-observation present for indicated parameter.

Day 91 individual histopathology findings in treated skin

Sex	Male			Female		
Group	1	3	4	1	3	4
Number examined	3	3	3	3	3	3
Epidermal hyperplasia	(3)	(3)	(3)	(3)	(3)	(3)
-minimal	-	1	-	1	3	3
-mild	3	1	2	1	-	-
-moderate	-	1	1	1	-	-
Hyperkeratosis	(3)	(2)	(3)	(3)	(0)	(0)
-minimal	1	-	-	2	-	-
-mild	2	2	3	1	-	-
-moderate	-	-	-	-	-	-
Adnexal hyperplasia	(3)	(3)	(3)	(2)	(2)	(2)
-minimal	1	1	-	1	2	1
-mild	2	2	2	1	-	1
-moderate	-	-	1	-	-	-

Note : () = number affected. – = no-observation present for indicated parameter.

Conclusion: Based on study results, dermal application of 1% Clindamycin in CTG Vehicle or Velac Gel vehicle for up to 90 days was well tolerated systemically as there were no test article-related mortalities or evidence of systemic toxicity. However,

dermal responses at the site of application produced both macroscopic and microscopic lesions indicative of dermal irritation more in the CTG vehicle (Group 1) and 1% Clindamycin in CTG vehicle (Groups 3 and 4) treated groups through the first 21 days of dosing. Clipping the animals either 1 or 3 days prior to the first dermal dose did not appear to impact the irritation potential as the dermal reactions of Groups 3 and 4 animals were similar. A less notable dermal response was observed in the Velac Gel Vehicle (Group 2) and 1% Clindamycin in Velac Gel Vehicle (Group 5) treated animals during this period. Continuation of dosing in the CTG Vehicle (Group 1) and 1% Clindamycin in CTG Vehicle (Groups 3 and 4) animals through 90 days resulted in the development of tolerance to the dermal dose as the macroscopic and microscopic lesions were generally reduced in severity.

7 Genetic Toxicology

The genotoxicity of Velac Gel and the two active ingredients have been reviewed in depth (IND 65,369, 2-06-2003) and previously summarized (NDA 50-803, 5-16-2005) but will be briefly described below.

The sponsor has conducted several *in vitro* genotoxicity studies. No evidence of mutagenicity was observed in bacterial reverse mutation assays with clindamycin phosphate in water, in Velac Gel vehicle or with the gel vehicle containing either tretinoin alone or in combination with clindamycin phosphate. The testing of the gels is somewhat unusual since this assay is primarily used for individual ingredients and not formulations. Clindamycin phosphate alone or tretinoin alone did not cause increases in chromosomal aberrations in cultured human lymphocytes. The clindamycin phosphate/tretinoin gel caused an increase in chromosomal aberrations in cultured human lymphocytes in one experiment but this was not repeated in a second experiment so the result is equivocal. POE 4, an excipient in the Velac Gel, was negative for inducing revertants in bacteria in the presence and absence of metabolic activation, nor did it exhibit genotoxicity in Chinese hamster ovary cells.

The sponsor has submitted a published study (Juhl *et al.*, Mutation Research, 58:317-320, 1978) which showed that vitamin A acid (presumably *trans*-retinoic acid) caused an approximately two fold increase in sister chromatid exchanges in human diploid fibroblasts. An *in vitro* Ames assay of tretinoin was conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 (Kamm, 1982). Doses of up to 2 mg/plate were tested, with and without metabolic activation. Tretinoin was negative for genotoxicity in this assay. Retinoic acid was evaluated in the CHO HGPRT assay as part of a published study investigating the ability of retinoic acid and retinol to inhibit DMBA- induced genotoxicity (Budroe *et al.*, 1988). Retinoic acid alone did not cause an increase in mutations in the presence or absence of metabolic activation. Concentrations of up to 25 μM were tested with metabolic activation and concentrations up to 100 μM were tested without metabolic activation. Retinoic acid was not toxic to the CHO cells with metabolic activation up to the highest dose of 25 μM . In the absence of metabolic activation, the high dose of 100 μM retinoic acid produced about an 80% reduction in survival of the CHO cells.

The information submitted indicates that tretinoin, clindamycin phosphate and the gel vehicle are not genotoxic in *in vitro* studies.

No additional genetic toxicology studies were conducted with the CT Gel and none are considered necessary for this NDA submission..

8 Carcinogenicity

Clindamycin:

The sponsor has conducted a 26-week dermal carcinogenicity study in Tg.AC mice with clindamycin phosphate in the Velac Gel vehicle (Study No. AA81EW.7D8T.BTL; reviewed previously, NDA 50-803, 5-16-2005). Under the conditions of the test there was a vehicle effect on papilloma formation which was accentuated by the administration of clindamycin phosphate in the Velac Gel vehicle at 3% and 5%. The incidence of papillomas was comparable in the vehicle control and the 1% group. The sponsor analyzed the predictive power and relative risk of dermal site of application (SOA) irritation for papilloma formation. Dermal SOA irritation was found to be highly correlated with, and predictive of, papilloma formation. The risk of developing a papilloma in this study was determined to be 144 times greater when dermal SOA irritation was present than when irritation was not present prior to papilloma formation. The sponsor believes that tumorigenesis in Tg.AC mice may be a nonspecific response to dermal insult. However, the eCAC was aware of other studies in Tg.AC mice in which irritation alone was not sufficient to cause papillomas. The eCAC concluded that the Velac Gel vehicle is at best, a tumor promoter, and at worst, a complete carcinogen. These findings formed the basis of an action letter (June 10, 2005) in which the Velac Gel was not approved.

Tretinoin:

The carcinogenic potential of topically applied tretinoin has been examined in two National Cancer Institute sponsored studies. The results demonstrated that tretinoin is a weak promoter in mice, but it is also capable of reducing 12-O-tetradecanoyl-phorbol 13-acetate (TPA)-inducing tumor promotion. In female CD-1 mice initiated with 7,12-dimethylbenz[α]anthracene (DMBA), multiple papillomas were observed following topical application of tretinoin for 20 weeks (Hennings *et al.*, 1982). In female SENCAR mice initiated with DMBA, promotion of tumors by TPA or mezerein was reduced by co-application with tretinoin (Tennenbaum *et al.*, 1998). In a chronic, 2-year bioassay topical treatment of tretinoin (three times per week, 0.1%) resulted in generalized amyloid deposition in the basal layer of tretinoin-treated skin, but no carcinogenicity (Tsubura and Yamamoto, 1979). The potential of systemically absorbed tretinoin to promote tumor formation will be reflected in the eventual drug label.

Topical tretinoin was tested for its ability to enhance experimental photocarcinogenicity in mice (Forbes, 1979). Hairless albino mice received daily topical applications of vehicle (Group A), 0.001% tretinoin (Group B), or 0.01% tretinoin (Group C) in methanol

beginning at 7 ± 1 week of age for 30 weeks. One group of mice was treated with 0.1% croton oil (Group D) in methanol daily. Beginning on the first day of treatment with croton oil and on the 15th day of treatment with tretinoin, each application was preceded by a 2 hour exposure to simulated sunlight. Tumors appeared at 38, 21, 20, and 26 weeks in Groups A, B, C, and D, respectively. After week 35, tumor incidence and yield in both Groups B and C were significantly greater than in Group A. At week 55, animals in Groups A, B, C, and D had 0.65, 6.3, 9.22, and 0.89 tumors/survivor, respectively. The ability of tretinoin to enhance photocarcinogenesis was assessed in another study of lightly pigmented mice (Kligman and Kligman, 1981). One group of mice was treated with UV light and topical tretinoin concomitantly, while the other group was treated with UV light to induce tumors prior to treating the animals with topical tretinoin. Tretinoin did not enhance photocarcinogenesis in either group. The ambiguity of these studies on the photocarcinogenic potential of tretinoin, is most likely attributable to variables within the study designs.

Study title: A two-year carcinogenicity study of clindamycin administered dermally (skin painting) to mice

Study no.: NPB00012

Study report location: SDN42: Volume 4, page 1

Conducting laboratory and location:



Date of study initiation: 10-26-06

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: 1% clindamycin in CTG vehicle, XBA-C, purity = 101.3%
 1% clindamycin in CTG vehicle, ZLX-C, purity = 100.8%
 CTG vehicle, WMB-C
 CTG vehicle, ZLU-C

Reviewer's comment: The test article was protected from white light until administered to the animals, with dispensing under yellow light. The control article was not protected from white light while stored. The 12-hour light/12-hour dark cycle provided yellow lighting.

CAC concurrence: Yes. The eCAC concurred with the protocol and dose (August 29, 2006; discussed under IND 65,369). The eCAC concurred that the study was acceptable

(February 2, 2010). Refer to Attachment for a copy of the respective eCAC meeting minutes.

Key Study Findings

Based on the results of this study, once daily dermal administration of 1% clindamycin in CTG vehicle (32 mg/kg/day) to CD-1 mice for up to two years did not produce evidence that the test article was tumorigenic in this species. Therefore 1% clindamycin in CTG vehicle was not considered a dermal carcinogen under the conditions of this study.

Adequacy of Carcinogenicity Study

The carcinogenicity study appeared adequate.

Appropriateness of Test Models

The test model was appropriate.

Evaluation of Tumor Findings

No treatment-related findings were noted in this study.

Methods

Doses: 0 (sham control), 0 (CTG vehicle), 32 mg/kg/day (1% clindamycin in CTG vehicle)

Frequency of dosing: daily
 Dose volume: 80 µL/day to ~10% body surface area
 Route of administration: Skin painting (topically to clipped dorsal skin; not covered)
 Formulation/Vehicle: 1% clindamycin in CTG vehicle (the clinical vehicle)
 Basis of dose selection: MTD
 Species/Strain: CD-1 mice
 Number/Sex/Group: 60/sex/group
 Age: ~8 weeks
 Animal housing: Housed individually
 Paradigm for dietary restriction: NA
 Dual control employed: Yes, sham and vehicle controls
 Interim sacrifice: No
 Satellite groups: toxicokinetics
 Deviation from study protocol: None, however, the original protocol was amended to give Group 3 TK female #L517 a dosing holiday from Days 137 to 139 due to notable dermal irritation with a heightened sensitivity to touch within the test site.

Group #	# of Animals				Test Material	Clindamycin Dose Level (mg/kg/day) ^a	Form. Dose Volume (µL)	Clindamycin Dose Conc. (mg/mL)	Treatment Area (cm ²)
	Carcinogenicity (Contamination control)		Toxicokinetic (Contamination control)						
	Male	Female	Male	Female					
1	60 (60)	60 (60)	-	-	Sham control	0	0	0	0
2	60	60	42 (42)	42 (42)	CTG vehicle	0	80	0	~2 x 2
3	60	60	42	42	1% clindamycin in CTG vehicle	32	80	10	~ 2 x 2

Note: Form = formulation, Conc = concentration

^aBased on a 25 gram mouse.

Observation times

Mortality: general health/mortality and moribundity checks were performed twice daily.

Clinical signs: detailed clinical observations were performed on Day -6 and once during each study week thereafter (prior to dosing on dosing days). In addition, each animal was observed for dermal findings once during each study week (prior to dosing). Slight, moderate, or severe redness or swelling as well as other dermal changes were noted if present. Beginning during Week 26, detailed clinical observations included a palpable mass examination (including the occurrence, size, location, and description of palpable masses). The persistence or disappearance of these masses was documented at the next weekly clinical examination.

Body weights: individual body weights were recorded on Day -6, weekly for the first 13 weeks, and once every 4 weeks thereafter. A final in-life body weight was recorded during Week 104 for Groups 1 and 2, and during Week 98 for the Group 3 males and Week 103 for the Group 3 females.

Food consumption: weekly for the first 13 weeks, and once every 4 weeks thereafter on the same days as body weights were measured.

Histopathology: The following tissues were collected from all carcinogenicity animals, preserved and examined microscopically: adrenal gland, aorta, bone (femur), bone (sternum), bone marrow (sternum), bone marrow smear, brain (cerebrum, cerebellum, brain stem), cervix, epididymis, esophagus, eye, gallbladder, Harderian gland, heart, intestine (cecum, colon, duodenum, ileum with Peyer's patch, jejunum, rectum), kidney, liver, lung, lymph node (mandibular, mesenteric), mammary gland, nerve (optic, sciatic), ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skeletal muscle (thigh), skin (mammary), skin (treated, untreated), spinal cord (cervical, thoracic, lumbar), spleen, stomach (glandular, nonglandular), testis, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, gross lesions, tissue masses suspect tumors.

Peer review: All tissues from 10% of the animals randomly selected from the control and high-dose groups and all proliferative (pre-neoplastic and neoplastic) lesions from all animals in all groups were peer reviewed. Other selected tissues were examined at the discretion of the reviewing pathologist.

Toxicokinetics: Treatment for the toxicokinetic phase was 180 days. Blood samples (~1 mL/sample) were collected on Days 1 and 180 (Group 2) and Days 2 and 181 (Group 3) at 0, 1, 2, 4, 8, and 24 hours postdosing via percutaneous cardiocentesis (under carbon dioxide anesthesia) into tubes containing K₂EDTA as the anticoagulant, immediately chilled in a commercial tube cooler, and protected from light. Following refrigerated centrifugation, the TK plasma samples were transferred to labeled tubes and stored in a -70°C freezer. The plasma samples were subsequently shipped overnight on dry ice to the bioanalytical laboratory for analysis.

Results

Mortality

Final survival data is summarized below in a table taken directly from the study report (Text Table 20 Survival).

Text Table 20 Survival

Best Available Copy

Sex	Males (N=60/Group)			Females (N=60/Group)		
Group	1	2	3	1	2	3
Dose (mg/kg/day)	0 (Sham)	0	32	0 (Sham)	0	32
Scheduled Euthanasia	28	26	15	24	21	15
Early Deaths	32	33	44	36	39	45
Accidental Deaths	0	1	1	0	0	0
Total	60	60	60	60	60	60

Test article administration reduced the overall survival rate of the Group 3 (32 mg/kg/day) males and females to ~25% and reached the level of statistical significance in both sexes by the end of the study with respect to the sham control. Additionally the survival rate of the Group 3 males was significantly less than that in CTG vehicle control group as well.

Survival at Study Week 90 is summarized below in a table taken directly from the study report (Text Table 15 Survival at Study Week 90).

Group	Males	%	Females	%
Group 1 (Sham Control)	44	73	38	63
Group 2 (CTG Control)	39	65	39	65
Group 3 (32 mg/kg/day)	26	43	35	58

Reviewer's comment: The life span of mice is about 1.5 to 2 years.

As per eCAC advice, dosing was discontinued in Group 3 males on Day 645 (Week 93) when the number of surviving mice in that sex/group reached 20 or less and this group was terminated during Week 98, when the number of surviving mice reached 15 in that sex/group. Similarly, dosing was discontinued on Day 683 (Week 98) for the Group 3 females and this group was terminated during Week 103.

Clinical Signs

Findings expected in any 2-year study occurred in all groups and included unkempt appearance (particularly in males), hair loss, and ocular lesions. Findings which appeared to have some relationship to treatment with the test article included scabs and

slight to moderate redness and desquamation at the site of application, distended abdomen and these were more apparent in Group 3 mice.

Summary of clinical observations

Observation	Group 1	Group 2	Group 3
Males (total observations / # animals affected)			
Scab(s)	140/19	280/22	373/29
Distended abdomen	112/14	289/27	262/28
Site of Application, Redness – slight	0/0	49/10	188/35
Site of Application, Redness- moderate	0/0	0/0	7/4
Desquamation	0/0	7/2	14/4
Females (total observations / # animals affected)			
Scab(s)	36/17	63/12	84/24
Distended abdomen	48/10	217/24	198/34
Site of Application, Redness – slight	2/2	20/7	322/51
Site of Application, Redness- moderate	0/0	1/1	32/4
Desquamation	0/0	13/5	33/9

There were no differences in the number of palpable masses noted or the number of animals affected between the male or female sex groups. (The following tables have been taken directly from the study report.)

Text Table 16 Number of Mice Denoted with At Least One Large Palpable Mass

Best Available Copy

Group	Males	Females
Group 1 (Sham Control)	8	1
Group 2 (Control)	13	2
Group 3 (32 mg/kg/day)	7	5

Text Table 17 Number of Mice Denoted with At Least One Palpable Mass of Any Size

Group	Males	Females
Group 1 (Sham Control)	17	17
Group 2 (Control)	28	18
Group 3 (32 mg/kg/day)	26	17

Body Weights

Although there were statistically significant mean body weight changes for the Group 3 males at specific time points (increases: Weeks 3-4, 9-10, 37-41; decreases: Weeks 17-21, 29-33), there were no statistically significant differences detected in mean body weight over the course of the study. Statistically significant differences were detected in mean body weight for Group 2 females by Study Week 65 and for Group 3 females by

Study Week 4. The increased body weight differences were maximal at Study Week 97 and were 9.8% and 12.9% for Groups 2 and 3, respectively.

Feed Consumption

Group 3 males had ~ 6% increase in food consumption values relative to male controls. In females, food consumption varied throughout the study with overall food consumption values for Groups 1 and 3 being quite similar, but values for Group 2 females were about 8% less than of Group 1.

Gross Pathology

There were no unusual gross pathology findings in mice euthanized at the end of the study. There was a low incidence of kidney findings in all groups. In general the treatment-related gross lesions correlated to treatment-related microscopic findings. There were no unusual observations noted for the gross pathology examination of the found dead mice. Wet carcass, body fat depletion, and urinary bladder distension (in males) were common findings in all treated groups.

Histopathology

Neoplastic

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Text Table 21 Incidence of Proliferative Changes in the Skin

Sex	Males (N=60/Group)			Females (N=60/Group)		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Dose (mg/kg/day)	0 (Sham)	0	32	0 (Sham)	0	32
Skin, Treated						
Fibrosarcoma	0	0	0	0	1	0
Papilloma, squamous	0	1	0	0	0	0
Squamous Cell Carcinoma	0	1	0	0	0	0
Skin, Untreated						
Fibrosarcoma	0	0	0	0	0	0
Papilloma, squamous	0	0	0	0	0	0
Squamous Cell Carcinoma	0	0	0	0	0	0

The only treatment site specific tumors were seen in the CTG Vehicle treated group (Group 2) and included a squamous papilloma and a squamous cell carcinoma in the males and a fibrosarcoma in a female. These tumors were of an incidence and character of those seen spontaneously in control CD-1 mice, but since they were treatment site specific they were likely secondary to the locally chronic irritant effects of the CTG vehicle to the skin. No treatment-related tumors were seen in the skin or other tissue locations among CD-1 mice treated with 1.0% clindamycin in the CTG vehicle. The other neoplastic findings (pleomorphic malignant lymphoma of the adrenal cortex,

brain, esophagus, intestine, etc.) were considered spontaneous, incidental lesions commonly observed in aging control CD-1 mice of this stock and are not considered test article related. The test article was not tumorigenic.

Non Neoplastic

The treated skin was considered a target tissue in both sexes, but the non-neoplastic effects seen in the treated skin of the CTG vehicle group (Group 2) and the 1% clindamycin in the CTG vehicle group (Group 3) were similar in character, incidence, and severity. These local epidermal and dermal effects included: hyperkeratosis, epithelial hyperplasia, dermal fibrosis, and a chronic-active inflammation and could not be distinguished from those seen with the CTG vehicle alone. Occasional local erosions and ulcerations were seen. Most of these changes were graded minimal to mild and did not increase in severity or incidence with increasing exposure to the test article over time. No systemic toxic effects were associated with the test article.

Text Table 22 Incidence of Pathological Changes in the Skin

Best Available Copy

Sex	Males (N=60/Group)			Females (N=60/Group)		
	1	2	3	1	2	3
Group	1	2	3	1	2	3
Dose (mg/kg/day)	0 (Sham)	0	32	0 (Sham)	0	32
Skin, Treated						
Erosion, focal	0	2	1	0	2	3
Fibrosis, dermal	4	31	33	0	23	22
Hyperkeratosis	2	23	23	0	34	26
Hyperplasia, epithelial	0	21	17	0	25	14
Inflammation, chronic-active	1	17	6	0	19	24
Ulceration, chronic-active	1	0	1	0	1	0
Skin, Untreated						
Erosion, focal	0	0	0	0	0	0
Fibrosis, dermal	0	1	1	0	0	0
Hyperkeratosis	1	1	0	0	1	1
Hyperplasia, epithelial	0	1	0	0	0	0
Inflammation, chronic-active	1	4	0	0	2	0
Ulceration, chronic-active	0	1	0	0	0	0

Toxicokinetics: TK samples were analyzed for clindamycin and one metabolite, clindamycin sulfoxide. The lower limit of detection for both analytes was 50 pg/mL.

Clindamycin

Following once daily dermal dosing of clindamycin (32 mg/kg/day), measurable plasma concentrations of clindamycin were observed at the earliest post dosing collection time points. The T_{max} value of clindamycin was 1 hour in both sexes, indicating rapid absorption of clindamycin from the skin into the systemic circulation. Mean C_{max} values were 26,800 and 53,500 pg/mL for males and female mice, respectively. The

corresponding $AUC_{(0-24)}$ values were 125,000 pg•h/mL for male and 147,000 pg• h/mL for female mice.

After 180 days of dermal administration of clindamycin at 32 mg/kg/day, plasma exposures (C_{max} and AUC) in both male and female mice were higher than the exposures observed on Day 2. The mean C_{max} value was 131,000 pg/mL for both male and female mice. The $AUC_{(0-24)}$ values were 590,000 and 266,000 pg•h/mL for male and female mice, respectively.

Clindamycin sulfoxide

The systemic plasma exposure of clindamycin sulfoxide was higher than the plasma exposure of the parent compound in both males and females on Day 2 and in the females on Day 181. However, on Day 181, the plasma exposure of clindamycin sulfoxide in male mice was comparable to the exposure of the parent compound. Following once daily dermal administration (skin painting) of clindamycin doses at 32 mg/kg/day, measurable plasma concentrations of clindamycin sulfoxide were observed at the earliest post dosing collection time points. Clindamycin sulfoxide displayed a T_{max} value of 2 hours in both sexes indicating its rapid production following a single dermal exposure of clindamycin. Clindamycin sulfoxide mean C_{max} values were 61,600 and 72,000 pg/mL for male and female mice, respectively. The corresponding $AUC_{(0-24)}$ values were 259,000 pg•h/mL for male and 237,000 pg•h/mL for female mice. Following 180 days of dermal administration of clindamycin at 32 mg/kg/day, plasma exposures of clindamycin sulfoxide (C_{max} and $AUC_{(0-24)}$) in both male and female mice were higher than the exposures observed on Day 2. The mean C_{max} values of clindamycin sulfoxide were 118,000 and 293,000 pg/mL for male and female mice, respectively. The corresponding $AUC_{(0-24)}$ values were 532,000 and 455,000 pg•h/mL for male and female mice, respectively. Modest accumulation of clindamycin sulfoxide was observed in mouse plasma following repeated dermal administration of clindamycin for 180 days. (The following table was taken directly from the study report.)

Text Table 24 Summary of Plasma TK Parameters of Clindamycin and Clindamycin Sulfoxide in Mice Following 32 mg/kg/day Dermal Doses of Clindamycin

Analyte	Day	Parameter	Male	Female
Clindamycin	2	Cmax, pg/mL	26,800	53,500
		Tmax, h	1.00	1.00
		AUC(0-24), pg·h/mL	125,000	147,000
	181	Cmax, pg/mL	131,000	131,000
		Tmax, h	4.00	1.00
		AUC(0-24), pg·h/mL	590,000	266,000
		Cmax Ratio ^a	4.89	2.45
		AUC Ratio ^b	4.72	1.81
Clindamycin Sulfoxide	2	Cmax, pg/mL	61,600	72,000
		Tmax, h	2.00	2.00
		AUC(0-24), pg·h/mL	259,000	237,000
	181	Cmax, pg/mL	118,000	293,000
		Tmax, h	4.00	1.00
		AUC(0-24), pg·h/mL	532,000	455,000
		Cmax Ratio ^a	1.92	4.07
		AUC Ratio ^b	2.05	1.92
^a Cmax _{Day 181} /Cmax _{Day 2} ^b AUC(0-24) _{Day 181} /AUC(0-24) _{Day 2}				

Conclusion

Based on the results of this study, once daily dermal administration of 1% clindamycin in CTG vehicle (32 mg/kg/day) to CD-1 mice for up to two years did not produce evidence that the test article was tumorigenic in this species. Therefore 1% clindamycin in CTG vehicle was not considered a dermal carcinogen under the conditions of this study.

9 Reproductive and Developmental Toxicology

The sponsor conducted a limit teratology study of Velac Gel by the dermal route in rats (Study No. 93/BSM001/1174). This study was previously reviewed by Dr. Paul Brown (IND 65,369, 02-06-2003). The study concluded that treatment with Velac Gel did not result in adverse effects on fetal development. However, although no systemic tretinoin was detected, the abnormalities described in the drug-treated groups, such as the craniofacial and heart abnormalities, could be consistent with retinoid effects. The sponsor was informed that if no additional developmental toxicity studies are conducted with the drug product, then the results of this limit study and any other publicly available information would be incorporated into eventual labeling. In addition to this preliminary study, the sponsor submitted several literature references on the reproductive and developmental toxicity of either clindamycin or tretinoin. These references were previously reviewed (IND 65,369, 02-06-2003), but will be briefly summarized here.

Published literature on clindamycin showed that subcutaneous clindamycin phosphate at 100 and 180 mg/kg was not teratogenic and had no detrimental effect on reproduction in mice and rats (Bollert *et al.*, 1974). Oral clindamycin hydrochloride (rat: 50, 200 mg/kg; mouse: 20, 50, 200 mg/kg) and clindamycin palmitate (rat: 300, 600 mg/kg; mouse 150, 300, 600 mg/kg) did not show any signs of teratogenicity in mice and rats (Gray *et al.*, 1972). Sprague-Dawley rats given up to 60 mg/kg clindamycin hydrochloride or up to 300 mg/kg clindamycin palmitate in the diet conceived at a slightly lower rate and their young were slightly smaller at weaning than the untreated controls, but otherwise no effect on reproductive performance was noted.

The references submitted by the sponsor showed that tretinoin, like other retinoids, is teratogenic and embryotoxic in multiple species when administered at sufficient doses and at the vulnerable gestational time period (Kraft, *et al.*, 1987; Seegmiller, *et al.*, 1997; Shenefelt, 1972; Tembe *et al.*, 1996; Hendrickx and Hummler, 1992; Fantel *et al.*, 1977). In the absence of maternal toxicity, significantly more rat pups of Wistar dams receiving 5 mg/kg/day or greater on days 6 through 15 of gestation had supernumerary ribs and offspring of the 10 mg/kg/day treatment group had a greater incidence of cleft palate than controls (Seegmiller, *et al.*, 1997). The NOAEL was considered to be 1 mg/kg/day. In cynomolgus monkeys treated with tretinoin (5, 10, or 20 mg/kg/day) before and during early organogenesis there were no skeletal alterations at 5 mg/kg, but fetuses in the 10 mg/kg group had craniofacial defects (Hendrickx and Hummler, 1992). The one fetus in the 20 mg/kg group did not survive. Doses of tretinoin that do not cause morphological changes in offspring may cause behavioral effects in the developing animals. Topical application of tretinoin appears to be less likely to result in teratogenic or other effects, probably due to lower systemic absorption and embryo exposure to tretinoin by the topical route than by the oral route (Zbinden, 1975). However, experimental studies are limited by maternal toxicity, including skin irritation, at doses below those causing teratogenic effects by other routes of exposure (Nau, 1993). The sponsor also included references in which the effects of tretinoin on fertility and postnatal development have been assessed (Kamm, 1982). In a fertility study male and female rats were treated orally with 0, 0.5, 2 and 5 mg/kg/day. Males were treated for 60-80 days and females for 14 days prior to mating up to and including parturition. No adverse effects were observed on gonadal function, fertility, conception rate, gestation, parturition, or neonatal viability at doses up to 2 mg/kg/day. At 5 mg/kg/day neonatal survival was decreased. In a peri- and postnatal development study female rats were treated with 0, 2, 5 and 10 mg/kg/day from the last third of gestation through lactation and weaning. Doses of 5 mg/kg/day and above resulted in decreased survival of the pups during the first 23 days following birth.

No reproductive and developmental toxicology studies were included in this NDA submission.

10 Special Toxicology Studies

The sponsor has previously submitted (IND 65,369, 02-06-2003) special toxicology studies to determine the potential of a clindamycin-tretinoin gel to induce skin irritation/corrosion, phototoxicity, contact hypersensitivity, and photoallergenicity. The

studies were previously reviewed by Dr. Paul Brown and it was concluded that under the conditions of the tests, the clindamycin-tretinoin gel did not cause skin irritation/corrosion in rabbits, nor was it phototoxic or photoallergenic in guinea pigs. However, it was considered a weak sensitizer in the guinea pig Maximization test.

No special toxicology studies were included in this NDA submission.

11 Integrated Summary and Safety Evaluation

The sponsor has conducted subchronic toxicity tests in both a rodent and nonrodent model on the previous formulation, Velac Gel (1% clindamycin, 0.025% tretinoin) with acceptable results. Since these two active ingredients are both used individually in approved products at similar or greater concentrations, chronic toxicity testing was not required for the current drug product. Velac Gel was determined to be nonmutagenic in an *in vitro* Ames *Salmonella* reversion test and was tested without effect in a limit teratology test in rodents. However, Velac Gel was determined to have equivocal clastogenic activity in a chromosome aberration assay and when tested in a 26-week dermal carcinogenicity study in Tg.AC mice, the vehicle alone caused a statistically significant increased incidence of skin papillomas compared to the untreated controls. Clindamycin in the Velac Gel vehicle caused further significant dose-related increases in papillomas relative to the vehicle controls and untreated animals. These nonclinical findings formed the basis of an action letter (June 10, 2005) in which the drug was not approved. The sponsor has subsequently reformulated Velac Gel, (b) (4) and (b) (4) polyoxyethylene 4 monolauryl ether (POE 4) (b) (4) was necessary (b) (4). The reformulated clindamycin 1% - tretinoin 0.025% gel (CT Gel) contains the same concentration of active ingredients as found in the previous formulation and, with the exception of (b) (4) POE 4, is identical to Velac Gel.

Given the similarities between Velac Gel and CT Gel, the pharmacology, pharmacokinetics and toxicology are expected to be similar. However, in order to use the supportive nonclinical information previously reviewed for Velac Gel (chronic toxicology, genetic toxicology, reproductive and developmental toxicology) the sponsor has developed a nonclinical bridge between the Velac Gel and CT Gel formulations (e.g., *in vitro* skin penetration, dermal toxicity studies in rodents). These studies were reviewed in the appropriate sections of this document. Based on the results of these studies, dermal application of 1% clindamycin in CT Gel vehicle or Velac Gel vehicle for up to 90 days was well tolerated systemically as there were no test article-related mortalities or evidence of systemic toxicity. Although dermal responses at the site of application produced macroscopic and microscopic lesions indicative of dermal irritation more prominently in the CT Gel vehicle and 1% clindamycin in the CT Gel vehicle groups compared to the Velac Gel vehicle and 1% clindamycin in Velac Gel vehicle groups, the sponsor has developed an adequate nonclinical bridge to the previous toxicological information.

The nonclinical deficiencies noted in the action letter, specifically the positive carcinogenicity signal in the Tg.AC mouse dermal carcinogenicity model, have been addressed by reformulation. Clindamycin at the clinical concentration (1%) in the reformulated vehicle has been tested in a 2-year dermal carcinogenicity study (NPB00012; reviewed within) with adequate results. Consistent with the Agency's guidance (FDA Minutes of the Post-Action Meeting, September 15, 2005), the sponsor has obtained the right of reference to a 27-week dermal carcinogenicity study of clindamycin phosphate (0, 0.5, 1, and 2 %) in a different gel-formulation to provide evidence that clindamycin does not display carcinogenic potential across a range of concentrations bracketing the clinical concentration of the CT Gel product. Although the study did not indicate a carcinogenic concern for clindamycin, technical inadequacies precluded further evaluation of this study and the results will not appear in the label (see Section 3.2 Studies Not Reviewed). The testing of the clinical clindamycin concentration in the vehicle and the long history of safe use of clindamycin are considered to be sufficient to support its use in the CT Gel. The sponsor was previously informed that literature information describing both the carcinogenic and photocarcinogenic potential of tretinoin as well as the limitations of this information could appear in the drug product label (IND 65,369, review #1, 2-6-03). The following section is taken from a previous review (IND 65,369, 2-06-2003, Dr. Paul Brown):

The photocarcinogenic potential of tretinoin has been investigated in several published studies. Some of the published studies show enhancement while others show that tretinoin either inhibited photocarcinogenesis or had no effect. However, not all published studies were performed with concurrent or intercurrent exposure to the test article and solar simulation. The design of those studies showing inhibition of photocarcinogenesis generally involved a period of exposure to UV radiation, followed by a later period of tretinoin treatment without continued UV exposure. Appropriately designed studies, in which tretinoin exposure and solar simulation are concurrent or intercurrent, show enhancement of photocarcinogenesis. Some authors argue that the albino mice used in some photocarcinogenicity studies are particularly susceptible to photocarcinogenesis because of their lack of pigmentation and that these studies are not clinically relevant. However, a recent study by Halliday *et al.* (not submitted by the sponsor) demonstrated that dark pigmentation provided some protection, but was not enough to overcome the the augmentation of photocarcinogenesis by retinoic acid. Additional photocarcinogenicity studies were not considered necessary since positive results with tretinoin are already available. These studies have been used in part to devise the label.

The sponsor conducted a limit teratology study with the Velac Gel and although there were no adverse effects on fetal development (IND 65,369, 2-06-2003), abnormalities described in the drug-treated groups, such as craniofacial and heart abnormalities, could be consistent with retinoid effects. At the pre-IND meeting the sponsor was informed that if no additional reproductive toxicity studies are conducted with the drug product then these results would be incorporated into the label. They were also told that they could conduct a definitive dermal teratology study, incorporating methods to

restrict access of the animals to the treatment site to reduce oral consumption of the gel. In addition, it was recommended that this definitive study be conducted in a nonrodent species, with dosing conducted so that steady state levels are achieved during the critical periods of development.

The sponsor has chosen to use the limit teratology data and supplement this with literature data for the effects of clindamycin and tretinoin on reproductive and developmental toxicology. They referenced a paper (Bollert *et al.*) in which rats and mice were treated by subcutaneous injection with 100 and 180 mg/kg clindamycin during gestation days 6 through 15. There was no indication of teratogenic effects and no detrimental effect on reproduction. In addition, oral clindamycin hydrochloride and clindamycin palmitate did not show any signs of teratogenicity in mice and rats (Gray *et al.*, 1972). Sprague-Dawley rats given up to 60 mg/kg clindamycin hydrochloride or up to 300 mg/kg clindamycin palmitate in the diet conceived at a slightly lower rate and their young were slightly smaller at weaning than the untreated controls, but otherwise no effect on reproductive performance was noted. The sponsor also submitted references indicating that tretinoin, administered at sufficient doses and during critical periods of development, is teratogenic and embryotoxic. Although topical tretinoin exposure is less likely to achieve sufficient levels to affect reproductive and development parameters, the label will contain the standard cautionary wording reflected in the label of various retinoid drug products.

Reviewer's comment: The sponsor's proposed label referred to the Bollert et al. paper, but erroneously referred to the higher subcutaneous dose as [REDACTED] (b) (4), as opposed to 180 mg/kg. This has been corrected in the Agency's recommended labeling and exposure multiples have been developed using the appropriate dosing information.

Based on the information provided, Veltin Gel is approvable from a pharmacology/toxicology perspective.

12 Appendix/Attachments

Attachments Included in the Appendix

Attachment #1 – Chemistry memorandum to address acceptable levels of lincomycin

Attachment #2 – Chemistry memorandum [REDACTED] (b) (4)
[REDACTED] (b) (4)

Attachment #3 – Clinical Pharmacology memorandum to address increased systemic exposure to clindamycin for Veltin Gel compared to Velac Gel

Attachment #4 – Exec CAC minutes for dermal carcinogenicity protocol

Attachment #5 – Exec CAC minutes for dermal carcinogenicity study review

Attachment #6 – Recommended labeling changes

Attachment #7 – Sponsor proposed wording for the nonclinical portions of the label

1) Memorandum (sent to Shulin Ding, 2-17-10)

To: NDA 50-803

From: Jill C Merrill, Ph.D.

Re:

Drug: Veltin Gel (1% clindamycin, 0.025% tretinoin)

Sponsor: Stiefel

Review date: February 16, 2010

Background:

Pharmacology/toxicology has been asked to comment on the sponsor's safety evaluation of the related substances in the drug product (January 19, 2010, Shulin Ding to Jill Merrill). In Section 3.2.P.5 Control of Drug Product (Module 3) the sponsor proposes a (b) (4) limit of not more than (NMT) (b) (4) of the clindamycin phosphate label claim (LC) and an (b) (4) limit of NMT (b) (4) of the tretinoin LC.

Information Supplied by the Sponsor in Section 3.2.P.5:

Clindamycin Phosphate LC = 1%

(b) (4) = NMT (b) (4) Clindamycin LC

Daily formulation application rate = 2 mg formulation /cm²

Area of application = 500 cm²

2 mg formulation/cm² x 500 cm² = 1000 mg formulation/application

Given that the (b) (4) is NMT (b) (4) of the clindamycin phosphate LC (1%), the (b) (4) is NMT (b) (4). With an application rate of 1000 mg of formulation/day and assuming 100% dermal absorption, the maximum amount of (b) (4) absorbed (b) (4). Assuming a reduced average body weight of 50 kg per patient, the maximum systemic exposure of (b) (4) would be (b) (4). This is about 50,000 times lower than the no effect levels (b) (4) reported in the literature for systemic toxicities in various biological systems and about (b) (4) times lower than the reported lowest toxic dose in humans after systemic absorption (b) (4).

Reviewer's comment: The sponsor reports this as (b) (4) times lower than the lowest toxic dose in humans after systemic administration. This appears to be an arithmetic error, but does not weaken their argument.

(b) (4) is a tretinoin-related substance that is a drug substance impurity, a process impurity and a drug product degradation product. Although the limit (b) (4) in the tretinoin drug substance (as defined in the current USP) is NMT (b) (4), Stiefel proposes a

tighter acceptance criterion for the drug substance of NMT (b) (4). Based on data taken from four different registration lots, the sponsor proposes (b) (4) is formed as a process impurity. Long-term stability studies (25°C/60% relative humidity, 18 months) with these same four lots suggest (b) (4) is formed during the stability period. Consequently (b) (4) a total (b) (4) limit (b) (4) is proposed for the drug product.

Tretinoin LC = 0.025%

(b) (4)

The topical application of tretinoin from the drug product and its subsequent metabolism and/or isomerization (b) (4) make it difficult to quantify the total exposure to (b) (4) from the drug product. However, (b) (4) the presence (b) (4) as an impurity or degradant at up to (b) (4) of tretinoin LC does not have an identifiable safety risk.

Pharmacology/Toxicology Discussion

The sponsor's proposed limits for (b) (4) (NMT (b) (4) clindamycin LC) and (b) (4) (NMT (b) (4) tretinoin LC) have been reviewed and found to be acceptable.

Jill C. Merrill
Reviewing Toxicologist

2) Memorandum (sent to Shulin Ding, 3-4-10)

To: NDA 50-803

From: Jill C Merrill, Ph.D.

Re:

Drug: Veltin Gel (1% clindamycin, 0.025% tretinoin)
Sponsor: Stiefel

Review date: March 4, 2010

Background:

Pharmacology/toxicology has been asked to comment on the sponsor's response to the following CMC comment (CMC/Clinical IR issued on 01-26-10):

3. The degradation peak which is from the vehicle and elutes at the same retention time o [REDACTED] (b) (4) (Section 3.2.P.5.3.3.8.1) should be controlled by the drug product specification with an acceptance criterion of NMT [REDACTED] (b) (4)

Information Supplied by the Sponsor in their CMC Response Part 1 (02-16-10):

(b) (4)

Pharmacology/Toxicology Discussion

The sponsor's proposed limit of NMT [REDACTED] (b) (4) of the proposed [REDACTED] (b) (4) concentration [REDACTED] (b) (4) for the degradation peak [REDACTED] (b) (4) have been reviewed and found to be acceptable from a pharmacology/toxicology perspective.

Jill C. Merrill
Reviewing Toxicologist

3) Memorandum (sent to Chinmay Shukla, 2-22-10)

To: NDA 50-803

From: Jill C Merrill, Ph.D.

Re:

Drug: Veltin Gel (1% clindamycin, 0.025% tretinoin)

Sponsor: Stiefel

Review date: February 22, 2010

Background:

Pharmacology/toxicology has been asked to comment on the adequacy of the sponsor's nonclinical testing and whether or not it covers the greater exposure of clindamycin and clindamycin sulfoxide using the same clindamycin concentration (1%) but in the CT Gel compared to the Velac Gel (February 12, 2010, Chinmay Shukla to Barbara Hill, Jill Merrill).

Repeat-dose nonclinical testing:

The sponsor has conducted two subchronic (13 week) dermal toxicity studies with the Velac formulation: one in rats (0, 1, 3, 5 mg/kg/day, Study No. 3600.7) and one in minipigs (0, 0.2, 3, 5 mg/kg/day, Study No. 3600.10); both reviewed by Jill Merrill under NDA 50-803, 05-16-05. In the rat study the NOAEL was the highest concentration tested, 5% clindamycin gel. In the minipig study the NOEL was the highest exposure level, which was designed to deliver 25x the Velac Gel exposure (5% clindamycin formulated in a gel applied to 5x area, ~20% BSA). In addition, 1% clindamycin in the CT Gel (equivalent to the Veltin gel formulation) was tested for dermal carcinogenicity using the traditional 2-year mouse model (NPB00012). The results of this study were negative.

Pharmacology/Toxicology Discussion:

Although Veltin Gel provides 1.5 to 2 times higher exposure to clindamycin and clindamycin sulfoxide, the nonclinical studies were done at sufficient excess to cover this increase in bioavailability.

Jill C. Merrill

Reviewing Toxicologist

4) Exec CAC minutes for dermal carcinogenicity protocol
Executive CAC Meeting Minutes
Date of Meeting: August 29, 2006

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair
Joseph Contrera, Ph.D., OPS, Member
William Taylor, Ph.D., DSPTP, Alternate Member
Paul Brown, Ph.D., DDDP, Supervisor
Jill Merrill, Ph.D., DDDP, Presenting Reviewer

Author of Draft: Jill Merrill

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 65,369

Drug Name: TBD (formerly Velac Gel)

Sponsor: Connetics

Background:

Velac Gel (1% clindamycin, 0.025% tretinoin) was developed by the sponsor as a dermal treatment for patients with acne vulgaris. The division previously informed the sponsor that the carcinogenic potential of tretinoin could be supported by the literature, but that it would be necessary to assess the carcinogenic potential of clindamycin in the clinical vehicle. The sponsor then conducted a Tg.AC mouse dermal carcinogenicity study for Velac Gel in which the vehicle alone caused a statistically significant increased incidence of skin papillomas compared to the untreated control and clindamycin in the Velac Gel caused a further increase in papillomas. These findings formed the basis of a nonapprovable letter for the associated NDA (50-803). The sponsor has subsequently modified the vehicle (b) (4) and (b) (4) polyoxyethylene 4 monolaurate ether (POE 4) (see below).

Composition of Velac and CT Gel (minus tretinoin)

Formulations	Velac	CT Gel
Active ingredients	Amount (% w/w)	
Clindamycin phosphate, USP	1.00	1.00
Inactive ingredients		
Butylated hydroxytoluene, NF	(b) (4)	
Carbomer 940 (b) (4), NF	(b) (4)	
Citric acid, USP	(b) (4)	
Edetate disodium, USP	(b) (4)	
Methylparaben, NF	(b) (4)	
Polyoxyethylene 4 Monolaurate Ether (POE 4)	(b) (4)	
(b) (4)	(b) (4)	
Propylene Glycol, USP	(b) (4)	
Tromethamine, USP	(b) (4)	
Purified water, USP	(b) (4)	
Total:	100.0	100.0

Note: Although the clinical formulation also contains tretinoin (0.025%), only clindamycin phosphate is being assessed in the proposed carcinogenicity study.

The sponsor plans to meet the carcinogenicity testing requirement for the clindamycin portion of their reformulated product by right-of-reference to an existing Tg.AC study. To meet the carcinogenicity testing requirement for their reformulated vehicle, the sponsor intends to conduct a 2-year dermal carcinogenicity study in CD-1 mice and has submitted results from a 90-day dose feasibility study in CD-1 mice.

Dermal Mouse Carcinogenicity Study Protocol and Dose Selection

The sponsor proposed a 2-year carcinogenicity study in mice with the following parameters:

Species/strain: CD-1 mice
 Number/sex/dose: 60
 Route: dermal application to shaved dorsal skin

Male and Female

Doses proposed (clindamycin): 0 (untreated control), 0 (80 µL vehicle), and 8, 16, 32 mg/kg (based on a 25 g mouse body weight) administered daily as 20, 40, and 80 µL of 1% clindamycin in the CTG vehicle

Complete histopathology is proposed for all groups.

- The sponsor has conducted a 13-week dose feasibility study in mice with clindamycin 1% in CTG vehicle (the clinical vehicle). Male and female mice were treated with 0, 20, 40 mg/kg (based on a 20 g mouse body weight) clindamycin in CTG vehicle in volumes of 40, 40 and 80 µL, respectively. Assessment of toxicity was based on mortality, clinical observations, dermal observations, body

weights, feed consumption, gross necropsy and limited histopathology. There were no test article-related mortalities or indications of systemic toxicity noted during the 90 days of dosing. Some mice administered 80 µL/day of 1% clindamycin in CTG vehicle had clinical signs of moderate to marked dermal erythema. No other adverse signs were observed clinically or at gross necropsy in mice administered 40 or 80 µL/day. There were no treatment-related effects on weight gain or feed consumption. Histologically, mice administered 40 or 80 µL/day of 1% clindamycin in CTG vehicle had minimal to moderate signs of dermal irritation characterized by epidermal hyperplasia, hyperkeratosis, and superficial dermatitis. The maximum tolerated dose in CD-1 mice was determined to be 80 µL/day of 1% clindamycin in CTG vehicle (~ 40 mg/kg clindamycin), based on these dermal findings.

Executive CAC Recommendations and Conclusions:

* The committee recommends that the sponsor perform a three armed study with dermal application of: 1) an untreated control; 2) 80 µL vehicle control; and 3) a 32 mg/kg arm (80 µL of reformulated vehicle containing 1% clindamycin). The other arms originally proposed are not needed, because the clindamycin was at the same concentration (1%) in all arms, and thus would not be exposing the skin to different doses. The protocol as recommended by FDA will examine the skin effects of the vehicle and clinical concentration of clindamycin phosphate, which is the purpose of the study.

* Hematology is not required for a carcinogenicity study.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\n
/Division File, DDDP
PBrown/Supervisor, DDDP
JMerrill/Reviewer, DDDP
MOwens/PM, DDDP
/ASeifried, OND IO

5) Exec CAC minutes for dermal carcinogenicity study review

Executive CAC Meeting Minutes **Date of Meeting:** February 2, 2010

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair
Paul Brown, Ph.D., OND IO, Member
David Joseph, Ph.D., DGP, Alternate Member
Barbara Hill, Ph.D., DDDP, Pharm Tox Supervisor
Jill Merrill, Ph.D., DDDP, Presenting Reviewer

Author of Draft: Jill Merrill

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 50-803

Drug Name: Veltin™ Gel (reformulation of Velac Gel)

Sponsor: Stiefel Laboratories Inc.

Background

Velac Gel (1% clindamycin, 0.025% tretinoin) was developed by the sponsor as a dermal treatment for patients with acne vulgaris. The Division of Dermatology and Dental Products previously informed the sponsor that the carcinogenic potential of tretinoin could be supported by the literature, but that it would be necessary to assess the carcinogenic potential of clindamycin in the clinical vehicle. The sponsor then conducted a Tg.AC mouse dermal carcinogenicity study for Velac Gel in which the vehicle alone caused a statistically significant increased incidence of skin papillomas compared to the untreated control. Clindamycin in the Velac Gel vehicle caused a further increase in papillomas. These findings formed the basis of a nonapprovable letter for the associated NDA (50-803). The sponsor subsequently modified the vehicle

(b) (4)

of polyoxyethylene 4 monolaurate ether. The present study (NPB00012) evaluates the carcinogenicity of the clinical concentration of clindamycin only in the reformulated vehicle.

Mouse Carcinogenicity Study

This study was designed to assess the carcinogenic potential of clindamycin with daily application to the skin of CD-1 mice for up to 104 weeks. The study groups (60 mice/sex/group) included the following: a sham treatment group; the clinical vehicle group (denoted CTG - clindamycin tretinoin gel without either clindamycin or tretinoin); 1% clindamycin in CTG vehicle (32 mg/kg/day). Dosing of either sex in the clindamycin-treated group was discontinued if the number of survivors in that sex reached 20 or less. Treatment of other groups continued. Any given treatment group of either sex was terminated and subjected to a complete necropsy if the number of surviving animals in that group declined to 15. Therefore, dosing was discontinued on Day 645 (Week 93) for clindamycin treated males and on Day 683 (Week 98) for clindamycin treated females. These groups were euthanized during Week 98 and Week 103, respectively.

The only treatment site specific tumors seen in the CTG vehicle-treated group included a benign squamous papilloma and a squamous cell carcinoma in the males and a fibrosarcoma in a female. These tumors were of an incidence and character of those seen spontaneously in control CD-1 mice at the testing facility. No treatment-related tumors were seen in the skin or in other tissue locations among mice treated with 1%

clindamycin in CTG vehicle. The other neoplastic findings were considered spontaneous, incidental lesions commonly observed in aging control CD-1 mice of this stock and are not considered test article related.

Executive CAC Recommendations and Conclusions:

- The Committee agreed that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concluded that the study was negative for drug related neoplasms.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\

/Division File, DDDP
/B Hill, DDDP
/JMerrill, DDDP
/CAttinello, DDDP
/ASeifried, OND IO

6) Recommended labeling changes

The sponsor of VELTIN Gel submitted an NDA that contains proposed labeling consistent with the physician's-labeling-rule (PLR) format. The portions of the label that pertain to nonclinical are provided after the discussion about the multiples of human exposure calculations used for the nonclinical sections of the label.

The recommended clinical dose is 1 gram daily and I am taking a conservative approach and using the average weight of 50 kg to protect teenage girls rather than the standard 60 kg.

VELTIN Gel : 1% clindamycin, 0.025% tretinoin

1% = 10 mg clindamycin / gram formulation

10 mg clindamycin / 50 kg = 0.2 mg clindamycin / kg body weight

0.025% tretinoin = 0.25 mg tretinoin/gram formulation

250 µg tretinoin

250 µg/50 kg = 5 µg/kg

Calculations for limit teratology study (rat)

2 mL/kg ~ 2 gram/kg

Safety margin for Clindamycin

$$20 \text{ mg/kg} \times 6/37 = 3.24 \text{ mg/kg} = \text{HED}$$

$$3.24 \text{ mg/kg} \div 0.2 \text{ mg/kg} = 16.2$$

Safety margin for Tretinoin

$$0.5 \text{ mg/kg} \times 6/37 = 0.081 \text{ mg/kg} = \text{HED}$$

$$81 \text{ } \mu\text{g/kg} \div 5 \text{ } \mu\text{g/kg} = 16.2 \text{ safety margin}$$

Calculations for clindamycin teratology

Oral dosing (Gray et al, TAP 21: 516-531, 1972)

$$600 \text{ mg/kg} \times 6/37 = 97.3 \text{ mg/kg HED (rat)}$$

$$600 \text{ mg/kg} \times 3/37 = 49 \text{ mg/kg HED (mouse)}$$

Safety margins:

$$\text{rat } 97.3 \text{ mg/kg} \div 0.2 \text{ mg/kg} = 486$$

$$\text{mouse } 49 \text{ mg/kg} \div 0.2 \text{ mg/kg} = 245$$

Subcutaneous dosing (Bollert et al, TAP 27: 322-329, 1974)

$$180 \text{ mg/kg} \times 6/37 = 29 \text{ mg/kg HED (rat)}$$

$$180 \text{ mg/kg} \times 3/37 = 14.6 \text{ mg/kg HED (mouse)}$$

Safety margins:

$$\text{rat } 29 \text{ mg/kg} \div 0.2 \text{ mg/kg} = 145$$

$$\text{mouse } 14.6 \text{ mg/kg} \div 0.2 \text{ mg/kg} = 73$$

Calculations for tretinoin teratology

Oral Wistar rat dosing (Seegmiller et al, JAAD 36 (3 Pt 2):S60-66, 1997)

$$\text{NOAEL } 1 \text{ mg/kg} \times 6/37 = 0.162 \text{ mg/kg (HED)}$$

Safety margin:

$$\text{rat } 162 \mu\text{g/kg} \div 5 \mu\text{g/kg} = 32$$

Oral monkey dosing (Hendrickx and Hummler, Teratology 45 (1):65-74,1992)

$$\text{NOAEL } 5 \text{ mg/kg} \times 12/37 = 1.621 \text{ mg/kg (HED)}$$

Safety margin:

$$\text{monkey } 1621 \mu\text{g/kg} \div 5 \mu\text{g/kg} = 324$$

Calculations for clindamycin mouse dermal carcinogenicity study

$$\text{NOAEL } 32 \text{ mg/kg} \times 3/37 = 2.59 \text{ mg/kg (HED)}$$

Safety margin:

$$2.59 \text{ mg/kg} \div 0.2 \text{ mg/kg} = 12.95$$

Calculations for tretinoin mouse dermal carcinogenicity study (Hennings et al., Cancer Lett 16(1):1-5, 1982)

$$5.1 \mu\text{g} / 0.025 \text{ kg} = 200 \mu\text{g/kg (dose)}$$

$$200 \mu\text{g/kg} \times 3/7 = 85 \mu\text{g/kg (three times weekly)}$$

$$85 \mu\text{g/kg} \times 3/37 = 6.95 \mu\text{g/kg (HED)}$$

Safety margin:

$$6.95 \mu\text{g/kg} \div 5 \mu\text{g/kg} = 1.4$$

Calculations for fertility section

Clindamycin (Gray et al., TAP 21:516-531, 1972):

$$300 \text{ mg clindamycin/kg} \times 6/37 = 48.65 \text{ mg/kg (HED)}$$

Safety margin:

$$48.65 \text{ mg/kg} \div 0.2 \text{ mg/kg} = 243$$

Tretinoin (Kamm, JAAD; 6(4 Pt 2 S):652-659, 1982):

NOAEL 2 mg tretinoin/ kg x 6/37 = 0.32 mg/kg (HED)

Safety margin:

$$320 \mu\text{g/kg} \div 5 \mu\text{g/kg} = 64$$

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C.

There are no well-controlled studies in pregnant women treated with VELTIN Gel. VELTIN Gel should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

A limit teratology study performed in Sprague Dawley rats treated topically with VELTIN Gel or 0.025% tretinoin gel at a dose of 2 mL/kg during gestation days 6 to 15 did not result in teratogenic effects. Although no systemic levels of tretinoin were detected, craniofacial and heart abnormalities were described in drug-treated groups. These abnormalities are consistent with retinoid effects and occurred at 16 times the recommended clinical dose assuming 100% absorption and based on body surface area comparison. For purposes of comparisons of the animal exposure to human exposure, the recommended clinical dose is defined as 1 g of VELTIN Gel applied daily to a 50 kg person.

Clindamycin

Reproductive developmental toxicity studies performed in rats and mice using oral doses of clindamycin up to 600 mg/kg/day (480 and 240 times the recommended clinical dose based on body surface area comparison, respectively) or subcutaneous doses of clindamycin up to 180 mg/kg/day (140 and 70 times the recommended clinical dose based on body surface area comparison, respectively) revealed no evidence of teratogenicity.

Tretinoin

Oral tretinoin has been shown to be teratogenic in mice, rats, hamsters, rabbits, and primates. It was teratogenic and fetotoxic in Wistar rats when given orally at doses greater than 1 mg/kg/day (32 times the recommended clinical dose based on body surface area comparison). However, variations in teratogenic doses among various strains of rats have been reported. In the cynomolgous monkey, a species in which tretinoin metabolism is closer to humans than in other species examined, fetal malformations were reported at oral doses of 10 mg/kg/day or greater, but none were observed at 5 mg/kg/day (324 times the recommended clinical dose based on body surface area comparison), although increased skeletal variations were

observed at all doses. Dose-related teratogenic effects and increased abortion rates were reported in pigtail macaques.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Clindamycin

[See Microbiology (12.4).]

Tretinoin

Although the exact mode of action of tretinoin is unknown, current evidence suggests that topical tretinoin decreases cohesiveness of follicular epithelial cells with decreased microcomedone formation. Additionally, tretinoin stimulates mitotic activity and increased turnover of follicular epithelial cells causing extrusion of the comedones.

12.4 Microbiology

Clindamycin binds to the 50S ribosomal subunit of susceptible bacteria and prevents elongation of peptide chains by interfering with peptidyl transfer, thereby suppressing protein synthesis. Clindamycin has been shown to have *in vitro* activity against *Propionibacterium acnes* (*P. acnes*), an organism that has been associated with acne vulgaris; however, the clinical significance of this activity against *P. acnes* was not examined in clinical studies with VELTIN Gel. *P. acnes* resistance to clindamycin has been documented. Resistance to clindamycin is often associated with resistance to erythromycin.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of VELTIN Gel or the effect of VELTIN Gel on fertility.

VELTIN Gel was negative for mutagenic potential when evaluated in an *in vitro* Ames *Salmonella* reversion assay. VELTIN Gel was equivocal for clastogenic potential in the absence of metabolic activation when tested in an *in vitro* chromosomal aberration assay.

Clindamycin

Once daily dermal administration of 1% clindamycin as clindamycin phosphate in the VELTIN Gel vehicle (32 mg/kg/day, 13 times the recommended clinical dose based on body surface area comparison) to mice for up to 2 years did not produce evidence of tumorigenicity.

Fertility studies in rats treated orally with up to 300 mg/kg/day of clindamycin (240 times the recommended clinical dose based on a body surface area comparison) revealed no effects on fertility or mating ability.

Tretinoin

In two independent mouse studies where tretinoin was administered topically (0.025% or 0.1%) three times per week for up to two years no carcinogenicity was observed, with maximum effects of dermal amyloidosis. However, in a dermal carcinogenicity study in mice, tretinoin applied at a dose of 5.1 µg (1.4 times the recommended clinical dose based on body surface area comparison) three times per week for 20 weeks acted as a weak promoter of skin tumor formation following a single application of dimethylbenz[α]anthracene (DMBA).

In a study in female SENCAR mice, papillomas were induced by topical exposure to DMBA followed by promotion with 12-O-tetradecanoyl-phorbol 13-acetate or mezerein for up to 20 weeks. Topical application of tretinoin prior to each application of promoting agent resulted in a reduction in the number of papillomas per mouse. However, papillomas resistant to topical tretinoin suppression were at higher risk for pre-malignant progression.

Tretinoin has been shown to enhance photo-co-carcinogenicity in properly performed specific studies, employing concurrent or intercurrent exposure to tretinoin and UV radiation. The photo-co-carcinogenic potential of the clindamycin tretinoin combination is unknown. Although the significance of these studies to humans is not clear, patients should avoid exposure to sun.

The genotoxic potential of tretinoin was evaluated in an *in vitro* Ames *Salmonella* reversion test and an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells. Both tests were negative.

In oral fertility studies in rats treated with tretinoin, the no-observed-effect-level was 2 mg/kg/day (64 times the recommended clinical dose based on body surface area comparison).

7) Sponsor proposed wording for the nonclinical portions of the label

The sponsor proposed wording for the nonclinical portions of the label are provided below for reference purposes.

2 Pages of Draft Labeling have been Withheld in Full as B4(CCI/TS) Immediately Following this Page

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Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-50803

ORIG-1

STIEFEL A GSK CO Veltin

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/s/

JILL C MERRILL
04/15/2010

BARBARA A HILL
04/15/2010
I concur

Pharmacology/Toxicology Summary
NDA Mid-Cycle Review Meeting

NDA 50-803

Mid-Cycle Review Meeting

Date: 02-17-10

Drug: 1% clindamycin, 0.025% tretinoin

Drug name: Veltin Gel

Reviewer: Jill C. Merrill

Background:

Veltin Gel is a reformulation of Velac Gel which was tested in the Tg.AC mouse dermal carcinogenicity model. The vehicle alone caused a statistically significant increased incidence of skin papillomas compared to the untreated control and clindamycin in the vehicle caused a further increase in papillomas. These findings formed the basis of a nonapprovable letter for the associated NDA (50-803). The sponsor subsequently modified the vehicle by (b) (4)

polyoxyethylene 4 monolaurate ether. The clinical concentration of clindamycin, formulated in the new vehicle but without tretinoin, was tested for dermal carcinogenicity in a 2-year mouse dermal model (NPB00012). This study was reviewed by the eCAC (February 2, 2010) which concluded that the study was acceptable and negative for drug neoplasms.

The sponsor obtained right-to-reference for an additional Tg.AC mouse dermal study evaluating clindamycin (0.5%, 1.0%, 2.0%) in a topical gel containing 17.3% acrylate copolymer. This study was confounded by the reallocation of previously randomized females with more severe dermal abrasions to the positive control group. Randomization of animals assigned to experimental groups is necessary to ensure that underlying variables do not skew the data for each of the groups and is a fundamental aspect of experimental design. This was discussed at the eCAC meeting and it was agreed that this study will not be reviewed and that no further testing is necessary for the current drug product.

By prior agreement, the sponsor has provided literature data to address the carcinogenic potential of tretinoin.

Review status:

Under review. Projected completion date: 03-02-10.

Review issues:

Pharmacology/toxicology has responded to issues posed by the Chemistry reviewer concerning the adequacy of the sponsor's drug product specifications via email on 2-17-10.

The issues posed by the BA/BE study are currently being reviewed from a Pharmacology/Toxicology perspective.



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: **50-803**
SERIAL NUMBER: **000**
DATE RECEIVED BY CENTER: **08/26/04**
PRODUCT: **Velac Gel (clindamycin 1%, tretinoin 0.025%)**
INTENDED CLINICAL POPULATION: **Patients with acne vulgaris**
SPONSOR: **Connetics Corporation**
DOCUMENTS REVIEWED: **Vol. 1-9**
REVIEW DIVISION: **Division of Dermatological and Dental Drug
Products (HFD-540)**
PHARM/TOX REVIEWER: **Jill C Merrill**
PHARM/TOX SUPERVISOR: **Paul C Brown**
DIVISION DIRECTOR: **Dr. Jonathan Wilkin**
PROJECT MANAGER: **Margo Owens**

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	5
2.6.1 INTRODUCTION AND DRUG HISTORY	5
2.6.2 PHARMACOLOGY.....	9
2.6.2.1 Brief summary.....	9
2.6.2.2 Primary pharmacodynamics.....	9
2.6.2.3 Secondary pharmacodynamics.....	10
2.6.2.4 Safety pharmacology.....	10
2.6.2.5 Pharmacodynamic drug interactions.....	10
2.6.3 PHARMACOLOGY TABULATED SUMMARY	11
2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....	11
2.6.4.1 Brief summary.....	11
2.6.4.2 Methods of Analysis.....	11
2.6.4.3 Absorption.....	11
2.6.4.4 Distribution.....	12
2.6.4.5 Metabolism.....	12
2.6.4.6 Excretion.....	13
2.6.4.7 Pharmacokinetic drug interactions.....	14
2.6.4.8 Other Pharmacokinetic Studies.....	14
2.6.4.9 Discussion and Conclusions.....	14
2.6.4.10 Tables and figures to include comparative TK summary.....	14
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	15
2.6.6 TOXICOLOGY.....	15
2.6.6.1 Overall toxicology summary.....	15
2.6.6.2 Single-dose toxicity.....	19
2.6.6.3 Repeat-dose toxicity.....	19
2.6.6.4 Genetic toxicology.....	29
2.6.6.5 Carcinogenicity.....	31
2.6.6.6 Reproductive and developmental toxicology.....	41
2.6.6.7 Local tolerance.....	42
2.6.6.8 Special toxicology studies.....	42
2.6.6.9 Discussion and Conclusions.....	43
2.6.6.10 Tables and Figures.....	44
2.6.7 TOXICOLOGY TABULATED SUMMARY.....	44
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	44
APPENDIX/ATTACHMENTS	46

EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability – Not approvable based on a positive signal in the Tg.AC mouse carcinogenicity study.
- B. Recommendation for nonclinical studies – No additional nonclinical studies are recommended for Velac Gel at this time.
- C. Recommendations on labeling - NA

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings - A dosage level of 5 mg/kg/day clindamycin gel, the highest dose tested, was considered to be the NOAEL following 90 days of topical application in male and female rats. Following dermal application in Hanford minipigs for 13 weeks, the systemic NOEL was 500 mg/kg/day Velac Gel and the local/dermal NOEL could not be established. Velac Gel was determined to be nonmutagenic in an *in vitro* Ames *Salmonella* reversion test. The non-compendial excipients, POE 4 (b) (4), have been tested as components of the gel vehicle in repeat dose toxicity tests and in the limit teratology study. They have also been tested for *in vitro* genotoxicity (Ames and chromosome aberration assays). No toxic or genotoxic signal was detected in these tests. Velac Gel was determined to have equivocal clastogenic activity in a chromosome aberration assay. However, when tested in a 26-week dermal carcinogenicity study in Tg.AC mice, the vehicle alone caused a statistically significant increased incidence of skin papillomas compared to the untreated controls and clindamycin in the Velac Gel vehicle caused further significant dose-related increases in papillomas relative to the vehicle controls and untreated animals. The sponsor has argued that dermal site of application irritation is a predictor for papilloma formation and as such the skin tumors are a nonspecific response to dermal insult. However, the eCAC is aware of other studies in Tg.AC mice in which irritation alone was not sufficient to cause papillomas. It is concluded that the vehicle is at best, a promoter, and at worst, a complete carcinogen.
- B. Pharmacologic activity – Clindamycin phosphate is a lincosamide antibiotic; Tretinon is a retinoid.
- C. Nonclinical safety issues relevant to clinical use - In general, the most pronounced toxicity associated with clindamycin has been pseudomembranous colitis. This is believed to be caused by an overgrowth of a toxin producing *Clostridium difficile*. However, because systemic exposure following topical application of clindamycin is low, it is not anticipated that subjects receiving topical clindamycin will be affected. A warning about this

adverse effect is included in the labels of all currently approved formulations of clindamycin. Tretinoin, like other retinoids, is teratogenic and embryotoxic in multiple species when administered at sufficient doses and at the vulnerable gestational time period. Doses of tretinoin that do not cause morphological changes in offspring may cause behavioral effects in the developing animals. Topical application of tretinoin appears to be less likely to result in teratogenic or other effects probably due to lower systemic and embryo exposure to tretinoin by the topical route than by the oral route.

Topical application of clindamycin in the Velac Gel vehicle caused an increase in papilloma formation in all treatment groups, including the vehicle control, when compared to the untreated controls in a Tg.AC mouse model for carcinogenicity. These findings indicate the drug product is, at best, a tumor promoter, and at worst, a complete carcinogen. A 2-year mouse dermal carcinogenicity study would distinguish between the two, but neither are acceptable for a topical drug for acne, where application to initiated skin can be expected.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 50-803

Review number: 1

Sequence number/date/type of submission: SN000/8-23-04/original NDA submission

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: Connetics Corporation

Manufacturer for drug substance:

Reviewer name: Jill C. Merrill

Division name: Dermatological and Dental Drug Products

HFD #: 540

Review completion date:

Drug:

Trade name: Velac Gel

Generic name: Clindamycin phosphate and tretinoin (retinoic acid, all-*trans*-retinoic acid)

Chemical name: Clindamycin phosphate: methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo*- α -D-galactooctopyranoside 2-(dihydrogen phosphate)

Tretinoin: *all*-E 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid

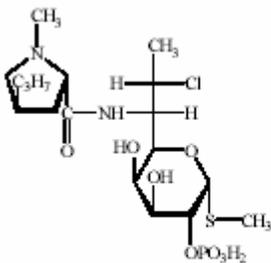
CAS registry number: clindamycin phosphate: 24729-96-2; tretinoin: 302-79-4

Molecular formula/molecular weight:

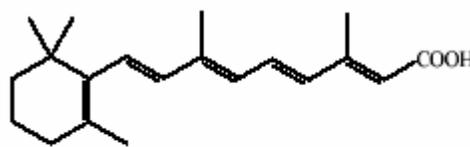
Clindamycin phosphate: $C_{18}H_{34}ClN_2O_8PS$ / MW = 504.97

Tretinoin: $C_{20}H_{28}O_2$ / MW = 300.44

Structure:



Clindamycin phosphate



Tretinoin

Relevant INDs/NDAs/DMFs:

IND 65,369

Drug class: antibiotic and retinoid

Intended clinical population: patients with acne vulgaris

Clinical formulation:

Quantitative composition of Velac Gel

Ingredients	Concentration (% w/w)
Clindamycin Phosphate	1.0 (expressed as clindamycin)
Tretinoin	0.025
Butylated Hydroxytoluene	(b) (4)
Carbomer 940 (b) (4)	(b) (4)
Citric Acid, anhydrous	
Edetate Disodium	
Methylparaben	
(b) (4)	
Polyethylene 4 Monolauryl Ether (POE 4) (b) (4)	
(b) (4)	
Propylene Glycol	
Tromethamine	
Purified Water	
(b) (4)	

Route of administration: topical to the skin

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

Fileability Status: This NDA is being filed under section 505(b)2 of the federal FD&C Act because it is supported in part by reference to published literature for fertility and peri-/postnatal development for which Connetics Corporation does not have right of reference to the underlying data.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 50-803 are owned by Connetics Corporation or are data for which Connetics Corporation has obtained a written right of reference. Any information or data necessary for approval of NDA 50-803 that Connetics Corporation does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug’s approved labeling. Any data or information described or referenced below from a previously approved application that Connetics Corporation does not own (or from FDA reviews or summaries of a previously

approved application) is for descriptive purposes only and is not relied upon for approval of NDA 50-803.

Studies reviewed within this submission:

In vitro skin penetration testing of Velac topical formulations (Study No. SL04-35).
A 90-day dermal toxicity study in rats with clindamycin (Study No. 3600.7)
A 13-week dermal toxicity study in rats with clindamycin (Study No. 3600.9)
26-Week dermal carcinogenicity study in Tg.AC mice (Study No. AA81EW.7D8T.BTL)

Studies not reviewed within this submission:



An investigation in the rabbit of the comedolytic activity of a gel containing tretinoin 0.025% and clindamycin phosphate 1.2% (Study no. 92014). This study was previously submitted/reviewed under IND 65,369 (SN000).

Assessment of acute oral toxicity with clindamycin-tretinoin-gel in the mouse (Study no. 081361). This study was previously submitted/reviewed under IND 65,369 (SN000) as Study No. 93004.

Assessment of acute oral toxicity with clindamycin-tretinoin-gel in the rat (Study no. 081359). This study was previously submitted/reviewed under IND 65,369 (SN000) as Study No. 93003.

Salmonella – Escherichia coli/mammalian –microsome reverse mutation assay with polyethylene-4 monolauryl ether. (Study no. 24043-0-409OECD). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Chromosome aberration in Chinese hamster ovary (CHO) cells with polyethylene-4 monolauryl ether. (Study no. 24043-0-437OECD). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Salmonella – *Escherichia coli*/mammalian –microsome reverse mutation assay with Glycerox L15. (Study no. 24042-0-409OECD). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Chromosomal aberrations in Chinese hamster ovary (CHO) cells with Glycerox L15. (Study no. 24300-0-437OECD). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Evaluation of the mutagenic activity of vehiculum clindamycin-tretinoin gel in the Ames *Salmonella*/microsome test (with independent repeat). (Study no.094782). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Evaluation of the mutagenic activity of tretinoin gel in the Ames *Salmonella*/microsome test (with independent repeat). (Study no.094771). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Evaluation of the ability of tretinoin to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat). (Study no. 187537). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Evaluation of the mutagenic activity of clindamycin phosphate in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay. (Study no. 187559). This study was previously reviewed under IND 65,369 (SN026) by Dr. Paul Brown.

Evaluation of the ability of clindamycin phosphate to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat). (Study no. 187548). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Evaluation of the mutagenic activity of clindamycin-tretinoin gel in the Ames *Salmonella*/microsome test (with independent repeat). (Study no. 094793). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

In vitro assessment of the clastogenic activity of clindamycin-tretinoin gel in human lymphocytes. (Study no. 94/BSM004/0576). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Limit teratology study in the rat by the dermal route. (Study no. 93/BSM001/1174). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Primary skin irritation/corrosion study with clindamycin-tretinoin-gel in the rabbit (4-hour semi-occlusive application). (Study no. 081372). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Determination of phototoxicity with clindamycin-tretinoin-gel in albino guinea pigs. (Study no. 081394). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Assessment of contact hypersensitivity to clindamycin-tretinoin-gel in albino guinea pigs (Maximization test). (Study no. 081383). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

Determination of photoallergenicity with clindamycin-tretinoin-gel in albino guinea pigs. (Study no. 351494). This study was previously submitted/reviewed under IND 65,369 (SN000) by Dr. Paul Brown.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Clindamycin phosphate:

Clindamycin binds to the 50S subunit of bacterial ribosomes and thereby interferes with bacterial protein synthesis. Clindamycin is primarily bacteriostatic. Clindamycin is active against gram positive cocci and most anaerobic gram negative organisms. Its activity against the anaerobe *Propionibacterium acnes* may account for its effectiveness in the treatment of acne vulgaris. Clindamycin decreases the percentage of skin surface free fatty acids, and decreases the number of *Propionibacterium acnes* in comedones.

Tretinoin:

Tretinoin, also known as all- trans- retinoic acid, is a member of the retinoid family of compounds and is an endogenous metabolite of vitamin A. Tretinoin, like other retinoids, regulates gene transcription through interaction with intracellular retinoic acid receptors. Retinoids impact a variety of cellular and physiologic processes. Although the exact mechanism by which retinoids are beneficial in acne is unknown, current evidence suggests that topical tretinoin decreases cohesiveness of follicular epithelial cells and decreases microcomedone formation. Additionally, tretinoin stimulates mitotic activity and increases turnover of follicular epithelial cells causing the stratum corneum to become thinner and extrude comedones. Tretinoin has also been shown to suppress sebum gland activity, most probably by virtue of its antiproliferative effect on sebum producing cells.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

The combination of clindamycin and tretinoin in Velac Gel potentially offers comedolytic, anti-bacterial, and anti-inflammatory pharmacological properties that should be effective in the treatment of acne.

Drug activity related to proposed indication:

The sponsor has submitted a study entitled “An investigation in the rabbit of the comedolytic activity of a gel containing tretinoin 0.025% and clindamycin phosphate 1.2%” (Study no. 92014). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. A summary of this previously reviewed study appears below:

This study addresses the comedolytic activity of three gels. One is a gel containing tretinoin (0.025%) and clindamycin phosphate (1.2%) in a base that is slightly different than the proposed product. The other two gels contain 0.025% tretinoin only; one in the same base as the clindamycin/tretinoin gel and the other is a reference product. In this study rabbits were treated with coal tar on both ears for two weeks to induce comedones. Then only the left ear was treated with one of the gels. The animals were then sacrificed and the epidermis and dermis of the ears were separated and comedones were counted. The clindamycin/tretinoin gel caused a 53% decrease; tretinoin in the same base caused a 42% decrease and the reference tretinoin gel a 14% decrease in comedones compared to the corresponding right ears. The submitted study suggests that a gel containing clindamycin and tretinoin is comedolytic. The study also suggests that the combination of clindamycin and tretinoin may be more comedolytic than tretinoin alone.

2.6.2.3 Secondary pharmacodynamics

No studies to address secondary pharmacodynamics were included in this submission.

2.6.2.4 Safety pharmacology

Although the sponsor has not submitted nonclinical safety pharmacology studies, transient neuromuscular blockade is a recognized side effect of clinical use of antibiotics, including clindamycin. Extensive analysis of the blockade has led to the conclusion that clindamycin exerts its main effect post-synaptically at the neuromuscular junction, with a minor component of the inhibition also occurring pre-synaptically. The basis for these effects has been determined to be the lipophilic nature of the structure of clindamycin, which allows the molecule to compete with calcium for entry into nerve terminals, resulting in interference with nerve transmission. The effect of clindamycin on neuromuscular transmission has potential relevance to gastrointestinal smooth muscle function and the development of enterocolitis. However, because systemic exposure following topical application of clindamycin is low, it is not anticipated that patients receiving treatment with Velac Gel will be affected.

2.6.2.5 Pharmacodynamic drug interactions

No studies to address pharmacodynamic drug interactions were included in this submission. However, Velac Gel should not be used in combination with photosensitizing drugs because of the possibility of tretinoin-augmented phototoxicity. Additionally patients should not expose Velac gel-treated skin to certain compounds

because of a possible interaction with tretinoin. These include concomitant topical medications, soaps/cosmetics with a drying effect, products with a high concentration of alcohol, astringents, spices, or lime. Velac Gel should be used with caution in patients receiving neuromuscular blocking agents because clindamycin has been shown to enhance their action.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

This section is not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The pharmacokinetic characteristics of both tretinoin and clindamycin by multiple routes of administration have been investigated for over three decades and are well characterized. ADME information available in the public domain indicates that systemic exposure to topical tretinoin or clindamycin is limited when compared to systemic levels observed following parenteral administration. It is also recognized that the presence of cutaneous lesions and the possibility of topical drug ingestion by study animals could produce greater systemic exposure than would be seen via strictly percutaneous routes. Despite these factors, systemic exposure following topical application was significantly lower in the majority of nonclinical studies when compared to other routes of administration.

2.6.4.2 Methods of Analysis

This section is not applicable.

2.6.4.3 Absorption

Clindamycin:

Nonclinical studies describing clindamycin absorption following oral, i.v., s.c., i.m., i.p., and topical administration have been described in published literature. Absorption of clindamycin phosphate, clindamycin HCl, and clindamycin palmitate is accompanied by activation to clindamycin and its derivatives. Topical administration of clindamycin results in measurable quantities of clindamycin locally (in the eye, or skin, with the epidermis having a higher absorption than the dermis), but systemic absorption is minimal compared to that observed following oral, i.v., i.m., i.p., or s.c. dosing (Devlin *et al.*, 1978; Mercer *et al.*, 1978; Gray *et al.*, 1983).

Tretinoin:

Absorption following topical administration of tretinoin has been studied in rats, hamsters, rabbits, and monkeys (Chou *et al.*, 1997; Franz and Lehman, 1990; Wilhite *et*

al., 1990; Christian *et al.*, 1997). Confounding factors in the interpretation of some of these studies include the presence of serious cutaneous lesions at the site of application that allow increased absorption (Franz and Lehman, 1990) and the potential for tretinoin ingestion by test animals from the application sites, urine or feces (Wilhite *et al.*, 1990). However, despite these caveats, the majority of studies demonstrated limited or no measurable absorption of tretinoin following topical application (Chou *et al.*, 1997; Franz and Lehman, 1990; Christian *et al.*, 1997). In one hamster study that did not preclude significant oral intake (Wilhite *et al.*, 1990), substantial absorption was reported, but C_{max} values were still low compared to those observed following oral administration of an equivalent dose (Howard *et al.*, 1989). Because these topical studies demonstrated limited absorption of tretinoin via the percutaneous route, no new tretinoin absorption studies have been conducted by Connetics.

2.6.4.4 Distribution

Clindamycin:

Nonclinical distribution studies following oral, i.v., i.m., or i.p. dosing show that once absorbed, clindamycin is rapidly distributed in the blood and tissues, including the lung, skin, skeletal muscle, liver, heart, pancreas, bones, and spleen (Brown *et al.*, 1975; Imoto *et al.*, 1981; Mader *et al.*, 1989; Brown *et al.*, 1990). Although topical dosing results in very low levels of absorbed drug, distribution patterns of absorbed drug do not differ significantly from that observed following systemic dosing, except for enhanced levels locally in the skin or the eye following dermal (Gray *et al.*, 1983) or ocular administration (Mercer *et al.*, 1978), respectively.

Tretinoin:

Tretinoin is distributed in the plasma, kidney, small intestines, conceptus, liver, heart, fat, and brain (Howard *et al.*, 1989; Kalin *et al.*, 1981; Le Doze *et al.*, 2000). Tretinoin and its metabolites have also been detected in the muscle, lung, adrenals, uterus, and bladder. Nonclinical distribution studies following topical administration of tretinoin have not been performed due to low levels of absorption, but any absorbed tretinoin would be expected to demonstrate distribution patterns similar to profiles observed for tretinoin in the systemic circulation, except at much lower levels. Distribution studies have demonstrated rapid and efficient transfer of tretinoin and its metabolites, particularly the *trans* metabolites, to the embryo following oral dosing (Collins *et al.*, 1994; Gunning *et al.*, 1993; Kraft *et al.*, 1987; Kraft *et al.*, 1989; Howard *et al.*, 1989; Tzimas *et al.*, 1995; Ward and Morriss-Kay, 1995).

2.6.4.5 Metabolism

Clindamycin:

Metabolic studies have been conducted following oral, i.v., and i.m. administration of clindamycin. Although no studies on the metabolism of clindamycin have been

conducted following topical dermal administration, metabolic pathways would be expected to be similar to that observed following systemic dosing, but at much lower levels. Metabolism studies have demonstrated that clindamycin is metabolized in the liver, and is excreted primarily in the bile and secondarily in the urine as free clindamycin and bioactive and inactive metabolites (Brown *et al.*, 1975; Brown *et al.*, 1990). These metabolites include clindamycin sulphoxide, clindamycin glucuronide, and N-demethylclindamycin (Onderdonk *et al.*, 1981; Sun F.F., 1973; Berg-Candolfi and Candolfi, 1996). Chronic administration of clindamycin does not appear to modify metabolism of the drug (Sun F.F., 1973).

Tretinoin:

Metabolism studies in a number of species have been conducted following oral, i.v., s.c., and i.p. administration. These studies have shown that the metabolism of tretinoin is dependent on schedule, dose, and route of administration (Collins *et al.*, 1995; Tzimas *et al.*, 1997; Swanson *et al.*, 1981). In general the oral route favors metabolism via *B*-glucuronidation, whereas elimination by this mechanism is less important following s.c administration. Six major metabolites of tretinoin have been observed following systemic dosing: all-*trans*-4-oxo-retinoic acid (AT-4-oxo-RA), all-*trans*-retinoyl-*B*-glucuronide (AT-RAG), 13-*cis*-retinoic acid (13-*cis*-RA), 13-*cis*-4-oxo-retinoic acid (13-*cis*-4-oxo-RA), 13-*cis*-retinoyl-*B*-glucuronide (13-*cis*-RAG), and retinyl palmitate/oleate (Collins *et al.*, 1995; Tzimas *et al.*, 1994). Because the metabolism of tretinoin is well characterized in published studies and absorption is low following topical dosing, no new studies on the metabolism of tretinoin have been conducted by Connetics. It is not anticipated that any percutaneously absorbed tretinoin will be metabolized significantly differently than what is described in the literature.

2.6.4.6 Excretion

Clindamycin:

Clindamycin and its metabolites are excreted via urine and feces (Sun F.F., 1973; Sun and Hsi, 1973). Excretion rates may differ with route of clindamycin administration (Budsberg *et al.*, 1992; Lavy *et al.*, 1999; Sun and Hsi, 1973) and maybe affected by the presence of liver disease (Brown *et al.*, 1975). Excretion studies following topical application of clindamycin have not been conducted due to low levels of systemic absorption. It is anticipated that any absorbed drug will exhibit elimination patterns similar to those following systemic dosing.

Tretinoin:

Tretinoin and its metabolites are excreted mainly in the bile and urine (Kalin *et al.*, 1981; Swanson, *et al.*, 1981), with the percentage excreted in the urine decreasing with increasing doses, indicating saturable renal excretion. Damage to the liver may affect tretinoin clearance (Swanson, *et al.*, 1981). Elimination studies have not been performed following topical administration of tretinoin, except for a single hamster study in which

increased systemic exposure may have occurred due to oral ingestion (Wilhite *et al.*, 1990). In this study, elimination was dose-dependent but slower than that observed following i.v. administration in the same species. Because percutaneous absorption is low following topical application, no new elimination studies on tretinoin have been conducted by Connetics.

2.6.4.7 Pharmacokinetic drug interactions

No studies to address pharmacokinetic drug interactions were included in this submission.

2.6.4.8 Other Pharmacokinetic Studies

The sponsor has submitted a study entitled “*In vitro* skin penetration testing of Velac topical formulations” (Study No. SL04-35). Velac drug substance was formulated into a gel vehicle (Lot # SIAC-1C), two cream formulations and a lotion formulation and these four formulations were tested in an *in vitro* human skin penetration model using a finite dose technique. Human abdomen skin without obvious signs of disease obtained from four different donors during elective surgery was dermatomed to approximately 0.25 mm thickness and stored at ~-80°C until the day of the experiment. For the experiment, the skin was mounted in specially designed diffusion chambers maintained at a temperature and humidity matching typical *in vivo* conditions. A finite dose (4-6 mg/cm²) of the test article was applied to the outer surface of the skin for 24 hours during which time the receptor fluid was sampled every 4 hours and saved for subsequent analysis. Drug absorption was measured by monitoring the rate of appearance of clindamycin and tretinoin in the receptor fluid using HPLC. Skin content was determined by extraction and analysis of clindamycin and tretinoin from the epidermis and dermis.

Velac formulations delivered significant amounts of clindamycin phosphate into the epidermis and dermis. The amount of clindamycin phosphate could be assessed from receptor fluid samples, whereas that of tretinoin could not be calculated because the sample concentrations were below the limit of detection for the analysis. All four Velac formulations delivered similar amounts of clindamycin phosphate and tretinoin into the epidermis and dermis.

2.6.4.9 Discussion and Conclusions

Literature studies to address the nonclinical pharmacokinetics of the combined drug product, Velac Gel, are not available. It is not expected that the combination of clindamycin phosphate and tretinoin would lead to significantly different, or new, ADME characteristics.

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:

Clindamycin:

The most pronounced toxicity associated with systemic exposure to clindamycin is pseudomembranous colitis (Feingold *et al.*, 1979; Carlstedt-Duke *et al.*, 1985). While the rat and dog do not demonstrate this toxicity, it has been observed in hamsters (Feingold *et al.*, 1979) and in humans (Freeman and Wilcox, 1999). It is believed that the ability of clindamycin to suppress the growth of indigenous flora of the gut and to interfere with intestinal motility and ion transport may contribute to the colonization of the gut by toxigenic bacteria, including *Clostridium difficile* (Percy and Christensen, 1985; Goldhill *et al.*, 1996). Production of toxins by *C. difficile* leads to the development of enterocolitis. Discontinuation of clindamycin administration and therapy with other antibiotics known to inhibit *C. difficile* growth, such as vancomycin or tetracycline, has proven to be an effective means to treat clindamycin-induced enterocolitis (Fisher, 1983; George, 1984). A warning about this adverse effect is included in the labels of currently approved formulations of clindamycin and labeling for Velac Gel will include warnings regarding discontinuation of treatment in the event that diarrhea or other gastrointestinal symptoms occur.

The maximum tolerated dose of clindamycin HCl published for a one-year study in dogs was between 300 and 600 mg/kg (Gray *et al.*, 1972). Clindamycin palmitate at oral doses of 100, 300, and 600 mg/kg was well-tolerated by rats in a 6-month study. Toxicity associated with repeated topical application of clindamycin was evaluated in rats and pigs over a period of 22 days and resulted in no cutaneous irritation in either species (Gray *et al.*, 1983).

Tretinoin:

Multi-dose studies with topically applied tretinoin have been conducted in mice, rats, hamsters, and rabbits for periods of up to 13 weeks. Because the skin is a primary target of tretinoin in normal physiology, it is not surprising that direct, chronic application on the skin resulted in extensive changes in these experiments. These changes, which included erythema, edema, eschar, acanthosis, hyperkeratosis, hyperplasia and desquamation, were dependent upon dose and duration of exposure (Wilhite *et al.*, 1997; Christian *et al.*, 1997; Herold *et al.*, 1975). A study in mice where only 0.025% tretinoin was topically applied showed a lack of toxicity for up to 63 weeks (Kligman *et al.*, 1992). Upon topical application of higher doses of tretinoin, both systemic effects and skin

changes were observed. These effects included body weight loss, vaginal bleeding, changes in liver and thymus weights, and increased mortality (Wilhite *et al.*, 1997; Christian *et al.*, 1997; Seegmiller *et al.*, 1990). Although interpretation of some of these studies was confounded by failure to occlude application sites or restrain animals, a carefully conducted study in rabbits dosed with 0.5 mg/kg/day tretinoin showed that the development of incapacitating cutaneous lesions in response to tretinoin could lead to seriously compromised health status and occasionally death (Christian *et al.*, 1997).

Genetic toxicology:

Clindamycin:

No published papers regarding the genotoxicity of clindamycin were identified.

Tretinoin:

The effects of tretinoin on aflatoxin B₁-induced mutagenesis was studied in the Ames assay using *Salmonella typhimurium* (TA98 and TA100 strains) (Raina and Gurtoo, 1985). Tretinoin, either alone or in combination with menadione (an inhibitor of microsomal oxidase), had <6% effect on the growth of bacteria and no effect on the frequency of spontaneous revertants. Tretinoin over a wide range of concentrations (2 x 10⁻⁸ to 2 x 10⁻⁶ M) inhibited up to 50% of microsome-mediated mutagenesis of AFB₁ in TA98, but not in TA100 strains.

Non-Compendial Excipients:

The majority of excipients in Velac Gel are well known pharmaceutical ingredients and have been used for extended periods in other dermal products without safety concerns. However, (b) (4) excipients, polyethylene 4 monolauryl ether (POE 4) and (b) (4) are non-compendial. POE 4 (also known as laureth-4), is listed on the FDA Inactive Ingredient List up to a concentration of 5% in topical products. (b) (4), as well as POE 4, (b) (4). This group is listed on the FDA Inactive Ingredient List with a maximum potency of 1.9% in topical emulsion or cream formulations and up to 7% for s.c. and i.m. injections. Concentrations of both POE 4 (b) (4) in the Velac Gel formulation (b) (4). Connetics evaluated both POE 4 (b) (4) for *in vitro* genotoxicity (Ames and chromosome aberration assays); these studies have been previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. The results indicate that neither agent is mutagenic.

Carcinogenicity:

Clindamycin:

The sponsor has conducted a 26-week dermal carcinogenicity study in Tg.AC mice with clindamycin phosphate (Study No. AA81EW.7D8T.BTL). Under the conditions of the

test there was vehicle effect on papilloma formation which was accentuated by the administration of clindamycin phosphate in the Velac Gel vehicle at 3% and 5%. The incidence of papillomas was comparable in the vehicle control and the 1% group. The sponsor has analyzed the predictive power and relative risk of dermal SOA irritation for papilloma formation. Dermal SOA irritation was found to be highly correlated with, and predictive of, papilloma formation. The risk of developing a papilloma in this study was determined to be 144 times greater when dermal SOA irritation was present than when irritation was not present prior to papilloma formation. The sponsor believes that tumorigenesis in Tg.AC mice may be a nonspecific response to dermal insult. However, the eCAC was aware of other studies in Tg.AC mice in which irritation alone was not sufficient to cause papillomas. They concluded that the Velac Gel vehicle is at best, a tumor promoter, and at worst, a complete carcinogen.

The Division is aware of 2-year dermal carcinogenicity study conducted in mice with a clindamycin 1% gel product. Carcinomas of the parotid salivary gland were seen in 1 male treated with 2.7 mL/kg/day and 1 male treated with 15 mL/kg/day clindamycin 1% gel. These are considered rare spontaneous tumors and were not seen in control CD-1 mice in the study. Despite the rarity of these tumors, it is considered that these tumors cannot be attributed to an effect from clindamycin 1% gel. Overall the total incidence of tumors and animals with tumors varied between groups but did not show a relationship to the clindamycin 1% gel. The executive carcinogenicity assessment committee met on June 6, 2000 and discussed the results of this 2-year study. The committee concluded that the study was adequate for the evaluation of a topical clindamycin phosphate drug product. The committee recommended that the study be described in the label of this other product as showing no significant increase in tumors.

Tretinoin:

The carcinogenic potential of topically applied tretinoin has been examined in two National Cancer Institute sponsored studies. The results demonstrated that tretinoin is a weak promoter in mice, but it is also capable of reducing tetradecanylphorbol-13-acetate-inducing tumor promotion. In female CD-1 mice initiated with 7,12-dimethylbenz[α]anthracene (DMBA), multiple papillomas were observed following topical application of tretinoin for 20 weeks (Hennings *et al.*, 1982). In female SENCAR mice initiated with DMBA, promotion of tumors by TPA or mezerein was reduced by co-application with tretinoin (Tennenbaum *et al.*, 1998). In a chronic, 2-year bioassay topical treatment of tretinoin (three times per week, 0.1%) resulted in generalized amyloid deposition in the basal layer of tretinoin-treated skin, but no carcinogenicity (Tsubura and Yamamoto, 1979). The potential of systemically absorbed tretinoin to promote tumor formation will be reflected in the eventual drug label.

Topical tretinoin was tested for its ability to enhance experimental photocarcinogenicity in mice (Forbes, 1979). Hairless albino mice received daily topical applications of vehicle (Group A), 0.001% tretinoin (Group B), or 0.01% tretinoin (Group C) in methanol beginning at 7 ± 1 week of age for 30 weeks. One group of mice was treated with 0.1% croton oil (Group D) in methanol daily. Beginning on the first day of treatment with

croton oil and on the 15th day of treatment with tretinoin, each application was preceded by a 2 hour exposure to simulated sunlight. Tumors appeared at 38, 21, 20, and 26 weeks in Groups A, B, C, and D, respectively. After week 35, tumor incidence and yield in both Groups B and C were significantly greater than in Group A. At week 55, animals in Groups A, B, C, and D had 0.65, 6.3, 9.22, and 0.89 tumors/survivor, respectively. The ability of tretinoin to enhance photocarcinogenesis was assessed in another study of lightly pigmented mice (Kligman and Kligman, 1981). One group of mice was treated with UV light and topical tretinoin concomitantly, while the other group was treated with UV light to induce tumors prior to treating the animals with topical tretinoin. Tretinoin did not enhance photocarcinogenesis in either group. The ambiguity of these studies on the photocarcinogenic potential of tretinoin, is most likely attributable to variables within the study designs.

Reproductive toxicology:

The sponsor conducted a limit teratology study of the combination gel by the dermal route in rats. The study report concluded that treatment with this gel did not result in adverse effects on fetal development. However, although no systemic tretinoin was detected, the abnormalities described in the drug-treated groups, such as the craniofacial and heart abnormalities, could be consistent with retinoid effects. In addition to this preliminary study, the sponsor submitted several literature references on the reproductive and developmental toxicity of either clindamycin or tretinoin. Published literature on clindamycin showed that subcutaneous clindamycin phosphate at 100 and 180 mg/kg was not teratogenic and had no detrimental effect on reproduction in mice and rats (Bollert *et al.*, 1974). Oral clindamycin hydrochloride and clindamycin palmitate did not show any signs of teratogenicity in mice and rats (Gray *et al.*, 1972). Sprague-Dawley rats given up to 60 mg/kg clindamycin hydrochloride or up to 300 mg/kg clindamycin palmitate in the diet conceived at a slightly lower rate and their young were slightly smaller at weaning than the untreated controls, but otherwise no effect on reproductive performance was noted.

The references submitted by the sponsor showed that tretinoin, like other retinoids, is teratogenic and embryotoxic in multiple species when administered at sufficient doses and at the vulnerable gestational time period (Kraft, *et al.*, 1987; Seegmiller, *et al.*, 1997; Shenefelt, 1972; Tembe *et al.*, 1996; Hendrickx and Hummler, 1992; Fantel *et al.*, 1977). Doses of tretinoin that do not cause morphological changes in offspring may cause behavioral effects in the developing animals. Topical application of tretinoin appears to be less likely to result in teratogenic or other effects probably due to lower systemic and embryo exposure to tretinoin by the topical route than by the oral route (Zibinden, 1975). The sponsor also included references in which the effects of tretinoin on fertility and postnatal development have been assessed (Kamm, 1982.). In a Segment I study male and female rats were treated orally with 0, 0.5, 2 and 5 mg/kg/day. Males were treated for 60-80 days and females for 14 days prior to mating up to and including parturition. No adverse effects were observed on gonadal function, fertility, conception rate, gestation, parturition, or neonatal viability at dose up to 2 mg/kg/day. At 5 mg/kg/day neonatal survival was decreased. In a Segment III study female rats were treated with 0, 2, 5 and

10 mg/kg/day from the last third of gestation through lactation and weaning. Doses of 5 mg/kg/day and above resulted in decreased survival of the newborns during the first 23 days following birth.

Reproductive and developmental toxicology conclusions:

The only reproductive toxicity study conducted by the sponsor was the dose ranging teratology study in rats. The only other reproductive or developmental toxicity information submitted is that obtained from the literature. The sponsor was previously told that this teratogenicity study may be sufficient if the results are incorporated into the label. In submission SN009 to IND 65,369, the sponsor stated that they intended to include a summary of information from the literature regarding the effects of tretinoin on fertility and early embryonic development and on peri- and post-natal development. The sponsor included that literature information in SN016.

Special toxicology:

The sponsor has previously submitted (IND 65,369, SN000) special toxicology studies to determine the potential of a clindamycin-tretinoin gel to induce skin irritation/corrosion, phototoxicity, contact hypersensitivity, and photoallergenicity. The studies were previously reviewed by Dr. Paul Brown and it was concluded that under the conditions of the tests, clindamycin-tretinoin gel did not cause skin irritation/corrosion in rabbits, nor was phototoxic or photoallergenic in guinea pigs. It was considered a weak sensitizer in the guinea pig Maximization test.

2.6.6.2 Single-dose toxicity

The sponsor has submitted a study entitled “Assessment of acute oral toxicity with clindamycin-tretinoin-gel in the mouse” (Study no. 081361). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown as Study No. 93004. No drug related findings were observed in mice treated with a single gavage dose of 5000 mg/kg clindamycin- tretinoin gel.

The sponsor has submitted a study entitled “Assessment of acute oral toxicity with clindamycin-tretinoin-gel in the rat” (Study no. 081359). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown as Study No. 93003. No drug related findings were observed in rats treated with a single gavage dose of 2000 mg/kg clindamycin- tretinoin gel.

2.6.6.3 Repeat-dose toxicity

Study Title: A 28-day repeated dose dermal toxicity study in FVB/N mice

Laboratory Study no.: Study No. AA81EW.2D32.BTL

A draft report of this study was previously reviewed under IND 65,369 (SN026) by Dr. Paul Brown. At that time, the only tissues examined histologically were the skin, both

from the site of application and from a site other than application. In the current submission, the final, audited report is included and the only substantive change is that histopathology of all tissues was included. An updated summary of the report appears below:

Under the conditions of the test, 1, 3, and 5 % concentrations of clindamycin phosphate in Velac Gel vehicle are well tolerated by FVB/N mice. Local effects at the site of application were relatively mild. Treatment related histopathological findings were limited to an increased incidence of epidermal hyperplasia at the site of application in all test article-treated females and in the mid- and high-dose males. The other lesions (i.e., cystic endometrial hyperplasia in the uterus, cytoplasmic vacuolization of the zona reticularis of the adrenal glands) were observed in both control and high dose animals and are not considered test article related. There was no significant systemic toxicity, although toxicokinetic data indicated that treatment with all three concentrations resulted in systemic exposure. A maximum tolerated dose does not appear to have been achieved either by systemic toxicity or local toxicity criteria.

Study title: A 90-day dermal toxicity study in rats with clindamycin

Key study findings: Based on the results of this study, dermal administration of 1% and 5% clindamycin gel was well tolerated in male and female rats and did not cause any remarkable dermal irritation or signs of systemic toxicity. Therefore, the NOAEL for males and females in this study was 5 mg/kg/day clindamycin dose.

Laboratory Study no.: 3600.7

Module #, and Volume #: module 4, volume 2

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: January 6, 2003

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Clindamycin gel 1%, batch # SIAB-C, 1%
Clindamycin gel 5%, batch # C2M001, 5.34%
Velac gel placebo, batch # SIAA-1C, clindamycin

phosphate described as absent

Methods

Doses: see study design

Species/strain: rat / CrI:CD@ (SD)IGS BR

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

Route, formulation, volume, and infusion rate: test articles (1% or 5%

clindamycin) or vehicle control were administered by dermal application once daily, for ~ 6 hours per day, up to the day prior to scheduled termination.

Satellite groups used for toxicokinetics or recovery: see study design

Age: ~ 9 weeks

Weight: males: 273-350 g; females: 200-241 g

Sampling times:

Unique study design or methodology: on day -1 the dorsal skin of each rat was clipped and the site was reclipped as necessary throughout the study. Test substance was administered by inunction with a glass rod to distribute substance over the designated area. The dose volumes for groups 1 and 3 was 0.3 mL/kg/day and the dose volume for groups 2 and 4 was 0.1 mL/kg/day. A cervical collar was placed on the animals prior to dose administration to prevent ingestion. Following the exposure interval (~6 hours), the cervical collar was removed and the test site was wiped with gauze soaked in reverse osmosis water. Any residual test substance in the animal's cage was removed with gauze soaked in reverse osmosis water.

Study Design:

Group	No. of Animals ^a		Clindamycin Dosage Level (mg/kg/day)	Test Material Dose Formulation
	Males	Females		
1	10	10	0	0
2	10 (9)	10 (9)	1	1%
3	10 (9)	10 (9)	3	1%
4	10 (9)	10 (9)	5	5%

^a Animals designated in parentheses were utilized for a toxicokinetic phase of the study

Observations and times:

Mortality: twice daily

Clinical signs: detailed clinical observation performed weekly

Dermal irritation: application sites were examined on day 0 prior to application and once weekly prior to dosing for erythema, edema, desquamation, and other changes. A final dermal observation was made on the day of termination.

Body weights: weekly

Feed consumption: weekly

Ophthalmoscopy: prior to in-life initiation (day -7) and during week 12. Eyes were dilated with 0.5% Mydriacyl® ophthalmic solution prior to examination.

EKG: not performed

Hematology: blood samples were collected on the day of scheduled euthanasia (days 90 and 91) for evaluation of the following hematology and coagulation parameters: erythrocyte count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelet count, reticulocyte count, total and differential leukocyte counts, aPTT, prothrombin time.

Clinical chemistry: blood samples were collected from the orbital sinus while animals were under light isoflurane anesthesia on the day of scheduled euthanasia (days 90 and 91) for evaluation of the following clinical chemistry parameters: alanine aminotransferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, calcium, cholesterol, blood creatinine, gamma glutamyl transferase, globulin, glucose, electrolytes (sodium, potassium, chloride), phosphorus, total bilirubin, total serum protein, triglycerides, urea nitrogen.

Urinalysis: urine samples were collected overnight prior to scheduled euthanasia (days 90 and 91) for the evaluation of the following parameters: bilirubin, color and appearance,

glucose, ketones, leukocytes, microscopy of spun deposit, occult blood, overnight volume, nitrites, pH, protein, specific gravity, urobilinogen

Gross pathology: rats were euthanized by carbon dioxide inhalation followed by exsanguination and subjected to a complete necropsy which included evaluation of all external surfaces of the body and viscera

Organ weights: organ weights were obtained at scheduled euthanasia for the liver, kidneys, adrenal glands, spleen, testes, epididymides, ovaries, brain, uterus, thymus, and heart.

Histopathology: The following organs/tissues were preserved in 10% neutral buffered formalin for possible histopathological examination: accessory genital organs (epididymides, seminal vesicles and prostate or uterus and vagina), adrenals, all gross lesions, aorta, brain (including sections of 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), lungs (infused with formalin) with bronchi, mammary gland, mandibular lymph node, mediastinal lymph node, mesenteric lymph node, pancreas, peripheral nerve (sciatic), pituitary gland, rectum, skeletal muscle (thigh), treated skin (dorsal back), untreated skin (hip region), spinal cord (cervical, midthoracic and lumbar), spleen, sternum with bone marrow, stomach (glandular/nonglandular), submaxillary salivary gland, testes/ovaries, thymus, thyroid/parathyroid, trachea, urinary bladder. All tissues collected at necropsy from the control and high-dose animals and the gross lesions and treated and untreated skin sites from all animals in groups 2 and 3 were processed for histopathological examination.

Adequate Battery: yes (x)

Results

Mortality: all animals survived until scheduled euthanasia.

Clinical signs: clinical signs observed during the study were similar between the control, 1, 3, and 5 mg/kg/day groups and included hairloss, scabs, dark material around the eyes and/or nose and ocular discharge. These findings were associated with the cervical collars placed on the animals prior to dose administration.

Dermal irritation: there was a low incidence of grade 1 erythema (very slight) in the control, 1, 3, and 5 mg/kg/day female dose groups and a single incidence of grade 1 eschar (focal and/or pinpoint areas up to 10% of test site) in one control and one 3 mg/kg/day female. A low incidence of desquamation was also observed in a few females in the control and 3 mg/kg/day dose groups. No erythema or edema was observed in any male animals.

Body weights: mean body weights for males and females in the 1, 3, and 5 mg/kg/day groups were comparable to controls throughout the study.

Feed consumption: no statistically significant or toxicologically significant differences were observed in mean feed consumption values (grams/animal/day) between the control, 1, 3, and 5 mg/kg/day groups.

Ophthalmoscopy: no test article-related ocular findings were observed

Hematology: although within the range of the lab's historical control values there was an increase in platelets for males in the 3 and 5 mg/kg/day groups; a decrease in monocytes in the 5 mg/kg/day males; an increase in eosinophils in the 3 mg/kg/day males; a decrease in prothrombin time in females in the 1 mg/kg/day dose group.

Clinical chemistry: although within the range of the lab's historical control values there was a decrease in globulin and A/G ratio in the 3 mg/kg/day group males; an increase in the globulin and A/G ratio in 5 mg/kg/day males; AST was decreased in the 1 mg/kg/day males; an increase in the A/G ratio in 1 and 3 mg/kg/day females.

Urinalysis: no statistically significant or toxicologically meaningful differences were observed in the urinalysis data.

Gross pathology: no remarkable findings were observed at gross necropsy

Organ weights: the only statistically significant differences were a decrease in absolute and relative adrenal weight in males treated with 5 mg/kg/day. The toxicological significance of these findings is unknown since they did not correlate with any abnormal histopathology.

Histopathology: There were no test article-related lesions observed in the tissues examined, including the treated skin.

Adequate Battery: yes (x)

Toxicokinetics:

Dosage (mg/kg/day)	Study Day:	AUC ₀₋₂₄ (ng•h/mL)		Cmax (ng/mL)		Tmax (h)	
		0	89	0	89	0	89
1	Male	0.00	0.00	0.00	0.00	ND	ND
	Female	92.3	157	23.5	20.2	6	8
3	Male	4.87	56.3	2.43	11.0	10	0.5
	Female	421	597	93.8	98.1	6	8
5	Male	34.8	122	11.9	12.4	10	10
	Female	762	847	172	87.2	6	8

The increases in AUC₀₋₂₄ and Cmax values were generally greater than dose proportional. AUC₀₋₂₄ increased with the duration of dosing. This increase was greater in the males than in the females. Cmax values were comparable on both study days. There was no apparent systemic exposure in the males at the low dose. Female rats experienced significantly greater exposure to clindamycin than male rats.

Other:

Based on the results of this study, dermal administration of 1% and 5% clindamycin gel was well tolerated in male and female rats and did not cause any remarkable dermal irritation or signs of systemic toxicity. Therefore, the NOAEL for males and females in this study was 5 mg/kg/day clindamycin dose.

Study Title: A preliminary dermal dose range-finding study in Hanford minipigs with Velac Gel

Laboratory Study no.: 3600.9

Hanford minipigs were treated daily with 0, 100, 300, or 500 mg/kg/day of Velac Gel (1/sex/group) for 14 days to set dosage levels for a subsequent 13-week dermal toxicity study. All animals survived until scheduled euthanasia. There were no test article-related clinical signs of toxicity during the study or differences among the groups in mean body weights, body weight changes, or gross necropsy findings. Based on the results of this preliminary study, the study design and dosage levels selected for the 13-week dermal study are presented in the following table:

Group	Total Velac Gel exposure	Test Material Formulation	Exposure Area	Clindamycin Dosage Level (mg/kg/day)	Tretinoin Dosage Level (mg/kg/day)	Formulation Dosage Level (mg/kg/day) ^b	Final Formulation Dose Volume (mL/kg/day)
1	0	0	5x	0.0	0.0	0	0.1
2	0	5x	5x	0.0	0.125	500	0.1
3	1x	1x	1x	0.2	0.005	20	0.02
4	15x	3x	5x	3.0 ^a	0.075	300	0.1
5	25x	5x	5x	5.0 ^a	0.125	500	0.1

^aAlthough the final study report describes the clindamycin dosage level for group 4 and 5 as 1.0 and 3.0 mg/kg/day, respectively, given that they represent 15x and 25x Velac Gel exposure then these values must be 3.0 (0.2 x 15 = 3.0) and 5.0 (0.2 x 15 = 5.0) mg/kg/day. Therefore the above table depicting the study design has been modified.

^bThis value depicts a theoretical formulation dosage level and as such represents a multiple of the exposure area times the formulation strength.

Study title: A 13-week dermal toxicity study in Hanford minipigs with Velac® Gel

Key study findings: Under the conditions of this test, the systemic no-observed-effect level (NOEL) was 500 mg/kg/day Velac Gel and the local/dermal NOEL could not be established.

Laboratory Study no.: 3600.10

Module #, Volume #: module 4, volume 4

Conducting laboratory and location: (b) (4)

Date of study initiation: 15 April, 2003

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity:

1x Velac Gel (1% clindamycin phosphate, 0.025% tretinoin; verified to be within range by CoA), lot# SIAC-1C,

3x Velac Gel (3% clindamycin phosphate, 0.075% tretinoin, verified to be within range by CoA), lot # C3B003, C3E006

5x Velac Gel (5% clindamycin phosphate, 0.125% tretinoin, verified to be within range by CoA), lot # C3C004, C3E007

5x Tretinoin (0.125% tretinoin, verified to be within range by CoA), lot # C3D005, C3E008

Velac Gel Placebo, lot # SIAA-1C, CoA verifies no clindamycin or tretinoin present

Methods

Doses: see study design

Species/strain: Hanford minipigs

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

Route, formulation, volume, and infusion rate: test articles or vehicle control was administered once daily to the clipped dorsal surface of each animal. Test substance was applied directly to skin and spread in a uniform layer by unction with a glass stirring rod. Target exposure levels were achieved by varying the dose formulation and the area of skin treated. Each site was covered by a gauze bandage secured with tape and a stockinette sleeve (non-occlusive binding). On each day of dosing, residual material was removed by gently wiping the site with gauze soaked in reverse osmosis water.

Satellite groups used for toxicokinetics or recovery: NA

Age: 8-13 weeks

Weight: males: 4.94 – 18.77 kg; females: 5.53 – 9.75 kg

Justification of dose level: local exposure was increased by using enriched formulations (3x and 5x Velac Gel). Systemic exposure was increased by increasing the area of skin treated by 5x (the maximum feasible area of exposure, ~ 20% BSA). Group 3 achieved the human clinical dose. Group 5 achieved the maximum level and dose based on a rangefinding study ((b) (4) Study No. 3600.9). A targeted amount of formulation (2 mg/cm²) was applied to all dose groups.

Group	Total Velac Gel exposure	Test Material Formulation	Exposure Area	Clindamycin Dosage Level (mg/kg/day)	Tretinoin Dosage Level (mg/kg/day)	Formulation Dosage Level (mg/kg/day) ^b	Final Formulation Dose Volume (mL/kg/day)
1	0	0	5x	0.0	0.0	0	0.1
2	0	5x	5x	0.0	0.125	500	0.1
3	1x	1x	1x	0.2	0.005	20	0.02
4	15x	3x	5x	3.0 ^a	0.075	300	0.1
5	25x	5x	5x	5.0 ^a	0.125	500	0.1

^aAlthough the final study report describes the clindamycin dosage level for group 4 and 5 as 1.0 and 3.0 mg/kg/day, respectively, given that they represent 15x and 25x Velac Gel exposure then these values must be 3.0 ($0.2 \times 15 = 3.0$) and 5.0 ($0.2 \times 15 = 5.0$) mg/kg/day. Therefore the above table depicting the study design has been modified.

^bThis value depicts a theoretical formulation dosage level and as such represents a multiple of the exposure area times the formulation strength.

Observations and times:

Mortality: checks performed twice daily

Clinical signs: all animals examined for overt toxic effects after daily dosing; detailed clinical observation performed weekly

Dermal observations: dermal scoring performed at least once per week

Body weights: recorded on day -1 and weekly during study period with a final body weight recorded on day of scheduled euthanasia

Feed consumption: not measured

Ophthalmoscopy: prior to in-life initiation (day -10) and just prior to the end of dosing (day 83). Eyes were dilated with 0.5% Mydracyl® ophthalmic solution prior to examination. Examinations were performed using a hand-held slit lamp and indirect ophthalmoscope.

EKG: not performed

Hematology: blood was collected from the vena cava from overnight fasted animals once prior to in-life initiation (day -8/-7) and near the conclusion of the dosing phase (day 87) for evaluation of the following hematology and coagulation parameters: erythrocyte count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelet count, reticulocyte count, total and differential leukocyte counts, aPTT, prothrombin time.

Clinical chemistry: blood was collected from the vena cava from overnight fasted animals once prior to in-life initiation (day -8/-7) and near the conclusion of the dosing phase (day 87) for evaluation of the following clinical chemistry parameters: alanine aminotransferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, calcium, cholesterol, creatinine, creatinine phosphokinase, globulin, glucose, electrolytes (sodium, potassium, chloride), phosphorus, total bilirubin, total serum protein, urea nitrogen.

Urinalysis: not performed

Toxicokinetics: blood samples were collected from the vena cava on Days 0 and 89 at 0 (pre-dose), 1, 4, 8, and 24 hours post-dose. Blood was collected into plastic tubes containing K₂EDTA as the anticoagulant, chilled and centrifuged. Frozen plasma was shipped on dry ice (b) (4) for analysis.

Gross pathology: all animals were fasted overnight and subjected to a complete gross necropsy at the time of death or scheduled euthanasia by an IV overdose of sodium pentobarbital followed by exsanguination.

Organ weights : fresh organ weights were obtained at scheduled euthanasia for the adrenal glands, brain, heart, kidneys, liver, ovaries, thyroid, pituitary, spleen, testes. Paired organs were weighed together.

Histopathology: With the exception of bone marrow smears, the following organs/tissues from all animals were preserved in 10% neutral buffered formalin for histopathological examination: accessory genital organs (epididymides, seminal vesicles and prostate or uterus and vagina), adrenals, all gross lesions, aorta, brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex), cecum, colon, duodenum, esophagus, eyes (including optic nerve), femur (including articular surface and bone marrow), bone marrow smear – rib (scheduled necropsies only), gallbladder, heart, ileum, jejunum, kidneys, liver (three sections), lungs (infused with formalin) with bronchi, mammary gland, mandibular lymph node, mediastinal lymph node, mesenteric lymph node, pancreas, peripheral nerve (sciatic), pituitary gland, rectum, skeletal muscle (thigh), treated skin (dorsal back), untreated skin (hip region), spinal cord (cervical, midthoracic and lumbar), spleen, sternum with bone marrow, stomach, submaxillary salivary gland, testes/ovaries (including oviducts), thymus, thyroid/parathyroid, trachea, urinary bladder.

Adequate Battery: yes (x)

Results

Mortality: One male (#S1859) in the 20 mg/kg/day Velac Gel group was found dead on Day 23 and one female (#S1869) in the 500 mg/kg/day tretinoin group was found dead on Day 89. Microscopic evaluation of the tissues/organs from these animals suggested that hemorrhage and sepsis after blood collection were partly the cause of death. All other animals survived to scheduled euthanasia.

Clinical signs: no significant clinical observations were noted in the test-article treated animals during the study.

Dermal observations: dermal irritation was observed in all groups, including the control. The incidence and severity of dermal findings were generally similar between the 500 mg/kg/day tretinoin and 300 and 500 mg/kg/day Velac Gel groups, with no clear dose response between the 300 and 500 mg/kg/day Velac Gel groups. Dermal irritation was noted in the 500 mg/kg/day group after 1 to 4 weeks of dosing and in the 300 and 500 mg/kg/day Velac Gel groups after 1 to 2 weeks of dosing. These findings persisted until study termination. In contrast, dermal findings in the 20 mg/kg/day Velac Gel group persisted until termination, but they were less severe.

The following table summarizes the irritation incidence for each dose group:

Treatment	Incidence of Erythema Scores*			
	Grade 1	Grade 2	Grade 3	Grade 4
Males				
Placebo Velac Gel	2	1		
Tretinoin		1		3
20 mg/kg/day Velac Gel	4			
300 mg/kg/day Velac Gel				4
500 mg/kg/day Velac Gel	1	1		2
Females				
Placebo Velac Gel		1		
Tretinoin	1	3		
20 mg/kg/day Velac Gel	2	1		1
300 mg/kg/day Velac Gel		2		2
500 mg/kg/day Velac Gel				4

Grade 1= very slight erythema; Grade 2 = well-defined erythema; Grade 3 = severe erythema; Grade 4 = maximized erythema; *= highest score reported for each animal

Body weights: there were no toxicologically significant differences in mean body weights or body weight gains

Organ weights: there were no statistically significant or toxicologically meaningful differences observed in mean absolute organ weights or organ-to-body weight ratios.

Ophthalmoscopy: no abnormalities were observed during the ocular examination performed on day 83.

Hematology and coagulation parameters: there were no statistically significant or toxicologically meaningful differences in hematology or coagulation parameters.

Clinical chemistry: there were no statistically significant or toxicologically meaningful differences in clinical chemistry parameters.

Gross pathology: at necropsy the most notable gross findings included scabbing of the treated skin for males in the 500 mg/kg/day Tretinoin and 300 and 500 mg/kg/day Velac Gel groups and for females in the 20, 300, and 500 mg/kg/day Velac Gel groups, and reddening of the treated skin for one female in the 20 mg/kg/day Velac Gel group and one male in the 500 mg/kg/day Velac Gel group. Dark red areas on the lungs of males in the 20, 300, and 500 mg/kg/day Velac Gel groups and females in the placebo control, 500 mg/kg/day Tretinoin and 20 and 500 mg/kg/day Velac Gel groups were observed.

Organ weights: there were no statistically significant or toxicologically meaningful differences in mean absolute organ weights or organ-to-body weight ratios

Histopathology: Microscopic evaluation of skin samples from the treated sites revealed test article-related microscopic changes (associated with Tretinoin exposure) in the 500

mg/kg/day Tretinoin group and the 20, 300, and 500 mg/kg/day Velac Gel groups. The incidence of lesions was evenly distributed throughout the groups with most animals being affected, but no dose response was evident. The microscopic changes in the skin samples included minimal to mild, chronic and chronic/active inflammation with accompanying epidermal changes, including minimal acantholysis, minimal to moderate hyperkeratosis, minimal to severe epithelial hyperplasia, minimal to moderate microabscesses, minimal to mild hyperkeratosis, and minimal to mild spongiosis (intracellular edema).

Toxicokinetics: Following dermal application of Velac Gel at clindamycin dosage levels of 0.2, 1.0, and 3.0 mg/kg for 90 days, negligible plasma concentrations of clindamycin were detected. No toxicokinetics were determined given that all but one sample was below the limit of detection for the assay (<20.0 ng/mL). A clindamycin plasma concentration of 24.1 ng/mL was reported for one male minipig in the highest dose group, 3.0 mg/kg, 4 hours after dosing on Day 89.

Following dermal application of Velac Gel at tretinoin dosage levels of 0, 0.005, 0.075, and 0.125 mg/kg, dose-related increases in C_{max} and AUC₍₀₋₂₄₎ were not observed after either single or multiple doses. No apparent gender differences were observed for tretinoin toxicokinetics in the minipig.

Other: Dermal administration of Velac Gel for 13 weeks was well tolerated in Hanford minipigs at dosage levels of 20, 300, and 500 mg/kg/day. No evidence of systemic toxicity was noted at any dose level. Microscopic changes in the treated skin (minimal to mild, chronic and chronic/active inflammation with accompanying epidermal changes, including minimal acantholysis, minimal to moderate hyperkeratosis, minimal to severe epithelial hyperplasia, minimal to moderate microabscesses, minimal to mild parakeratosis, and minimal to mild spongiosis) were observed at a similar incidence in the 500 mg/kg/day tretinoin group and the 20, 300, and 500 mg/kg/day Velac Gel groups. These changes were attributed to Tretinoin and not clindamycin. Based on these data, the systemic no-observed-effect level (NOEL) was 500 mg/kg/day Velac Gel and the local/dermal NOEL could not be established.

2.6.6.4 Genetic toxicology

The sponsor has submitted a study entitled “*Salmonella – Escherichia coli*/mammalian – microsome reverse mutation assay with polyethylene-4 monolauryl ether.” (Study no. 24043-0-409OECD). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Polyethylene-4 monolauryl ether was negative for inducing revertants in *Salmonella* and *E. coli* in the presence and absence of metabolic activation.

The sponsor has submitted a study entitled “Chromosome aberration in Chinese hamster ovary (CHO) cells with polyethylene-4 monolauryl ether.” (Study no. 24043-0-437OECD). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Polyethylene-4 monolauryl ether was negative for inducing chromosomal aberrations in CHO cells in the presence and absence of metabolic activation.

The sponsor has submitted a study entitled “*Salmonella* – *Escherichia coli*/mammalian – microsome reverse mutation assay with Glycerox L15.” (Study no. 24042-0-409OECD). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Glycerox L15 was negative for inducing revertants in *Salmonella* and *E. coli* in the presence and absence of metabolic activation.

The sponsor has submitted a study entitled “Chromosomal aberrations in Chinese hamster ovary (CHO) cells with Glycerox L15.” (Study no. 24300-0-437OECD). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Glycerox L15 was negative for inducing chromosomal aberrations in CHO cells in the presence and absence of metabolic activation.

The sponsor has submitted a study entitled “Evaluation of the mutagenic activity of vehiculum clindamycin-tretinoin gel in the Ames *Salmonella*/microsome test (with independent repeat).” (Study no. 094782). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. The vehicle for the clindamycin/tretinoin gel was negative for inducing revertants in *Salmonella* in the presence and absence of metabolic activation.

The sponsor has submitted a study entitled “Evaluation of the mutagenic activity of tretinoin gel in the Ames *Salmonella*/microsome test (with independent repeat).” (Study no. 094771). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. The gel containing tretinoin only was negative for inducing revertants in *Salmonella* in the presence and absence of metabolic activation at up to 33 µL of the gel per plate.

The sponsor has submitted a study entitled “Evaluation of the ability of tretinoin to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat).” (Study no. 187537). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Tretinoin did not cause an increase in chromosomal aberrations in cultured human lymphocytes at doses up to 75 µg/mL in the presence of metabolic activation or up to 100 µg/mL in the absence of metabolic activation.

The sponsor has submitted a study entitled “Evaluation of the mutagenic activity of clindamycin phosphate in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay.” (Study no. 187559). This study was previously reviewed under IND 65,369 (SN026) by Dr. Paul Brown. Clindamycin phosphate was not mutagenic in a reverse mutation assay conducted in *Salmonella typhimurium* and *Escherichia coli*.

The sponsor has submitted a study entitled “Evaluation of the ability of clindamycin phosphate to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat).” (Study no. 187548). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Clindamycin phosphate did not cause an

increase in chromosomal aberrations in cultured human lymphocytes at doses up to 1778 µg/mL in either the presence or absence of metabolic activation.

The sponsor has submitted a study entitled “Evaluation of the mutagenic activity of clindamycin-tretinoin gel in the Ames *Salmonella*/microsome test (with independent repeat).” (Study no. 094793). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. The clindamycin/tretinoin gel was negative for inducing revertants in *Salmonella* in the presence and absence of metabolic activation.

The sponsor has submitted a study entitled “In vitro assessment of the clastogenic activity of clindamycin-tretinoin gel in human lymphocytes.” (Study no. 94/BSM004/0576). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. A statistically significant increase in chromosomal aberrations was observed in human lymphocytes treated for 19 hours in the absence of metabolic activation at doses of 650, 1300, and 1950 µg/mL. However, this result was not repeated in a second experiment and so the clastogenicity of the gel is equivocal.

2.6.6.5 Carcinogenicity

Study title: 26-Week dermal carcinogenicity study in Tg.AC mice

Key study findings: Under the conditions of the test there was a vehicle effect on papilloma formation which was accentuated by the administration of clindamycin phosphate in Velac Gel vehicle at 3% and 5%. The incidence of papillomas was comparable in the vehicle control and 1% groups.

Adequacy of the carcinogenicity study and appropriateness of the test model:
eCAC meeting minutes (12-16-03) for protocol and dose selections have been appended.

Evaluation of tumor findings: Statistical analysis comparing the incidence of animals exhibiting at least one papilloma by Week 27 in the vehicle control group with the incidence in the clindamycin groups revealed no increase in the low-dose animals, a significant increase in the high-dose males, and a significant increase in the mid- and high-dose females. A similar analysis, performed using the incidence in the untreated animals for comparison, revealed a significant increase in all treatment groups including the vehicle control group.

Laboratory Study no.: AA81EW.7D8T.BTL

Volume #, and page #: vol 7, page 1

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: November 19, 2003

GLP compliance: YES

QA report: yes (X) no ()

Drug, lot #, and % purity: 1% clindamycin phosphate, SIAB-C, 1.01% purity
3% clindamycin phosphate, C3J011, 2.9% purity

5% clindamycin phosphate, C3J010, 4.9% purity

CAC concurrence: provided on December 16, 2003 (meeting minutes provided as an attachment)

Methods

Doses:

Group	Test Article	Volume (mL/day)	Dose clindamycin phosphate (mg/kg)
1	Velac gel vehicle control	0.1	0
2	Shaved, untreated control	0	0
3	50 µg TPA in Velac gel vehicle	0.1 mL 3x/week	0
4	1% clindamycin phosphate in Velac gel vehicle	0.1	40
5	3% clindamycin phosphate in Velac gel vehicle	0.1	120
6	5% clindamycin phosphate in Velac gel vehicle	0.1	200

TPA = 12-O-tetradecanoylphorbol 13-acetate

Basis of dose selection: range finding study (AA81EW.2D32.BTL); 5% is the maximum feasible dose

Species/strain: mouse/Tg.AC (FVB/N Tac-TgN(v-Ha-ras))

Number/sex/group: 20/sex/group, except TPA positive control which was 15/sex

Route, formulation, volume: topically to the clipped skin, 0.1 mL/6 cm²

Frequency of dosing: daily, except for TPA which was applied 3x/week (M,W,F)

Age: ~7-8 weeks of age

Animal housing: individually in polycarbonate cages with Sani-Chip Hardwood bedding to absorb liquids

Restriction paradigm for dietary restriction studies: none

Drug stability/homogeneity: samples of each Velac gel formulation (1%, 3%, 5%) and placebo were taken at the beginning and end of the study and sent to the sponsor for analysis. Each met Connetics' specifications ($\leq \pm 10\%$).

Dual controls employed: no

Interim sacrifices: no

Deviations from original study protocol: protocol specified relative humidity was 30% to 70% and it fell to 20% for a 22 hour period.

Observation times

Mortality: 2x daily for moribundity and mortality

Clinical signs: a detailed (hands-on) examination for all clinical signs of toxicity and carcinogenicity (including observation of the site of application) was performed once prior to dosing and then weekly thereafter. The number of tumors at the site of application and non-site of application were recorded for each animal each week.

Evaluation of dermal application site: tissue masses were scored as latent papillomas when they reached 2 mm in diameter and protruded from the surface of the skin. If they remained scorable for 3 consecutive weeks then they were scored as actual papillomas. Once scored as a papilloma it was included in the count even if it disappeared, animal died, or papilloma converted to a carcinoma.

Body weights: prior to dosing, once weekly to Week 13, and biweekly thereafter

Feed consumption: not performed

Hematology: not performed

Gross pathology: all surviving animals were sacrificed by CO₂ asphyxiation after the last treatment and, except for positive control animals, were necropsied. The necropsy procedure involved examination and dissection of the animal's viscera and carcass, and preservation of required tissues in 10% neutral buffered formalin (NBF).

Histopathology: Histopathology was performed on the following tissues from all animals (except positive control animals): adrenal glands, aorta, bone (femur and sternum), bone marrow (femur and sternum), brain, epididymides, esophagus, eyes, gall bladder, gross lesions, Harderian glands, heart, spinal cord (cervical, thoracic, and lumbar), kidneys, large intestine (cecum, colon, and rectum), liver, lungs with bronchi, lymph nodes (mesenteric and mandibular), mammary gland, nasal cavity, ovaries, pancreas, parathyroid glands, pituitary glands, prostate gland, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin at non-site of application (taken with mammary gland), skin at site of application, small intestine (duodenum, jejunum, and ileum), spleen, stomach, testes, thymus, thyroid glands, trachea, urinary bladder, uterus, vagina.

Peer review: of histopathological observations in the skin was performed

Toxicokinetics: not performed

Organ weights: The brain, heart, liver, kidneys, lungs, thymus, spleen, and testes/ovaries were collected and weighed from all animals at time of necropsy (excluding Group 3 positive control animals). Paired organs were weighed together except if a gross lesion was present in one.

Results

Mortality: male mortality prior to terminal sacrifice (including animals either sacrificed in moribund condition, sacrificed after attaining 20 or more papillomas, or found dead) was 1, 2, 13, 1, 3, and 2 animals from Groups 1-6, respectively. Female mortality prior to terminal sacrifice was 1, 4, 12, 1, 5, and 2 animals from Groups 1-6, respectively.

Mortality was not significantly increased in any test article-related group of either sex when compared to vehicle control. The elevated mortality in Group 3 in both sexes was a reflection of the high tumor burden observed in the positive control group (21.1 and 20.4 mean actual tumors per tumor-bearing animal for males and females, respectively) and the sacrifice of those animals.

Clinical signs: Dermal irritation at the SOA was noted in 11/20, 0/20, 14/15, 13/20, 16/20, and 17/20 males in Groups 1-6, respectively. Dermal irritation at the SOA was noted in 6/20, 0/20, 15/15, 9/20, 12/20, and 16/20 females in Groups 1-6, respectively.

Desquamation was noted in 3/20 and 2/20 mid- and high-dose males and in 1/20 and 2/20 low and mid-dose females, but this incidence was not statistically different from the vehicle or untreated control in either sex. Desquamation was not noted in any vehicle or untreated control group animals.

Dermal irritation at SOA in male mice:

Group	#1, Vehicle control	#2, Untreated control	#3 Positive control	#4	#5	#6
Dose	0 mg/kg/day	0 mg/kg/day	50 µg TPA/0.1 mL vehicle	40 mg/kg/day	120 mg/kg/day	200 mg/kg/day
# of mice	20	20	15	20	20	20
# of observations ^a	92	-	122	128	153	209
# of animals	11*	-	14*	13*	16*	17*,**
Days from – to ^b	36 182	-	43 182	43 182	64 183	29 183

^a“# of observations” is the total number of times an observation was recorded in each group.

^b“Days from – to” indicates the first day the observation was recorded within the group and the last day the observation was recorded within the group.

* $p \leq 0.05$, Fisher’s Exact Test, when compared to the untreated control group (Group 2).

** $p \leq 0.05$, Fisher’s Exact Test, when compared to the vehicle control group (Group 1).

Dermal irritation at SOA in female mice:

Group	#1, Vehicle control	#2, Untreated control	#3 Positive control	#4	#5	#6
Dose	0 mg/kg/day	0 mg/kg/day	50 µg TPA/0.1 mL vehicle	40 mg/kg/day	120 mg/kg/day	200 mg/kg/day
# of mice	20	20	15	20	20	20
# of observations ^a	40	-	171	66	104	170
# of animals	6*	-	15*,**	9*	12*	16*,**
Days from – to ^b	71 184	-	43 182	64 184	43 185	29 185

^a“# of observations” is the total number of times an observation was recorded in each group.

^b“Days from – to” indicates the first day the observation was recorded within the group and the last day the observation was recorded within the group.

* $p \leq 0.05$, Fisher’s Exact Test, when compared to the untreated control group (Group 2).

** $p \leq 0.05$, Fisher’s Exact Test, when compared to the vehicle control group (Group 1).

Body weights: although there were sporadic instances of significant differences in body weights, there were no statistically significant differences in total body weight gains.

Organ weights: there was a statistically significant decrease in the absolute and relative liver weights (7.9% and 9.8%, respectively) in the high-dose males when compared to the vehicle control. Also the relative liver weights of the vehicle control males were significantly increased when compared to the untreated control. However, histopathological evaluation of the male vehicle and high dose group livers did not reveal any corroborating lesions, so these findings are not considered test article-related. Statistically significant differences that occurred sporadically included: an increase in absolute heart weights in mid-dose females when compared to vehicle control; decreased relative liver weights in the low-dose females when compared to vehicle control; decreased relative lung weights in the mid-dose females when compared to the untreated control; decreased absolute heart weights in the vehicle control males when compared to the untreated control.

Tumor counts: The following tables give latent and actual tumor incidence at the end of the study (Week 27).

Table 8: Summary of Tumor Incidence and Burden

CONNETICS STUDY AA81EW.7D8T.BTL

Week 27

SKIN PAPILOMA DATA

SOA= Site of Application

NA = Not Applicable

NSOA=Non-SOA

Group #	Sex(MorF)	Incidence (Latent)			Burden (Latent)
		Animals bearing at least one latent papilloma			Latent papillomas per latent papilloma bearing animal
		per effective # of Animals(% incidence)			SOA only
		SOA	NSOA	Anywhere	
1	M	2/20 (10)	0/20 (0)	2/20 (10)	1.0
1	F	1/20 (5)	2/20 (10)	3/20 (15)	1.0
2	M	0/18 (0)	1/18 (6)	1/18 (6)	0.0
2	F	0/16 (0)	2/16 (13)	2/16 (13)	0.0
3	M	NA			NA
3	F	NA			NA
4	M	3/20 (15)	1/20 (5)	4/20 (20)	1.0
4	F	2/20 (10)	0/20 (0)	2/20 (10)	1.5
5	M	5/20 (25)	1/20 (5)	6/20 (30)	2.6
5	F	5/20 (25)	1/20 (5)	5/20 (25)	1.0
6	M	6/20 (30)	1/20 (5)	6/20 (30)	1.7
6	F	5/20 (25)	2/20 (10)	7/20 (35)	1.8

Nominal Dose:

Group 1 – Velac Gel vehicle control

Group 2 – Untreated Control

Group 3 – 50 ug TPA/0.1 ml vehicle

Group 4 – 40 mg/kg/day

Group 5 – 120 mg/kg/day

Group 6 – 200 mg/kg/day

Table 8: Summary of Tumor Incidence and Burden (continued)

CONNETICS STUDY AA81EW.7D8T.BTL

Week 27

SKIN PAPILOMA DATA

SOA= Site of Application

NSOA=Non-SOA

Group #	Sex(MorF)	Incidence (Actual)						Burden (Actual)
		Animals bearing at least one actual papilloma						Actual papillomas per actual papilloma bearing animal
		per effective # of Animals(% incidence)						
		SOA	NSOA	Anywhere			SOA only	
1	M	8/20 (40)	5/20 (25)	11/20 (55)			1.8	
1	F	5/20 (25)	1/20 (5)	5/20 (25)			1.8	
2	M	0/18 (0)	3/18 (17)	3/18 (17)			0.0	
2	F	0/16 (0)	4/16 (25)	4/16 (25)			0.0	
3	M	14/14 (100)	3/14 (21)	14/14 (100)			21.1	
3	F	15/15 (100)	1/15 (7)	15/15 (100)			20.4	
4	M	11/20 (55)	5/20 (25)	14/20 (70)			2.1	
4	F	5/20 (25)	2/20 (10)	6/20 (30)			1.4	
5	M	13/20 (65)	5/20 (25)	14/20 (70)			2.1	
5	F	10/20 (50)	1/20 (5)	10/20 (50)			1.7	
6	M	14/20 (70)	3/20 (15)	14/20 (70)			3.2	
6	F	14/20 (70)	3/20 (15)	15/20 (75)			1.9	

Nominal Dose:

Group 1 – Velac Gel vehicle control

Group 2 – Untreated Control

Group 3 – 50 ug TPA/0.1 ml vehicle

Group 4 – 40 mg/kg/day

Group 5 – 120 mg/kg/day

Group 6 – 200 mg/kg/day

Table 8: Summary of Tumor Incidence and Burden (continued)

CONNETICS STUDY AA81EW.7D8T.BTL

Week 27

SKIN PAPILOMA DATA

SOA= Site of Application

NA = Not Applicable

NSOA=Non-SOA

Group #	Sex(MorF)	Incidence (All)						Burden (All papillomas)	
		Animals bearing at least one latent or actual papilloma						All papillomas per papilloma bearing animal	All papillomas per effective animal
		per effective # of Animals(% incidence)							
		SOA	NSOA	Anywhere		SOA only	SOA only		
1	M	9/20** (45)	5/20 (25)	12/20 (60)		1.8	0.8		
1	F	5/20** (25)	3/20 (15)	6/20 (30)		2.0	0.5		
2	M	0/18 (0)	4/18 (22)	4/18 (22)		0.0	0.0		
2	F	0/16 (0)	6/16 (38)	6/16 (38)		0.0	0.0		
3	M	NA						NA	NA
3	F	NA						NA	NA
4	M	12/20** (60)	5/20 (25)	15/20 (75)		2.2	1.3		
4	F	6/20** (30)	2/20 (10)	7/20 (35)		1.7	0.5		
5	M	15/20** (75)	6/20 (30)	16/20 (80)		2.7	2.0****		
5	F	12/20* ** (60)	2/20 (10)	12/20 (60)		1.8	1.1****		
6	M	16/20* ** (80)	4/20 (20)	16/20 (80)		3.4	2.8****		
6	F	16/20* ** (80)	5/20 (25)	16/20 (80)		2.2	1.8****		

* p ≤ 0.05, Fisher's Exact Test, when compared to the vehicle control group (Group 1).

** p ≤ 0.05, Fisher's Exact Test, when compared to the untreated control group (Group 2).

*** p ≤ 0.05, ANOVA and Dunnett's t-test, when compared to the vehicle control group (Group 1).

**** p ≤ 0.05, ANOVA and Dunnett's t-test, when compared to the untreated control group (Group 2).

Nominal Dose:

Group 1 – Velac Gel vehicle control

Group 2 – Untreated Control

Group 3 – 50 ug TPA/0.1 ml vehicle

Group 4 – 40 mg/kg/day

Group 5 – 120 mg/kg/day

Group 6 – 200 mg/kg/day

None of the untreated animals (either sex) developed SOA latent and/or actual papillomas. By Week 27, 9 vehicle control males and 5 vehicle control females (45% and 25%, respectively) had developed papillomas. The incidence was 100% in the positive control animals. At study termination, the incidence of papillomas was 60%, 75%, and 80% in the males and 30%, 60%, and 80% in the females from the low-, mid-, and high-dose groups, respectively. Comparing the incidence of animals exhibiting at least one papilloma by Week 27 in the vehicle control group with the incidence in the

clindamycin groups revealed no increase in the low-dose animals, a significant increase in the high-dose males, and a significant increase in the mid- and high-dose females. Using the untreated animals for comparison, there was a significant increase in all treatment groups including the vehicle control group. These was also a dose-response effect.

Assessment of tumor burden (multiplicity) revealed a statistically significant increase in the number of papillomas per effective animal (i.e., the number of animals alive when the first papillomas appeared in the group) in the high-dose group when compared to the vehicle control group in both sexes and in the mid- and high-dose groups when compared to the untreated control group in both sexes. The low-dose group did not show a significant difference in tumor burden when compared to either the vehicle control or untreated control group. No significant differences were noted when comparisons for multiplicity were made between the tumor-bearing vehicle control animals and the tumor-bearing clindamycin-treated animals at Week 27.

The mean latency period (i.e., time to first appearance of papillomas) for actual papillomas per actual tumor-bearing animal was 17.5, 18.6, 18.2, and 16.6 weeks for the males and 20.4, 18.0, 18.2, and 17.4 weeks for the females in the vehicle, low-, mid-, and high-dose groups, respectively. There was no significant difference between any of the groups.

The FDA statistical reviewer, Steve Thomson, performed an independent analysis of the data. (His report is available in DFS). He concluded that 'for both genders, there was statistically significant evidence of an increasing response to dose in terms of time to tumor development, number of animals with tumors, and number of tumors per animal.'

Gross pathology: all gross lesions (except those noted at the skin site of application) are considered to be spontaneous or background lesions in this strain of mice and therefore not considered vehicle or test article related.

Histopathology:

Non-neoplastic: other organs examined which exhibited histopathological changes included the mandibular and maxillary bones, nasal cavity, mandibular lymph nodes, liver, spleen, stomach, lungs, ovaries and uteri, salivary glands, and eyes. Findings in these organs were determined to have occurred spontaneously, to be distributed evenly among both control and treated animals, or to be a commonly reported finding in Tg.AC mice.

Neoplastic:

Table 1: Male Groups Correlation of Papillomas Scored In-Life and Microscopic Findings

Number of Mice in Group (skin papillomas submitted)	1	2	4	5	6
	20(20 ¹)	20(18 ¹)	20(20 ¹)	20(20 ¹)	20(20 ¹)
Determined papillomas	8	0	10	13	14
Determined hyperplasia	8	NA	9	11	11 ²
Determined inverted papillomas	0	NA	0	1	4
Determined keratoacanthoma	0	NA	1 ³	0	0
Determined normal skin	0	NA	0	1 ³	1 ^{3,4}
Percent correlation	100%	NA	100%	92%	86%

Appears this way on original

Group 1 = Vehicle Control
 Group 2 = Untreated Control
 Group 4 = 40 mg/kg/day
 Group 5 = 120 mg/kg/day
 Group 6 = 200 mg/kg/day
 NA = Not Applicable

- 1 Effective number of animals- the number of animals alive when the first papilloma appeared in the group
- 2 Animal number 112 had two papillomas.
- 3 Keratoacanthomas, inverted papillomas and papillomas are virtually indistinguishable in-life and therefore a microscopic determination of a keratoacanthoma or inverted papilloma will be considered as a confirmation of in-life scoring when calculating the correlation percentage. These findings occurred in the papillomatous area of the SOA
- 4 Animal number 105 had a keratoacanthoma as well as hyperplasia.

Table 2: Female Groups Correlation of Papillomas Scored In-Life and Microscopic Findings

Number of Mice in Group (skin lesion submitted)	1	2	4	5	6
	20(20*)	20(16*)	20(20*)	20(20*)	20(20*)
Determined papillomas	3	0	6	9	14
Determined hyperplasia	3	NA	6	8	14
Determined hyperplasia	0	NA	0	1	0
Percent correlation	100%	NA	100%	89%	100%

Group 1 = Vehicle Control
 Group 2 = Untreated Control
 Group 4 = 40 mg/kg/day
 Group 5 = 120 mg/kg/day
 Group 6 = 200 mg/kg/day
 NA = Not Applicable

- * Effective number of animals- the number of animals alive when the first papilloma appeared in the group

There was a high overall correlation between SOA papillomas scored in-life and the microscopic findings. A vehicle-related increase in the number of papillomas was observed in all treatment groups of both sexes when compared to the untreated control.

Epidermal hyperplastic lesions at skin SOA were noted during the histopathological analysis in 10/20, 0/20, 7/20, 11/20, and 9/20 males in Groups 1, 2, 4, 5, and 6,

respectively. Similar lesions were also noted in 5/20, 0/20, 12/20, 8/20 and 1/20 females in Groups 1, 2, 4, 5, and 6, respectively. In the males the incidence of hyperplastic lesions was significantly increased in the vehicle and in all three test article-treated groups when compared to the untreated control group (Group 2). In the females, the incidence of hyperplastic lesions was significantly increased in the vehicle control group and in the low and mid-dose groups (but not the high dose group) when compared to the untreated control group (Group 2).

There were no vehicle or treatment-related effects on the other tissues examined.

Addendum:

The sponsor has prepared and submitted an addendum to Study No. AA81EW.7D8T.BTL to analyze the predictive power and relative risk of dermal SOA irritation for papilloma formation. Of the 160 animals analyzed (40 animals per treatment group), 100 (63%) had dermal SOA irritation and 91 (57%) had a SOA papilloma. Of the 91 animals with papillomas, 87 had dermal SOA irritation prior to papilloma formation. The evaluation of dermal SOA irritation as a predictor for papilloma formation yielded sensitivity, specificity, and overall predictive power of 0.99, 0.86, and 0.93, respectively. Further evaluation using a time-to-event model confirmed that the risk of developing a papilloma was 144 times greater when dermal SOA irritation was present than when irritation was not present. The sponsor believes that tumorigenesis in Tg.AC mice may be a nonspecific response to dermal insult.

Reviewer's comments: It is well known that Tg.AC mice are 'genetically initiated' for tumor formation (Leder et al., Proc. Natl. Acad. Sci. USA, 1990; 87(23):9178-82). However, irritation alone is not sufficient to induce papilloma formation. These findings indicate that the vehicle is at best, a tumor promoter, and at worst, a complete carcinogen.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

No studies to address fertility and early embryonic development have been submitted to this NDA. The sponsor has provided literature references which were reviewed above.

Embryofetal development

The sponsor has submitted a study entitled "Limit teratology study in the rat by dermal route." (Study no. 93/BSM001/1174). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. The study report concludes that the treatments did not result in adverse effects on fetal development. However, although no systemic tretinoin was detected, the abnormalities described in the drug-treated groups such as craniofacial and heart abnormalities could be consistent with retinoid effects.

Prenatal and postnatal development

No studies to address prenatal and postnatal development have been submitted to this NDA. The sponsor has provided literature references which were reviewed above.

2.6.6.7 Local tolerance

No local tolerance studies were submitted with this NDA.

2.6.6.8 Special toxicology studies

The sponsor has submitted a summary of preclinical pilot experiments meant to assess an administration scheme for testing multiple dose applications of Velac Gel on dermal toxicity. Initially a study was conducted in rabbits, but they developed severe irritation and it was decided that the rabbit was not a suitable model for testing multiple dose applications of the gel. The rat then proved to be less sensitive with respect to erythema, but more vulnerable to eschar formation. However, when Velac Gel was applied only on alternate days, rat skin showed no or only slight erythema without eschar formation. It was concluded that the latter application scheme is suitable for testing multiple dose applications of Velac Gel.

The sponsor has submitted a study entitled "Primary skin irritation/corrosion study with clindamycin-tretinoin-gel in the rabbit (4-hour semi-occlusive application)." (Study no. 081372). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Although the formulation tested was not provided, the gel contained 1% clindamycin and 0.025% tretinoin. It was applied to the shaved skin of three male albino rabbits under semi-occlusive conditions for 4 hours, followed by observations at approximately 1, 24, 48, and 72 hours after removal of the dressings and remaining test substance. Under the conditions of the test, no skin irritation was caused by clindamycin-tretinoin-gel and no corrosive effect occurred on the skin in any of the three rabbits.

The sponsor has submitted a study entitled "Determination of phototoxicity with clindamycin-tretinoin-gel in albino guinea pigs." (Study no. 081394). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Although the formulation tested was not provided, the gel contained 1% clindamycin and 0.025% tretinoin. Hair was clipped from the backs of 15 guinea pigs and then each of ten animals received a single application of the four test articles (100%, 30%, 10%, and 3% gel) to each flank. Five animals were treated with ethanol as control. Fifteen minutes after test article application, the left flank was exposed to 20 J/cm² UVA light. Treated areas were examined for signs of erythema and edema at 24, 48 and 72 hours after application of the test articles. There were no signs of erythema or edema in any animal. It is concluded that under the conditions of the test, clindamycin-tretinoin-gel did not cause a phototoxic reaction in the guinea pig.

The sponsor has submitted a study entitled “Assessment of contact hypersensitivity to clindamycin-tretinoin-gel in albino guinea pigs (Maximization test).” (Study no. 081383). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Although the formulation tested was not provided, the gel contained 1% clindamycin and 0.025% tretinoin. Experimental animals were intradermally injected with three pairs of materials: 1) the gel diluted to 5% with saline; 2) Freund’s Complete Adjuvant diluted 50:50 with water; 3) the gel diluted to 10% with saline then mixed 50:50 with Freund’s Complete Adjuvant. Seven days after the injections animals were topically exposed to the undiluted test substance (0.5 mL) between the two sets of injection sites. The patch was held in place with tape and an elastic bandage for 48 hours. Twenty animals were induced with the test article and another ten control animals were treated in the same way except no test article was used. Two weeks after the topical application all animals were challenged with test substance concentrations of 25%, 10%, and 5% and the vehicle (distilled water). The challenge reactions were evaluated for erythema and edema 24 and 48 hours after patch removal. One animal showed some erythema in response to challenges to all three concentrations of the gel tested (25%, 10%, and 5%). Two other animals had scattered red spots in response to challenge with the 25% concentration, but because a control animal also had scattered red spots in response to the 25% concentration, none of the results at 25% were considered positive. The report concluded that only one animal (1/20) had a positive response at the 5 and 10% concentrations. According to the criteria listed by the sponsor, a test article with a 5% response rate (1/20) is considered to be a weak sensitizer.

The sponsor has submitted a study entitled “Determination of photoallergenicity with clindamycin-tretinoin-gel in albino guinea pigs.” (Study no. 351494). This study was previously reviewed under IND 65,369 (SN000) by Dr. Paul Brown. Although the formulation tested was not provided, the gel contained 1% clindamycin and 0.025% tretinoin. On day one of the study, the hair was clipped from the backs of 30 female Himalayan spotted guinea pigs. Four injections of Freund’s complete adjuvant mixed 1: 1 with saline were made at the corners of a 6-8 cm² site. A thin layer of the undiluted test article was then spread over the test site in 20 of the animals. The test site was then irradiated with 1.8 J/ cm² UVB and 10 J/ cm² UVA from the following lamps: Philips Actinic “TLD” lamp (36/ 08), 320- 400 nm and Philips UVB Sunlamp TL 20W/ 12, 280- 320 nm. The topical application of the test article and irradiation was repeated on days 3, 5, 8 and 10. Control animals (n= 10) received the adjuvant injections but were otherwise not treated. Three weeks after the start of the induction phase the animals were challenged. Hair was clipped from the flanks of the animals, undiluted test article was applied to each flank and then the left flank only was irradiated with 10 J/ cm² UVA. The test sites of each animal was assessed for erythema and edema at 24, 48 and 72 hours after exposure. Similar erythema reactions were observed at irradiated and nonirradiated sites in both control and test groups. This suggests that the test article does not possess photoallergenic potential.

2.6.6.9 Discussion and Conclusions

A dosage level of 5 mg/kg/day clindamycin gel, the highest dose tested, was considered to be the NOAEL following 90 days of topical application in male and female rats. Following dermal application in Hanford minipigs for 13 weeks, the systemic NOEL was 500 mg/kg/day Velac Gel and the local/dermal NOEL could not be established. Velac Gel was determined to be nonmutagenic in an *in vitro* Ames *Salmonella* reversion test. Velac Gel was determined to have equivocal clastogenic activity in a chromosome aberration assay. The non-compendial excipients, POE 4 (b) (4), have been tested as components of the gel vehicle in repeat dose toxicity tests and in the limit teratology study. They have also been tested for *in vitro* genotoxicity (Ames and chromosome aberration assays). No toxic or genotoxic signal was detected in these tests. However, when tested in a 26-week dermal carcinogenicity study in Tg.AC mice, the vehicle alone caused a statistically significant increased incidence of skin papillomas compared to the untreated controls and clindamycin in the Velac Gel vehicle caused further significant dose-related increases in papillomas relative to the vehicle controls and untreated animals. The sponsor has argued that dermal site of application irritation is a predictor for papilloma formation and as such the skin tumors are a nonspecific response to dermal insult. However, the eCAC is aware of other studies in Tg.AC mice in which irritation alone was not sufficient to cause papillomas. It is concluded that the vehicle is at best, a promoter, and at worst, a complete carcinogen. Although a 2-year mouse dermal carcinogenicity study would distinguish between the two, neither are acceptable for a topical drug for acne, where application to initiated skin can be expected. For these reasons, Velac Gel is considered to be nonapprovable.

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:

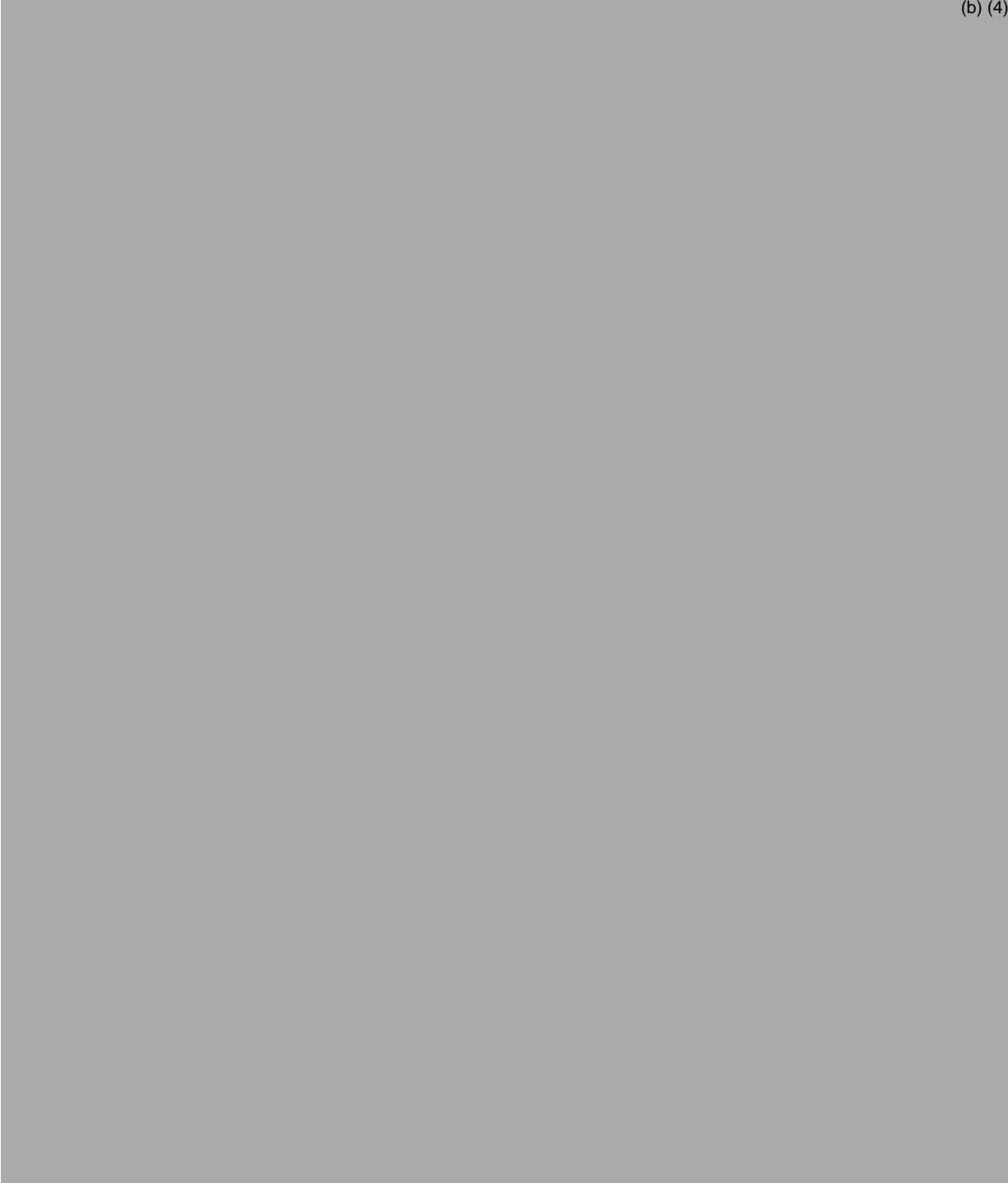
Velac Gel is considered to be nonapprovable because of a positive response in a 26-week dermal carcinogenicity study in Tg.AC mice in which there was a vehicle effect on papilloma formation. It is concluded that the vehicle is at best, a promoter, and at worst, a complete carcinogen. Although a 2-year mouse dermal carcinogenicity study would distinguish between the two, neither are acceptable for a topical drug for acne, where application to initiated skin can be expected. For these reasons, Velac Gel is considered to be not approvable.

Unresolved toxicology issues (if any):

There are no unresolved toxicology issues for Velac Gel.

Recommendations: Not approvable.

Sponsor proposed labeling : Although the Pharmacology/Toxicology recommendation is not to approve NDA 50-803, the sponsor's proposed labeling is included here for completeness.



(b) (4)



Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Copy of minutes of 12/16/03 Executive Carcinogenicity Assessment Committee meeting
Copy of minutes of 3/29/05 Executive Carcinogenicity Assessment Committee meeting
References

EXECUTIVE CAC

Date of Meeting: December 16, 2003

Committee: Abby Jacobs, Ph.D., HFD-024, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
Jeri El Hage, Ph.D., HFD-510, Alternate Member
Robert Osterberg, Ph.D., HFD-520, Alternate Member
Barbara Hill, Ph.D., HFD-540, Acting Pharm/Tox Supervisor
Paul Brown, Ph.D., HFD-540, Presenting Reviewer

Author of Draft: Paul Brown

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review (IND 65,369 SN 026).

The committee did not address the sponsor’s proposed statistical evaluation for the carcinogen bioassay, as this does not affect the sponsor’s ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 65,369

Drug Name: Clindamycin phosphate

Sponsor: Connetics Corp.

Background:

Clindamycin is an antibacterial. It has been used topically for the treatment of acne vulgaris in several approved drug products. The sponsor is developing a new gel formulation of 1% clindamycin phosphate/0.025% tretinoin. The sponsor was previously told that an NDA for their product could be supported by carcinogenicity data for clindamycin phosphate and literature information for tretinoin.

Transgenic Tg.AC Mouse Carcinogenicity Study Protocol:

The sponsor proposed a 26-week study in which Tg.AC mice (20/sex/group) will be treated daily by topical application of 0.1 mL of a gel containing 0, 1, 3 or 5% clindamycin phosphate. Mice will have hair clipped from the backs one to two days before the start of the study and weekly thereafter. A positive control group (15/sex) will have 50 µg TPA in the vehicle gel applied three times per week. An untreated control group will also be included. Animals will be housed individually and feed will be available *ad libitum*. Histopathology is planned for the following tissues from all animals except positive control: skin at the site of application (selected papillomas), skin at the site of application (non-lesioned skin), skin at a site other than the site of application and, all gross lesions.

Dose Selection:

The sponsor conducted a 28-day dose range finding study in FVB/N mice. This study evaluated daily application of 1, 3 or 5% clindamycin phosphate in the sponsor's gel vehicle. Essentially no local or systemic toxicity was observed in this study. Therefore, all of these doses may be tolerated for a 26-week study. The sponsor states that 5% clindamycin phosphate is the maximum concentration achievable in the formulation. Therefore, while the proposed carcinogenicity study may not result in the use of a maximum tolerated dose, it appears to be using the maximum feasible dose.

Executive CAC Recommendations and Conclusions:

- The Committee concurred with sponsor's proposed groups treated with 0.1 ml of a gel containing 0, 1, 3, or 5% clindamycin phosphate (equivalent to approximately 0, 40, 120, and 200 mg/kg) to be administered topically for 26 weeks and of an untreated control group.
- It is not possible, however, to concur with the use of 50 µg TPA in the gel vehicle as the positive control without data showing that this dose of TPA in this vehicle produces a satisfactory positive response in Tg.AC mice. The sponsor will assume some risk by using this dose without such preliminary data.
- The committee recommended that histopathology be performed on all tissues and organs for all dose groups (except positive control) rather than the limited number suggested in the protocol. This will help characterize the possible systemic effects of this drug product.

Abby Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:

HFD-540/Pharm Tox /Hill
 HFD-540/Pharm Tox /Brown
 HFD-024/Exec. Sec./Seifried
 HFD-540/PM/Owens

Executive CAC

Date of Meeting: March 29, 2005

Committee: Abby Jacobs, Ph.D., HFD-024, Acting Chair
 Joseph Contrera, Ph.D., HFD-901, Member
 Lois Freed, Ph.D., HFD-120, Alternate Member
 Paul Brown, HFD-540 Supervisor
 Jill Merrill, HFD-540 Presenting Reviewer

Author of Draft: Jill Merrill

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA 50-803

Drug Name: Velac Gel® (clindamycin phosphate 1%, tretinoin 0.025%)

Sponsor: Connetics Corporation

Background: Velac Gel® contains the antibiotic clindamycin phosphate and the retinoid tretinoin in a gel vehicle and is being considered for the topical treatment of acne vulgaris. The protocol for the Tg.AC mouse study for clindamycin phosphate was reviewed and concurred with by the Executive CAC (12-16-03). Results from the 26-week Tg.AC mouse dermal carcinogenicity study (b) (4), AA81EW.7D8T.BTL) were received as part of the NDA submission.

Tg.AC Mouse Carcinogenicity Study:

DOSING COMMENTS: 5% is the maximum feasible dose

NUMBER OF MICE: 20/sex/group, except TPA positive control which was 15/sex

MOUSE DOSE LEVELS: see table

Group	Test Article	Volume (mL/day)	Dose clindamycin phosphate (mg/kg)
1	Velac gel vehicle control	0.1	0
2	Shaved, untreated control	0	0
3	50 µg TPA in Velac gel vehicle	0.1 mL 3x/week	0
4	1% clindamycin phosphate in Velac gel vehicle	0.1	40
5	3% clindamycin phosphate in Velac gel vehicle	0.1	120
6	5% clindamycin phosphate in Velac gel vehicle	0.1	200

TPA = 12-O-tetradecanoylphorbol 13-acetate

MOUSE TUMOR FINDINGS: At the end of the study (week 27)

Group #	Sex (M or F)	Incidence	Burden (all papillomas)	
		Animals bearing at least one latent or actual papilloma per effective # of animals (% incidence)	All papillomas per papilloma bearing animal	All papillomas per effective animal
		SOA	SOA only	SOA only
1	M	9/20 ** (45)	1.8	0.8
1	F	5/20 ** (25)	2.0	0.5
2	M	0/18 (0)	0.0	0.0
2	F	0/16 (0)	0.0	0.0
3	M	NA	NA	NA
3	F	NA	NA	NA
4	M	12/20 ** (60)	2.2	1.3
4	F	6/20 ** (30)	1.7	0.5
5	M	15/20** (75)	2.7	2.0****
5	F	12/20 * ** (60)	1.8	1.1****
6	M	16/20 * ** (80)	3.4	2.8 *** ****
6	F	16/20 * ** (80)	2.2	1.8 *** ****

* $p \leq 0.05$, Fisher's exact Test, when compared to vehicle (Group 1)

** $p \leq 0.05$, Fisher's exact Test, when compared to untreated (Group 2)

*** $p \leq 0.05$, ANOVA and Dunnett's t-test, when compared to vehicle (Group 1)

**** $p \leq 0.05$, ANOVA and Dunnett's t-test, when compared to untreated (Group 2)

NOTE: Papillomas were scored as "latent" after attaining a size of 2 mm in diameter and protruding from the skin. Papillomas were scored as "actual" when they remained countable for 3 consecutive weekly scoring sessions. Group 3 (positive control) incidence is listed as NA since by week 27 all animals in this group were removed from the study due to tumor burden.

At study termination, the incidence of papillomas was 45%, 0%, 60%, 75%, and 80% in the males and 25%, 0%, 30%, 60%, and 80% in the females from the vehicle control, untreated control, low-, mid-, and high-dose groups, respectively. The incidence was 100% in the positive control animals. These results indicate a positive, statistically significant response in the incidence of papillomas in all treatment groups, including the vehicle control.

Executive CAC Recommendations and Conclusions:

* The Committee had previously agreed on the doses, and the Committee agreed that the study was adequate.

* The Committee concurred that the vehicle of Velac gel alone caused a statistically significant increased incidence of skin papillomas compared to the untreated controls and that clindamycin in Velac gel caused further significant dose-related increases in papillomas relative to the vehicle controls and untreated animals.

The Committee noted that positive results in the Tg.AC assay indicate that a substance may be either a promoter or a complete carcinogen.

* It was noted that the sponsor believes that application site irritation led to papilloma formation. However, the Committee is aware of other studies in Tg.AC mice in which irritation alone was not sufficient to cause papillomas. Therefore, the Committee cannot concur with the sponsor's position that the increase in papillomas with vehicle and clindamycin represents a nonspecific response to irritation.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
NDA 50-803/ Division File, HFD-540
P Brown/ Team leader, HFD-540
J Merrill/ Reviewer, HFD-540
M Owens/ PM, HFD-540
A Seifried, HFD-024

References:

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/s/

Jill Merrill
5/16/05 04:07:08 PM
PHARMACOLOGIST

added sentence re: sponsor's proposed labeling

Paul Brown
5/16/05 05:10:58 PM
PHARMACOLOGIST

**Division of Dermatologic and Dental Drug Products (HFD-540)
Pharmacology/Toxicology Checklist for NDA Filing Meeting**

Date: 10/13/04

Reviewer: Jill Merrill/Paul Brown

NDA Number: (b) (4)

Drug Name: Velac (clindamycin phosphate 1%, tretinoin 0.025%) gel

CAS Number: Clindamycin phosphate: 24729-96-2 ; Tretinoin: 302-79-4

Drug Class: antibiotic and retinoid

Indication: acne vulgaris

Route of Administration: topical to the skin

Date CDER Received: August 25, 2004

User Fee Date: June 25, 2005

Date of Draft Review: April 2, 2005

Sponsor: Connetics Corp.

Fileability:

On initial overview of the NDA application:

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

Yes

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

Yes

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

Yes

(4) Are all required (*) and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity, teratogenicity*, effects on fertility*, juvenile studies, acute studies*, chronic studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetic studies, etc)?

Yes

(5) If the formulation to be marketed is different from the formulation used in the toxicology studies, has the Sponsor made an appropriate effort to either repeat the studies using the to be marketed product or to explain why such repetition should not be required?

Not all nonclinical information is from studies conducted with the clinical formulation since some of the information is from the literature. However, it appears that nonclinical studies were conducted with the clinical formulation when appropriate.

(6) Are the proposed labeling sections relative to pharm/tox appropriate (including human

dose multiples expressed in either mg/m² or comparative serum/plasma levels) and in accordance with 201.57?

It does not appear that the sponsor included information on fertility and peri-/post-natal development in the labeling. The literature information will be reviewed to determine if it can be incorporated into the labeling. It appears that the dose multiples are appropriately calculated although these will be checked in the review.

(7) Has the Sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the Sponsor?

Yes

(8) On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the Sponsor submitted a rationale to justify the alternative route?

The appropriate route appears to have been used in the relevant studies.

(9) Has the Sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?

It appears that the submitted pivotal studies have GLP compliance statements when appropriate.

(10) Has the Sponsor submitted the data from the nonclinical carcinogenicity studies, in the STUDIES electronic format, for the review by Biometrics?

Electronic copies of the data appear to have been submitted. The appropriateness of the format will be determined by the statistical reviewer.

(11) From a pharmacology perspective, is this NDA fileable? If "no", please state below why it is not.

Yes

(13) If the NDA is fileable, are there any issues that need to be conveyed to Sponsor? If so, specify:

None at this time.

(14) Issues that should not be conveyed to the Sponsor:

None at this time.

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/s/

Jill Merrill
10/18/04 01:28:48 PM
PHARMACOLOGIST

filing memo

Paul Brown
10/19/04 01:26:31 PM
PHARMACOLOGIST