CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-400

PHARMACOLOGY REVIEW(S)

AUG 22 1994

Review and Evaluation of Pharmacology and Toxicology Data
Division of Topical Drug Products, HFD-540

NDA#: 20-400

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Sponsor: Penederm Inc.
320 Lakeside Drive, Suite A
Foster City, CA 94404

Name of Drug: Acticin Gel 0.025%
Generic Name: Tretinoin Gel 0.025%
Chemical Name: All-trans retinoic acid
Names Used in Studies:

Identifying Name	Other Names
Acticin 0.025% Gel	PDT 004-002
Acticin Gel Vehicle	PDT 004-006
Acticin 0.1% Cream	PDT 004-046
Acticin 0.05% Cream	PDT 004-045
Acticin 0.025% Cream	PDT 004-044
Acticin Cream Vehicle	PDT 004-054
Retinoic Acid Solution 0.2%	PDT 004-055
Retin-A® 0.025% Gel	PDT 004-003
Retin-A® 0.1% Cream	PDT 004-031
Polyolprepolymer-2	PDT 002-001, PDT 002-002, TopiCare Delivery Compound, TopiCare 35A

Pharmacological Category: Retinoid

Indication: Acne vulgaris

Route of Administration: Topical dermal

Recommended Dosage: Once nightly, "thin layer over effected area", multiple 6-week treatments may be necessary, exposure is considered chronic

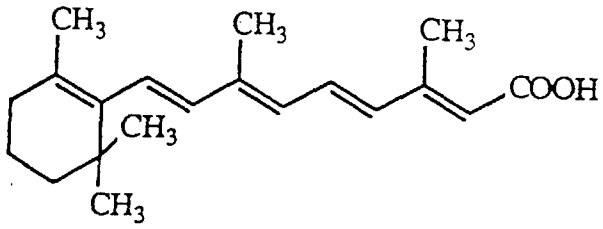
Related IND:

Related NDA: Acticin Cream Formulations: NDA 20-404

Related ANDA:

Related Drugs: IND
NDAs 17-340-001 (emulsion, cream), 17-522-001 (emulsion, cream), 17-579-001 (gel); Renova® (NDA 19-963)

Structural Formula:



Formulation:

Ingredient	% w/w
Alcohol,	
Polyolprepolymer-2	
Hydroxypropyl cellulose	
Tretinoin USP	
Butylated hydroxytoluene, NF or F.C.C.	

The formulation is virtually identical to Retin-A®, except for the addition of polyolprepolymer-2. Other formulations were sometimes used in testing; they are presented in Appendix A.

Index of Preclinical Studies:

The following studies were not reviewed previously and are presented in detail.

In Vitro Percutaneous Absorption Study
Toxicity/Irritation Tests:
- Acute Toxicity (LD_{50})
- 14-Day Dermal Toxicity and Irritation in Mice
- 7-Day Dermal Irritation in Guinea Pigs
- Primary Skin Irritation in Guinea Pigs
- Primary Eye Irritation in Rabbits
- 13-Week Dermal Toxicity and Irritation Test in Rabbits
- 91-Day Dermal Irritation and Toxicity Study

Reproduction Studies:
- Pilot Teratology Study in Rabbits
- Segment II Teratology in Rabbits

Mutagenicity Tests:
- Ames Assay
- Mouse Lymphoma Assay
- Mouse Micronucleus Bone Marrow Erythrocyte Assay *(In Vivo)*

The following studies were reviewed previously by Dr. Syed Alam and are summarized in this review:

In Vitro Percutaneous Absorption Studies in Human Cadaver Skin
Primary Skin Irritation Studies in Rabbits
Repeat Skin Irritation Studies in Guinea Pigs
Skin Hypersensitivity Studies in Guinea Pigs
Comedogenicity Study in Rabbits
28-Day Percutaneous Studies in Rabbits

Introduction:

The active ingredient in Acticin 0.025% Gel is tretinoin, a natural metabolite of Vitamin A (retinol) that has been used for over 20 years for acne vulgaris; the dermal form is currently on the market as Retin-A® gel or cream. The exact mechanism of action for tretinoin remains unknown, although evidence suggests a three-fold effect: the drug decreases cohesiveness of follicular epithelial cells and thus decreases microcomedo formation; tretinoin stimulates mitotic activity and increased turnover of follicular epithelial cells, which tends to cause extrusion of comedones; and third, tretinoin decreases sebum production.

Several adverse reactions are associated with Retin-A® use. The drug may cause skin irritation so severe that use must be discontinued, although the skin reactions are reversible. Retin-A® is also considered teratogenic in animals (pregnancy category C). Carcinogenicity testing for Renova® (another tretinoin formulation) was negative, although the assay for photocarcinogenicity in CD-1 mice was positive.

Acticin 0.025% Gel is identical to Retin-A® 0.025% Gel, except for the addition of polyolprepolymer-2, a new and unknown ingredient that adds emollient qualities and reduces irritation (according to the sponsor). Polyolprepolymer-2 is a polyurethane glycol moiety formed by the linkage of polypropylene glycol units with dicyclohexylmethane di-isocyanate. The sponsor did not report the structure(s) of polyolprepolymer-2, although the formula is reported as $HO(C_3H_6O)_{12}[C_{15}H_{22}N_2O_2(C_3H_6O)_{12}]_mH$, where m = 1 to 4, predominately. The material is identified chromatographically as 5 different peaks with an average molecular weight of 4,000, although the range is approximately to more than daltons. Polyolprepolymer-2 is a new entity that has never been through the IND/NDA safety review process.

Pharmacokinetics: The sponsor performed no original pharmacokinetics preclinical studies. The sponsor submitted one journal article as a review of retinoid pharmacokinetics: Allen, JG, and Bloxham, DP, The Pharmacology and Pharmacokinetics of the Retinoids. *Pharmac. Ther.* 40:1-27, 1989. The article presents absorption, transport, distribution, metabolism, and pharmacokinetics associated with most retinoid compounds. Of specific concern to this NDA review is the section reviewing pharmacokinetics of tretinoin. The following section is taken from the article.

Pharmacokinetics

Studies in mammals have indicated that the relative amounts of the metabolites produced from all-*trans*-retinoic acid also depends upon the dose administered. Thus Roberts and Frolik (1979) reported that the ratio of 13-*cis*-isomer (isotretinoin) to 4-*oxo*-retinoic acid formed by tissues *in vitro* decreases as the concentration of tretinoin is increased. Similarly Swanson *et al.* (1981) and Zile *et al.* (1982) reported that, when tretinoin is given intravenously to rats, the proportion that is converted to the glucuronide metabolite increases with increasing dose. This suggests that some of the other metabolic pathways have been saturated. Such effects could explain why non-linear pharmacokinetics were seen when rats were given i.v. doses between 0.015 and 5 mg/kg (Swanson *et al.*, 1981). Thus, the initial rates of elimination reduced significantly with dose, but these deviations from first-order kinetics decreased with time, and all doses ultimately showed terminal half-lives of approximately 20 min. Similar non-linearities of pharmacokinetics were reported for mice given 10 mg/kg oral doses (Munsell *et al.*, 1987) but not in anaesthetised dogs dosed i.v. at 3 mg/kg (Patel *et al.*, 1982b).

Similar pharmacokinetic studies have not apparently been performed with tretinoin in humans (Verweij *et al.*, 1985). Chiang (1980) did administer a single 0.5 mg dose, intravenously to a male volunteer, as a control for a topical absorption study. Following the i.v. dose, plasma concentrations of 14.6 and 5.4 ng/ml were found after 5 min and 1 hr respectively. (Based on these concentrations Lucek and Colburn (1985) estimated that the volume of distribution for tretinoin in man was approximately 30 l.) Following topical application, tretinoin levels were reported to be non-measurable (<2 ng/ml) even after 28 days of continuous application of 0.025% cream to the arms and legs, and also following a single whole-body inunctiation with a 1% cream. These findings are consistent with data obtained following a single application of radioactive drug which showed that following seven days pretreatment with unlabelled tretinoin, only approximately 6% of the labelled dose could be recovered in urine and faeces (Lucek and Colburn, 1985).

Preclinal Studies

The following studies have not been reviewed previously.
All studies appear to have been done under GLP guidelines, except for the *in vitro* percutaneous absorption studies.

Percutaneous Absorption, *In Vitro*

Laboratory: The sponsor

Materials tested: Acticin 0.025% Gel (PDT 004-002), Retin-A® 0.025% Gel (PDT 004-003, both US and Foreign versions, although the sponsor has not explained how the two formulations differ)

Amount applied: 10 mg. to each 0.74 cm^2 diffusion cell

Time length of test: 48 hours

Method: Dermatomed human skin was placed on standard Bronaugh (Franz) flow-through percutaneous diffusion cells to evaluate the penetration potential of the test materials. The tretinoin formulations were spiked with tritiated tretinoin. The receptacle fluid beneath the skin was collected every 6 hours and counted for the absorbed amount of radio-labelled test material. At 48 hours, the skin surface was washed and the washes, epidermis, and dermis were also counted.

Results:

Percutaneous Absorption (% of Applied Dose; mean ± SD, n = 14)

Formulation	Receptor Fluid	Epidermis	Dermis	Total Recovery
Acticin Gel (PDT 004-002)	0.22 ± 0.04	0.58 ± 0.19 †	0.26 ± 0.10	93.5 ± 3.7
Retin-A® Gel-US (PDT 004-003)	0.28 ± 0.06 *	1.76 ± 0.82 *	0.28 ± 0.16	101.9 ± 5.6
Retin-A® Gel-Foreign (PDT 004-003)	0.22 ± 0.07	1.91 ± 0.70	0.21 ± 0.09	99.9 ± 3.0

* Statistically different from Acticin gel and "foreign" gel
† Statistically different from US and foreign Retin-A®

Under the conditions of this assay, Acticin Gel (0.025%) had less than 1% penetration into the epidermis, dermis, and receptor fluid. Penetration of Retin-A® gel (in both formulations) was only slightly greater.

Toxicity and Irritancy Tests

Acute Toxicity (LD_{50})

Laboratory:

Number of Animals: 10 (5/sex)

Animal Strain: Sprague Dawley rats

Formulations: Acticin 0.025% Gel (PDT 004-002)

Route: Oral

Results:

Material Tested	Route	LD_{50}
PDT 004-002	p.o.	> 5.0 g/kg

Results: No deaths occurred. Materials with an LD_{50} greater than 5.0 g/kg are considered to be of very low toxicity.

14-Day Dermal Toxicity Study in Mice

Laboratory:

Number of Animals/Group: 8/group (4/sex)

Animal Strain: Crl:CD®(ICR) BR mice

Materials Tested: Acticin 0.025% Gel (PDT 004-002), Acticin 0.05% Cream (PDT, 004-045), Acticin 0.10% Cream (PDT 004-046)

Dose levels/Study Design:

Group	Formulation	Dose level (mg/kg/day)	Concentration (% Tretinoin)	Dose Volume (ml/kg)
1	PDT 004-054 (cream vehicle)	0	0	5
2	PDT 004-045 (Acticin 0.05% cream)	2.28	0.05	5
3	PDT 004-046 (Acticin 0.10% cream)	4.55	0.10	5
4	PDT 004-006 (gel vehicle)	0	0	5
5	PDT 004-002 (Acticin 0.025% Gel)	0.52	0.025	5
6	PDT 004-002 (Acticin 0.025% Gel)	1.04	0.025	5

Route: Dermal

Methods: Test materials were administered to the clipped back every day for two weeks. Following six hours of exposure, residual test materials were wiped off. Animals were euthanized after 14 days of treatment.

Results:

Mortality: All animals survived to scheduled euthanasia.

Body Weights: Mean body weights and body weight gains were statistically comparable across all groups, although animals treated with the high-dose cream had somewhat lower body weights and body weight gains.

Clinical Observations: Males and females both exhibited test-article build up. Dermal findings were normal in the two control groups, except the cream vehicle mice (7/8) were noted to have stained exposure sites. The Acticin gel- and cream-treated animals exhibited a variety of findings at the exposure site, including slight-to-moderate erythema, slight-to-moderate atonia, slight-to-moderate desquamation, skin thickening, and whitening at the test

site. Blanching was noted only in males treated with the high-dose gel. Eschar was noted in the males treated with the cream formulations and in the females in both the gel and cream formulations. No other clinical observations were made that were considered treatment related.

Necropsy: Animals treated with the low-dose gel formulation were noted at necropsy with a dilated kidney pelvis (one male) and ovarian cysts (two females); all other findings were unremarkable.

7-Day Dermal Irritation Study in Guinea Pigs

Laboratories: The Sponsor (report preparation),

Animals: 25 (13 males, 12 females)

Animal Strain: guinea pigs

Test Materials: All formulations contain 0.025% tretinoin: Acticin Gel (PDT 004-002), Retina-A® Gel (PDT 004-003), Acticin "Research" Cream (PD 46-1360-A), Retin-A® Cream (PDT 004-024)

Dose Levels: 50 or 100 mg twice/day

Study Design: This study was conducted under GLP conditions, although the design of the study is somewhat unusual. Six separate skin sites were designated on each animal. Each site was treated with the same compound, and thus every compound was tested with n=6 (where n = the number of skin sites tested). Each test material was tested on six different animals (but at single test sites). One animal served as an untreated control. Thus, the sponsor tested a large number of compounds while using a relatively small number of guinea pigs. The table on the following page summarizes the study design.

Formulation	PDT #	Dose (mg/animal/treatment)
Acticin 0.025% Gel	004-002	50
Retin-A® 0.025% Gel	004-003	50
Sham Treatment	none	50
Acticin 0.025% Gel	004-002	100
Retin-A® 0.025% Gel	004-003	100
Sham Treatment	none	100
Sham Treatment	none	50
Retin-A® 0.025% Cream	004-024	50
Acticin "Research" Cream Vehicle	46-1360-C	50
Acticin "Research" 0.025% Cream	46-1360-A	50

Methods: Test articles were administered to the clipped backs of guinea pigs, and the test skin sites were observed clinically for dermal erythema or edema. If sufficient erythema was noted, a guinea pig would skip one or more dosings. The skin was evaluated daily, and following euthanasia, skin samples were evaluated histopathologically.

Route and Duration: Dermal application twice per day for 7 days at unoccluded skin sites. Test materials were left on the animals for approximately 6 hours following each exposure.

Results:

Mortality: All animals survived to scheduled euthanasia.

Dermal Clinical Observations: Beginning one day post-treatment, animals treated with Acticin 0.025% Gel (50 and 100 mg) were noted to have greater erythema and edema than the sham controls. At 50 mg, differences were statistically significant on days 2, 6, 7, and 8 for erythema and days 5, 6, 7, and 8 for edema. At 100 mg, differences were statistically significant on days 3 through 8 for erythema and days 5, 6, 7, and 8 for edema. Retin-A® generally caused erythema more quickly and to a greater degree than Acticin. The sponsor statistically compared the Acticin-related effects to Retin-A® effects. Animals treated with 50 mg Acticin exhibited significantly less erythema than the Retin-A®-treated (50 mg) animals on days 3, 4, 5, 6, and 8; and significantly less edema on days 3 through 8. The severity

for all of the observations ranged from very slight to moderate for both erythema and edema observations; severity tended to increase over time.

The cream formulations (50 mg), which consisted of Retin-A®, the Acticin research vehicle, and the research cream, caused statistically significantly greater erythema and edema than control values throughout the study (i.e. days 2 to 8). Retin-A® generally caused more severe effects than Acticin, although for all compounds the severity ranged from very slight to moderate with severity increasing over time.

Histopathology: Histopathological evaluation of the skin revealed that treatment-related effects were more severe in the Retin-A®-treated than in the Acticin-treated animals. Animals treated with Acticin (50 and 100 mg) gel were noted to have numerous changes in the epidermis and dermis when compared to control. Changes of note include epidermal parakeratosis, hyperplasia, thickening, mitotic rate, hyperkeratosis, and inflammatory infiltrates in the dermis. Animals treated with Retin-A® presented with a similar histomorphology, although it tended to occur more rapidly and to a greater degree. The sponsor compared the Retin-A® and the Acticin groups statistically; the Retin-A® group had statistically greater severity of hyperplasia, thickening, and mitotic rate at 50 and 100 mg, and greater severity of epidermititis, crust formation, and vasodilation and inflammatory infiltrates of the dermis.

The Acticin cream (50 mg) effects were similar to the gel, although the effects were more severe in the cream and also caused epidermal hypergranulsis, epidermititis, crust formation, spongiosis, and slight vasodilation in the dermis. Once again, the Retin-A® product (in this case a cream) caused the same sort of effects, although they were somewhat more severe. (However, the only statistical difference between Retin-A® cream and Acticin cream was noted in vasodilation of the dermis.)

Acticin cream and gel are moderate irritants which cause classic tretinoin-related histomorphologic changes in guinea pig skin. Under the conditions of this test, it appears that the irritation and histomorphologic changes are less severe following treatments of Acticin gel than Acticin cream, and that both Acticin treatments cause less severe effects than Retin-A® products.

Primary Skin Irritation Study in Rabbits

Laboratory:

Number of Animals: 6 (3/sex)

Animal Strain: New Zealand White

Formulation: Acticin 0.025% Gel (PDT 004-002)

Dose Level: 0.05 ml

Administration: Dermal

Duration: 24 hours

Methods: The test material was applied to 1 X 1" gauze squares on intact and abraded skin on each animal. Sites were occluded and animals were restrained to protect the application sites. At 24 hours, the sites were cleaned and skin irritation was evaluated at 24 hours and 72 hours. The animals were scored by the method of Draize.

Dermal Evaluation: The primary irritation index, based on scores of edema and erythema, was 4.0, and thus Acticin 0.025% Gel was found not to be a primary irritant. No corrosion of the skin was noted.

Primary Eye Irritation Study

Laboratory:

Number of Animals: 12 (3/sex/group)

Animal Strain: New Zealand White Rabbits

Formulation: Acticin 0.025% Gel (PDT 004-002)

Dose Level: 0.1 ml to one eye/animal

Duration: 6 animal eyes were not rinsed, 6 animal eyes were rinsed with lukewarm tap water for 1 minute following 30 seconds of sample contact.

Method: Rabbit eyes were administered Acticin 0.025% Gel, and then rinsed (6 animals) or not rinsed (6 animals). At 24 hours, all rabbit eyes were rinsed with physiological saline. Eyes were examined and graded by the Draize method for ocular reactions at 24, 48, and 72 hours following dose administration.

Ophthalmic Evaluation Results: Acticin 0.025% Gel produced corneal opacity (maximum score 2) in rinsed and unrinsed eyes that persisted up to day 10 in unrinsed eyes and up to day 7 in rinsed eyes. In the conjunctiva, erythema, swelling, and discharge were noted up to day 7 in rinsed and unrinsed eyes, although the findings were generally longer lasting and more severe (maximum score 2) in unrinsed eyes. No corrosion was seen in rinsed or non-rinsed eyes. The material was considered an irritant in unrinsed eyes.

13-Week Dermal Irritation and Toxicity Study

Laboratory:

Animal Strain: New Zealand White rabbits, approximately 15 weeks old, 2.2 to 2.6 kg.

Animals: 7/sex/group (42 total)

Test Materials: Polyolprepolymer-2 (PDT 002-002)

Route: Dermal

Duration: at least 13 weeks, dosing 5 days/week

Study Design & Dose Levels:

Group	Treatment	Dose level (mg/kg)	Dose Level (ml/kg)
1	Vehicle Control	125	0.125
2	polyolprepolymer-2 (PDT 002-002)	200	0.200
3	polyolprepolymer-2 (PDT 002-002)	500	0.500

Methods: The appropriate test material was applied to two application sites clipped of fur on each animal: an abraded or a non-abraded site. Test materials were applied over the skin site and occluded. Animals were collared; after approximately 6 hours of exposure, occlusions were removed, remaining test material was wiped off, and the collars were removed.

Results:

Mortality: All animals survived to scheduled euthanasia.

Clinical Observations: No compound-related changes were noted, except for changes in the skin (discussed below).

Body Weights and Food Consumption: Body weights and body weight gains were slightly and sporadically depressed in the treated groups, but none of the differences were statistically significant. Food consumption was similarly decreased; the decrease was statistically significant during week 7 for males in both treated groups.

Dermal Irritation Results: Occasional desquamation and slight erythema was noted in males and females in the low and high dose groups (1-3 males per day, 1-5 females per day). High dose animals were noted with slight erythema earlier and in more animals compared to low dose incidence. One male in the high dose was noted with pustules/papules throughout most of the study. Based on these findings, polyolprepolymer-2 appears to be a slight dermal irritant.

Ophthalmic Observations: No visible lesions were noted at the end of the study.

Hematology (16 parameters): High-dose males had statistically significantly increased red blood cell count (RBC) and hematocrit percentile (HCT%) as compared to control males following 13 weeks of treatment. Other hematology values for high-dose males and females varied slightly from controls, but the difference did not appear to be related to the treatment.

Clinical Chemistry (16 parameters): No significant differences were noted between control and treated groups. As with hematology, some variances were noted between high and control groups, but the changes were not clearly related to treatment.

Organ and Organ-to-Body Weights: No significant differences were noted between groups, except for high-dose females. This group had significantly greater ovary, ovary-to-body, and ovary-to-brain weights.

Gross Necropsy Observations: One high-dose male had a mottled lung; all ovaries appeared normal. No other findings were noted.

Histopathology: Only the control and high-dose animals were examined histopathologically. In the lungs, one control male and one control female had acute alveolar/bronchiolar inflammation; one high-dose male had congestion and three high-dose females had chronic inflammation. Liver infiltrate was noted once each in high-dose male, high-dose female, and control female. One high-dose female was noted to have chronic inflammation (papillary dermis) on abraded skin, and one high-dose female had chronic inflammation (papillary dermis), epidermal hyperplasia, and hyperkaretenosis. In the urinary bladder, calcereous material was noted in two animals in all groups, except for the high-dose females noted with three incidence. One high dose male had an atrophied/degenerated testis. None of the findings appear to be treatment-related. All other findings were not remarkable.

91-Day Dermal Irritation and Toxicity Study

Laboratory:

Animal Strain: Mice

No. of Animals: 20/sex/group

Test Materials: Acticin 0.025% Gel (PDT 004-002)

Route: Dermal

Duration: At least 91 days

Study Design & Dose Levels:

Group	Formulation	Dose of Tretinoin (mg/kg/day)	Dose Conc. (% of Tretinoin)	Dose Volume (ml/kg)
1 (control)	Gel Vehicle (PDT 004-006)	0	0	3.33
2 (low dose)	Acticin 0.025% Gel (PDT 004-002)	0.07	0.025	0.33
3 (mid dose)	Acticin 0.025% Gel (PDT 004-002)	0.25	0.025	1.17
4 (high dose)	Acticin 0.025% Gel (PDT 004-002)	0.70	0.025	3.33

Methods: The appropriate test material was applied to the back (clipped of fur) on each animal every day for 13 weeks. The exposure area constituted at least 10% of the animal body surface area. After approximately 6 hours of exposure all remaining test material was wiped off.

Results:

Mortality: One mid-dose male was found dead; all other mice survived to scheduled euthanasia.

Clinical Observations: In male mice (all groups) and one mid-dose female, palpable masses in the urogenital area were noted. All other findings were in the range of normal for mice, except for findings in the skin (detailed below).

Body Weights: Low dose male body weights were significantly below control on days 71 and 91 and generally lagged below control values following 1 week of treatment. High-dose male

body weight gains were significantly less than control in week 8. All differences, however, were minimal (less than 10%). All other body weights and all body weight gains in male mice were similar. Female body weights were similar for all groups. Female body weight gains were significantly below control for the low-dose during week 3; for the mid-dose during weeks 2 and 6, and for the high dose in weeks 3 and 6.

Food Consumption: Food consumption was significantly below control values for mid-dose males in weeks 7 and 10, and in high-dose males for weeks 7, 10, 11, 12, and 13. High dose female values were significantly below control in weeks 2, 4, 6, 9, 10, 11, 12, and 13.

Dermal Irritation Results: Compound-related findings were noted in both the males and the females. Observations included erythema, edema, atonia, desquamation, fissuring (males only), eschar areas, thickened skin, and skin whitening (males only). These findings increased in quantity and severity with increasing dose-level.

Ophthalmic Observations: Ophthalmological examination revealed corneal crystals in the week 13 examination. The reviewing ophthalmologist (David A. Wilkie DVM, MS, Diplomate ACVO; Ohio State University) did not consider the changes to be related to compound treatment.

Hematology (16 parameters): In low-dose males, leukocytes and segmented neutrophils were significantly decreased when compared to control values. In low-dose females, reticulocytes were significantly decreased compared to control values. All other values were comparable; no findings were considered treatment related.

Clinical Chemistry (6 parameters): In all treated animals (low-, mid-, and high dose males and females), mean aspartate transferase (AST) values were significantly greater than control values. Glucose was significantly decreased in mid-dose males, and urea nitrogen was significantly increased in low and high-dose females in comparison to control values. (Mid-dose females were also increased, although the difference was not statistically significant.)

Organ, Organ-to-Body, and Organ-to-Brain Weights: Differences between control mean values and mean values from the Acticin-treated animals are summarized on the following page.

Organ	MALES			FEMALES		
	Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
Kidney Wt.	↓*	↓*	↓			
Kidney:Brain	↓*	↓*	↓			
Heart Wt.	↓*	↓ (slt)	↓ (slt)			
Heart:Body	↓*	↓	↓	↓		
Heart:Brain	↓*	↓*	↓*			
Liver Wt.	↓*	↓	↓ (slt)	↓	↓*	
Liver:Body	↓*	↓	↑	↓*	↓*	↓ (slt)
Liver:Brain	↓*	↓*	↓			
Brain Wt.						
Brain:Body	↑	↑	↑*			

↓ or ↑ indicate a decrease or increase, respectively, from mean control values; * indicates change was statistically significant; slt is the abbreviation for a slight change; dose levels with no entries were virtually equal to control values

Although numerous changes are noted, particularly in the kidney, heart and liver, consideration should be given to the fact that in both males and females the changes decrease or even disappear in the high dose (except in male heart:brain weight).

Gross Necropsy Observations: At necropsy, three high-dose males had enlarged mandibular lymph nodes. Male mice also had enlarged inguinal lymph nodes in low- (1 observation), mid- (2), and high-dose (10) animals; and enlarged axillary lymph nodes in mid- (3) and high-dose (10) mice. Female mice had enlarged inguinal lymph nodes in the low (10) and high (3) doses; and enlarged axillary lymph nodes in the mid- (1) and high- (3) dose groups. Other findings did not appear to be treatment related.

Histopathology: Only low- and high-dose tissues were evaluated from the brain, heart, kidneys, liver, lungs, skin, thymus, and gross lesions. The reviewing pathologist (Robert G. Geil, DVM, Diplomate) considered treatment-related findings in the skin to include acanthosis, hyperkeratosis, and chronic dermatitis. The changes did not increase with dose level. Outside of the skin area, the only other possibly treatment related effect was in the thymus where a diffuse necrosis of individual lymphocytes was noted. The pathologist, however, noted that the condition could be stress related. All other microscopic findings were consistent with normal background lesions for this age and strain of mice.

Reproduction Studies

Dermal Teratology Pilot Study

Laboratory:

Number of Animals/Group: 6/group

Animal Strain: New Zealand White female rabbits

Dose levels/Study Design:

Group	Compound	Dosage Level (mg/kg/day)	Dosage Volume (ml/kg)
1	Distilled Water	0	2.0
2	PDT 002-002	1000	1.0
3	PDT 002-002	2000	2.0

Route of Administration: Dermal

Methods: Test materials were placed on the clipped backs of rabbits on gestation days 6-15. A collar was placed on each animal prior to dosing and for 6 hours post dosing. When the collar was removed, the application site was wiped with gauze to remove any remaining test material. Fetal evaluations for malformations and variations was done by external evaluation only.

Results:

Mortality: All females survived to scheduled death

Pregnancy Rate: 100% for all groups

Clinical Observations: Slight desquamation was noted at a slightly higher rate in the treated females when compared to controls. The high dose group was noted to have a high incidence of unkempt appearance. The sponsor theorized that this was because the high dose compound was extremely sticky. Other findings were considered within the range of normal.

Body Weight Gain: Body weights and body weight gains were comparable between groups.

Maternal Necropsy: At necropsy, one rabbit in the mid-dose had a dilated pelvis of the kidney. The high dose rabbits had no significant changes noted.

Reproduction Parameters: No statistically significant differences were noted between groups. The high-dose group had fewer total mean implantation sites and a greater pre-implantation loss than the control and low-dose groups. The low-dose group had a greater number of early resorptions and post-implantation loss.

Fetal Malformations and Variations: All fetuses appeared normal; no differences were noted.

Segment II Teratology Study

Laboratory:

Number of Animals/Group: 18/group

Animal Strain: New Zealand White female rabbits

Animal Age & Weight: Approximately 6 months old, from 3.1 to 4.5 kg

Dose levels/Study Design:

Group	Compound	Tretinoin Dose (mg/ kg/day)	Dosage Conc. - % Active	Dosage Volume (ml/kg)
1	PDT 004-006 (gel vehicle)	0	0	2
2	Sham control	0	0	0
3	PDT 004-002 (Acticin gel)	0.42	0.025	2
4	PDT 004-003 (Retin-A® gel)	0.40	0.025	2
5	PDT 004-031 (Retin-A® cream)	1.82	0.100	2
6	PDT 004-046 (Acticin cream)	1.82	0.100	2

Route of Administration: Dermal

Methods: Test materials were applied dermally beginning on gestation day 5 on the clipped backs in three areas that were rotated for treatment every 3 days. Collars were placed on the rabbits immediately prior to dosing, and were left on the females for 6 hours. At 6 hours,

any remaining test material was wiped off, the area was washed, and the collars were removed. Animals that aborted were euthanized. All surviving females were euthanized on gestation day 29 and fetuses were evaluated for visceral and morphologic abnormalities. Does were grossly evaluated at necropsy; uterine contents were evaluated and implantation sites were counted.

Results:

Mortality: Group 3 (treated with Acticin gel formulation) had four unscheduled deaths: three females that aborted and 1 moribund animal were euthanized. The other treated groups had one unscheduled death each. Group 4 and 6 (treated with Retin-A® gel or the Acticin cream formulation, respectively) each lost a female due to abortion; Group 5 (treated with the Retin-A® cream product) had one unscheduled death due to an accidental injury.

Pregnancy Rate: Pregnancy rates ranged from 100% in Group 1, to 94% in Groups 2, 3, 4, and 5, to 89% in Group 6. The pregnancy rate did not appear to be decreased by tretinoin treatment.

Clinical Observations: Several clinical observations were noted that indicated the animals were discomforted by the tretinoin-containing treatments. Observations included vocalization and animals struggling during dosing or rinsing; these observations were particularly high in Group 5. Other observations were considered within the range of normal.

Dermal Evaluation: Clinical observations at the exposure sites included slight to moderate erythema, slight desquamation, and multiple red areas in the Group 1 gel control; Group 2 sham controls had no abnormal dermal observations. Animals in the treated groups had a large number of observations indicating severe irritation at the treatment sites; observations included slight to severe erythema, slight edema, atonia, desquamation, fissuring, eschar areas, thickened skin, bleeding, and red raised areas. Additionally, despite washing, residual test material was often noted at or around the treated site.

Body Weight Gain and Food Consumption: Body weights were similar for all groups throughout gestation. However, Groups 5 and 6 (the two cream formulations) had significantly lower body weight gains during days 9-12 and 6-19, and all treated animals had decreased body weight gains to some extent. Food consumption was also lower for treated groups and for Groups 5 and 6 it was significantly less than control during days 9-12.

Maternal Necropsy: Observations at necropsy were not considered out of the ordinary for this size and sex of animal undergoing dermal dosing.

Reproduction Parameters: All tretinoin-treated animals (except for the commercial gel formulation, Group 4) had an increase in the mean number of late resorptions. Group 5 females also had a slightly greater pre-implantation loss, fewer viable fetuses, and larger fetal weights; none of these findings, however, were statistically significant. Statistically

significant findings consisted of greater post-implantation loss in Group 3 and significantly fewer male fetuses in Group 5. All other gravid females generally were comparable across groups for reproduction parameters.

Fetal Malformations: Fetal malformations are summarized in the table on the following two pages. Several findings in the treated fetuses appear to be drug related. Groups 3, 4, 5, and 6 had a higher incidence of domed head; this difference was statistically greater than controls for Group 4 only. Treated groups also had a higher incidence of cleft palate, flexed paw, and omphalocele, although these differences were not statistically significant. In the visceral examination, Groups 3, 4, and 5 had a statistically significant greater incidence of hydrocephaly; Group 6 also had a higher incidence of hydrocephaly, although it was not statistically significant. Other visceral findings did not appear to be drug related. In the skeletal examination, Group 5 had a significantly greater number of skull anomalies when compared to control. Total malformations consisted of a significantly greater number of soft tissue malformation for Group 3, greater number of external tissue malformations for Group 4, and a greater number of external, skeletal, and total malformations for Group 5. Group 6 also was noted to have a greater number of malformations, although the findings were not statistically significant.

SLS STUDY NO.: 3313.3
CLIENT: PENEDERM INC.
CLIENT NO.: TOX 004-026

TABLE 11
DERMAL TERATOLOGY STUDY IN RABBITS WITH PDT 004 COMPOUNDS
SUMMARY OF FETAL OBSERVATIONS - VARIATIONS

	FETUSES / LITTERS					
GROUP:	1	2	3	4	5	6
LEVEL (MG/KG/DAY):	0	0	0.42	0.40	1.82	1.82
	GEL VEHICLE (PDT 004-006)	SHAM CONTROL	GEL PRODUCT FORMULATION (PDT 004-002)	COMMERCIAL GEL PRODUCT (PDT 004-003)	COMMERCIAL CREAM PRODUCT (PDT 004-031)	CREAM PRODUCT FORMULATION (PDT 004-046)
NUMBER EXAMINED EXTERNALLY	109/ 18	121/ 17	78/ 13	105/ 16	67/ 15	95/ 15
NUMBER WITH FINDINGS	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0
NUMBER EXAMINED VISCERALLY	109/ 18	121/ 17	78/ 13	105/ 16	67/ 15	95/ 15
HEMORRHAGIC RING AROUND THE IRIS	2/ 2	1/ 1	1/ 1	1/ 1	1/ 1	0/ 0
RETROCAVAL URETER	1/ 1	4/ 4	4/ 3	4/ 3	0/ 0	1/ 1
MAJOR BLOOD VESSEL VARIATION	8/ 5	2/ 2	4/ 2	2/ 2	6/ 2	5/ 4
TRACHEA ANOMALY	1/ 1	0/ 0	2/ 2	0/ 0	3/ 2	2/ 1
NUMBER EXAMINED SKELETALLY	109/ 18	121/ 17	78/ 13	105/ 16	67/ 15	95/ 15
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE	32/ 14	43/ 11	19/ 8	41/ 15	16/ 8	25/ 10
13TH RUDIMENTARY RIB(S)	31/ 14	26/ 15	16/ 8	16/ 10	9/ 5*	13/ 11
7TH CERVICAL RIB(S)	3/ 3	2/ 2	0/ 0	0/ 0	10/ 5	1/ 1
HYOID ARCH(ES) BENT	9/ 7	9/ 4	5/ 3	14/ 8	8/ 5	5/ 4
13TH FULL RIB(S)	41/ 13	42/ 14	24/ 9	57/ 15	33/ 12	44/ 14
27 PRESACRAL VERTEBRAE	16/ 8	23/ 14*	18/ 8	22/ 10	20/ 7	29/ 11
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	4/ 3	5/ 2	0/ 0	3/ 3	0/ 0	1/ 1
ACCESSORY SKULL BONE(S)	1/ 1	0/ 0	0/ 0	3/ 3	1/ 1	2/ 2
SPHERICAL ENLARGEMENT OF THE RIB(S)	1/ 1	1/ 1	1/ 1	1/ 1	0/ 0	1/ 1
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	0/ 0	0/ 0	3/ 3	2/ 1	1/ 1	1/ 1
25 PRESACRAL VERTEBRAE	0/ 0	0/ 0	1/ 1	0/ 0	1/ 1	0/ 0
REDUCED OSSIFICATION OF THE SKULL	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05

SLS STUDY NO.: 3313.3
CLIENT: PENEDERM INC.
CLIENT NO.: TOX 004-026

TABLE 10
DERMAL TERATOLOGY STUDY IN RABBITS WITH PDT 004 COMPOUNDS
SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

	FETUSES/LITTERS					
GROUP:	1	2	3	4	5	6
LEVEL (MG/KG/DAY):	0	0	0.42	0.40	1.82	1.82
	GEL VEHICLE (PDT 004-006)	SHAM CONTROL	GEL PRODUCT FORMULATION (PDT 004-002)	COMMERCIAL GEL PRODUCT (PDT 004-003)	COMMERCIAL CREAM PRODUCT (PDT 004-031)	CREAM PRODUCT FORMULATION (PDT 004-046)
NUMBER EXAMINED EXTERNALLY	109/ 18	121/ 17	78/ 13	105/ 16	67/ 15	95/ 15
DOMED HEAD	1/ 1	1/ 1	9/ 5	14/ 6*	9/ 5	8/ 4
CLEFT PALATE	1/ 1	0/ 0	3/ 2	1/ 1	6/ 3	3/ 2
FLEXED PAW	1/ 1	0/ 0	1/ 1	3/ 1	5/ 4	4/ 3
OMPHALOCELE	0/ 0	0/ 0	0/ 0	1/ 1	1/ 1	0/ 0
HIGH-ARCHED PALATE	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
OPEN EYELID(S)	0/ 0	0/ 0	1/ 1	0/ 0	1/ 1	0/ 0
MICROGNATHIA (MAXILLARY OR MANDIBULAR)	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
GASTROSCHISIS	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
KINKED TAIL	1/ 1	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0
NUMBER EXAMINED VISCERALLY	109/ 18	121/ 17	78/ 13	105/ 16	67/ 15	95/ 15
HYDROCEPHALY	1/ 1	2/ 1	12/ 6*	15/ 7*	10/ 6*	9/ 4
HEART AND/OR GREAT VESSEL ANOMALY	1/ 1	1/ 1	1/ 1	0/ 0	0/ 0	0/ 0
ADRENAL(S) ANOMALY	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
TRANSPOSITION OF THE GREAT VESSELS	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
NUMBER EXAMINED SKELETALLY	109/ 18	121/ 17	78/ 13	105/ 16	67/ 15	95/ 15
APPENDICULAR SKELETAL DEFECT	0/ 0	0/ 0	2/ 2	1/ 1	3/ 3	0/ 0
EXTRA SITE OF OSSIFICATION ANTERIOR TO STERNEBRA #1	2/ 2	1/ 1	0/ 0	1/ 1	5/ 3	1/ 1
SKULL ANOMALY	0/ 0	0/ 0	0/ 0	0/ 0	12/ 4*	4/ 2
RIB ANOMALY	0/ 0	1/ 1	0/ 0	0/ 0	2/ 2	0/ 0
COSTAL CARTILAGE ANOMALY	0/ 0	0/ 0	2/ 2	1/ 1	2/ 2	1/ 1
STERNEBRA(E) MALALIGNED (SEVERE)	0/ 0	1/ 1	0/ 0	0/ 0	1/ 1	0/ 0
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	0/ 0	0/ 0	2/ 2	1/ 1	3/ 2	1/ 1

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05

TABLE 10
DERMAL TERATOLOGY STUDY IN RABBITS WITH PDT 004 COMPOUNDS
SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

	FETUSES / LITTERS					
GROUP:	1	2	3	4	5	6
LEVEL (MG/KG/DAY):	0	0	0.42	0.40	1.82	1.82
	GEL VEHICLE (PDT 004-006)	SHAM CONTROL	GEL PRODUCT FORMULATION (PDT 004-002)	COMMERCIAL GEL PRODUCT (PDT 004-003)	COMMERCIAL CREAM PRODUCT (PDT 004-031)	CREAM PRODUCT FORMULATION (PDT 004-046)
TOTAL MALFORMATIONS						
NUMBER WITH EXTERNAL MALFORMATIONS	2/ 1	1/ 1	9/ 5	14/ 6*	11/ 7*	9/ 4
NUMBER WITH SOFT TISSUE MALFORMATIONS	2/ 2	3/ 2	13/ 7*	15/ 7	10/ 6	9/ 4
NUMBER WITH SKELETAL MALFORMATIONS	2/ 2	3/ 3	4/ 4	3/ 2	23/ 9*	6/ 3
TOTAL NUMBER WITH MALFORMATIONS	5/ 4	6/ 5	15/ 9*	16/ 8	29/ 10*	16/ 6

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = $P<0.05$

Mutagenicity Studies

Ames (Bacteria) Mutagenicity Assay *(In Vitro)*

This assay was performed at PDT 002-002 was tested using the Salmonella/mammalian-microsome plate incorporation assay at concentrations of 667, 1000, 3333, 6667, and 10,000 μg/plate. Generally, this assay evaluates the mutagenicity potential of the test article for its ability to induce back mutations at selected loci in several strains of Salmonella.The tester strains used in the study consist of TA-98, TA-100, TA1535, TA1537, and TA1538. The assay was performed in triplicate and DMSO was used as the vehicle. A positive control was included to verify all tests.

All concentrations of PDT 002-002 were negative for mutagenicity in this assay.

Mouse Lymphoma Mutagenicity Assay *(In Vitro)*

This assay was performed at PDT 002-002 was tested using in the L5178Y TK+/- mouse lymphoma mutagenicity assay with and without S-9 (which adds some metabolizing capabilities). This assay tests for specific locus mutations in a mouse lymphoma cell line. Without S-9 activation, PDT 002-002 was tested at 0.05, 0.1, 0.5, 1.0 , and 5.0 μl/ml. With activation, tests were performed at 0.1, 0.8 1.4, 2.1, and 2.9 μl/ml. (In an initial toxicity test, PDT 002-002 was found to be toxic at 50 μl/ml for all cultures.) DMSO served as the solvent for all materials tested. Three counts per plate were made and the median value was reported. A positive control was included to verify all tests. A substance is considered a positive mutagen in this test if there is a positive dose response and the mutant frequency is double the background growth.

Assays performed at the described dose levels were negative for mutagenicity.

Mouse Micronucleus Bone Marrow Erythrocyte Assay *(In Vivo)*

This assay was performed at PDT 002-002 was tested in the micronucleus bone marrow erythrocyte assay for chromosome-breaks or mitotic spindle damage. The test is evaluated through erythroblasts undergoing final DNA replication and mitosis before the main nucleus is expelled. When chromosome breaks or spindle abnormalities occur during erythrocyte division, abnormal chromosomes and their fragments are left in the daughter cells. These are counted as micronuclei. The assay of PDT 002-002 first included a dose range-finding study dose levels of 313, 625, 1250, 2500, and 5000 mg/kg for two days (3/sex/group). This was followed by a sponsor-described "definitive" assay at 1250, 2500, and 5000 mg/kg/day (10/sex/group). Both the range-finding and the definitive test included control mice dosed with corn oil; the definitive test also included a positive control group dosed with benzene. Mice were evaluated by clinical observations, cytotoxicity, and micronucleus formation in bone marrow erythrocytes. A maximum tolerated dose level (MTD) should be reached in the mice, and the data should be

expressed as a ration of polychromatic erythrocytes (PCEs) to total erythrocytes (RBCs). A compound would be considered positive for genotoxicity if they have a larger number of micronuclei than noted in the negative controls and if the number increases with increasing dose level.

In the pilot study, male mice were noted to have fight wounds, scabs, and rough fur. The PCEs were comparable to controls at all dose levels. In the definitive assay, male mice were again noted to have fight wounds, scabs, and rough fir. One male mouse dosed with benzene was found dead on day 2, although its death was thought to be fight related. At both the 24 and 48 hour harvest no significant differences were noted between the negative control animals and the PDT 002-002 animals in evaluation of PCEs. The benzene-treated group had significantly increased PCEs, approximately 5 to 10 times greater than negative controls. PDT 002-002 may be considered negative for genotoxicity in mice based on the time and conditions of this test.

The following studies were previously reviewed by Dr. Syed Alam. The studies are summarized below.

***In Vitro* Percutaneous Absorption Studies in Human Cadaver Skin**

The table on the following page summarizes the findings of nine studies using the Franz assay system to test several formulations. In brief, the studies demonstrate that Acticin seems to be absorbed somewhat less than Retin-A®, although less than 5% of both compounds penetrated to the receptor fluid under all conditions of the assays.

Primary Eye Irritation in Rabbits

The sponsor performed nine primary eye irritation studies in rabbits. The studies are summarized on the table below. For all studies, gross observations were performed under white light and longwave UV light subsequent to fluoresceine staining. Eyes were scored by the method of Draize. In brief, none of the materials were corrosive and all signs of conjunctivitis cleared with 48 hours.

Primary Skin Irritation Potential in Rabbits

New Zealand White rabbits (6) were given 0.5 ml Acticin 0.25% gel (PDT 004-002) or Retin-A® Gel (PDT 004-003). Materials were applied to the clipped and abraded skin under occlusion for 24 hour. Observations for erythema and edema were performed at 24 and 72 hours after application. PDT 004-002 and Retin-A® produced a skin irritation score of 3.1 and 3.8, respectively, and therefore are not considered primary skin irritants.

Material Tested	Dose (mg/cm^2)	Duration (hours)	Method of Quantification	Results
PDT 002-001 45% w/w in 95% ETOH	225	24	UV Spec.	No compound was detectable in the receptor fluid; minimum sensitivity level was 50μg.
PDT 002-002 neat in ETOH	45-140	48	Radio-label with ^{14}C; scintillation count and gel permeation chromatography	Highest penetration was in the 25% petrolatum based ointment; epidermal levels reached approximately 7%, dermal levels were 1.2%. Lowest penetration used 100% PDT 002-002 with 1.1% in the epidermis and 0.2 % in the dermis. All receptor levels were < 1%.
Retin-A®, PDT 004-002, PDT 004-007 (no polyolprepolymer-2)	3.18	40	same as above	Retin-A® penetrated at 18.3%, 0.2% and 0.4% ; PDT 004-002 at 27.4%, 0.4%, and 0.4%; and PDT 004-007 (no polyolprepolymer-2) at 65%, 1.3%, and 0.3% into the SC/epidermis, dermis, and receptor fluid, respectively.
Retin-A® Cream, PDT-004,044, 045, and 046 (Acticin creams)	10/0.64	48	same as above	For all test materials, 99.1 $\pm$ 1.3% of the applied radioactivity was removed from the skin surface by ethanol wash. The absorption of the 0.025% formulation was comparable between Acticin and Retin-A® formulations. The absorption of the 0.05% Acticin was somewhat greater than the control formulation. But at 0.1%, absorption from Retin-A® was significantly greater than the Acticin formulation.
Tretinoin Ointment (0.1%, 0.3%, 0.5%) , 0.1% Retin-A® cream, and two "Frost & Weinstein" formulations	3.2/0.64	24	same as above, but used ^{3}H-tracer	The percent of applied dose for high-dose PDT 004-070 was 1.86 $\pm$ 0.54, 1.08 $\pm$ 0.68, and 0.79 $\pm$ 0.31 for the epidermis, dermis, and receptor fluid, respectively. No statistical differences were noted between 0.5% tretinoin test formulation and the 0.3% Frost and Weinstein preparation.

Material Tested	Dose (ml)	No. of Animals	Duration	Method	Results
PDT 004-002	0.1	9	1 exposure	6 eye unrinsed, 3 rinsed following 30 second exposure	The material was considered a severe irritant in both rinsed and non-rinsed eyes. No corrosion was noted.
Retin-A® 0.025% Gel	0.1	9	1 exposure	6 eye unrinsed, 3 rinsed following 30 second exposure	The material was considered a severe irritant in both rinsed and non-rinsed eyes. No corrosion was noted.
in petrolatum: PDT 002-001 (10%) and PDT 002-002 (10%, 20%)	0.1	6	1 exposure	6 eyes with and without rinsing	The materials produced no corneal opacity, iritis, or significant conjunctivitis. No corrosion was observed. The materials were "practically non-irritating."
in petrolatum: PDT 002-001 (10%) and PDT 002-002 (10 & 20%)	0.1	6	1 exposure	6 eyes without rinsing, evaluations to 3 days	The 10% formulations produced conjunctival irritation clearing in 24 hours; 25% formulation required 3 days to clear.
Acticin 0.1% Cream (PDT 004-046)	0.1	6	1 exposure	6 eyes rinsed after 30 seconds, 6 eyes unrinsed	All eyes cleared by 48 hours; no corneal involvement was noted, the compound was considered "practically non-irritating."
Retin-A® 0.1% Cream	0.1	6	1 exposure	6 eyes rinsed after 30 seconds, 6 eyes unrinsed	All eyes cleared by 48 hours; no corneal involvement was noted, the compound was considered "practically non-irritating."
0.2% retinoic acid solution	0.1	6	1 exposure	all eyes (6) rinsed 24 hours post exposure	Irritation cleared by 48 hours. No corrosion was noted. The material was considered a "mild irritant."
tretinoin ointment (0.1, 0.3, 0.5%; PDT 004-068, 069, and 070)	0.1	6	1 exposure	all eyes (6) rinsed 24 hours post exposure	All formulations were "virtually non-irritating" to the eye. There was no evidence of irritation to either the iris or cornea in any animal in any formation.

Repeat Skin Irritation Test in Guinea Pigs

This study was performed at the

NOTE: This laboratory was found to be out of regulatory compliance (profoundly) on 11/26/91 and thus this study is considered unacceptable. Penederm was informed by letter that the studies performed by this laboratory will not be considered in support of an application for research or marketing.

Acute Toxicity (LD_{50})

Acticin 0.025% Gel (PDT 004-002) and Retin-A® 0.025% Gel (PDT 004-003) was given to 10/group Sprague Dawley rats orally. The LD_{50} was greater than 5.0 g/kg, and is thus considered to be of very low toxicity.

An LD_{50} was performed in BALB/C mice using polyolprepolymer-2 (PDT 002-001) in 2% ethanol. It is not clear whether this study was performed at and is therefore not acceptable. It is included here for completeness. Deaths occurred within 15 minutes of injection; observations included quivering and convulsions followed by death. LD_{50} was estimated to be "approximately" 3.7 g/kg.

Polyolprepolymer-2 (PDT 002-002) was given to 10 Sprague Dawley rats by gavage. Two rats died on the day of dosing. At necropsy, no gross pathology was reported in any rat. The LD_{50} was estimated at 5.0 g/kg, and is thus considered to be of very low toxicity.

Hypersensitivity Test in Guinea Pigs

Ten Hartley guinea pigs were given a 10% concentration of polyolprepolymer-2 (PDT 002-002) in a GLP hypersensitivity study (Maguire, 1973, modified, J. Soc. Cosm. Chem., 24, 151-162. Prince and Prince, 1977, Cosmetic Toiletries, 92, 53-58). No allergic contact dermatitis was noted in the guinea pigs.

Comedogenicity Study in Rabbits

New Zealand White rabbits (6) had applied 0.2 ml of polyolprepolymer-2 (25% w/v) on the inner surface at the basal portion of the right ear once daily, five days per week, for three weeks. The test materials were found to be non-comedogenic; evaluation included histopathology of the ear skin.

28-Day Percutaneous Toxicity Study in Rabbits

New Zealand Albino rabbits [20/group (10/sex)] were given Polyolprepolymer-2 (100, 25, or 10%) on the clipped dorsal skin under occlusion once daily for a total of 28 doses. The test material was left on the skin for 6-8 hours. Smaller adrenal glands were noted in males dosed at 25%; no other significant group differences were reported. In the histopathological

exam, chronic inflammation was noted in the low-dose animals. All other findings were considered incidental and not related to treatment.

Summary and Conclusion

Penederm Inc., Foster City, CA has filed this NDA for approval of Acticin 0.025% Gel, a retinoid compound (tretinoin, all-*trans*-retinoic acid) to be applied dermally for the treatment of acne vulgaris. A second ingredient in the formulation is polyolprepolymer-2, which according to the sponsor adds qualities to their formulation that makes it superior to the already-marketed Retin-A® products. Polyolprepolymer-2 is the only difference in formulation between Retin-A® 0.025% Gel and Acticin 0.025% Gel. Polyolprepolymer-2 is a new entity that has never been through the IND/NDA safety review process.

The sponsor presented results from an assortment of formulations in the IND and NDA: studies of Acticin cream products, Retin-A® Creams and/or Gels, along with an array of other formulations. The only data summarized below, however, is what directly supports this NDA for Acticin 0.25% Gel, and includes solely (1) Acticin 0.025% Gel, (2) Retin-A® 0.025% Gel, (3) polyolprepolymer-2 (PDT 002-002), and (4) appropriate controls. All else is considered background.

For clarity the summary is divided into two sections: data from polyolprepolymer-2 studies, and data from the Acticin/Retin-A® studies.

Polyolprepolymer-2 Summary:

In the non-GLP *in vitro* **percutaneous absorption studies** in human cadaver skin (the Franz system), polyolprepolymer-2 (PDT 002-002) in ethanol had receptor rates up to 1.2% with 7% epidermal levels at the high concentration (25%). This appears to be a relatively low level of absorption, however, no standard positive control was tested in this assay. According to Dr. Thomas Franz (personal communication) percutaneous assays usually include the positive control tritiated water. But the sponsor did include Retin-A® 0.025% Gel as a positive control, which as expected exhibited low levels of absorption. Thus, the assay appears to at least be qualitatively acceptable, and supportive of the sponsor's claim that polyolprepolymer-2 has low absorption potential into human skin.

In **primary eye irritation studies** in rabbits, polyolprepolymer-2 (0.1ml, 25% concentration in petrolatum) applied without rinsing caused conjunctival irritation that cleared within 3 days; a similar experiment involving rinsed eyes was graded "practically nonirritating." In acute toxicity tests, polyolprepolymer-2 (PDT 002-002) had an LD_{50} greater than 5.0 g/kg, which indicates very low toxicity. Polyolprepolymer-2 also was tested for **hypersensitivity in guinea pigs, and in rabbits for codedogencity and in a 28-day percutaneous toxicity study.** Polyolprepolymer-2 was found to be negative for allergic dermatitis and

comedogenicity. In the toxicity study, no findings were clearly dose-related. These irritation, acute and subacute toxicity studies submitted by the sponsor support the claim that polyolprepolymer-2 is a slight irritant of low toxicity.

The longest study done for polyolprepolymer-2 alone was a **13-week dermal irritation and toxicity study** in New Zealand White rabbits. No compound-related changes were noted, except for changes in the skin. Occasional desquamation and slight erythema were noted in males and females in the low and high dose groups (1-3 males per day, 1-5 females per day). High dose animals were noted with slight erythema earlier and in more animals compared to low dose incidence. One high-dose male was noted with pustules/papules throughout most of the study. Based on these findings, polyolprepolymer-2 appears to be a slight dermal irritant. No other visible lesions were noted at necropsy. In hematology and clinical chemistry tests, some variances were noted between high-dose and control groups, but the changes were not clearly related to treatment. High-dose females had significantly greater ovary, ovary-to-body, and ovary-to-brain weight. The finding, however, appears to be unimportant because no ovarian lesions were noted in the gross necropsy and the histopathology. (Only the control and high-dose animals were examined histopathologically.) One high-dose female was noted to have chronic inflammation (papillary dermis) on abraded skin, and one high-dose female had chronic inflammation (papillary dermis), epidermal hyperplasia, and hyperkaretenosis. Several lesions were noted in the lung, liver, and bladder, but they did not appear in a dose-related manner, and thus do not appear to be treatment-related. All other findings were not remarkable. In conclusion, under the conditions of this study, polyolprepolymer-2 was not systemically toxic and caused slight skin irritation.

The sponsor performed a **dermal teratology pilot study** in New Zealand White female rabbits using polyolprepolymer-2 (1000, 2000 mg/kg/day). Fetal evaluations for malformations and variations was done by external evaluation only. Slight desquamation was noted at a slightly higher rate in the treated females when compared to controls. The high-dose group was noted to have a high incidence of unkempt appearance, possibly because the formulation was extremely sticky. Other findings were considered within the range of normal. No statistically significant differences were noted between groups for pregnancy parameters. The high-dose group had fewer total mean implantation sites and a greater pre-implantation loss than the control and low-dose groups. The low-dose group had a greater number of early resorptions and post-implantation loss. These findings may indicate that the compound is feto-toxic, especially because no maternal toxicity was noted. These data are from a pilot study with no follow-up study. As such, the findings are equivocal. All fetuses appeared normal; no differences were noted. Under the conditions of this pilot study, polyolprepolymer-2 is not teratogenic.

The sponsor submitted three mutagenicity assays. In the ***in vitro*** **Ames assay**, polyolprepolymer-2 was negative for mutagenicity. In an ***in vitro*** **mouse lymphoma assay**, polyolprepolymer-2 was tested using in L5178Y TK+/- mouse lymphoma cells (without S-9 activation at 0.05, 0.1, 0.5, 1.0 , and 5.0 μl/ml and with activation at 0.1, 0.8 1.4, 2.1, and

2.9 μl/ml). The assays were negative for mutagenicity. The assays, however, would have been more meaningful if the sponsor had tested one or two dose levels between 5.0 μl/ml and 50μl/ml. As the test was performed, the dose levels have simply portrayed the no-effect level and the toxic level; the value of the assay (as performed by the sponsor) to test the mutagenicity potential of PDT 002-002 is an unknown.

Polyolprepolymer-2 was evaluated in an ***in vivo*** **mouse micronucleus bone marrow erythrocyte assay** for chromosome-breaks or mitotic spindle damage (1250, 2500, and 5000 mg/kg/day, 10/sex/group). Both the range-finding and the definitive test included control mice dosed with corn oil; the definitive test also included a positive control group dosed with benzene. Mice were evaluated by clinical observations, cytotoxicity, and micronucleus formation in bone marrow erythrocytes. A maximum tolerated dose level (MTD) generally should be reached, and although the sponsor did not reach the MTD in dose levels for this test, the high dose level of 5,000 mg/kg/day is considerable; testing beyond that level often is beyond the volume capacity of the mouse. Thus, polyolprepolymer-2 may be considered negative for genotoxicity in mice based on the time and conditions of this test.

Acticin 0.25% Gel Summary:

The following studies were reviewed previously in the original IND and supplements. These studies are separately summarized because they included results from a different Acticin formulation (presented in Appendix A) from what has been submitted for NDA approval.

In the non-GLP ***in vitro*** **percutaneous absorption** studies in human cadaver skin (the Franz system) Retin-A® 0.025% Gel and Acticin 0.025% Gel penetrated less than 1% into the receptor fluid and 18 and 27%, respectively into the epidermis. In another formulation of Acticin Gel made up without polyolprepolymer-2, less than 1% reached the receptor fluid and 65% was found in the epidermis. As noted above for polyolprepolymer-2, this assay serves as qualitative evidence that Acticin 0.025% Gel has a relatively low absorption rate.

In **primary eye irritation studies** in rabbits, Acticin 0.025% Gel and Retin-A® 0.025% Gel were graded severe irritants; no corneal corrosion was noted (0.1 ml dose, rinsed and unrinsed eyes). In a **primary skin irritation study** in rabbits with Acticin 0.025% Gel and Retin-A® 0.025% Gel applied to clipped and abraded skin, both compounds were not considered primary irritants. In acute toxicity tests for LD_{50}, Acticin 0.025% Gel and Retin-A® 0.025% Gel both had LD_{50} greater than 5.0 g/kg, which indicates very low toxicity.

The following studies had not been previously reviewed.

These studies were done with the formulation of Acticin 0.025% Gel that is proposed for NDA approval. Except for the *in vitro* percutaneous absorption assay, all of the following studies appear to have been completed under GLP guidelines.

In a non-GLP *in vitro* **percutaneous absorption study** of Acticin 0.025% Gel and Retin -A® 0.025% Gel (10 mg. to each 0.74 cm^2 diffusion cell), Acticin Gel (0.025%) had less than 1% penetration into the epidermis, dermis, and receptor fluid. Penetration of Retin-A® Gel (in both formulations) was only slightly greater. As noted above, this assay serves as qualitative evidence that Acticin 0.025% Gel has a relatively low absorption rate.

For acute/subacute toxicity testing, the sponsor submitted an **oral acute toxicity** test in Sprague Dawley rats. Acticin 0.025% Gel (PDT 004-002) was found to have an LD_{50} of approximately 5.0 g/kg, i.e. a substance of low acute toxicity. In a **14-day dermal toxicity study** with Acticin 0.025% Gel (0.52 and 1.04 mg/kg/day), mice were evaluated for body weights, clinical observations, dermal evaluation, and gross necropsy observations. Acticin gel was found to be an irritant with no systemic toxic effects.

In a **7-day dermal irritation study** in Hartley guinea pigs, Acticin Gel and Retin-A® Gel (0.25%, 50 or 100 mg) was administered twice/day to the clipped backs of guinea pigs, and the test skin sites were observed clinically for dermal erythema or edema. Following euthanasia, skin samples were evaluated histopathologically. Beginning one day post-treatment, animals treated with Acticin (50 and 100 mg) were noted to have greater erythema and edema than the sham controls. Retin-A® generally caused more severe effects than Acticin, although for all compounds the severity ranged from very slight to moderate with severity increasing over time. Acticin tested as a moderate irritant that caused classic tretinoin-related histomorphologic changes in guinea pig skin. Under the conditions of this test, it appears that the irritation and histomorphologic changes are less severe following treatments of Acticin gel than Retin-A® products.

Rabbits exposed dermally to Acticin 0.025% Gel (0.05 ml) under occlusion for 24 hours in a **primary skin irritation study**. The primary irritation index, based on scores of edema and erythema, was 4.0, and thus Acticin 0.025% Gel was not found to be a primary irritant. No corrosion of the skin was noted.

In a **primary eye irritation study** in New Zealand White rabbits, Acticin 0.025% Gel (0.1 ml to one eye/animal), Acticin 0.025% Gel produced corneal opacity (maximum score 2) in rinsed and unrinsed eyes that persisted up to day 10 in unrinsed eyes and up to day 7 in rinsed eyes. In the conjunctiva, erythema, swelling, and discharge were noted up to day 7 in rinsed and unrinsed eyes, although the findings were generally longer lasting and more severe (maximum score 2) in unrinsed eyes. No corrosion was seen in rinsed or non-rinsed eyes. The material was considered an irritant in rabbit eyes.

The longest study performed for Acticin 0.025% Gel was a **91-day dermal irritation and toxicity study** in mice. Acticin (0.07, 0.25, and 0.70 mg/kg/day) was applied to the clipped back (10% of body surface area) for 6 hours every day for 13 weeks. One mid-dose male was found dead; all other mice survived to scheduled euthanasia. In male mice (all groups) and one mid-dose female, palpable masses in the urogenital area were noted. All other findings were in the range of normal for mice, except for findings in the skin (detailed

below). Body weights (total or as ratios) and/or food consumption occasionally were significantly below control for treated males and females. Compound -related skin observations included erythema, edema, atonia, desquamation, fissuring (males only), eschar areas, thickened skin, and skin whitening (males only). These findings increased in quantity and severity with increasing dose-level. The ophthalmological examination was negative for compound-related changes. In the hematology evaluation, no findings were considered treatment related. In all treated animals (low-, mid-, and high dose males and females), mean aspartate transferase (AST) values were significantly greater than control values. Glucose was significantly decreased in mid-dose males, and urea nitrogen was significantly increased in low and high-dose females in comparison to control values. (Mid-dose females were also increased, although the difference was not statistically significant.) Numerous changes were noted in organ weights, particularly in the kidney, heart and liver, although consideration should be given to the fact that in both males and females the changes decrease or even disappear in the high dose (except in male heart:brain weight). At necropsy, the only possibly treatment-related finding was increased lymph node weights in treated males and females. Only low- and high-dose tissues were evaluated from the brain, heart, kidneys, liver, lungs, skin, thymus, and gross lesions. The reviewing pathologist considered treatment-related findings in the skin to include acanthosis, hyperkeratosis, and chronic dermatitis. The changes did not increase with dose level. Outside of the skin area, the only other possibly treatment related effect was in the thymus where a diffuse necrosis of individual lymphocytes was noted. The pathologist, however, noted that the condition could be stress related. All other microscopic findings were consistent with normal background lesions for this age and strain of mice.

The dermal application of all levels of Acticin 0.025% Gel resulted in skin irritation with the expected accompanying histomorphological changes in the skin. A confounding factor throughout this study is the lack of restraint during the period the compound was on the mice skin. (The sponsor was not acting irresponsibly; it is a weakness in the study design despite the fact that this is a commonly performed study.) The amount of Acticin ingested by the animals is unknown. In support of this compound, and despite possible oral ingestion, few findings in any observation outside of the skin can be clearly labelled compound related. The changes in liver and kidney enzymes and body weights are of concern, although the observations are not supported by histopathological findings. (A lifetime chronic study would allow us to fully evaluate the effect of the drug to liver and kidney.) Thus under the conditions of this study, Acticin is considered not systemically toxic, although it is clearly a skin irritant.

Of greatest concern of all the preclinical studies are the findings in the **Segment II teratology study** in New Zealand White female rabbits. The sponsor tested just one dose level of Acticin Gel 0.025%, as well as one dose level of Retin-A® 0.025% Gel. (Acticin creams were also tested in this study; those results will be considered in support of the NDA for the creams, number 20-404.) This dose is approximately 25 times the dose expected to be applied to humans, assuming a 1 cc dose to a 70 kg individual. Test materials were applied dermally for 6 hours to collared rabbits beginning on gestation day 5. The Acticin-treated

group had four unscheduled deaths: three females that aborted and one moribund animal were euthanized. The Retin-A® group had one unscheduled death due to abortion. Pregnancy rates were 100% in the control group and, 94% in the Acticin and Retin-A® groups.

Several clinical observations were noted that indicated the animals were discomforted by the tretinoin-containing treatments, in particular vocalization and animals struggling during dosing or rinsing. Clinical observations at the exposure sites included slight to moderate erythema, slight desquamation, and multiple red areas in the gel control. Animals in the treated groups had a large number of observations indicating severe irritation at the treatment sites (slight to severe erythema, slight edema, atonia, desquamation, fissuring, eschar areas, thickened skin, bleeding, and red raised areas.) Additionally, despite washing, residual test material was often noted at or around the treated site. Body weights and food consumption were similar for all groups throughout gestation. Observations at necropsy were not considered out of the ordinary for this size and sex of animal undergoing dermal dosing.

Acticin-treated animals had an increase in the mean number of late resorptions and a statistically significant number of greater post-implantation loss. All other gravid females generally were comparable across groups for reproduction parameters.

Several findings in the treated fetuses appear to be drug related. Acticin and Retin-A® Gel-treated groups had a higher incidence of domed head; this difference was statistically greater than controls for the Retin-A® group only. Treated groups also had a higher incidence of cleft palate, flexed paw, and omphalocele, although these differences were not statistically significant. In the visceral examination, Acticin and Retin-A®-treated groups had a statistically significant greater incidence of hydrocephaly. Other visceral findings did not appear to be drug related. Total malformations consisted of a significantly greater number of soft tissue malformations for the Acticin group and a greater number of external tissue malformations for the Retin-A® group. For the sake of comparison, the following table presents the abnormal findings as percents and includes historical control data from MARTA (Middle Atlantic Reproduction and Teratology Association) published in September, 1993. The percentage of hydrocephaly and domed head in the Acticin and Retin-A® groups is almost 10 times higher than that seen in the control values in this NDA.

Comparison of Teratogenicity Findings (Fetus/Litter)
(percent of mean)[1]

	External Anomaly: Domed Head (%)	Visceral Anomaly: Hydrocephalus (%)
MARTA Control Values (Summary of all Studies)	Avg: 0.06/0.32	Avg: 0.05/0.24 Max: 3.03/16.67
NDA 20-400 Vehicle Control	0.9/5.6	0.9/5.6
Acticin 0.025% Gel	11.5/38.5	15.4/46.2
Retin-A® 0.025% Gel	13.3/38.0	14.3/44.1

In addition, this study is of an unusual design: the sponsor chose to test different compounds rather than several doses of Acticin gel. The study design prevents us from investigating an expected attribute of teratogens: increasing severity with increasing dose. A further confounding factor is that animals may have received a higher dose than intended through oral ingestion of dosing material left on the animals fur and through the tretinoin-irritated skin. Also of concern is feto-toxicity: the statistically significant increases in late resorptions and post-implantation loss in the Acticin-gel treated group combine with the changes seen in the polyolprepolymer-2 pilot teratogenicity study to suggest fetal viability effects. (Polyolprepolymer-2 treated animals in the high dose had fewer mean implantation sites and an increased number of pre-implantation loss; the low-dose had an increased number of early resorptions and post-implantation losses.)

As this study stands, Acticin 0.025% Gel must be considered definite teratogen and a possible feto-toxicant in rabbits.

Recommendations:

Approval, with the following caveats:

1. The sponsor must complete a one-species carcinogenicity test because of the addition of polyolprepolymer-2, a previously untested compound. This test will also better clarify the findings in increased liver and kidney enzymes and increased lymph node size found in the 91-day dermal study. (The sponsor has sent a letter dated 6/9/94 agreeing to a carcinogenicity study for the gel to begin within four months of final approval of the gel and

[1] Percent of mean = total occurrences of malformations in fetuses of litters / total number of fetuses or litters * 100

cream formulations.)

2. Acticin 0.025% gel must be given a pregnancy category C.

3. Product labelling has not been submitted and thus cannot be reviewed. Labelling must be submitted and reviewed prior to approval.

/S/

Hilary V. Sheevers, Ph.D.

ORIG

cc:
HFD-540
HFD-540/PHARM/HSHEEVERS
HFD-540/MO/SLIFMAN
HFD-540/CHEM/REJALI
HFD-540/PMS/CHAPMAN

Concurrence Only:
HFD-540/DD/JWilkin 8/22/94
HFD-540/SPHARM/SALAM 8/24/94

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Appendix A

PDT 004-002 - 0.025% Retinoic Acid Gel (from jacket Volume 1.1)
This formulation was utilized in the studies reviewed by Dr. Alam.

Component	Grams/100 g
All-trans-retinoic acid	
Ethanol	
PDT 002-002	
Hydroxypropyl cellulose	
Butylated hydroxytoluene	

PDT 004-006 - Gel Vehicle (Vol. 1.1)

Component	Grams/100 g
Ethanol	
PDT 002-002	
Hydroxypropyl cellulose	
Butylated hydroxytoluene	

Tretinoin Ointment Formulations

Component	PDT 004-068	PDT 004-069	PDT 004-070
Tretinoin, USP	0.12	0.36	0.60
Butylated hydroxytoluene, NF (BHT)			
Polyolprepolymer-2			

Formulations of Acticin Creams

Component	PDT 004-046	PDT 004-044	PDT 004-055	PDT 004-054
Tretinoin	0.12	0.030	0.24	0.00
Purified Water				
Stearic Acid NF				
Polyolprepolymer-2 (PDT 002-002)				
Isopropyl myristate NF				
Polyoxyethylene 40 stearate NF				
Propylene glycol USP				
Stearyl alcohol NF				
Xanthan gum, Food Grade				
Sorbic acid NF				
Butylated hydroxytoluene				

Review and Evaluation of Pharmacology and Toxicology Data
Division of Dermatologic and Dental Drug Products, HFD-540

Carcinogenicity Assessment Committee (CAC/CAC-EC) Report

Review of Study Design/Dose Selection Proposals

Application Number: NDA 20.400

Drug Name: Acticin (tretinoin) Gel 0.025%

Applicant: Penederm
Date Submitted: 12/26/95
Date Division Received: 2/6/96
Division Submitted to: Derm. & Dental Drug Products (HFD-540)
Review Completed: February 13, 1996
Primary Pharm Reviewer: Hilary V. Sheevers, PhD
Team Leader Pharmacologist: Abigail Jacobs, PhD
Date of CAC Review: 2/20/96
CAC Members: Joe DeGeorge, Charles Resnick, Conrad Chen

Summary of Dose-Finding Study
Species, Strain: mice
Number/sex/dose:

Group	No./Sex	Tretinoin Gel Conc. (w/w)	Volume (ml/kg)	Tretinoin dose (mg/kg)
control	20	0	3.33	0
low	20	0.0250	0.33	0.07
mid	20	0.0250	1.17	0.25
high	20	0.0250	3.33	0.70

Duration: 13 weeks
Route: Topical dermal
Major Findings: The skin exhibited the expected signs of retinoid exposure, including erythema, edema, eschar, desquamation, and fissuring. The findings increased in occurrence and severity with dose level. Histopathological changes in the skin included acanthosis, hyperkeratosis, and chronic dermatitis. The severity did not increase with dose. Indications of liver toxicity noted in clinical chemistry values and organ weight differences were not noted

at histopathology. Dose-related changes in the thymus, which may have been stress related, were also noted.

Summary of Proposed Carcinogenicity Study

Species, Strain/Source: Mice (6-8 weeks of age)
Number/sex/dose:

Group	No./Sex	Tretinoin Gel Conc. (w/w)	Volume (ml/kg)	Tretinoin dose (mg/kg)
control	50	0	0.9	0
low	50	0.0005	0.9	0.004
mid	50	0.0075	0.9	0.056
high	50	0.0250	0.9	0.187

Duration: 5 days/week for 104 weeks
Route: Topical dermal
Methods: Treatments will be applied daily to the dorsal skin, which will be clipped of hair weekly. Tretinoin and major metabolite blood levels will be evaluated on day 14 and week 52 (prior to dosing, and at 0.5, 1, 2, 6, and 12 hours after dosing).

Basis of Dose Selection:
No toxicokinetic data were collected for this topical compound. The dose selection was based on the irritancy potential of the drug. Tretinoin is a known irritant, and previous studies utilizing tretinoin or other retinoids have sometimes been ended early due to high mortality related to skin irritation.

The clinical dose of Acticin gel is 240 mg/day, or 0.0012 mg/ kg. The high dose (0.025%) in the proposed mouse study (0.187 mg/kg) is approximately 150 times the human dose on a mg/kg basis. The mid-dose (0.0075%) is 1/3 of the high dose, and the sponsor based this on the expectation that the dose would not cause severe irritation. The low-dose (0.0005%) was chosen based on the sponsor's belief that this was equivalent to the clinical dose.

Suggested Conclusion:

Dose levels: The sponsor is fundamentally mistaken that the dose levels they have chosen should be compared to the clinical formulation based on a mg/kg (or surface area) basis. The dose levels for this dermal carcinogenicity study (in which we are concerned about changes in

the skin) should be based primarily on a concentration basis, and secondarily on a volume basis.

The high-dose level is thus approximately equivalent to (and not 150 times higher than) the clinical dose of 0.025%. We do agree, however, that the dose is at the maximum feasible level, given the clinical signs of increasing inflammation in the 90-day study.

We recommend that the mid-dose group be 1/3 the high-dose group (0.008%), and that the low dose group be 1/9 the high dose (0.003%).

Other Recommendations:

1. The sponsor should consider adding 2 additional control groups: an untreated vehicle control and a vehicle control group that does not include the previously untested excipient, polyoprepolymer-2. An untreated control group will enable the sponsor to clearly establish the appropriate background skin tumor level. The excipient-free control group may help the sponsor avoid additional studies if the vehicle control gel proves to be as tumorigenic as the three tretinoin-treated groups.
2. All formulations should be prepared in the clinically used vehicle (except for the above mentioned excipient-free control group).
3. Physical exams should be performed prior to study initiation
4. The sponsor should clearly state the minimal survival rate needed before the study is terminated early.
5. Since blood samples are already being collected by the sponsor, we suggest that they include clinical pathology parameters of ALT, AST, glucose, and BUN (recommended collection days: 13 weeks, 52 weeks, and termination). These suggestions are based on changes seen in the 90-day study; and are made with the assumption that the additional tests can be run on the blood already being sampled.
6. Also because of the 90-day study findings, the sponsor should examine lungs, liver, kidneys, heart, thymus, and skin (treated and untreated) **from all** groups, not just low and mid-dose animals. We also suggest that all harvested organ samples from the control and high-dose be examined histopathologically.

Review of 90-Day Dermal Irritation and Toxicity Study

Laboratory:

Animal Strain: Crl:CD-1®(ICR) BR Mice

No. of Animals: 20/sex/group

Test Materials: Acticin 0.025% Gel (PDT 004-002)

Route: Dermal

Duration: At least 91 days

Study Design & Dose Levels:

Group	Formulation	Dose of Tretinoin (mg/kg/day)	Dose Conc. (% of Tretinoin)	Dose Volume (ml/kg)
1 (control)	Gel Vehicle (PDT 004-006)	0	0	3.33
2 (low dose)	Acticin 0.025% Gel (PDT 004-002)	0.07	0.025	0.33
3 (mid dose)	Acticin 0.025% Gel (PDT 004-002)	0.25	0.025	1.17
4 (high dose)	Acticin 0.025% Gel (PDT 004-002)	0.70	0.025	3.33

Methods: The test material was applied to the back (clipped of fur) on each animal every day for 13 weeks. The exposure area constituted at least 10% of the animal body surface area. After approximately 6 hours of exposure all remaining test material was wiped off.

Results:

Mortality: One mid-dose male was found dead; all other mice survived to scheduled euthanasia.

Clinical Observations: In male mice (all groups) and one mid-dose female, palpable masses in the urogenital area were noted. All other findings were in the range of normal for mice, except for findings in the skin (detailed below).

Dermal Irritation Results: Compound-related findings were noted in males and the females. Observations included erythema, edema, atonia, desquamation, fissuring (males only), eschar areas, thickened skin, and skin whitening (males only). These findings increased in quantity and severity with increasing dose-level.

Body Weights & Food Consumption: Low-dose male body weights were significantly below control on days 71 and 91 and generally lagged below control values following 1 week of treatment. High-dose male body weight gains were significantly less than control in week 8. All differences, however, were minimal (less than 10%). All other body weights and all body weight gains in male mice were similar. Female body weights were similar for all

groups. Female body weight gains were significantly below control for the low-dose during week 3; for the mid-dose during weeks 2 and 6, and for the high dose in weeks 3 and 6. Food consumption was significantly below control values for mid-dose males in weeks 7 and 10, and in high-dose males for weeks 7, 10, 11, 12, and 13. High dose female values were significantly below control in weeks 2, 4, 6, 9, 10, 11, 12, and 13.

Ophthalmic Observations: Ophthalmological examination revealed corneal crystals in the week 13 examination. The reviewing ophthalmologist did not consider the changes to be related to compound treatment, and the findings did not appear in a dose-related manner.

Hematology (16 parameters): In low-dose males, leukocytes and segmented neutrophils were significantly decreased when compared to control values. In low-dose females, reticulocytes were significantly decreased compared to control values. All other values were comparable; no findings were considered treatment related.

Clinical Chemistry (6 parameters): In all treated animals (low-, mid-, and high dose males and females), mean aspartate transferase (AST) values were significantly greater than control values. Glucose was significantly decreased in mid-dose males, and urea nitrogen was significantly increased in low and high-dose females in comparison to control values. (Mid-dose females were also increased, although the difference was not statistically significant.)

Organ, Organ-to-Body, and Organ-to-Brain Weights: Differences between control mean values and mean values from the Acticin-treated animals are summarized below.

Organ	MALES			FEMALES		
	Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
Kidney Wt.	↓ *	↓ *	↓			
Kidney:Brain	↓ *	↓ *	↓			
Heart Wt.	↓ *	↓ (slt)	↓ (slt)			
Heart:Body	↓ *	↓	↓	↓		
Heart:Brain	↓ *	↓ *	↓ *			
Liver Wt.	↓ *	↓	↓ (slt)	↓	↓ *	
Liver:Body	↓ *	↓	↑	↓ *	↓ *	↓ (slt)
Liver:Brain	↓ *	↓ *	↓			
Brain Wt.						
Brain:Body	↑	↑	↑ *			

↓ or ↑ indicate a decrease or increase, respectively, from mean control values; * indicates

change was statistically significant; slt is the abbreviation for a slight change; dose levels with no entries were virtually equal to control values

Although numerous changes are noted, particularly in the kidney, heart and liver, consideration should be given to the fact that in both males and females the changes decrease or even disappear in the high dose (except in male heart:brain weight).

Gross Necropsy Observations: At necropsy, three high-dose males had enlarged mandibular lymph nodes. Male mice also had enlarged inguinal lymph nodes in low- (1 observation), mid- (2), and high-dose (10) animals; and enlarged axillary lymph nodes in mid- (3) and high-dose (10) mice. Female mice had enlarged inguinal lymph nodes in the low (10) and high (3) doses; and enlarged axillary lymph nodes in the mid- (1) and high- (3) dose groups. Other findings did not appear to be treatment related.

Histopathology: Only low- and high-dose tissues were evaluated from the brain, heart, kidneys, liver, lungs, skin, thymus, and gross lesions. Treatment-related findings in the skin included acanthosis, hyperkeratosis, and chronic dermatitis. The changes did not increase between the mid- and high-dose levels. Outside of the skin area, the only other possibly treatment related effect was in the thymus where a diffuse necrosis of individual lymphocytes was noted. The pathologist, however, noted that the condition could be stress related. All other microscopic findings were consistent with normal background lesions for this age and strain of mice.

Regulatory Conclusion:

Summary of Recommendations to CAC

The sponsor should be informed of the following comments. (The reviewing pharmacologist would like to be present at the telecon. Following the telecon, the recommendations should be faxed to the sponsor.)

The Division and the Exec. CAC committee recommend the following doses: 0.025%, 0.0083%, and 0.003% for both male and female mice.

The basis for this recommendation is that the highest dose is the maximum tolerated dose based on irritation and histopathology noted in the 90-day study; lower two doses (1/3 and 1/9) are calculated conservatively (i.e. to ensure survival) so that there is a sufficiently high survival rate at 104 weeks.

1. The sponsor should consider adding 2 additional control groups: an untreated vehicle control and a vehicle control group that does not include the previously untested excipient, polyoprepolymer-2. An untreated control group will enable the sponsor to clearly establish the appropriate background skin tumor level. The excipient-free control group may help the

sponsor avoid additional studies if the vehicle control gel proves to be as tumorigenic as the three tretinoin-treated groups.
2. All formulations should be prepared in the clinically used vehicle (except for the above mentioned excipient-free control group).
3. Physical exams should be performed prior to study initiation
4. The sponsor should clearly state the minimal survival rate needed before the study is terminated early.
5. Since blood samples are already being collected by the sponsor, we suggest that they include clinical pathology parameters of ALT, AST, glucose, and BUN (recommended collection days: 13 weeks, 52 weeks, and termination). These suggestions are based on changes seen in the 90-day study; and are made with the assumption that the additional tests can be run on the blood already being sampled.
6. Also because of the 90-day study findings, the sponsor should examine lungs, liver, kidneys, heart, thymus, and skin (treated and untreated) **from all** groups, not just low and mid-dose animals. We also suggest that all harvested organ samples from the control and high-dose be examined histopathologically.

/S/ 2/21/96

Hilary V. Sheevers, Ph.D.

cc:
HFD-340
HFD-540
HFD-540/PHARM/Sheevers
HFD-540/MO/Toombs
HFD-540/CHEM/DeCamp
HFD-540/PM/Cook

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Concurrence Only:
HFD-540/DD/Wilkin 3/3/96
HFD-540/TLTOX/Jacobs 2/21/96

Review and Evaluation of Pharmacology and Toxicology Data
Division of Dermatologic and Dental Drug Products (HFD-540)

NDA: 20-400.AZ (Amendment, dated 7/12/96)

Drug Name: Avita^{R} (Tretinoin Gel 0.025% Topical)

Category: Retinoid

Indication: Treatment of acne vulgaris

Sponsor: Penederm, Inc., Foster City, CA

Number of Vols.: 10

Date CDER Received: 7/15/96

Date Assigned: 7/17/96

Date Review Started: 7/30/96

Date 1st Draft Completed: 7/30/1996

Date Review Accepted by Supervisor:

Related Submissions: IND NDA 20-404

Review Objective: To review adequacy of the Sponsor's response to preclinical deficiencies enumerated in the FDA letter of 6/26/96.

Preclinical Studies: None submitted in this amendment.

Comments:

In the FDA nonapprovable letter of June 26, 1996, 8 recommendations from the CDER Cancer Assessment Committee (CAC) on the Sponsor's proposed protocol for a phase 4 dermal 2-year carcinogenicity study were conveyed. The Sponsor has accepted all the recommendations and has agreed to change the protocol accordingly. Some comments have been made about some of the

recommendations which are presented below.

CAC comment # 1. Please submit data supporting the claims that the dose levels chosen should be compared to the clinical formulation based on a mg/kg (or surface area) basis. The dose levels for the dermal carcinogenicity study (which is primarily concerned about changes in the skin) should be based primarily on a concentration basis, and secondarily on a volume basis.

Sponsor's response: The Sponsor agreed to base the dose levels on concentrations of tretinoin. Further comments are quoted: "There are multiple ways of addressing the degree of exaggeration of the conditions defined in this proposed protocol:

- The Sponsor agrees that there is no exaggeration in the proposed high concentration to be employed in the carcinogenicity study relative to the clinical concentration of 0.025% tretinoin. The concentrations are equivalent.
- The Sponsor believes, however, that the proposed test conditions will provide a significant exaggeration of the dose of tretinoin per cm^2, to provide a cutaneous exaggeration.
- The Sponsor also believes that the proposed test conditions will provide a significant exaggeration of the dose per kg body weight, to provide a systemic exaggeration."

The proposed formulation volume to be administered is 0.9 ml/kg (or 0.765 mg formulation/kg, based on a formulation density of 0.84). For a 40 gm mouse the dose will be about 30 mg applied to an area of 2 cm^2 or 15 mg/cm^2. The proposed clinical dose is 0.5 to 1.0 mg/cm^2.

The sponsor has pointed out a typographical error in the FDA letter. The low dose should be

⅜ instead of %.

The exaggeration over the clinical dose is shown in Table 1.

Table 1. Summary of Exaggeration in Dose Groups for Proposed Carcinogenicity Study

Treatment Group	Proposed Tretinoin Concentration (% w/w)	Exaggeration Multiple vs. Clinical Dose		
		Tretinoin Conc (vs. clinical dose of 0.025% w/w)	Application Density of Tretinoin (vs. clinical dose of 0.00025 mg/cm^2)	Tretinoin Dose (vs. clinical dose of 0.0012 mg tretinoin/kg)
High	0.0250	1.00	15	156
Mid	0.008	0.32	4.8	50
Low	0.003	0.12	1.8	19

The low dose to be used now would be 1/9 of the high dose or 0.003% tretinoin formulation.

Evaluation:

There are no further outstanding preclinical issues for the approval of this application.

The proposed dermal carcinogenicity protocol is acceptable since it now complies with all the CAC recommendations.

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HFD-540/DD/Concur/Wilkin 8/2/96

HFD-540/TL/Concur/Jacobs 7/31/96

cc:
NDA 20-400

HFD-340/

HFD-540/

HFD-540/Pharm/Alam

HFD-540/SPharm/Jacobs

HFD-540/MO/Labib

HFD-540/Chem/Rejali

HFD-540/CSO/Blay