CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-112

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: fluocinolone acetonide, hydroquinone, tretinoin, depigmentation
Reviewer Name: Amy Nostrandt
Division Name: Division of Dermatologic and Dental Drug Products
HFD# 540
Review Completion Date: 9/27/99

OCT 31 1999

Review number: 1
IND/NDA number: NDA 21-112
Serial number/date/type of submission: original NDA, received 3/22/99
Information to sponsor: Yes (X) No ( )
Sponsor (or agent): Hill Dermaceuticals, Inc.
Manufacturers for drug substance:

fluocinolone acetonide - [redacted]
hydroquinone - [redacted]
tretinoin - [redacted]

Drug:

Code Name: [redacted] (fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%)

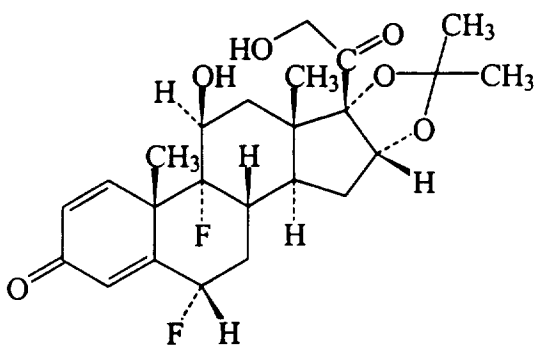
Generic/Chemical Names: fluocinolone acetonide; Pregna-1,-4-diene-3,20-dione, 6,9-difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-; 6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone
hydroquinone; 1,4-benzenediol
tretinoin; retinoic acid; all-*trans*-retinoic acid; (*all-E*)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid

Trade Name: [redacted] fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%)

CAS Registry Numbers:

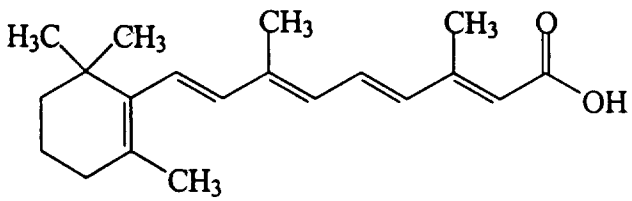
fluocinolone acetonide	67-73-2
hydroquinone	123-31-9
tretinoin	302-79-4

Molecular Formula/ Molecular Weight/Structure:
fluocinolone acetonide, $C_{24}H_{30}F_2O_6$, MW=452.50



hydroquinone, $C_6H_6O_2$, MW=110.11

tretinoin, $C_{20}H_{28}O_2$, MW=300.44



Relevant INDs/NDAs/DMFs:

Drug Class: depigmenting agent

Indication: cutaneous melanosis in skin types II and III

Clinical formulation:

Ingredient	mg per gram	percent (w/w)
Tretinoin, USP	0.5	0.05
Fluocinolone Acetonide, USP	0.1	0.01
Hydroquinone, USP	40	4.00
magnesium aluminum silicate, NF		
butylated hydroxytoluene, NF		
cetyl alcohol, NF		
stearic acid, NF		
stearyl alcohol, NF		
methylparaben, NF		
propylparaben, NF		
methyl gluceth-10		
glycerin, USP		
citric acid, USP		
sodium metabisulfite, NF		
purified water, USP		

Route of administration: topical to the skin

Proposed clinical protocol or Use:

The draft labeling directs the patient

Previous clinical experience:

Subjects in clinical trials applied the material once daily before bedtime for 8 weeks, followed by open label continuation of dosing until complete clearing, followed by 12 weeks of tapered maintenance dosing (to once daily three times per week). Adverse events included burning, stinging, rosacea, telangiectasia, and perioral dermatitis. One patient treated with tretinoin and hydroquinone without the steroid developed atrophy. Other adverse events reported included itching, acne, dryness of the skin and papules, cheilitis, and swelling of the face. One of the investigators, Dr. Willis (who also has some financial interest in the product), did not report adverse events that he considered to be expected, including irritation, erythema, and peeling. During the maintenance period adverse events reported included erythema, peeling, telangiectasias, and repigmentation. The Applicant states that most patients dropped out of the study during this time because of repigmentation that was seen as early as two weeks into the maintenance period.

Disclaimer : Note that some information may come directly from the sponsor's submitted material.

Introduction and drug history:

The proposed drug product contains three active ingredients, fluocinolone acetonide, hydroquinone and tretinoin. Each is marketed individually in a topical formulation. Only hydroquinone is indicated for the treatment of hyperpigmented lesions. The Applicant proposes their combination based on a 1975 paper by Kligman and Willis in which a combination of 0.1% tretinoin, 5.9% hydroquinone, and 0.1% dexamethasone was more effective in depigmentation of skin of African-American subjects "with naturally dark skin" than any of the individual components alone. The authors hypothesized an additive or synergistic effect of the active ingredients.

The following information is from labels of marketed products containing one of the proposed drug substances:

Fluocinolone acetonide is a fluorinated corticosteroid, and is currently available in topical formulations at concentrations up to 0.5 mg/g (0.05%) to be applied as a thin film 2-4 times per day. The compound is absorbed when applied topically, with increased absorption when under an occlusive dressing, and may be highly plasma protein bound. The drug primarily is metabolized in the liver and excreted via the kidneys. Adverse events in the skin reported with this drug substance alone include stinging or burning, itching, irritation, dryness, folliculitis, hypertrichosis, acne, hypopigmentation, perioral dermatitis, allergic contact dermatitis, maceration of the skin, secondary infection, skin atrophy, striae, and miliaria. Systemic effects may occur if the product is under an occlusive dressing, including suppression of the hypothalamic-pituitary-adrenal axis. Prolonged use may result in manifestations of Cushing's syndrome, hyperglycemia, glucosuria, increased susceptibility to infection, and growth retardation. No mutagenicity, carcinogenicity, fertility or teratogenicity studies, or evaluation of

excretion of the drug in milk have been reported for the marketed topical products. However, steroids may have adverse effects on reproduction and development.

Tretinoin (all trans retinoic acid) is currently available in formulations containing up to 0.1% active ingredient, to be applied once per day, in the evening. The drug undergoes hepatic metabolism and biliary and urinary excretion. Adverse effects include irritation, desquamation, stinging, dryness, and thinning of the stratum corneum. (Reviewer's note: It is unclear whether or not the thinning of skin resulting from the prolonged use of tretinoin or corticosteroids could result in increased systemic absorption of those or other compounds). Dermal carcinogenicity studies of tretinoin have been considered negative, but properly performed photo co-carcinogenicity studies indicate that tretinoin may enhance sunlight-induced carcinogenesis. Tretinoin is a known teratogen at when administered orally, and studies in animals appear to indicate that developmental effects after topical exposure may be possible. It is unknown if the drug is excreted in milk.

Hydroquinone is a depigmenting agent, available by prescription as a 4% preparation to be applied twice daily. This drug substance inhibits tyrosinase (blocks oxidation of tyrosine to dopa) and suppresses other melanocyte metabolic processes. Depigmentation resulting from this product is reversible. Adverse side effects include localized contact dermatitis. No data is provided in the prescription label on carcinogenic or mutagenic potential, reproductive toxicity, secretion in milk, ADME, or pediatric use.

Reviewer's comment: It has been reported that prolonged use may result in paradoxical hyperpigmentation or exogenous ochronosis. Studies of genotoxicity, carcinogenicity, and reproductive/developmental toxicity have since been performed. NTP data indicates that hydroquinone was positive for genotoxicity in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation and that hydroquinone induced sister chromatid exchanges in CHO cells with and without metabolic activation and chromosomal aberrations in the presence of activation. The conclusions of NTP carcinogenicity studies of hydroquinone administered by gavage were that there was some evidence of carcinogenic activity in F344/N rats (marked increases in tubular cell adenomas of the kidney in males and mononuclear cell leukemia in females) and female B6C3F$_1$ mice (increases in hepatocellular neoplasms, mostly adenomas). There was no evidence of carcinogenic activity in male B6C3F$_1$ mice. Associated effects included thyroid follicular cell hyperplasia in male and female mice, and anisokaryosis, multinucleated hepatocytes and basophilic foci of the liver in male mice.

Studies reviewed within this submission:

No nonclinical studies were performed or submitted. The sponsor has provided studies from the literature using combinations of either the active ingredients or drugs of the same classes. None of the submitted journal articles describe the use of these three active ingredients in combination in either humans or animals. The articles are briefly summarized below.

Studies <u>not</u> reviewed within this submission: none

PHARMACOLOGY:

No nonclinical pharmacology studies were performed. The Applicant provided a two-page summary of nonclinical pharmacology and toxicology of the individual drug substances. That information is summarized in the toxicology section of this review and below:

The Applicant states that animal tests are available to demonstrate differences between systemic and topical potency through anti-inflammatory responses and to determine the relative

potency of corticosteroids. They also state that antimetabolic effects have been demonstrated, e.g. the thinning effect of topical corticosteroids on the epidermis of mouse tail.

The Applicant states that the depigmenting activity of hydroquinone has been assessed in hairless mice, and the possible relationship of that compound to melanin has been investigated.

They state that the exact mechanism of action of tretinoin on depigmentation is vaguely understood. They propose three theories on the possible mechanism of action:

1. inhibition of tyrosinase (necessary for formation of melanin)
2. desquamation induced by keratolytic action.
3. interference with melanosome transfer in keratinocytes.

The Applicant also provided a list of 20 journal articles describing use or effects of one or more of the active ingredients or drug(s) of the same class. The reference list does not match the articles actually provided. Of the articles provided, those related to nonclinical evaluation of pharmacology of the drug substances are summarized below.

1. Menter JM and Willis I. Interaction of several mono- and dihydroxybenzene derivaties of various depigmenting potencies with L-3,4-dihydroxyphenylalanine-melanin. Archives of Biochemistry and Biophysics 244:846-56, 1986.

In a model system, the authors demonstrated that a number of depigmenting compounds, including hydroquinone may directly interact chemically with melanin. The kinetics of reduction of the model's electron acceptor, ferricyanide, were consistent with "a mechanism involving noninteractive binding of both depigmenting agent and ferricyanide to melanin prior to coupled electron transfer through the melanin backbone. Using the collected data, the authors derived an enhancement factor (EF) that appeared to be directly related to depigmenting potency.

2. Nair X and Tramposch KM. UVB-induced pigmentation in hairless mice as an in vivo assay for topical skin-depigmenting activity. Skin Pharmacol. 2:187-97, 1989.

The authors developed a model of hyperpigmentation in Skh:HR2 mice. The mice were exposed to progressively increasing doses of UVB radiation with selection after the first week of exposure for animals responding by increasing pigmentation. This approach minimized skin irritation, which was evident as erythema. The model was used to evaluate compounds with known depigmenting activity, including hydroquinone (HQ), 4-hydroxyanisole (4-HA), and tretinoin (ATRA). Topical application of 5% 4-HA or 0.1% ATRA inhibited the increase in pigmentation over 5 weeks of treatment. 5% HQ also resulted in grossly observable reduction in pigmentation, but was not significantly different from vehicle control. Tretinoin produced slight to moderate irritation at the treatment site and at remote sites. Those areas also developed hyperpigmented patches that were darker than normal skin. Slight erythema was noted with HQ and 4-HA. Histologically, there was a decrease in melanocyte density and pigment in skin treated with 4-HA. In skin treated with HQ or ATRA, pigment and melanocyte distribution was similar to the vehicle control group. In ATRA-treated skin, there were areas with little or no melanocytes or pigment and other areas with a high density of melanocytes and pigment; high density regions also had pigment in the dermis. It was postulated that part of the mechanism of action of ATRA was by exfoliation, which would account for a lack of histological evidence of decreased melanocyte or pigment density.

3. Kligman L. Effects of all-trans-retinoic acid on the dermis of hairless mice. J. Am. Acad. Dermatol. 15:779-85, 1986.

Skh:HR1 hairless mice were irradiated three times per week with FS20 sunlamps (280-370 nm, peak emission at 313 nm, 3.5 J/cm^2) for 10 weeks in order to induce damage to the dermis. After this phase, animals were treated with topical 0.05% tretinoin or vehicle for 5 or 10 weeks. Hairless mouse skin after 10 weeks of irradiation showed mild elastic fiber hyperplasia with modest thickening and twisting of the fibers. Small scattered foci in the collagen matrix lacked affinity for van Gieson's stain, indicating mild damage to mature collagen. After 10 weeks of treatment with 0.05% tretinoin, a wide region of new subepidermal collagen was evident that was significantly wider than the corresponding repair zone in vehicle-treated or untreated animals. Staining with van Gieson's stain revealed dense parallel arrays of new mature collagen bundles. New elastic fibers were noted within the repair zone, and the number and size of fibroblasts were increased relative to controls. Results were similar after five weeks, but not as pronounced. Similar treatment with nonspecific irritants had no effect on the repair zone. In a second experiment, the effects of different concentrations of tretinoin were examined. A dose-response was seen in the width of the repair zone. The author hypothesizes that the enhanced repair of UV-damaged dermis by tretinoin is the result of increased synthesis of new collagen by hyperactive fibroblasts. Additionally, findings of other work are discussed that were considered to contribute to tretinoin's effect, including stimulation of collagen synthesis, inhibition of collagenase activity, and dermal angiogenesis. The author also reported that application of tretinoin to nonirradiated skin for 20 weeks resulted in no delineated repair zone and only a slight increase in dermal cellularity and collagen density.

4. Yaar M et al. Retinoic acid delays the terminal differentiation of keratinocytes in suspension culture. J. Invest. Dermatol. 76:363-6, 1981.

In cultured guinea pig keratinocytes, retinoic acid inhibited cell differentiation as determined by a decrease in the percent of cells that developed disulfide cross-linked keratin and cornified envelopes. The effect was maximal at 5 μg/ml of retinoic acid on day 3 of a 5-day culture. Concentrations as low as 0.005 μg/ml were also inhibitory. Fifty μg/ml of retinoic acid accelerated cross-linking of keratin, but this was associated with cytotoxicity. Cell proliferation was not affected at these concentrations. A higher percentage of cells treated with retinoic acid were able to exclude trypan blue dye. The authors hypothesized that retinoic acid acts by stabilizing the cell membrane, thereby delaying transition from a living epidermal cell to a dead cornified cell. To support this, they demonstrated that the inhibitory effect of retinoic acid on cornified envelope formation was rapidly reversed by agents that permeabilized the membrane (ionophore X537A or Triton-X 100). These findings were consistent with the in vivo effects of increased number of nucleated cells and thickened granular cell layer after treatment with vitamin A or vitamin A acid previously reported in the literature.

5. Viluksela M. Characteristics and modulation of dithranol (anthralin)-induced skin irritation in the mouse ear model. Arch. Dermatol. Res. 283:262-8, 1991.

The effects of several pharmacological agents on dithranol-induced skin irritation were assessed. A single topical application of dithranol resulted in dose-dependent irritation with peak responses on days 1-2 and on days 6-10 after application. Topical corticosteroids were effective and persistent in inhibition of irritation, but their effect was slightly diminished during the second peak irritation. Retinoic acid did not inhibit the ear swelling.

SAFETY PHARMACOLOGY:

No studies were performed.

PHARMACOKINETICS/TOXICOKINETICS:

No studies were performed in animals. An in vitro absorption study was performed using human skin evaluating 24 hours of exposure. Hydroquinone achieved steady-state flux across the skin into the receptor fluid within 8 hours. Steady-state flux ranged from 1.5-5 $\mu g/cm^2/hr$ in the eighteen skin samples (from three donors). After 24 hours, tretinoin and fluocinolone were detected in the receptor fluid of skin samples from one of three skin donors. The LOQ's are not stated. Fluocinolone was detected in the epidermal and dermal layers after 24 hours, reaching levels of 20-115 ng/cm^2. The Applicant stated that this represents 2-8% absorption into the skin, but was an underestimate, since steady-state flux was not reached. The Applicant states that tretinoin is minimally absorbed at best and that 80% of the applied drug remained on the skin surface. However, that leaves a possible 20% that may be absorbed to some degree, and the Applicant's proposed mechanism of action is based on actions on viable and metabolically active cells, indicating absorption at least that far into the skin. Repeated dosing would be more likely to result in systemic exposure.

The following reference was also provided:

1. Wester RC et al. Human in vivo and in vitro hydroquinone topical bioavailability, metabolism, and disposition. J. Toxicol. Env. Health, Part A 54:301-317, 1998.

A series of experiments were performed in human subjects to evaluate systemic ADME of hydroquinone. In the first, the in vivo bioavailability for a 24-hour application in six volunteers treated on a 25 cm^2 site on the forehead was determined. Urine samples were collected for 4 days. The bioavailability of hydroquinone was approximately 45% of a 0.125 g (2.5 mg hydroquinone) dose of a 2% cream containing ^{14}C-hydroquinone, based on urinary recovery of radioactivity. Most of the radioactivity was excreted within the first 24 hours. Another 5% of administered radioactivity was detected in the wash from the treated site at 24 hours. The rest was presumed to have been rubbed off during sleep. Extraction of urine from volunteers treated in vivo showed nearly all radioactivity to be only water soluble, free hydroquinone following glucuronidase treatment, with a minor amount of benzoquinone present.

In the second experiment, four volunteers were treated on five sites of one cm^2 each on the ventral forearm. Each site was treated with 0.1 mg hydroquinone in 0.005 g cream. At 0, 1, 3, 6, and 24 hours, a site was washed with soap and water and tape stripped. From these timed skin wash and tape stripping, it was determined that there was rapid and continuous movement of hydroquinone into the stratum corneum.

In the third experiment, four volunteers were treated with 2.5 mg hydroquinone in 0.125 g cream on a 25 cm^2 area on the left ventral forearm. Blood samples were taken from the ipsi- and contralateral arms. Plasma levels were detected at the first sampling at 0.5 hours, and the hydroquinone concentration in the sample drawn from the ipsilateral arm was slightly greater than that from the contralateral arm. Subsequent paired samples contained nearly identical concentrations of the drug. Peak drug concentrations were seen at 4 hours. The application site was washed at 8 hours, and urine was collected at 24, 48, 72, and 96 hours after dosing. In the first 8 hours, 8% of the dose was excreted in the urine. In the urine, approximately 3-6% of the dose was found as free hydroquinone, approximately 28-31% was found after glucuronidase treatment, and approximately 2-5% was found after sulfatase treatment.

An in vitro percutaneous absorption study was performed with and without pretreatment with the metabolic inhibitor sodium azide (inhibits cytochrome oxidase). Approximately 43% of

the dose penetrated the skin. Pretreatment with sodium azide had no effect on percutaneous absorption. The receptor fluid and skin extractions had a peak on thin layer chromatography corresponding to hydroquinone and a second tentatively identified as benzoquinone (decreased with azide pretreatment). At 24 hours, 34% of the dose had permeated through the skin and 9% was in the skin. After a lag time of approximately 8 hours (artifact of the system), the flux was 2.9 $\mu g/cm^2$/hour; this was unchanged by sodium azide pretreatment. In comparison, the flux through skin in vivo after half the dose was applied on a per cm^2 basis was 1.9 $\mu g/cm^2$/hour for forehead and 1.0 $\mu g/cm^2$/hour for forearm.

TOXICOLOGY:

No nonclinical toxicology studies were performed. The Applicant provided a two-page summary of nonclinical pharmacology and toxicology of the individual drug substances. That information is summarized in the pharmacology section of this review and below:

The Applicant states that the individual drug substances have been in existence for decades and that information regarding them as individual entities is available. They then state that, "In as much as the investigational drug is a single formulation (considered new) of 3 actives combined, literature reports on this product are harder to find." The Applicant did not perform nonclinical studies of the proposed drug product to fill this apparent information gap.

The Applicant also provided a list of 20 journal articles describing use or effects of one or more of the active ingredients or drug(s) of the same class. The reference list does not match the articles actually provided. Of the articles provided, those related to nonclinical evaluation of toxicologic effects are summarized below. None of the articles are particularly relevant to describing the toxicological profile of the proposed combination drug formulation.

1. McLean HM et al. Novel fluorinated antiinflammatory steroid with reduced side effects: Methyl 9α-fluoroprednisolone-16-carboxylate. J. Pharmaceutical Sci. 83:476-480, 1994.

A fluorinated analog of a carboxylic acid ester of prednisolone was tested in the acute rat croton oil-induced ear edema model. It was found to be twice as potent in topical antiinflammatory activity as the non-fluorinated drug or prednisolone, as determined by comparison of ID_{50} values. When topical dosing at the respective ID_{50} values was repeated for five days to the right ear, systemic effects associated with prednisolone (decrease thymus and adrenal weights, decreased body weight gain, and decreased plasma corticosterone concentrations) were not seen with either the carboxylic acid ester or the fluorinated analog.

A receptor binding study was performed in rat hepatoma cell culture. IC_{50} values were determined from competitive binding curves with dexamethasone for the glucocorticoid receptors. Fluorination of the parent drug enhanced receptor binding affinity, but the affinity of both carboxylic acid esters were still considerably lower than for prednisolone.

In the tyrosine aminotransferase (TAT) assay, EC_{50} values for induction of that enzyme were determined for the fluorinated analog and for prednisolone as a measure of biological activity. The fluorinated compound was demonstrated to induce tyrosine aminotransferase with approximately one-tenth the potency of prednisolone, while the non-fluorinated analog did not induce TAT activity.

The authors state that, in general, fluorination at the 9α position enhances biological activity of glucocorticoids.

2. Spearman RIC and Jarrett A. Bio-assay of corticosteroids for topical application. British J. Dermatol. 92:581-4, 1975.

Five corticosteroid creams were assessed in the mouse tail test for epidermal thinning. The preparations were applied daily to the tail skin of male "to" mice for three weeks. Epidermal thickness measurements were made on histological examination. All of the tested drugs produced significant thinning of the epidermis. The rank order for relative percent thinning was prednisolone stearoylglycolate 1.9% > fluocinolone acetonide 0.025% > triamcinolone acetonide 0.1% > betamethasone valerate 0.1% > hydrocortisone 1.0%. Additionally, triamcinolone acetonide 0.1% applied with vitamin A was shown to result in less skin thickening than with vitamin A alone.

3. Milioni C et al. Pharmacological study in vivo of the new topical anti-inflammatory steroid 21-thiol-9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dione-21-acetylamino cysteine.

The carrageenin-induced granuloma model and the cotton pellet granuloma test in rats were used to assess the antiinflammatory activity of 21-thiol-9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dione-21-acetylamino cysteine (CMJ). In the first experiment, topically applied CMJ at 0.075% or 0.75% caused slight but nonsignificant reduction in the granuloma, while dexamethasone treatment resulted in significant granuloma reduction. Dexamethasone caused significant reduction in thymus weight while CMJ did not. Neither drug resulted in a significant change in adrenal weight. In the cotton pellet-induced granuloma test, the ED_{50} values were 7.0 mg/pellet for dexamethasone and 67.5 mg/pellet for CMJ (pellets were impregnated with drug prior to subcutaneous implantation, indicating a 10-fold lower potency for local effects of CMJ relative to dexamethasone. Systemic potency was evaluated by the weight of the granuloma around a contralateral vehicle treated pellet. Systemic activity was approximately 1000-fold less for CMJ relative to dexamethasone. CMJ again failed to cause reduction in thymus or adrenal weights. Body weights were decreased in dexamethasone treated rats in both tests, but weight gain was observed in CMJ treated animals. In summary, CMJ was found to have some, although lower, anti-inflammatory potency than dexamethasone, but was without the systemic adverse effects of the latter.

4. Deakin MJ. Current dangers and problems in the topical use of steroids. Med. J. Aust. 1:120-121, 1976.

The effects of topical corticosteroids, and particularly fluorinated corticosteroids were reviewed. Absorption of these compounds can be transdermal, via the hair follicles, or both. Occlusion can significantly enhance cutaneous penetration. Assays for corticosteroid potency include the human vasoconstriction assay and an assay for antimitotic activity on the skin of hairless mice. Complications of topical steroid use include secondary infection, miliaria, and epidermal atrophy. Prolonged occluded exposure of intertriginous areas may result in epidermal atrophy, painful non-healing fissures, pruritus, and telangiectasiae. Adrenal suppression may occur when occlusive methods are used over large areas. Corticosteroids are thought to increase epidermal aryl hydrocarbon hydroxylase (AHH) activity, which is involved in hydrocarbon carcinogenesis in humans, and thus may increase the risk of cancer. Corticosteroids may be procarcinogenic by suppression of immunological defense systems in the epidermis. Application to "solar type malignancies" may result in epidermal atrophy and diffuse spread of the malignant lesion. Application of fluorinated corticosteroids to the face may result in a rosacea-like syndrome characterized by persistent erythema, telangiectatic vessels, papulopustules and atrophy, or in perioral dermatitis. Other potential complications include steroid acne and tinea incognito.

CARCINOGENICITY:

No studies were submitted. There is a statement in the toxicology summary that hydroquinone is mutagenic but has not been tested for carcinogenic potential. The Applicant has made it clear that continued long-term use of the drug is necessary to maintain depigmentation; dermal carcinogenicity studies have not been performed to support that use.
Reviewer's comment: Oral carcinogenicity studies have been performed by the NTP. The conclusions of NTP carcinogenicity studies of hydroquinone administered by gavage were that there was some evidence of carcinogenic activity in F344/N rats (marked increases in tubular cell adenomas of the kidney in males and mononuclear cell leukemia in females) and female B6C3F$_1$ mice (increases in hepatocellular neoplasms, mostly adenomas). There was no evidence of carcinogenic activity in male B6C3F$_1$ mice. Associated effects included thyroid follicular cell hyperplasia in male and female mice, and anisokaryosis, multinucleated hepatocytes and basophilic foci of the liver in male mice.

The Applicant also provided the following journal articles addressing the carcinogenic potential of tretinoin and corticosteroids in support of this application.

1. Thorne EG. Long-term clinical experience with tretinoin. British J. Dermatol. 127 (suppl. 41):31-6, 1992.

In this review article, the author discusses two long-term studies in mice. In the first, a lifetime exposure study performed in Japan by Tsubura and Yamamoto (primary reference not provided), 0.03 ml of 0.1% tretinoin cream or lotion was applied to ICR mice once or three times daily for two years. The treatment was well tolerated, and there was no evidence of tumorigenesis. In the second, performed by Kligman et al. (unpublished data), 0.025% tretinoin was applied to hairless albino mice three times weekly for 98-103 weeks. No effects on tumor incidence or mortality were reported.

The author also discusses tretinoin's enhancement of UV-induced carcinogenesis in SKh-1 hairless albino mice. The author states that a total of 12 such studies have been performed. Tretinoin enhanced UV-induced carcinogenesis in six of those, inhibition of tumor formation was seen in three, and no effect was seen in the remaining three. (*Reviewer's comment: There is no discussion of the relationship of study design to the results seen. In general, studies in which exposure to tretinoin and UV radiation is concurrent, enhancement of UV-induced carcinogenesis is seen, while inhibition or no effect is seen when mice are irradiated for a period of time, e.g. six weeks, followed by a period of time of tretinoin treatment.*)

2. Epstein JH. All-*trans* retinoic acid and cutaneous cancers. J. Am. Acad. Dermatol. 15:772-778, 1986.

This review article describes effects of tretinoin on UVB-induced cutaneous tumors. The author cites a number of studies in which tretinoin was shown to enhance UVB-induced carcinogenesis. He concludes that "even nontoxic and essentially nonirritating concentrations of tretinoin could promote photocarcinogenesis in experimental animals. Other studies mentioned by the author showed no change or improvement when tretinoin was applied after UV irradiation.

The author cites evidence that tretinoin and other retinoids induced sister chromatid exchange in human diploid fibroblasts. He states that this finding has been correlated with carcinogenicity of substances. He also states that high doses of tretinoin have been associated with immunosuppression.

Possible mechanisms of inhibition of carcinogenicity are discussed. Alteration of enzyme activities, e.g. ornithine decarboxylase (ODC), altered DNA synthesis, immunostimulation at low doses, regulation of cell differentiation, stabilization of lysosomal membranes, and modulation of endogenous growth factors were proposed as potentially leading to such an effect.

The author states that there is no evidence of enhancement of carcinogenicity in humans. He points out evidence for the regression of actinic keratoses with tretinoin treatment, but with a high rate of recurrence. Tretinoin alone was effective on the face, but tretinoin in combination with 5-fluorouracil was more effective on the hands and forearms than tretinoin alone. He also cites either regression or no effect on basal cell epitheliomas. He describes treatment of melanoma metastases in two patients. Regression was seen in one with recurrence in 12 months after discontinuation of therapy. No effect was seen in the second patient.

3. Deakin MJ. Current dangers and problems in the topical use of steroids. Med. J. Aust. 1:120-121, 1976.

The effects of topical corticosteroids, and particularly fluorinated corticosteroids were reviewed. The author states that corticosteroids are thought to increase epidermal aryl hydrocarbon hydroxylase (AHH) activity, which is involved in hydrocarbon carcinogenesis in humans, and thus may increase the risk of cancer. Corticosteroids may be procarcinogenic by suppression of immunological defense systems in the epidermis. Application to "solar type malignancies" may result in epidermal atrophy and diffuse spread of the malignant lesion.

At the end of the summary section, the Applicant states, "The active ingredients present in the drug product have the potential to be carcinogenic and teratogenic in animals. The drug product has not been tested for carcinogenicity, mutagenicity or teratogenicity"

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

  No studies were performed to evaluate the combination drug product or the effects of the combination on the carcinogenic potential of any of the individual active ingredients. No studies were performed to evaluate the carcinogenic potential of novel degradation products found in the combination drug product. There is no information in the labels of marketed products containing either hydroquinone or fluocinolone acetonide regarding carcinogenic potential. Dermal carcinogenicity studies of tretinoin are described in a review article, but no primary references are provided. NTP studies of hydroquinone indicate that carcinogenicity is seen when that drug is administered orally.

- Evaluation of Tumor Findings:

  not applicable

Summary Conclusions and Recommendations

There is insufficient data to evaluate the proposed drug product for carcinogenic potential. It is recommended that the sponsor perform dermal carcinogenicity studies in two species using the clinical formulation of the combination drug product.

REPRODUCTIVE TOXICOLOGY:

No studies were performed. The Applicant has made a statement in the toxicology summary that fluocinolone acetonide and tretinoin are known teratogens and that hydroquinone has not been tested for reproductive or developmental toxicologic potential.

The Applicant also provided the following journal article addressing nonclinical testing of tretinoin for developmental toxicity.

1. Kistler A and Howard WB. Testing of retinoids for teratogenicity in vivo. Methods in Enzymology 190:433-437, 1990.

The authors advocate dosing (oral) of mice or rats only on day 9 or on days 8-10 of gestation, as these are the critical days for development of craniofacial abnormalities. They then advocate caesarean section on gestation day 20 and external examination only.

Summary and Evaluation:

In the Applicant's justification for full waiver of pediatric use information, they state that the drug is "designed to treat hyperpigmentation of the chloasmic or melasmic types that occurs only in sexually mature females." In their nonclinical pharmacology and toxicology summary, the only statement made to address reproductive/developmental toxicity of the proposed drug product is, "Teratogenicity in vivo has been proven with retinoids on rodent fetus. It is also claimed that retinoids can induce specific malformations in humans."

At the end of the summary section, the Applicant states, "The active ingredients present in the drug product have the potential to be carcinogenic and teratogenic in animals. The drug product has not been tested for carcinogenicity, mutagenicity or teratogenicity. Because of the teratogenic effect of tretinoin found in mouse fetus, a warning statement for pregnant women is appropriate."

Labeling Recommendations:

Since no studies have been performed with the drug product, and since drug products containing the individual drug substances are labeled pregnancy category C, then the same might be appropriate for this drug product. However, the Applicant's above statement indicates that they are aware that there are potential risks, but have made no effort to characterize them. They have also stated their intention that the drug be used to treat melasma, which may occur in pregnant women. It would seem appropriate that, at the very least, a statement similar to that in the Renova® label, that the drug should not be used in pregnancy, should be included. Designation as pregnancy category X may be warranted, as it is unknown whether or not the combination of drug substances might potentiate their reproductive effects. It is also unknown what untoward effects novel degradation products found in the mixture might have on reproduction or on embryofetal development.

GENETIC TOXICOLOGY:

No studies were performed. In the toxicology summary, the sponsor states that hydroquinone was found to be positive in genotoxicity studies.

The Applicant has provided the following journal articles in support of this application:

1. Gocke E et al. Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutation Research 90:91-109, 1981.

A number of cosmetic ingredients used in Europe were evaluated in the Salmonella/microsome (Ames) test, the Basc test in Drosophila, and the mouse micronucleus assay. Salmonella typhimurium strains used were TA1535, TA100, TA1538, TA98, and TA1537. Strains of Drosophila melanogaster used were the Berlin K (wild type) and Basc. The micronucleus assay was performed in NMRI mice. Hydroquinone was positive in the Ames and

micronucleus tests. The authors used 2 different media in the Ames test, ZLM, which is a minimally modified medium for E. coli, and Vogel-Bonner medium. They state that the positive result for hydroquinone would not have been detected if only the Vogel-Bonner medium had been used.

2. Islam S and Ahmad M. Mutagenic activity of aziridinyl steroids and their mechanism of action in biological systems. Mutagenesis 6:271-278, 1991.

Aziridinyl steroids were found to be mutagenic in the Ames assay. While mutagenicity was detected without metabolic activation, the effect was enhanced by metabolic activation. The authors state that these steroids appear to generate H_2O_2 as well as superoxide and hydroxyl radicals. They also found that structural features that appeared to be essential to mutagenic potential were i) a reactive N-aminophthalamide group at the (5,6b) position and ii) an acetyoxy/chlorine group at the third position of the steroid nucleus.

There does not appear to be any relevance of this article to the steroid included in the proposed combination drug formulation.

Summary:

Hydroquinone was positive in tests for genotoxicity. Fluocinolone acetonide has not been evaluated in genotoxicity tests. The Applicant has not provided information regarding the genotoxic potential of tretinoin.

Reviewer's comment: Hydroquinone was also positive for genotoxicity in the Ames assay in S. typhimurium strain TA97 with metabolic activation, and in strains TA98 and TA100 with and without metabolic activation (Lin and Lee, Mutat. Res. 269:217-224, 1992). NTP data indicates that hydroquinone was positive for genotoxicity in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation and that hydroquinone induced sister chromatid exchanges in CHO cells with and without metabolic activation and chromosomal aberrations in the presence of activation. One of the degradants (also a metabolite) of hydroquinone, benzoquinone, has been shown to be mutagenic in the Ames assay in S. typhimurium strains 104 (sensitive to oxidative mutagens, Hakura et al., Mutat. Res. 347:37-43, 1995), TA97, TA98, and TA100 (Lin and Lee, Mutat. Res. 269:217-224, 1992)

SPECIAL TOXICOLOGY STUDIES:

No studies were performed. Dermal irritation is expected due to the known effects of tretinoin. The Applicant states that this effect is mitigated by the anti-inflammatory effects of the glucocorticoid. Eye irritation would be assumed on the basis of positive dermal irritation results. Sensitization potential of the combination drug product or of novel degradation products was not assessed. Phototoxicity and photoallergenicity were not assessed. Sodium metabisulfite is an excipient in the formulation that may be a sensitizer in human patients.

OVERALL SUMMARY AND EVALUATION:

Introduction:

The proposed drug product is a combination of three active ingredients, fluocinolone acetonide, hydroquinone and tretinoin. Proposed treatment of hyperpigmented skin involves once daily application to the hyperpigmented areas and surrounding normally pigmented skin.

Safety Evaluation:

Each of the drug substances is currently marketed as one or more individual products at similar concentrations. Only hydroquinone is approved for the same clinical indication. There is not complete nonclinical safety information available for all of the individual drug substances or for the combination product. There has been no assessment of the combination for potential for genotoxicity, carcinogenicity, or reproductive/developmental effects. Additionally, there appear to be novel degradation products in the combination formulation that have not been evaluated for safety in experimental animals.

Clinical Relevance of Safety Issues:

It is unclear how these three drug substances might interact. One or more might potentiate the toxicity of the other(s), or effects could be additive. There is no information to extrapolate to clinical use of some of the drug substances or of the degradation products.

Other Clinically Relevant Issues: none

Conclusions:

From a pharmacology/toxicology standpoint, it is recommended that the application be not approvable. There is insufficient evidence of safety of the combination drug product or of novel degradants found in the combination.

Communication Review:

- **Labeling Review (NDA):**

The label should be revised to be consistent with those of products containing each of the three active ingredients. Corrections should be made to include only statements supported by submitted information and to reinforce the fact that there is no nonclinical safety data regarding the combination product.

Specifically, under "Clinical Pharmacology," speculatory statements about the mechanism of action of fluocinolone acetonide and tretinoin should be removed until data or literature references are provided to support them. In the section on tretinoin: the term "essential" should be removed from the description of the drug; the statement that blood levels of the drug are >75% tretinoin should be removed or data submitted to support it; the description of metabolism of tretinoin should include photoisomerization of tretinoin to isotretinoin and recognition of metabolism in and on the skin as well as the liver.

Under "Precautions," possible adverse effects from labels of all three individual active compounds should be noted, as well as a statement the interactions of the three drug substances and the effects of novel degradation products are unknown. The paragraph describing photo co-carcinogenicity should be limited to the first sentence only; the remaining sentences describe studies that were not designed to test for that effect. The positive genotoxic and carcinogenic effects of hydroquinone should be discussed, and the lack of genotoxicity data for fluocinolone acetonide, tretinoin, the combination drug product, and novel degradation products should be made clear. Under "Pregnancy," the statement describing topical tretinoin should be modified to state that topical tretinoin may be teratogenic in laboratory animals. It would be consistent with the label for Renova and the Applicant's comments in this NDA to state that the drug should not be used in pregnancy.

- **Investigator's Brochure/Informed consent review (IND):** not applicable

RECOMMENDATIONS:

Internal comments:

From a pharmacology/toxicology standpoint, it is recommended that this drug not be approved. No nonclinical evaluation of the combination product was performed to assess short or long-term toxicity, whether local or systemic. The Applicant has made it clear that the drug will be used long-term to maintain the effect. No evaluation was performed of carcinogenic potential, or of reproductive and developmental toxicity. Although some of this information is available from the labels of approved products, there is no information on the combined effects of these drugs. Additionally, there are novel degradation products in the combination formulation that have not been qualified in nonclinical studies.

External Recommendations (to sponsor)/Draft letter Content for Sponsor:

Future development or NDA issues: none

/S/ 10/6/99
Amy C. Nostrandt, D.V.M., Ph.D.
Pharmacologist/Toxicologist

cc:
NDA 21-112
HFD-340
HFD-540
HFD-540/PHARM/Nostrandt
HFD-540/TLPHARM/Jacobs
HFD-540/MO/Ko
HFD-540/CHEM/Pappas
HFD-540/PMS/~~Kozma-Fornarc~~ /S/
C:\word files\nda\n21112_000.doc
Draft date (# of drafts) 9/27/1999 (1)
HFD-725 /S/

Concurrence Only:
HFD-540/DD/WILKIN 10/21/99 OFS
HFD-540/TLPHARM/JACOBS o.j. for DFS 10/6/99

INFORMATION TO BE CONVEYED TO THE APPLICANT:

[redacted]

APPEARS THIS WAY
ON ORIGINAL

HFD 540

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: fluocinolone acetonide, hydroquinone, tretinoin, depigmentation
Reviewer Name: Amy Nostrandt
Division Name: Division of Dermatologic and Dental Drug Products
HFD# 540
Review Completion Date: 3/7/00 MAR 3 0 2000

Review number: 2
IND/NDA number: NDA 21-112
Serial number/date/type of submission: letters in response to NA letter, received 1/27/00 and 2/23/00

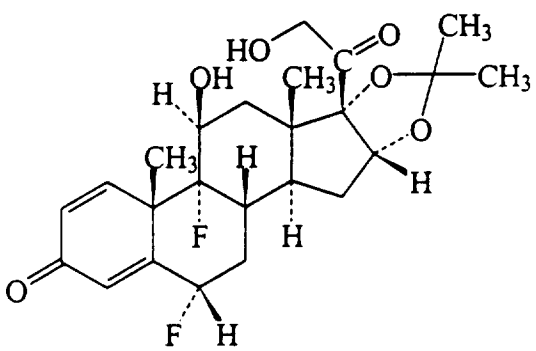
Information to sponsor: Yes (X) No ( )
Sponsor (or agent): Hill Dermaceuticals, Inc.
Manufacturers for drug substance:
fluocinolone acetonide -
hydroquinone -
tretinoin -

Drug:
Code Name: (fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%)
Generic/Chemical Names: fluocinolone acetonide; Pregna-1,-4-diene-3,20-dione, 6,9-difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-; 6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone
hydroquinone; 1,4-benzenediol
tretinoin; retinoic acid; all-*trans*-retinoic acid; (*all-E*)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid

Trade Name: (fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%)
CAS Registry Numbers:

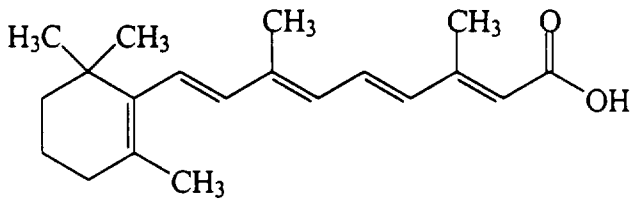
fluocinolone acetonide	67-73-2
hydroquinone	123-31-9
tretinoin	302-79-4

Molecular Formula/ Molecular Weight/Structure:
fluocinolone acetonide, $C_{24}H_{30}F_2O_6$, MW=452.50



hydroquinone, $C_6H_6O_2$, MW=110.11

tretinoin, $C_{20}H_{28}O_2$, MW=300.44



Relevant INDs/NDAs/DMFs:

Drug Class: depigmenting agent

Indication: cutaneous melanosis in skin types II and III

Clinical formulation:

Ingredient	mg per gram	percent (w/w)
Tretinoin, USP	0.5	0.05
Fluocinolone Acetonide, USP	0.1	0.01
Hydroquinone, USP	40	4.00
magnesium aluminum silicate, NF		
butylated hydroxytoluene, NF		
cetyl alcohol, NF		
stearic acid, NF		
stearyl alcohol, NF		
methylparaben, NF		
propylparaben, NF		
methyl gluceth-10		
glycerin, USP		
citric acid, USP		
sodium metabisulfite, NF		
purified water, USP	5	5

Route of administration: topical to the skin

Proposed clinical protocol or Use:

Previous clinical experience:

Subjects in clinical trials applied the material once daily before bedtime for 8 weeks, followed by open label continuation of dosing until complete clearing, followed by 12 weeks of tapered maintenance dosing (to once daily three times per week). Adverse events included burning, stinging, rosacea, telangiectasia, and perioral dermatitis. One patient treated with tretinoin and hydroquinone without the steroid developed atrophy. Other adverse events reported included itching, acne, dryness of the skin and papules, cheilitis, and swelling of the face. One of the investigators, Dr. Willis (who also has some financial interest in the product), did not report adverse events that he considered to be expected, including irritation, erythema, and peeling. During the maintenance period adverse events reported included erythema, peeling, telangiectasias, and repigmentation. The Applicant states that most patients dropped out of the study during this time because of repigmentation that was seen as early as two weeks into the maintenance period.

The sponsor states in their current submission that they have followed the safety of local adverse events in human subjects for six months, and that there have been no "unexpected" adverse events.

Disclaimer : Note that some information may come directly from the sponsor's submitted material.

Introduction and drug history:

The proposed drug product contains three active ingredients, fluocinolone acetonide, hydroquinone and tretinoin. Each is marketed individually in a topical formulation. Only hydroquinone is indicated for the treatment of hyperpigmented lesions. The Applicant proposes their combination based on a 1975 paper by Kligman and Willis in which a combination of 0.1% tretinoin, 5.9% hydroquinone, and 0.1% dexamethasone was more effective in depigmentation of skin of African-American subjects "with naturally dark skin" than any of the individual components alone. The authors hypothesized an additive or synergistic effect of the active ingredients.

The following information is from labels of marketed products containing one of the proposed drug substances:

Fluocinolone acetonide is a fluorinated corticosteroid, and is currently available in topical formulations at concentrations up to 0.5 mg/g (0.05%) to be applied as a thin film 2-4 times per day. The compound is absorbed when applied topically, with increased absorption when under an occlusive dressing, and may be highly plasma protein bound. The drug primarily is metabolized in the liver and excreted via the kidneys. Adverse events in the skin reported with this drug substance alone include stinging or burning, itching, irritation, dryness, folliculitis, hypertrichosis, acne, hypopigmentation, perioral dermatitis, allergic contact dermatitis, maceration of the skin, secondary infection, skin atrophy, striae, and miliaria. Systemic effects may occur if the product is under an occlusive dressing, including suppression of the hypothalamic-pituitary-adrenal axis. Prolonged use may result in manifestations of Cushing's syndrome, hyperglycemia, glucosuria, increased susceptibility to infection, and growth

retardation. No mutagenicity, carcinogenicity, fertility, or teratogenicity studies, or evaluation of excretion of the drug in milk have been reported for the marketed topical products. However, steroids may have adverse effects on reproduction and development.

Tretinoin (all trans retinoic acid) is currently available in formulations containing up to 0.1% active ingredient, to be applied once per day, in the evening. The drug undergoes hepatic metabolism and biliary and urinary excretion. Adverse effects include irritation, desquamation, stinging, dryness, and thinning of the stratum corneum. (*Reviewer's comment: It is unclear whether or not the thinning of skin resulting from the prolonged use of tretinoin or corticosteroids could result in increased systemic absorption of those or other compounds.*) Dermal carcinogenicity studies of tretinoin have been considered negative, but properly performed photo co-carcinogenicity studies indicate that tretinoin may enhance sunlight-induced carcinogenesis. Tretinoin is a known teratogen when administered orally, and studies in animals appear to indicate that developmental effects after topical exposure may be possible. It is unknown if the drug is excreted in milk.

Hydroquinone is a depigmenting agent, available by prescription as a 4% preparation to be applied twice daily. This drug substance inhibits tyrosinase (blocks oxidation of tyrosine to dopa) and suppresses other melanocyte metabolic processes. Depigmentation resulting from this product is reversible. Adverse side effects include localized contact dermatitis. No data is provided in the prescription label on carcinogenic or mutagenic potential, reproductive toxicity, secretion in milk, ADME, or pediatric use.
Reviewer's comment: It has been reported that prolonged use may result in paradoxical hyperpigmentation or exogenous ochronosis. Studies of genotoxicity, carcinogenicity, and reproductive/developmental toxicity have since been performed. NTP data indicates that hydroquinone was positive for genotoxicity in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation and that hydroquinone induced sister chromatid exchanges in CHO cells with and without metabolic activation and chromosomal aberrations in the presence of activation. The conclusions of NTP carcinogenicity studies of hydroquinone administered by gavage were that there was some evidence of carcinogenic activity in F344/N rats (marked increases in tubular cell adenomas of the kidney in males and mononuclear cell leukemia in females) and female B6C3F$_1$ mice (increases in hepatocellular neoplasms, mostly adenomas). There was no evidence of carcinogenic activity in male B6C3F$_1$ mice. Associated effects included thyroid follicular cell hyperplasia in male and female mice, and anisokaryosis, multinucleated hepatocytes and basophilic foci of the liver in male mice.

In the original submission, the Applicant states that the individual drug substances have been in existence for decades and that information regarding them as individual entities is available. They then state that, "In as much as the investigational drug is a single formulation (considered new) of 3 actives combined, literature reports on this product are harder to find." The Applicant did not perform nonclinical studies of the proposed drug product to fill this apparent information gap.

Studies reviewed within this submission:
No nonclinical studies were performed or submitted.
Studies not reviewed within this submission: none

OVERALL SUMMARY AND EVALUATION:

Introduction:

The proposed drug product is a combination of three active ingredients, fluocinolone acetonide, hydroquinone and tretinoin. Proposed treatment of hyperpigmented skin involves once daily application to the hyperpigmented areas and surrounding normally pigmented skin.

The sponsor's letters in the current submissions make the following arguments:

- The sponsor objects to performance of a chronic dermal application study in minipigs. They state that they have followed the safety of the drug product for local adverse effects in humans out to six months and that they propose use for only eight weeks. *Reviewer's comment: Systemic adverse events have not been monitored, but could be in a nonclinical study. Previous submissions have indicated that the product has been used in clinical trials for more than eight weeks and long-term tapered therapy has been necessary to maintain the effect.*
- The sponsor claims that they were informed in a teleconference on 6/18/97 that "short term treatment does not require toxicology studies" and that application of the material for less than six months constitutes short-term treatment. *Reviewer's comment: The teleconference to which the sponsor refers was not attended by a pharmacology reviewer, and no such statement was made. The sponsor may be confusing recommendations made at the time of review of the original IND, when the sponsor was advised that, " If prolonged use (for 6 months or longer at one time or cumulatively over a 10-year period) is anticipated, then carcinogenicity ... testing should be performed in laboratory animals."*
- The sponsor states that they would be willing to perform nonclinical studies in phase 4. This would be inconsistent with what has been required of other sponsors. Chronic and reproductive/developmental toxicology studies should be performed prior to NDA submission in order to provide adequate information for the label. The sponsor did include statements in the NDA that the active ingredients of the drug product "have the potential to be carcinogenic and teratogenic in animals."
- The sponsor states in the letter received 2/23/00 that the indication has been revised to state that the product is to be used for the temporary relief of melasma, used once daily for eight weeks. *Reviewer's comment: The sponsor indicates here that the drug product will be specifically aimed at treatment of a condition seen in pregnant women, but they do not want to perform reproductive/developmental toxicology studies prior to approval.*
- The sponsor states that information regarding degradants has been submitted and there are none that are present at >0.1% of the relevant drug substances.

Safety Evaluation:

Each of the drug substances is currently marketed as one or more individual products at similar concentrations for different clinical indications. There is not complete nonclinical safety information available for all of the individual drug substances or for the combination product. There has been no assessment of the combination for potential for genotoxicity, carcinogenicity, or reproductive/developmental effects.

Clinical Relevance of Safety Issues:

It is unclear how these three drug substances might interact. One or more might potentiate the toxicity of the other(s), or effects could be additive. There is no information to extrapolate to clinical use of some of the drug substances. Without assessment of the potential

of the combination drug product for reproductive/developmental effects, it is impossible to assign a pregnancy category.

Other Clinically Relevant Issues: none

Conclusions:

Systemic adverse effects, as well as potential for reproductive/developmental toxicity, mutagenicity, and carcinogenesis have not been characterized for all of the three active ingredients, or for the combination drug product. It is unknown whether or not interaction of the active ingredients of the combination may potentiate their toxicity. Evaluation of the drug product is important to characterize its systemic exposure and effects, to provide vital information for the label, and to be consistent with regulatory requirements as they are applied to all other applicants.

RECOMMENDATIONS:

Internal comments:

No nonclinical evaluation of the combination product was performed to assess short or long-term toxicity, whether local or systemic. The Applicant currently states that an individual course of therapy will be eight weeks, but previous submissions indicate that the drug may need to be used long-term to maintain the effect. No evaluation was performed of carcinogenic potential, or of reproductive and developmental toxicity. Although some of this information is available from the labels of approved products, there is no information on the combined effects of these drugs. The applicant was advised in the action letter to perform a chronic dermal application toxicology study in a non-rodent and reproductive and developmental toxicology studies. At the minimum, these studies should be performed prior to approval. A dermal carcinogenicity study may be warranted in phase 4.

A teleconference was held on March 7, 2000. The following was conveyed to the Applicant:

A chronic dermal application study in a nonrodent species would help evaluate not only local effects, but also systemic adverse effects. Since the interaction of the three active drug substances has not been evaluated, it is unknown how they might affect the systemic exposure and toxicity of each other. Similarly, studies of reproductive/developmental toxicology of the drug product would evaluate the interactions of the three active ingredients and their combined effects in this area. Performance of nonclinical toxicology and reproductive/developmental toxicology studies in phase 4 would result in omission of vital information from the drug label.

Reviewer's comment: The sponsor appears to believe that the drug must be labeled for six months of continuous use to be considered as used in a chronic indication, and that any drug used for less time does not require toxicological testing. Even if the use of the drug were limited to a shorter time period of use, toxicology studies of the combination product, and particularly reproductive and developmental toxicology studies of a product intended for use in pregnant women, would be recommended to support approval of an NDA.

)/Draft letter Content f

Future development or NDA issues: none

/S/ 3/13/00
Amy C. Nostrandt, D.V.M., Ph.D.
Pharmacologist/Toxicologist

cc:
NDA 21-112
HFD-340
HFD-540
HFD-540/PHARM/Nostrandt
HFD-540/TLPHARM/Jacobs
HFD-540/MO/Ko
HFD-540/CHEM/Pappas
HFD-540/PMS/Lutwak
C:\word files\nda\n21112_nc_012700.doc
Draft date (# of drafts): 3/7/00 (1)

Concurrence Only:
HFD-540/DD/WILKIN

HFD-540/TLPHARM/JACOBS

As above. The Sponsor has not adequately addressed:
1) the inherent likelihood for chronic use of a product, that is followed by rapid relapse, for a condition that is chronic, and
2) the principle of emerging properties when combining different pharmacologically active ingredients.

/S/ 5/30/00

INFORMATION TO BE CONVEYED TO THE APPLICANT:

[redacted]

APPEARS THIS WAY
ON ORIGINAL

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number:	21-112 (resubmission)
Review number:	3
Sequence number/date/type of submission:	NDA resubmission, response to non-approval letter, letter date July 20, 2001 N-000, BP, letter date 9/18/01 N-000, BZ, letter date 11/22/01
Information to sponsor: Yes (X) No ( )	
Sponsor and/or agent:	Hill Dermaceuticals, Inc., Sanford, FL
Manufacturer for drug substances:	(from original review)
fluocinolone acetonide -	[redacted]
hydroquinone -	
tretinoin -	[redacted]

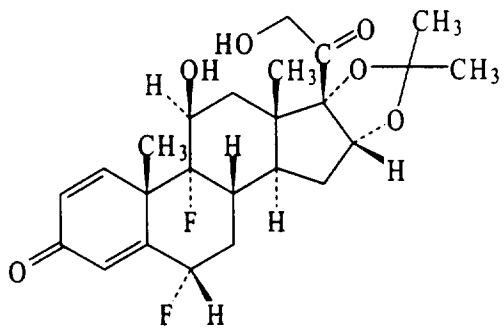
Reviewer name:	Amy C. Nostrandt, D.V.M., Ph.D.
Division name:	Division of Dermatologic and Dental Drug Products
HFD #:	540
Review completion date:	12/17/01

Drug:

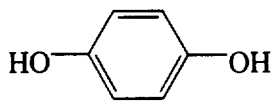
Trade name:	Tri-Luma
Generic names (list alphabetically):	fluocinolone acetonide; hydroquinone; tretinoin
Code name:	[redacted] fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%)
Chemical names:	fluocinolone acetonide: Pregna-1,-4-diene-3,20-dione, 6,9-difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-; 6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone hydroquinone: 1,4-benzenediol tretinoin: retinoic acid; all-*trans*-retinoic acid; (*all-E*)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid
CAS registry numbers:	fluocinolone acetonide 67-73-2 hydroquinone 123-31-9 tretinoin 302-79-4
Mole file numbers:	not provided

Molecular formulae/molecular weights/structures:

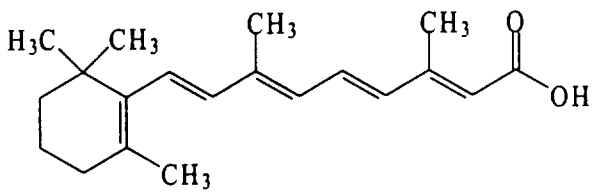
fluocinolone acetonide, $C_{24}H_{30}F_2O_6$, MW=452.50



hydroquinone, $C_6H_6O_2$, MW=110.11



tretinoin, $C_{20}H_{28}O_2$, MW=300.44



Relevant INDs/NDAs/DMFs:

Drug class: depigmenting agent (combination of a steroid and a retinoid with hydroquinone)

Indication: melasma

(Reviewer's comment: The term used to describe the clinical indication has changed from the description in the original submission, "cutaneous melanosis in skin types II and III."

Clinical formulation:

Ingredient	mg per gram	percent (w/w)
Tretinoin, USP	0.5	0.05
Fluocinolone Acetonide, USP	0.1	0.01
Hydroquinone, USP	40	4.00
magnesium aluminum silicate, NF		

butylated hydroxytoluene, NF
cetyl alcohol, NF
stearic acid, NF
stearyl alcohol, NF
methylparaben, NF
propylparaben, NF

methyl gluceth-10
glycerin, USP
citric acid, USP
sodium metabisulfite, NF
purified water, USP

Route of administration: topical to affected skin

Proposed use:

Previous clinical experience:

Subjects in the original clinical trials applied the material once daily before bedtime for 8 weeks, followed by open label continuation of dosing until complete clearing, followed by 12 weeks of tapered maintenance dosing (to once daily three times per week). Adverse events included burning, stinging, rosacea, telangiectasia, and perioral dermatitis. One patient treated with tretinoin and hydroquinone without the steroid developed atrophy. Other adverse events reported included itching, acne, dryness of the skin and papules, cheilitis, and swelling of the face. During the maintenance period adverse events reported included erythema, peeling, telangiectasias, and repigmentation. The Applicant states that most patients dropped out of the study during this time because of repigmentation that was seen as early as two weeks into the maintenance period.

The resubmission contains information on additional 8-week clinical trials, without maintenance therapy, irritation and sensitization studies, ongoing 12-month studies, and 8-week clinical pharmacology studies.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history:

The proposed drug product contains three active ingredients, fluocinolone acetonide, hydroquinone and tretinoin. Each is marketed individually in a topical formulation. Only hydroquinone is indicated for the treatment of hyperpigmented lesions. The Applicant proposes their combination based on a 1975 paper by Kligman and Willis in which a combination of 0.1% tretinoin, 5.9% hydroquinone, and 0.1% dexamethasone was more effective in depigmentation of skin of African-American subjects "with naturally dark skin" than any of the individual components alone. The authors hypothesized an additive or synergistic effect of the active ingredients.

The original NDA was submitted with no supporting nonclinical information for the combination drug product. The recommendation from a pharmacology/toxicology standpoint was that the application not be approved. A nonclinical development program was recommended to support safety of long-term use in clinical patients and provide adequate information for proposed labeling. Deficiencies outlined in the NA letter included a chronic toxicology study in a nonrodent species (minipig), and reproductive/developmental toxicology testing of the combination drug product.

Studies reviewed within this submission:

Pharmacokinetics/Toxicokinetics Studies:

1. Analytical determination of hydroquinone, fluocinolone acetonide, and tretinoin in mini-pig plasma.
2. Analytical determination of hydroquinone, fluocinolone acetonide, and tretinoin in rat plasma.
3. Analytical determination of hydroquinone, fluocinolone acetonide, and tretinoin in rabbit plasma.

General Toxicology studies:

1. Study no. 082-001: Range finder for dermal application toxicity study in minipigs
2. Study no. 082-002: 26 week chronic dermal application study in minipigs

Reproductive/developmental toxicology studies

1. Study no. 7169-103: Study of fertility and early embryonic development to implantation in rats

2. Study no. 7169-101: Dose range-finding developmental toxicity study in rats
3. Study no. 7169-104: Rat developmental toxicity study
4. Study no. 7169-102: Dose range-finding developmental toxicity study in rabbits
5. Study no. 7169-106: Rabbit developmental toxicity study
6. Study no. 7169-105: Study for effects on pre- and postnatal development, including maternal function, in the rat

Studies not reviewed within this submission:

A number of journal articles were provided. Relevant information is included in the appropriate sections below.

Executive Summary

I. Recommendations

A. Recommendation on Approvability

From a pharmacology/toxicology standpoint, the application is approvable.

B. Recommendation for Nonclinical Studies

A dermal carcinogenicity study should be performed in phase 4.

C. Recommendations on Labeling

In general, references to data derived from studies of other drug products containing only one of the active ingredients should be removed from the label. Only descriptions of nonclinical studies of the combination drug product are relevant. General statements about the known toxic potential of any one of the active ingredients are acceptable.

Under "Carcinogenesis, mutagenesis, impairment of fertility," the label should state the positive results of genetic toxicology tests of hydroquinone, the negative results of tretinoin in the Ames assay, and the lack of information for a complete genetic toxicology test battery for tretinoin or for fluocinolone acetonide.

The label should state that there have been no carcinogenicity studies of the drug product or of the combination of the three active ingredients. The potential risk for carcinogenicity of hydroquinone should be stated. The potential risk for photo co-carcinogenicity of tretinoin should also be stated.

Data from the sponsor's segment I rat study should not be included in the "Carcinogenesis, mutagenesis, impairment to fertility" section, as that study was not adequate to assess the effects of the undiluted clinical product. Wording in that section should state that no adequate study of fertility and early embryonic toxicity of the drug product have been performed. A statement should be included to mention the finding of severe hypospermia and testicular effects after treatment with the clinical formulation in the 6-month minipig study.

Discussion of the segment II data should be limited to treatment groups that were treated with the clinical formulation, with the understanding that dosing in these studies was not properly done and resulted in overestimation of the dose. Findings in those studies in rats and rabbits included increased pre- and post-implantation loss, decreased number of live fetuses, decreased fetal weights, skeletal effects, and in some cases retinoid-associated malformations. The consistent findings of embryofetal death and/or malformations warrant assignment of a Pregnancy Category X for this combination drug product for this indication.

Detailed description of the segment III study in rats should be omitted. However, it should be stated that while no adequate study of the late gestational and postnatal effects of the drug product has been performed, a study of a 10-fold dilution of the drug product in rats resulted in developmental delays and postnatal behavioral changes in offspring at all doses tested that are consistent with prenatal retinoid exposure.

The drug product was noted in each study to be a dermal irritant. Therefore it should be assumed to be an ocular irritant and appropriate wording added to the appropriate section of the label.

Specific wording is recommended at the end of this review.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Dermal irritation was seen in all studies at varying degrees of severity. The presence of a steroid in the formulation did not appear to mitigate this effect. Testicular adverse effects in the six-month minipig study and adverse effects on development and prenatal survival of offspring appear to be evidence of systemic exposure to one or more of the active ingredients. There is evidence from the published literature of genetic toxicity, carcinogenic potential, and enhancement of photocarcinogenicity from one or more components of the drug product.

B. Pharmacologic Activity

The sponsor claims that all three drug substances act to decrease skin pigmentation. No supporting nonclinical data were provided.

C. Nonclinical Safety Issues Relevant to Clinical Use

Findings in the 6-month minipig study indicate that there is a possibility of testicular toxicity consistent with systemic exposure to tretinoin.

Findings in reproductive and developmental toxicity studies indicate that systemic exposure from the clinical formulation may be sufficient to cause harm to the developing offspring in the form of toxicity, lethality, or teratogenicity.

Information supplied from the literature indicates that hydroquinone is a mutagen, a clastogen, and a possible carcinogen and that tretinoin enhances photo co-carcinogenicity.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc:
NDA 21-112
HFD-540/DD/WILKIN
HFD-540/SupPHARM/JACOBS
HFD-540/MO/Ko
HFD-540/CHEM/Pappas
HFD-540/PMS/Lutwak
C:\data\my documents\word files\nda\n21112_resub.doc

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

No pharmacology studies were submitted. A discussion of the pharmacology of the drug substances was previously reviewed in the original NDA. Additional information included in this submission is discussed below.

The sponsor has provided articles from the published literature. In one study (Kligman, J. Am. Acad. Dermatol 15:779-785, 1986), mice with pre-existing mild dermal photodamage were treated topically with tretinoin for 5 or 10 weeks. The author states that the subepidermal repair zone in tretinoin-treated mice was wider than in control mice and the effect was dose-dependent. The author also claims increased dermal vascularity.

An article was also supplied describing an *in vitro* study of retinoic acid in keratinocyte culture. Terminal differentiation of keratinocytes was inhibited, apparently by stabilizing the cell membrane and delaying the transition from a living cell to a dead cornified cell.

II. SAFETY PHARMACOLOGY:

No safety pharmacology studies were submitted.

III. PHARMACOKINETICS/TOXICOKINETICS:

No separate pharmacokinetics/toxicokinetics studies were submitted. Blood samples were taken during the minipig toxicology study and in segment II reproductive/developmental toxicology studies in rats and rabbits. Separate reports were provided and are discussed below.

In the 6-month minipig toxicology study, samples were obtained at weeks 4, 13, 26 and 30 (recovery animals) and sent away for analysis. ——— performed the analysis. Assay was by HPLC for hydroquinone with a calibration range of ——— (*Reviewer's comment: It would seem that this assay was not sensitive enough to have been of much use.*). Assays for fluocinolone acetonide and tretinoin were by ——— with the calibration range for each being ———. At week 4, values for all drug substances in all animals were reported as 0.0 (*Reviewer's comment: Since tretinoin occurs endogenously in plasma, concentrations should have been detectable, if not quantifiable.*). At week 13, values for hydroquinone were reported as 0.0 in all animals. Fluocinolone acetonide concentrations were detected, but below the limit of quantitation (BLQ) for one animal in each of the low and mid-dose groups, and were measurable in one control animal. Tretinoin concentrations were reported as 0.0 for all animals but one low dose male, in which the drug was detected but BLQ. At week 26, fluocinolone was again detected but was BLQ in the same control animal, and tretinoin was detected in three control animals, three low-dose animals, one mid-dose animal, and was measurable in two high-dose animals. At week 30, values for all drug substances in all recovery animals were reported as 0.0. Based on the finding of measurable or detectable drug concentrations of fluocinolone acetonide in control animals and the lack of detection of endogenous tretinoin in all animals, the validity of the pharmacokinetic information from this study appears questionable. No assay was performed for metabolites.

A pharmacokinetics report is provided for samples from rats that appear to have been obtained in the rat segment II study (study no. 7169-104). Samples were taken at a single time

point from five animals per dose group; no rationale for selection of that time point was provided. Assay was by HPLC for hydroquinone with a calibration range of [redacted] (*Reviewer's comment: It would seem that this assay was not sensitive enough to have been of much use.*). Assay for fluocinolone acetonide and tretinoin was by [redacted] with the calibration range for each being [redacted] Values for hydroquinone were reported as 0.0 for all animals. Fluocinolone acetonide was detectable and measurable in all high dose animals (treated with the clinical formulation) and in one mid-dose animal (treated with a 50% dilution of the clinical formulation). Tretinoin was detectable in all but the control animals and one low-dose animal treated with a 10-fold dilution of the drug product (*Reviewer's comment: Endogenous tretinoin should have been detected, if not quantifiable, in all animals.*). In all high dose animals (treated with the clinical formulation), concentrations of tretinoin ranged from [redacted] (mean 20.5 ng/mL), well above concentrations considered to be endogenous (<5 ng/mL). Tretinoin concentration was also quantifiable in one mid-dose animal treated with the 50% formulation ([redacted]). No assay was performed for metabolites.

A pharmacokinetics report is provided for samples from rabbits that appear to have been obtained in the rabbit segment II study (study no. 7169-106). Samples were taken at a single time point from five animals per dose group; no rationale for selection of that time point was provided. Assay was by HPLC for hydroquinone with a calibration range of [redacted] (*Reviewer's comment: It would seem that a more sensitive assay would be more useful.*). Assays for fluocinolone acetonide and tretinoin were by [redacted] with the calibration range for each being [redacted] Values for hydroquinone were reported as 0.0 for most animals. Hydroquinone was detected, but BLQ, in two animals in each in groups 3, 4, and 5 (treated with 25%, 50%, and the clinical formulation, respectively). It was quantifiable in one group 4 animal [redacted] and in three animals treated with the clinical formulation (range [redacted] ng/mL). Fluocinolone acetonide was detectable in two group 4 animals and was measurable in one of them [redacted]. Tretinoin was detectable in all but one control animal and was quantifiable only in one group 4 animal and one animal treated with the clinical formulation [redacted], respectively). No assay was performed for metabolites.

An article from the literature (J. Am. Coll. Toxicol. 13:167-230) is included in the current submission. It is an addendum to the Cosmetic Ingredient Review final report on the safety assessment of hydroquinone. It states that hydroquinone is absorbed through the skin from aqueous solutions and that absorption is even greater from an alcoholic vehicle.

Another article supplied by the sponsor from the published literature indicates that fluorinated steroids have a significant thinning action on the epidermis. This would potentially facilitate transcutaneous absorption of all three of the active ingredients.

PK parameters: Insufficient data were collected to calculate any PK parameters.

PK/TK conclusions: There appears to be potential for significant systemic exposure to the drug substances contained in the clinical formulation. Although the data do not appear to be entirely reliable, in some instances concentrations of the parent drug(s) were measurable and relatively high. Effects were seen in the toxicology study and in reproductive and developmental toxicology studies of the clinical formulation (i.e. 100% formulation), which would indicate significant systemic exposure to the drugs and/or their active metabolites.

IV. GENERAL TOXICOLOGY:

1. **Study title:** Range finder for dermal application toxicity study in minipigs

Key study findings: In a limited evaluation of the clinical formulation and a 50% dilution of the drug product, findings were limited to mild reversible erythema at the treatment site.

Study no: 082-001
Volume #, and page #: volume 2, page 5 0041
Conducting laboratory and location: ——
——

Date of study initiation: 8/2/2000
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: —— batch no. 98K073

Formulation/vehicle: clinical formulation and vehicle

Methods (unique aspects):
Dosing:

Species/strain:	Hanford minipig
#/sex/group or time point (main study):	1/sex/dose
Satellite groups used for toxicokinetics or recovery:	All animals appear to have been held for a two week recovery period.
Age:	young adult
Weight:	26.5-30.2 (presumably kg, units were not provided)
Doses in administered units:	100% and 50% (diluted in clinical vehicle)
Route, form, volume, and infusion rate:	4 mL applied topically to a 100 or 200 cm^2 area (The report states 100 cm^2 in one place and 200 cm^2 in another.) behind the neck of the pig, applied daily, 7 days per week. The vehicle was applied to a second area of the same size on the back of each pig.

Observations and times:

Clinical signs:	observed at least once daily; treatment sites were scored for dermal irritation daily.
Body weights:	obtained on the first day of dosing and weekly thereafter
Food consumption:	measured on the first day of dosing and weekly thereafter
Ophthalmoscopy:	not performed
EKG:	not performed
Hematology:	Blood was collected for hematology prior to first dosing and 28 days later.

Clinical chemistry:	Blood was collected prior to first dosing and 28 days later for clinical chemistry.
Urinalysis:	not performed
Gross pathology:	not performed
Organs weighed:	none
Histopathology:	not performed
Toxicokinetics:	not performed
Other:	none

Results:

Mortality: none

Clinical signs: No test article-related signs were observed. Grade 1 (barely perceptible) erythema was observed in both animals treated with the clinical formulation. Grade 2 (well-defined) erythema was observed in one animal beginning on day 14. One of the animals treated with the 50% dilution of the clinical formulation exhibited grade 1 erythema. Irritation subsided in all animals after a two-week recovery period. No irritation was noted at vehicle-treated sites.

Body weights: All animals gained weight. There were no differences between the two groups in body weight.

Food consumption: All animals consumed most or all of the food provided each day.

Ophthalmoscopy: not applicable

Electrocardiography: not applicable

Hematology: Data were similar between groups. There were no control animals in the study for comparison, and no range of normal values for the testing laboratory was provided. Mean hematocrit and hemoglobin appeared to be slightly lower on day 29 than on day 1.

Clinical chemistry: Data were similar between groups. There were no control animals in the study for comparison, and no range of normal values for the testing laboratory was provided. Mean glucose and BUN appeared to be slightly increased on day 29, relative to day 1.

Urinalysis: not applicable

Organ weights: not applicable

Gross pathology: not applicable

Histopathology: not applicable

Toxicokinetics: not applicable

Summary of individual study findings:

Findings in the range finding study in minipigs were limited to low levels of erythema at the treatment site. This sign of irritation was reversible after a two week recovery period.

2. Study title: 26 week chronic dermal application toxicity study in mini pigs

N.B. The complete and final study report was not provided in the resubmission, and was submitted 2 months later (N-000 BP) only after Agency request.

Key study findings: In all treated animals, dermal irritation, consisting of dose- and duration-related erythema and edema at and adjacent to the application site that persisted into the recovery period was seen, as was generalized lymphadenitis. Small testicles and severe hypospermia were seen in the only high dose male evaluated. The "high dose" animals were the only ones treated with the undiluted clinical formulation.

Study no: 082-002
Volume #, and page #: 9/18/01 submission
Conducting laboratory and location: [redacted]

Date of study initiation: 10/4/00
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity:

- fluocinolone acetonide [redacted] hydroquinone [redacted], tretinoin [redacted] concentration, lot #J000078
- fluocinolone acetonide [redacted], hydroquinone [redacted], tretinoin [redacted] concentration, lot #J000067
- fluocinolone acetonide [redacted] ydroquinone [redacted] tretinoin [redacted] concentration, lot #L990083
- vehicle lot #C000022 (mfg. lot #00C019)

Formulation/vehicle: proposed clinical formulation, with dilutions of active ingredients to achieve dose range.
Methods:
Dosing:

Species/strain: minipigs (The protocol lists the strain as [redacted] which is the name of a supplier of Göttingen minipigs.)
#/sex/group or time point (main study): 3
Satellite groups used for toxicokinetics or recovery: 1/sex/group for recovery
Age: young adult
Weight: approximately 13 kg at study week 1
Doses in administered units: 600mg/kg of respective formulation or vehicle to approximately 10% total body surface area.

*Reviewer's comment: The sponsor previously was advised to alter doses by **enriching** the formulation or by increasing the percent body surface area treated. Instead they chose to dilute the formulation. As a result, the study may provide information on dose-relationship to effects, but only the highest dose is relevant to the clinical formulation.*

Protocol Deviation #3 indicates that the test material was spread at the dosing site using a rubber glove rather than a glass rod. It is unclear whether or not more material would have remained on the glove that would have remained on the glass rod.

Route, form, volume, and infusion rate: Topical application was made to a shaved area of the back, once daily, seven days per week, for 26 weeks. A measured dose