

was applied and spread using a latex exam glove. The dose site was washed daily with water and a cotton cloth one hour prior to scoring of the site and administration of the next dose.

Observations and times:

Clinical signs:	once daily after dosing Dosing sites were scored daily (Draize).
Body weights:	obtained on first day of dosing and weekly thereafter
Food consumption:	not measured
Ophthalmoscopy:	prior to first day of dosing and during week 26
EKG:	prior to first day of dosing and during week 26
Hematology:	prior to first day of dosing, and at end of weeks 4, 13, 26, and 30
Clinical chemistry:	prior to first day of dosing, and at end of weeks 4, 13, 26, and 30
Urinalysis:	Urine was collected one day prior to necropsy.
Gross pathology:	3/sex were sacrificed at the end of week 26. 1/sex was sacrificed at the end of week 30 (recovery animals).
Organs weighed:	liver, heart, spleen, paired kidneys, gonads, thyroid, paired adrenals, brain, paired epididymides (<i>Reviewer's comment: The protocol also included thymus and pancreas, but these were omitted per Protocol Amendment #4.</i>)
Histopathology:	see table
Toxicokinetics:	There is no mention in the report of toxicokinetic evaluation. However, blood was drawn at sacrifice. There is a protocol deviation (#2) that specifies plasma collection after weeks 4, 13, 26, and 30 (in addition to blood for clinical pathological examination that is specified in the protocol) that was submitted to _____ for analysis, however, there is no other mention of those samples or their purpose. <i>Reviewer's comment: It was later confirmed by the sponsor in a submission sent on 11/22/01 (BZ) that those samples were for toxicokinetic analysis. The data were submitted in a separate report that did not specifically identify the study from which the samples came, but was followed by a summary with study information, but conflicting information regarding the data derived from the analysis. That report was in the 7/20 submission and again in the 11/22 submission.</i>
Other:	Bartlett's test was used to test for homogeneity of variance. If variances were homogeneous, then parametric tests were used for continuous data (ANOVA followed by Dunnett's multiple comparison test). If variances were not homogeneous, then nonparametric tests were used (Kruskal-Wallis test followed by Mann-Whitney U test for pairwise comparisons).

Reviewer's comment: Given the variability that was seen in the study animals, it is unlikely that significant differences would be detectable with so few animals per treatment group.

Results:

- Mortality:** none
- Clinical signs:** Animals in all groups exhibited darkening around the eyes and skin during the first week of the study, from which *Candida albicans* was cultured. The report cites a journal article to document that this is an endemic infection in this strain of animal. (*Reviewer's comment: A copy of this article was requested from the sponsor on 10/31/01, but was not provided in the sponsor's response on 11/22/01*) Animals were treated topically with nystatin/polymixin B as needed. (*Reviewer's comment: The additional treatment could have confounded the study.*)
- Observations at the dosing site included a dose-dependent incidence/severity of dark brown/red crust in test article-treated animals, which was softened with water and removed approximately every two weeks.
- Very slight erythema and/or edema (grade 1) were observed at the mid-dose beginning on day 20, which progressed to slight (grade 2) by day 38. In the recovery animals, erythema was resolved on day 189 (27 weeks) for males and day 191 (27 weeks, 2 days) for females.
- In the high dose group, grade 1 (very slight) erythema/edema was observed as early as day 7, progressing to grade 2 (slight) by day 20 and to grade 3 (moderate) by day 163. Erythema resolved by days 191-194.
- Body weights:** The report states that there was no significant difference in mean body weights between groups. However, the body weights of the animals in the high dose group appeared to lag behind those of the other groups. Body weight gains were not reported, and there was no graphical representation of body weights or body weight gains. These data were requested from the sponsor on 10/31/01, and the data provided on 11/22/01 indicated that body weight losses were seen in all groups at weeks 4, 13, 17, and 26.
- Food consumption:** not measured
- Ophthalmoscopy:** There were no treatment-related findings.
- Electrocardiography:** Sinus tachycardia was noted at the pre-study evaluation and at the end of the study and was considered to be stress-related.
- Hematology:** No significant differences in hematology or coagulation parameters were reported.
- Clinical chemistry:** No significant differences in clinical chemistry parameters were reported.
- Urinalysis:** No significant differences in specific gravity, pH, ketones, protein, bilirubin, glucose, and occult blood were reported. Data were not provided in the report, but were requested from the sponsor on 10/31/01. The data were provided in the 11/22/01 submission and there did not appear to be any biologically significant findings. It was notable that urine specific gravities for many animals in the treated and control groups were in the isosthenuric range.

Organ weights: No significant differences were reported. There did appear to be a dose-related decrease in testicular weight (absolute and relative) that was most prominent in the high dose group. The sponsor did not separate organ:body weight ratios for gonads by sex; those values should be separated by sex and the statistical analysis should be performed without pooling those values for both sexes within groups. The latter was requested on 10/31/01, and provided on 11/22/01. Decreases in relative testis:body weight ratios appeared to be dose dependent, and the relative ovary weight in the "high dose" females appeared to be low. No information on statistical differences was provided.

Gross pathology: Findings in control animals consisted of dark skin and crusting at or adjacent to the treatment site and dark lymph nodes. High dose animals had similar findings, but skin findings were increased in incidence and included yellow and red coloration in some animals. One high dose male had bilateral small testes; no data were recorded for the other high dose males.

No gross pathology data were reported for the low and mid-dose animals.

Histopathology: Full histopathologic evaluation was performed only for control and high dose groups. Skin and lymph nodes were evaluated in all groups.

Subacute dermatitis was noted in the skin at anterior and posterior areas of the application sites. This finding was dose-related in incidence and severity and persisted in the recovery period. Subacute dermatitis was present adjacent to the dose sites with similar or lesser severity. The incidence and severity appeared to be greater in females than in males. The sponsor considered the finding to be only significant in high dose males and mid- and high dose females. Subacute inflammation of inguinal skin in study animals was not dose-related and was not considered to be test-article related.

Lymphadenitis was considered to possibly be test article-related. It was present in all groups and in all lymph nodes, but might have been associated with *Candida* infection.

In the high dose group, one of the three males exhibited severe bilateral hypospermia, in association with the gross finding of small testicles (*Reviewer's comment: This finding is consistent with systemic retinoid exposure.*). No data were entered for the other two main study animals or the recovery animal for testicular findings.

Additional findings in both control and treated groups were not likely to be related to test article treatment. These included subacute inflammation of the esophagus and intestines (*Reviewer's comment: This might have been consistent with parasitism.*), subacute inflammation of the kidneys, and necrotizing myocarditis.

Reviewer's comment: At the time of protocol review, the sponsor was requested to include examination of the growth plate if animals were young enough to still have open growth plates at the start of the study. This request was not addressed.

Toxicokinetics: No data were reported, although it appears that samples were taken and sent for analysis.

A separate report was submitted for determination of blood concentrations of the active ingredients in minipig plasma. Neither the study number nor the group assignments of the animals were noted in the report. The report was followed by a summary that did identify the study and stated that concentrations of all three drug substances were either 0.0 or BLQ. This statement is not entirely accurate. 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 a control animal and the lack of detection of endogenous tretinoin in most animals at most time points, the validity of the pharmacokinetic information from this study appears questionable. No assay was performed for metabolites.

Summary of individual 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. Only the "high dose" was relevant to the proposed clinical use; others were dilutions of clinical formulation.

Toxicology summary:

Only one toxicology study was performed. That study was a topical application study in minipigs with treatments consisting of the clinical formulation and dilutions thereof. 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. The gonads of only one male treated with the clinical formulation were evaluated; findings in that animal included small testicles and severe hypospermia. Only the high dose in this study was relevant to the proposed clinical use; others were dilutions of clinical formulation.

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. The article states that hydroquinone is cytotoxic to rat hepatoma cells in culture and nephrotoxic to rats treated in vivo. Toxicity associated with oral administration includes dose-dependent mortality, lethargy, tremors, and increased liver and kidney weights. Topically, hydroquinone was associated with slight to severe irritation. The conclusion of the expert panel was that hydroquinone was safe at concentrations $\leq 1\%$ for aqueous formulations designed for discontinuous, brief use, followed by rinsing from the skin and hair. They stated that it should not be used in leave-on products.

Toxicology conclusions:

Adverse findings include local irritation of the skin at the site of application. Testicular effects in high dose male animals may indicate significant systemic exposure to the retinoid component of the formulation.

A literature review of hydroquinone provided by the sponsor states that hydroquinone is only safe at concentrations $\leq 1\%$ in aqueous formulations designed for discontinuous, brief use, followed by rinsing from the skin and hair. It stated that it should not be used in leave-on products. The proposed drug product would appear to fall in the latter category.

Histopathology Inventory for NDA #21-112

Study	082-002			
Species	mini pigs			
Duration	6 months			
Adrenals	X*			
Aorta	X			
Bone Marrow smear	X			
Bone (femur)	X			
Brain	X			
Cecum				
Cervix				
Colon	X			
Duodenum	X			
Epididymis				
Esophagus	X			
Eye	X			
Fallopian tube	X			
Gall bladder	X			
Gross lesions	X			
Harderian gland				
Heart	X			

Ileum	X			
Injection site				
Jejunum	X			
Kidneys	X			
Lachrymal gland				
Larynx				
Liver	X			
Lungs	X			
Lymph nodes, cervical	X ^a			
Lymph nodes mandibular	X ^a			
Lymph nodes, mesenteric	X ^a			
Mammary Gland				
Nasal cavity				
Optic nerves	X			
Ovaries	X			
Pancreas	X			
Parathyroid	X			
Peripheral nerve	X			
Pharynx				
Pituitary	X			
Prostate	X			
Rectum	X			
Salivary gland				
Sciatic nerve				
Seminal vesicles				
Skeletal muscle				
Skin	X ^b			
Spinal cord	X			
Spleen	X			
Sternum				
Stomach	X			
Testes	X			
Thymus	X			
Thyroid	X			
Tongue				
Trachea	X			
Urinary bladder	X			
Uterus	X			
Vagina				
Zymbal gland				

- Peritoneal cavity

Standard List				

X, histopathology performed

*, organ weight obtained

^a lymph nodes were examined, but the specific nodes are not identified.

^b two sites from dosing area and two sites adjacent to dosing area

V. GENETIC TOXICOLOGY:

No genetic toxicology studies were submitted.

Information from the published literature that was submitted to the NDA indicates that hydroquinone was mutagenic in the Ames assay in Salmonella strains TA 104 and TA 102. Treatment with hydroquinone resulted in increased DNA single strand breaks in rat hepatocytes, gene mutations, chromosomal aberrations and sister chromatid exchanges. In human cells *in vitro*, induction of DNA strand breaks, sister chromatid exchanges and chromosomal aberrations were seen. Hydroquinone induced micronuclei in human lymphocytes in the absence of metabolic activation. *In vivo*, in the mouse micronucleus assay, hydroquinone induced micronuclei and chromosomal aberrations in several studies. Hyperploidy and chromosome loss also were seen. One study indicated that hydroquinone could induce micronuclei in fetal liver cells after transplacental exposure.

Tretinoin was negative in the Ames test in the presence and absence of S9, using *Salmonella typhimurium* strains TA1535, TA 1537, TA 1538, TA98, and TA 100.

Genetic toxicology conclusions:

Hydroquinone has been demonstrated to be mutagenic and/or clastogenic in all of the assays that make up the ICH genetic toxicology test battery. Additionally, it may act as a mutagen in the developing fetus.

Labeling recommendations:

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.

VI. CARCINOGENICITY:

No carcinogenicity studies were submitted.

The sponsor has included several articles from the published literature that demonstrate carcinogenic potential of hydroquinone. Studies conducted by the National Toxicology Program have shown hydroquinone to produce renal tubule adenomas and to exacerbate spontaneous chronic progressive nephropathy in male F344 rats. Atypical tubule hyperplasias and adenomas were located in areas of severe chronic progressive nephropathy. In female rats, there was a significant increase in the incidence of mononuclear cell leukemias at 50 mg/kg (high dose), but this incidence was within the range of historical controls. The results in males were confirmed in

a second, independent study of dietary hydroquinone in F344 rats. Long-term feeding of hydroquinone to rats led to aplastic anemia, liver cord cell atrophy and gastric ulceration.

A review article stated that skin painting studies in mice showed hydroquinone to be inactive as an initiator of skin carcinogenesis. Bladder implantation studies of hydroquinone in mice demonstrated an increase in the incidence of bladder carcinomas. An NTP oral study in B6C3F₁ mice demonstrated increased relative liver weights in males at 50 and 100 mg/kg and in females at 100 mg/kg. The incidence of hepatocellular adenomas was significantly greater than controls at both doses in females. Dose-dependent hepatic morphological changes included anisokaryosis and increased frequency of multinucleated cells. Another study of dietary hydroquinone (0.8%) in mice demonstrated significantly increased incidence of hepatocellular adenomas in male B6C3F₁ mice and three renal adenomas in thirty treated male mice, but no increase in any tumor type in females. Hepatic centrilobular hypertrophy and forestomach hyperplasia were also seen.

IARC evaluation of studies of hydroquinone led to the conclusion that there was some evidence for carcinogenicity in experimental animals. It was also concluded that there was inadequate evidence of carcinogenicity in humans, and that hydroquinone was not classifiable as to its carcinogenicity to humans.

Another published study provided by the sponsor evaluated a metabolite of hydroquinone, 2,3,5-tris(glutathion-S-yl) hydroquinone, in Eker rats, which carry a mutation that predisposes them to renal tumors. In rats treated for four months, tubular dysplasias were found from which adenomas arose. After 10 months, there were significant increases in basophilic dysplasias, adenomas, and renal cell carcinomas in treated animals, relative to controls.

The sponsor has provided a copy of Forbes, et al. Cancer Letters 7:85-90, 1979. The article describes a photo co-carcinogenicity study of tretinoin in which the incidence of UV-induced skin tumors was increased, and the time of onset shortened, relative to control animals treated with UV irradiation alone.

Carcinogenicity conclusions:

There is evidence in animals that hydroquinone and/or its metabolites may be carcinogenic. In photo co-carcinogenicity studies conducted using concurrent or intercurrent solar simulated irradiation with test article application, tretinoin has been positive for enhancement of photocarcinogenesis.

Recommendations for further analysis:

Since the drug is proposed to be used clinically for long periods of time (>6 months), dermal carcinogenicity data should be provided for the clinical formulation.

Labeling Recommendations:

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.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Reviewer's comment: The sponsor was previously advised in several protocol discussions to increase doses in nonclinical studies by increasing the concentration of the drug substances in the test article or by increasing the surface area to be treated, rather than by increasing the volume of material applied to the same surface area. The sponsor chose to treat maximal surface area in all dose groups and to dilute the formulation to control dose-limiting irritation.

It was also suggested that the sponsor consider a combined segment I/II/III protocol or a combined segment I/II study in order to ensure sure steady-state systemic exposures would be achieved at the critical developmental time windows. Treatment in the definitive segment II developmental toxicity study in rats was begun on gestation day (GD) 4 instead of GD 6, which may have been sufficient.

1. Study title: Study of fertility and early embryonic development to implantation in rats

Key study findings: There was no effect of treatment with a 10-fold dilution of the drug product on copulation and fertility indices, sperm count and sperm motility. Possible effects were seen on estrous cycling and pre- and post-implantation loss.

Due to dilution of the drug product, overestimation of the doses, and limited duration of daily drug exposure, the study is inadequate to assess the effects of the full strength drug product on fertility and early embryonic development.

Study no.: 7169-103

Volume #, and page #: volume 3, page 5 0161

Conducting laboratory and location: _____

Date of study initiation: 07 December 2000

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: 10% dilution of clinical drug product, lot no. J000078

vehicle, lot no. C000022

Formulation/vehicle: 10% dilution of clinical formulation, clinical vehicle

Methods:

Species/strain: CrI:CD®(SD)IGS BR rats

Doses employed: 0.1, 0.2, 0.4, 0.8 g/kg/day of a 10% dilution of the drug product, control – 0.8 g/kg/day of vehicle

Route of administration: topical to a 4x5 cm shaved area on the back

*Reviewer's comment: Body weights ranged approximately from 225-300 g for females and 350-500g for males. It should be noted that the highest applied daily dose of 800 mg/kg * .225-.5 kg = 180-400 mg of test article. When applied to the clipped area, 180-400 mg/20 cm² = 9-20 mg/cm², this application would be 4.5-10 times as thick as the clinically applied formulation on human skin (2 mg/cm²). This results in an overestimation of the dose, since much of the applied material*

cannot diffuse through the thick layer of vehicle to contact the skin. Additionally, the material was diluted 10-fold from the clinical formulation, and was applied for a daily duration of 6 hours, rather than a clinically relevant 8-12 hours. For these reasons, this study did not result in sufficient exposure to the drug product to be adequate to assess effects on fertility or early embryonic development.

Study design: The dose was applied directly to the skin, spread with a glass rod, and covered with a semi-occlusive bandage. After 6 hours, the site was wiped with distilled water and dried with a towel. Males were dosed for 28 days prior to mating, through the mating period, and through the day prior to termination. Females were dosed at least 14 days prior to mating, through the mating period, and through gd 7. Rats were separated during the mating period for the 6-hour dosing interval.

Number/sex/group: 20

Parameters and endpoints evaluated: Rats were observed twice daily for mortality and clinical signs. Physical examinations were conducted at weighing intervals. Dermal irritation was scored at body weight intervals, just prior to application of the next dose. Males were weighed prior to initiation of dosing, twice weekly during treatment, and at termination. Females were weighed prior to initiation of dosing, twice weekly during the pre-mating and mating phases, and at GD 0, 3, 7, 10, and 13. Food consumption was measured weekly, and for females at body weight intervals during gestation.

During the mating period, females were examined daily for estrous cycle assessment and to detect evidence of mating. On GD 13, females were sacrificed and necropsied. Uteri were examined for implantation sites, live fetuses, early and late resorptions and any abnormalities of the placenta or amniotic sac. The ovaries were examined for corpora lutea. Uteri and ovaries were retained in formalin for animals that did not appear pregnant, but were not examined for implantation sites.

Males were sacrificed and necropsied at termination. Right epididymis, right testis, left epididymis, left testis, seminal vesicles (with coagulating gland), and prostate were weighed and preserved in formalin. The first ten surviving males per group were evaluated for reproductive capacity. For those animals, the right vas deferens was used for motility assessment and the right testis was fixed, cross-sectioned and stored for possible future spermatogenic staging.

Results:

Mortality: - One control male and one 0.4 g/kg/day male were found dead on days 18 and 43. Another 0.4 g/kg male was sacrificed *in extremis* on day 55, but the report states that clinical observations were limited to the intermittent appearance of sores from days 24-52. One moribund female at 0.4 g/kg/day was sacrificed on GD 6. Another 0.4 g/kg female was noted as a mistimed pregnancy and was sacrificed on GD 5.

Clinical signs: The female sacrificed on GD 6 exhibited hypoactivity, pale and thin appearance, hunched posture, cold to the touch, urine stains, and

chromodacryorrhea. One 0.1 g/kg/day female was noted to have red/black vaginal discharge on GD 0, but was pregnant and had viable fetuses at caesarean section.

— In males, dermal irritation (erythema) was dose-related in incidence and severity. Desquamation and scaling were seen in all treated groups, and fissuring was seen in a few animals at 0.4 and 0.8 g/kg/day. In females, very slight erythema, desquamation, and scaling were noted in all treated groups. Well-defined erythema and very slight edema were noted in one to two animals in the 0.4 g/kg/day group. Desquamation and scaling were seen in a few animals in all treated groups.

Body weight: Mean body weights were significantly lower than control in males at 0.4, and 0.8 g/kg/day, as a result of lower body weight gains during the treatment period.

In females, during the pre-mating period, there was a dose-related decrease in mean body weight gain at 0.2 and 0.4 g/kg/day and a mean body weight loss (less than 5%) at 0.8 g/kg/day. During gestation, mean body weights continued to be significantly lower than control at those three doses, although there were no differences in body weight gain from control during this period.

Food consumption: Food consumption was decreased in females at 0.8 mg/kg/day during the second week of the pre-mating period.

Toxicokinetics: not performed

In-life observations: Estrous cycling was reported to be unaffected by treatment with a 10-fold dilution of the drug product. However, one dam in each of the 0.1 and 0.2 g/kg/day groups and two in the 0.4 g/kg/day group were in prolonged estrus. All three were reported to be pregnant at necropsy. Summary estrous cycling data were not provided. Copulation and fertility indices were not affected by treatment.

Terminal and necroscopic evaluations: In males, a moderately dilated right renal pelvis was noted in one 0.4 g/kg/day rat, and an irregularly shaped right epididymis was noted in a 0.1 g/kg/day rat; both were considered to be incidental findings. Findings in females included pale spleen and kidney and empty and discolored stomach in the one dam sacrificed *in extremis* (This animal was found to be gravid), hydrometra in two 0.1 g/kg/day females and a moderately dilated left renal pelvis in a 0.8 g/kg/day female.

Mean organ weights were similar across groups, but relative testis and epididymis weights were increased in the 0.4 and 0.8 g/kg groups due to decreased mean final body weights.

— Caesarean section data revealed that pregnancy rates and the number of live fetuses were similar across groups. While not considered statistically significant, pre-implantation loss seemed to be greater in the 0.2 and 0.4 g/kg/day groups, and post-implantation loss (early resorptions) seemed to be increased in the 0.4 g/kg/day group. Sperm counts and motility were not affected by treatment. No histological examination was performed for male reproductive organs.

Summary of individual study findings:

The report states that the NOEL for male toxicity was 0.2 g/kg/day and the NOEL for female toxicity was 0.1 g/kg/day, based on body weight findings. The NOEL for implantation and embryo-fetal viability was reported as 0.8 g/kg/day of a 10-fold dilution of the drug product. However, there may have been an effect on estrous cycling at 0.1, 0.2, and 0.4 g/kg/day and a slight increase in pre- or post-implantation losses at 0.2 and 0.4 g/kg/day. No effects were noted on copulation and fertility indices, sperm counts, and sperm motility. No histopathological examination of male reproductive organs was performed. Because the product was diluted and the doses were grossly overestimated, and because evaluation was incomplete, the study is inadequate to assess the effects of the full strength drug product on fertility and early embryonic development.

2. Study title: Dose range-finding developmental toxicity study in rats

Key study findings: This study employed improper dosing and inadequate daily exposure duration to the drug product. Maternal toxicity was observed as body weight reductions, dermal irritation, and moribundity resulting in early sacrifice of some animals. Dose-related effects on the offspring included decreased fetal weight, pre- and post-implantation loss, decreased live fetuses, and retinoid-related malformations in groups treated with the clinical formulation and 75, 50, and 25% dilutions thereof.

Study no.: 7169-101

Volume #, and page #: volume 4, page 5 0529

Conducting laboratory and location: _____

Date of study initiation: 5 September 2000

GLP compliance: no, but performed in a GLP laboratory using those practices as a guide.

QA reports: yes () no (X)

Drug, lot #, radiolabel, and % purity: test article (fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05% - 100% concentration) lot #K990075; vehicle lot # 00C019

Formulation/vehicle: appears to be clinical formulation and vehicle

- 25% concentration: fluocinolone acetonide 0.0025%, hydroquinone 1.0%, tretinoin 0.0125% (clinical 100% formulation provided by the sponsor was diluted by the contract laboratory in vehicle provided by the sponsor.)
- 50% concentration: fluocinolone acetonide 0.005%, hydroquinone 2.0%, tretinoin 0.025%
- 75% concentration: fluocinolone acetonide 0.0075%, hydroquinone 3.0%, tretinoin 0.0375%
- 100% concentration: fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%

Methods:

Species/strain: CrI:CD®(SD)IGS BR rats

Doses employed: 2g/kg/day of vehicle, 25%, 50%, 75% or 100% clinical formulation

Route of administration: topical to a shaved area 4.5 x 5.5 cm, but the report later states that the area of application was only 2.5x4.5 cm (11.25 cm²).

*Reviewer's comment: Body weights at the beginning of the study were approximately 225 g. For the group treated with the clinical formulation, the applied daily dose of 2 g/kg * 0.225 kg = 0.45 g or 450 mg of drug product. When applied to the clipped area, 450 mg/11.25 cm² = 40 mg/cm², this application would be twenty times as thick as the clinically applied formulation on human skin (2 mg/cm²). This results in an overestimation of the dose, since much of the applied material cannot diffuse through the thick layer of vehicle to contact the skin. The relevant dose would be closer to 1/20 of that claimed, or approximately 0.1 g/kg (HED = 0.017 g/kg, or 0.6 times the sponsor's estimated clinical use for the high dose group only).*

Study design: Doses were applied once daily on GD 6-17. The test article was covered with gauze. After six hours, the area was wiped with wet gauze and dried with a towel.

Number/sex/group: five pre-mated female rats per group

Parameters and endpoints evaluated:

Rats were checked daily for survival and clinical observations. Dermal irritation scoring was performed prior to dose application on GD 6, 7, 9, 11, 13, 15, 17, and on GD 19 and 20. Body weights and food consumption were measured on GD 0, 4, 6, 8, 12, 16, 18, and 20. At necropsy on GD 20, uteri were examined for implantations, number of live and dead fetuses and resorptions, and any abnormalities of the placenta and amniotic sac. Ovaries were examined for number of corpora lutea. Live fetuses were sexed, weighed, and evaluated for external abnormalities.

Results:

Mortality: There were six unscheduled deaths. One moribund sacrifice was in group 4 (75% formulation), four moribund sacrifices were in group 5 (clinical formulation), and the remaining group 5 animal was sacrificed because it aborted.

Clinical signs: In groups 3 and 4 (50 and 75% formulations, respectively), thin appearance and red/black vaginal discharge were reported as compound related observations.

Dose-related dermal irritation (erythema) was reported. Desquamation and scaling were found in all treated animals, and fissuring was seen in all animals in groups 3, 4, and 5 (50%, 75%, and 100% formulations).

Body weight: Body weights were decreased in all treated groups by GD 12. All but the highest dose group appeared to maintain and/or exhibit a slight body weight gain for the rest of the study. A dose-related body weight loss was recorded for treated groups over the dosing period.

At termination, there was a dose-related decrease in mean gravid uterine weight, corrected body weight and net body weight change in treated animals.

Food consumption: Food consumption was somewhat less than control for most groups during the treatment period, but was without consistent dose relationship. The decreases were slight and appeared early in the treatment period.

Toxicokinetics: not performed.

In-life observations: see above

Terminal and necroscopic evaluations:

Dams: - Findings reported as treatment-related included small spleens in 3-4 animals in each of groups 3, 4, and 5 (50%, 75%, and 100% formulations). Sporadic findings of pale areas in the liver, thickened, empty or dark areas in the stomach, raised areas or discoloration in the kidneys, thickened uterus, and pale adrenals were reported in groups 4 and 5.

All rats were pregnant, but the one group 5 animal that aborted had no viable fetuses. Three of the four group 4 animals at termination had litters with no viable fetuses.

Offspring:

Increased post-implantation loss (increased early and late resorptions) and decreased live fetuses were seen in groups 3 and 4. Dose-related decreases in mean fetal weight were seen in all treated groups.

Fetal alterations included pale fetuses in all treated litters and a dose-related increase in external malformations including fetal edema, protruding tongue, cleft palate, and umbilical hernia. Litter incidences were 80-100% in groups 2 through 4.

Summary of individual study findings:

Maternal effects included dose-related effects on body weight, dermal irritation, and mortality. Systemic toxicity in dams appeared to be more severe than would be expected with similar concentrations of single active ingredients, in this reviewer's opinion. The one surviving animal in group 5 aborted and had no viable fetuses. Three of the four litters at termination in group 4 had no viable fetuses. External alterations including typical retinoid malformations were seen in litters from surviving treated animals. Decreased numbers of live fetuses were seen in groups treated with 50% and 75% concentrations of the drug product, and decreased fetal weight was observed in all treated groups.

This study employed improper dosing (dilution of test article and overly thick applications) and inadequate daily exposure duration to the drug product. Only the "high dose" group was exposed to the clinical formulation.

3. Study title: Rat Developmental toxicity study

Key study findings: At 0.4 g/kg/day of the clinical formulation, applied approximately 2.25 times as thick as described for clinical application, findings included decreased fetal weight, increased post-implantation loss, decreased number of live fetuses, decreased fetal weight, and retinoid-associated malformations. Maternal findings included dermal irritation, lower food consumption and lower body weight gains than controls.

Study no.: 7169-104

Volume #, and page #: volume 5, page 5 0675

Conducting laboratory and location:

Date of study initiation: 06 October 2000

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity:

- 10% formulation: fluocinolone acetonide 0.001%, hydroquinone 0.4%, tretinoin 0.005% (lot #J000078 provided by the sponsor.)
- 25% concentration: fluocinolone acetonide 0.0025%, hydroquinone 1.0%, tretinoin 0.0125% (lot #J000068 provided by the sponsor.)
- 50% concentration: fluocinolone acetonide 0.005%, hydroquinone 2.0%, tretinoin 0.025% (lot #J000067 provided by the sponsor.)
- 100% concentration: fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05% (lot #L99083 provided by the sponsor.)
- vehicle lot #00C019

Formulation/vehicle: appears to be clinical formulation (and dilutions thereof in clinical vehicle) and clinical vehicle

Methods:

Species/strain: pre-mated female Crl:CD®(SD)IGS BR rats

Doses employed: 0.4 g/kg/day of vehicle, 10%, 25%, 50% or 100% formulations, respectively

Route of administration: topical to a shaved area of skin 4 x 5 cm (20 cm²)

*Reviewer's comment: This report does not specify application of the test article to a smaller area within the shaved area as in the range-finding study. 400mg/kg * 0.225 kg (approximate starting weight) = 90 mg applied to 20 cm² = 4.5 mg/cm², for a layer approximately 2.25 times the thickness of the proposed clinical application. Correction for the thickness of application would result in a dose of 178 mg/kg (HED=30 mg/kg, or 1.1 times the clinical dose for the high dose group only). Only the high dose group is relevant to the clinical product, as the lower "doses" were achieved by dilution of the drug product.*

Study design: The test article was applied directly to the skin and spread with a glass rod once daily from GD 4 through GD 17. The site was then covered with a semi-occlusive bandage. After approximately six hours, the site was washed with distilled water and dried with a towel. On GD 20 all surviving females were sacrificed.

Number/sex/group: 25/group

Parameters and endpoints evaluated: Animals were observed at least twice daily for mortality and clinical signs. Dermal irritation was scored every other day beginning on GD 4 prior to administration of test article and on GD 20. Body weights were recorded on GD 0, 4, 6, 8, 10, 12, 14, 18, and 20, and food consumption was recorded at the same intervals, beginning on GD 4. On GD 17, the first five rats per group were bled at least 15 minutes after the completion of dosing for toxicokinetics (*Reviewer's comment: It is unclear if this time point is intended to represent time of peak concentration of any of the active ingredients.*). At sacrifice, dams were examined grossly, uteri were excised, weighed, and examined for implantation sites, live and dead fetuses, resorptions, and abnormalities of the placenta or amniotic sac. Ovaries were examined for the number of corpora lutea. Fetuses were sexed, weighed, and examined for external

alterations. One half of the fetuses were examined for soft tissue alterations by Wilson's technique, and the other half were examined for skeletal alterations using the Alizarin Red S staining method.

Results:

Mortality: There were no test article-related mortalities. One animal in the 50% group was sacrificed early following an injury to a paw.

Clinical signs: There was an increased incidence of thin appearance in the 100% group, although this finding was sporadic and limited to a few animals. Red/black vaginal discharge was noted in one animal in each of the 10% and 25% groups and in three animals in the 100% group. All of these animals still had viable fetuses at Caesarean section, although one of the 100% animals had nine late resorptions.

There was a dose-related increase in the incidence and severity of erythema, although it was limited to very-slight to well-defined in the majority of animals. Very slight to slight edema was noted in the 50% and 100% groups, desquamation and scaling were observed in all treated groups and in one control animal, and fissuring was observed in six rats in the 100% group.

Body weight: Mean maternal body weights in all treated groups were significantly lower than controls from GD 10 through GD 20. The differences were mostly due to lower weight gains in treated animals. Body weight gains were significantly lower than control in all treated groups and were dose-related. During the dosing period, a slight body weight loss was recorded in the 100% group for the early days of treatment, but body weight gains were made from GD 10 onward.

Gravid uterine weights were significantly decreased in the 25%, 50%, and 100% groups. Mean corrected terminal body weights were significantly decreased in all treated groups, however, the difference was less than 10% in the 10% treatment group.

Food consumption: There was a dose-related decrease in food consumption during the treatment period that was significant in the 25%, 50%, and 100% groups.

Toxicokinetics: These data were not included in the report, but were provided separately.

Assay was by HPLC for hydroquinone with a calibration range of 100-1250 ng/mL (*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 5.0-100 ng/mL. 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 _____ (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 (7.698 ng/mL). No assay was performed for metabolites.

In-life observations: see above

Terminal and necroscopic evaluations:

Dams: There were no abortions or early deliveries. There was an increase in post-implantation loss in the group treated with the clinical formulation that was associated with an increase in early and late resorptions and a decrease in the number of live fetuses.

Maternal necropsy findings included a shared placenta in one 25% rat, and a pale right kidney containing granular material and bladder calculi in a 50% rat.

Offspring: Dose-related decreases in mean fetal weights were significant in the 25%, 50%, and 100% groups. Fetal external variations were limited to observations of "pale fetus." There was an increased litter incidence at 25%, 50%, and 100%, which was significant at 100%, and fetal incidence was significant at all three doses. The incidence of total external malformations was increased in the 100% group. Malformations included fetal edema, protruding tongue, open eyes, anophthalmia, agnathia, astomia, aglossia, and umbilical hernia. Total soft tissue variations and the incidence of retinal folding were increased in 100% litters, and dilation of the third ventricle was observed in that group. Groups treated with the 25% dilution had single fetal incidences of situs inversus, ventricular septal defect and hermaphroditic fetus, each in different litters. The incidence of total soft tissue malformations was increased in groups treated with 50% (fetal) and 100% (fetal and litter) formulations. The fetal incidence of cleft palate was significantly increased at both of those doses, and the litter incidence was significantly increased at 100%. The fetal incidence of retinal dysplasia was increased significantly at 100%. The incidence of skeletal variations, consisting of incomplete or delayed ossification, was significantly increased in all treated groups. Skeletal malformations were increased in the 50% and 100% groups, but the difference was not statistically significant. These consisted of a missing skull bone at 100% and vertebral and sternebral anomalies.

Summary of individual study findings:

The sponsor states that the NOAEL for maternal effects was < 10%, the NOAEL for embryo-fetal toxicity was 10%, and the NOAEL for teratogenicity was 25%. However, dose groups were treated with dilutions of the clinical formulation were not relevant to the clinical formulation. Other inappropriate factors in the dosing regimen included application of too thick a layer to the treatment site and too short a daily duration of exposure.

At 0.4 g/kg/day of the clinical formulation, applied approximately 2.25 times as thick as described for clinical application, findings included decreased fetal weight, increased post-implantation loss, decreased number of live fetuses, decreased fetal weight, and retinoid-associated malformations. Maternal findings included dermal irritation, lower food consumption and lower body weight gains than controls.

4. Study title: Dose range-finding developmental toxicity study in rabbits

Key study findings: Adverse effects on fetal survival and growth in groups treated with the clinical formulation at full and half strength were observed in the absence of maternal toxicity. Gravid uterine weights and fetal body weights were decreased in both of those groups. In the group treated with the clinical formulation, increased postimplantation loss and the number of late resorptions were seen, and the number of live fetuses was decreased. One abortion was seen in the group treated with the clinical formulation.

Study no.: 7169-102

Volume #, and page #: volume 6, page 5 1140

Conducting laboratory and location: _____

Date of study initiation: 3 October 2000

GLP compliance: no, but performed in a GLP laboratory using those practices as a guide.

QA reports: yes () no (X)

Drug, lot #, radiolabel, and % purity: test article (fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05% - _____) lot #K990075; vehicle lot # 00C019

Formulation/vehicle: appears to be clinical formulation and vehicle

- 10% formulation: fluocinolone acetonide 0.001%, hydroquinone 0.4%, tretinoin 0.005% (clinical 100% formulation provided by the sponsor was diluted by the contract laboratory in vehicle provided by the sponsor.)
- 20% concentration: fluocinolone acetonide 0.002%, hydroquinone 0.8%, tretinoin 0.01%
- 50% concentration: fluocinolone acetonide 0.005%, hydroquinone 2.0%, tretinoin 0.025%
- 100% concentration: fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05%

Methods:

Species/strain: Hra:(NZW)SPF rabbits

Doses employed: daily dose of 1 g/kg of the respective formulation on GD 7-20; Group 1 – control; Groups 2-5 – 10, 20, 50, and 100% formulations, respectively. (Reviewer's comment: Only the high dose, the 100% formulation, is relevant to the clinical formulation.)

Route of administration: topical to clipped areas on the back approximately 10 cm x 10 cm in area (100 cm²). The test article was spread over the area with a glass rod and a stainless steel spatula.

*Reviewer's comment: The report states that this represents >10% total body surface area, but it is considerably less than 10% TBSA. Assuming TBSA for the average 1.8 kg rabbit = 0.15 m² * 10,000 cm²/m² = 1500 cm², then 10% TBSA = 150 cm². However, body weights in these animals were approximately 4 kg, and 100 cm² would be an underestimate of even greater magnitude in animals of this size.*

It should also be noted that the applied daily dose of $1 \text{ g/kg} * 4 \text{ kg} = 4 \text{ g}$ or 4000 mg of drug product. When applied to the clipped area, $4000 \text{ mg}/100 \text{ cm}^2 = 40 \text{ mg/cm}^2$, this application would be twenty times as thick as the clinically applied formulation on human skin (2 mg/cm^2). This results in an overestimation of the dose, since much of the applied material cannot diffuse through the thick layer of vehicle to contact the skin. The relevant dose would be closer to 1/20 of that claimed, or approximately 0.05 g/kg ($\text{HED} = 0.02 \text{ g/kg}$, or 0.75 times the sponsor's estimated clinical use for the high dose group only).

The rabbits were collared for a 6-hour exposure period. At the end of the six hours, the sites were washed with wet gauze and wiped dry.

Study design: The rabbits were sacrificed at GD 29 and necropsied.

Number/sex/group: 5 pre-mated females per group

Parameters and endpoints evaluated:

Animals were checked daily for survival and clinical observations.

Dermal irritation scoring was performed prior to dose application on GD 7, 9, 11, 13, 15, 17, and 19, and on GD 21, 23, 25, 27, and 29 prior to sacrifice. Body weights and food consumption were measured on GD 0, 4, 7, 9, 11, 13, 15, 17, 19, 21, 24, 27, and 29. At necropsy, uteri were examined for number of live and dead fetuses and resorptions, and ovaries were examined for number of corpora lutea. Live fetuses were sexed, weighed, and evaluated for external abnormalities (Reviewer's comment: Neither the protocol nor the report state that examination of the palate was performed.).

Results:

Mortality: One group 5 animal aborted and was sacrificed on GD 24 and evaluated at that time.

Clinical signs: Clinical observations were limited to sporadic observations of brown discoloration of the hair coat in 1-3 animals in groups 4 and 5 between GD 14 and GD 29.

Dermal observations included a dose-related increase in dermal irritation (erythema and edema). Atonia and desquamation were observed in all treated groups, and fissuring was seen in one group 3 animal and in all animals in groups 4 and 5.

Body weight: Body weights in all treated groups were within 5% of control. Body weight gains were slightly lower for groups 4 and 5 at the end of the treatment period (Reviewer's comment: Terminal body weights for these animals were not different from controls when corrected for uterine weight. This confirmed that the decrease in body weight gain was entirely accounted for by lower gravid uterine weights and lower fetal body weights in those groups).

Food consumption: Food consumption was similar in all groups.

Toxicokinetics: No data were provided.

In-life observations: see above

Terminal and necroscopic evaluations:

Dams: For the group 5 animal that aborted on GD 24, the report states that iatrogenic pulmonary lesions were found on necropsy. Since treatment in

- this study was topical, and no procedures are described in the report that
- might have pulmonary effects, it is unclear exactly what the sponsor means
- by this. One group 3 animal had a distended pericardial sac.

Mean gravid uterine weights were decreased in groups 4 and 5 by greater than 10%.

Offspring: Fetal body weights were decreased in groups 4 and 5. In group 5, mean postimplantation loss (25%) and the number of late resorptions were increased. The number of live fetuses in group 5 was decreased.

Summary of individual study findings:

Only group 5, the "high dose", was treated with the proposed clinical formulation; the remaining groups were treated with dilutions of that formulation. Therefore, only results seen in the "high dose" group are relevant to clinical use. Daily topical exposure was limited to six hours per day, which is likely to underestimate clinical daily topical exposure.

Adverse effects on fetal survival and growth in groups treated with the clinical formulation at full and half strength were observed in the absence of maternal toxicity. Gravid uterine weights and fetal body weights were decreased in both of those groups. In the group treated with the clinical formulation, increased postimplantation loss and the number of late resorptions were seen, and the number of live fetuses was decreased. The cause of one abortion in the group treated with the clinical formulation was not determined.

5. Study title: Rabbit developmental toxicity study

Key study findings: In the group treated with the clinical formulation, increased post-implantation loss, increased late resorptions, decreased number of live fetuses, decreased fetal weights, and increases in some skeletal variations were seen, in the absence of significant maternal toxicity. Fetal effects accounted for effects on maternal body weight at that dose. Two animals aborted, and another had no viable fetuses. Some evidence of treatment effects appeared at lower doses using dilutions of the clinical formulation.

Study no.: 7169-106

Volume #, and page #: volume 6, page 5 1285

Conducting laboratory and location:

Date of study initiation: 25 January 2001

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity:

- 10% formulation: fluocinolone acetonide 0.001%, hydroquinone 0.4%, tretinoin 0.005% (lot #J000078 provided by the sponsor.)
- 25% concentration: fluocinolone acetonide 0.0025%, hydroquinone 1.0%, tretinoin 0.0125% (lot #J000068 provided by the sponsor.)
- 50% concentration: fluocinolone acetonide 0.005%, hydroquinone 2.0%, tretinoin 0.025% (lot #J000067 provided by the sponsor.)
- 100% concentration: fluocinolone acetonide 0.01%, hydroquinone 4.0%, tretinoin 0.05% (lot #L99083 provided by the sponsor.)

- vehicle lot #C000022

Formulation/vehicle: appears to be clinical formulation (and dilutions thereof in clinical vehicle) and clinical vehicle

Methods:

Species/strain: Haz:(NZW)fBR rabbits

Doses employed: daily dose of 1 g/kg of the respective formulation on GD 7-20; Group 1 – vehicle control; Groups 2-5 – 10, 25, 50, and 100% formulations, respectively. (*Reviewer's comment: Only the high dose, the 100% formulation, is relevant to the clinical formulation.*)

Route of administration: topical to clipped areas on the back approximately 10 cm x 10 cm in area (100 cm²). The dose was spread with a glass rod.

*Reviewer's comment: The report states that this represents approximately 10% total body surface area, but it is considerably less than 10% TBSA. Assuming TBSA for the average 1.8 kg rabbit = 0.15 m² * 10,000 cm²/m² = 1500 cm², then 10% TBSA = 150 cm². However, body weights in these animals were approximately 4 kg, and 100 cm² would be an underestimate of even greater magnitude in animals of this size.*

*It should also be noted that the applied daily dose of 1 g/kg * 4 kg = 4 g or 4000 mg of drug product. When applied to the clipped area, 4000 mg/100 cm² = 40 mg/cm², for an application that would be twenty times as thick as the clinically applied formulation on human skin (2 mg/cm²). This results in an overestimation of the dose, since much of the applied material cannot diffuse through the thick layer of vehicle to contact the skin. The relevant dose would be closer to 1/20 of that claimed, or approximately 0.05 g/kg (HED = 0.02 g/kg, or 0.75 times the sponsor's estimated clinical use for the high dose group only).*

The rabbits were collared for a 6-hour exposure period. At the end of the six hours, the sites were wiped with distilled water and wiped dry with a towel.

Study design: The rabbits were sacrificed at GD 29 and necropsied.

Number/sex/group: 20 pre-mated females per group

Parameters and endpoints evaluated:

Animals were checked daily for survival and clinical observations. A physical examination was performed at each weighing interval. Dermal irritation scoring was performed prior to dose application on GD 7 at body weight intervals, and on GD 29 prior to sacrifice. Body weights were measured on GD 0, 4, 7, 9, 11, 13, 15, 17, 19, 21, 24, 27, and 29. Food consumption was measured every 2-3 days beginning on GD 4. Five animals per group were bled for toxicokinetic determinations at least 15 minutes after the dosing (*Reviewer's comment: It is not clear if this means 15 minutes after initial application of the dose or if it means 15 minutes after the end of the 6-hour dosing period. There is no indication that this timepoint would represent a time of peak concentrations for any of the three drug substances.*). At necropsy, uteri were examined for implantation sites, numbers of live and dead fetuses and resorptions, and ovaries were examined for number of corpora lutea. Live fetuses were sexed, weighed, and evaluated for external, visceral, and skeletal abnormalities.

Results:

Mortality: Four rabbits (one at 10%, one at 25%, and two at 100%) aborted between GD 19 and 27 and were sacrificed early. Another rabbit in the 25% group was sacrificed *in extremis* with findings including red/black vaginal discharge.

Clinical signs: One high dose rabbit had red fluid in the cage pan on GD 29, but had viable fetuses at caesarean section. The incidence and severity of dermal irritation in all treated groups was dose-related. Irritation consisted primarily of erythema; slight edema was seen in one animal at 10%. Desquamation was seen in all groups, and atonia and fissuring were seen at 25% and higher formulations.

Observations noted in animals that aborted included cool to the touch and few or no feces. The same were noted in the animal sacrificed *in extremis*, with the addition of hypoactivity, prostration, thin and pale appearance, and red/black vaginal discharge. The status of the pregnancy of that animal was not reported.

Body weight: Mean maternal body weight was decreased significantly on GD 24 and 29 in the high dose group, but mean body weights in all treated groups were within 10% of controls. There was a dose-related decrease in mean body weight gains over GD 7-21. Body weight gains were variable and without pattern in the post-dosing period. The body weights corrected for uterine weight were similar across groups, indicating that decreases were accounted for by decreased fetal weight and decreased number live fetuses.

Food consumption: No treatment-related effects were seen on food consumption.

Toxicokinetics: Data were not included in the report, and it was stated that they would be reported separately.

Assay was by HPLC for hydroquinone with a calibration range of 0.1 to 10 ng/mL (*Reviewer's comment: It would seem that a more sensitive assay would be more useful.*). Assays for fluocinolone acetonide and tretinoin were by HPLC with the calibration range for each being 0.1 to 10 ng/mL. 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 (0.1 ng/mL) and in three animals treated with the clinical formulation (range 0.1 to 0.5 ng/mL). Fluocinolone acetonide was detectable in two group 4 animals and was measurable in one of them (0.1 ng/mL). 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 (0.1 ng/mL, respectively). No assay was performed for metabolites.

In-life observations: see above

Terminal and necroscopic evaluations:

Dams:

Of the rabbits that aborted, one rabbit at 100% had no abnormal findings at necropsy. The other three had dark red gastric mucosa and pale kidneys. Other findings included thickened uterine horns in the rabbit at 10%, a pale liver in the rabbit at 25%, and a distended urinary bladder containing red fluid in the remaining

rabbit at 100%. All of these animals were reported as gravid in the summary pathology table.

The animal that was euthanized *in extremis* (25% group) had pale liver and kidneys, light tan gastric mucosa, and thickened uterine horns. The summary gross pathology table appears to indicate that this animal was gravid, but it does not indicate whether or not this animal was aborting. It does not appear that the pregnancy or fetuses were evaluated in this animal.

Findings in the remaining rabbits were reported to be sporadic. Pale kidneys were reported in one animal each at 10% and 25%, in addition to those mentioned above.

Gravid uterine weights were decreased at 25% and 100%. The sponsor attributes this finding to the fact that one litter in each group had no viable fetuses. Exclusion of these litters in calculations resulted in a significantly decreased gravid uterine weight in the 100% dose group.

Mean postimplantation loss and late resorptions were increased, and the number of live fetuses was decreased in the group treated with the clinical formulation. The sponsor states that the same was found in the 25% group, but that this finding is related to a lower mean number of implantation sites and would not have been affected by treatment (*Reviewer's comment: A lower number of implantation sites would imply pre-implantation loss, and should have no effect on the proportion of post-implantation losses. The sponsor appears to be arguing that a smaller denominator in the calculation would artificially inflate the proportion of post-implantation loss, but the argument is spurious. Fewer implantation sites in an unaffected animal would have post-implantation loss proportional to the number of implantation sites, and that would fall within a range of historical controls, if there were no effect of treatment.*) However, examination of the summary table of caesarean section data reveals that early resorptions appeared to be increased in the 25% group and that late resorptions appeared to be increased in the 50% dose group, as well as in the 100% dose group.

Offspring:

One litter in each of the 25% and 100% groups had no live fetuses.

Mean fetal body weight was decreased by more than 10% of control in the group treated with the clinical formulation (100%).

Litter or fetal incidence of some fetal skeletal alterations were increased in one or more of each of the treated groups. Overall, the greatest number of increased incidences of fetal skeletal variations was seen in the 100% group.

Fetal incidence of variations of the major vessels was increased in the 10%, 25%, and 50% groups. The incidence was not increased in the 100% group, but this may be a reflection of fetal wastage (i.e. fetuses with fatal developmental alterations may have been resorbed by the time of caesarean section and would not be included in the counts).

One 25% litter had one fetus with hydrocephaly. This and other sporadic malformations were considered unrelated to treatment, although hydrocephaly is a known retinoid effect. There appeared to be a slight increase in the fetal incidence of dilation of the lateral ventricles in the 25% and 100% dose groups.

Summary of individual study findings:

In the group treated with the clinical formulation at a dose that would be approximately equivalent to 0.75 times the proposed maximum clinical dose, increased postimplantation loss, increased late resorptions, decreased number of live fetuses, decreased fetal weights, and increases in some skeletal variations were seen, in the absence of significant maternal toxicity. Fetal effects accounted for effects on maternal body weight at that dose. It should be noted that the daily duration of treatment in this study was 6 hours, considerably less than the 8-12 hours that may be expected for clinical patients.

There was also some evidence of treatment effects at lower doses using dilutions of the clinical formulation. It is interesting that the group treated with the 25% formulation appeared to be affected similarly to the group treated with the clinical formulation. This may imply some error in the formulation or lack of homogeneity in the lot. Analysis of the test formulations was not provided in this report.

6. Study title: Study for effects on pre- and postnatal development, including maternal function, in the rat.

Key study findings: In the absence of significant maternal toxicity, there was an increased percent of litters with stillborn pups at 0.4 g/kg/day of the 10-fold diluted drug product. In F1 pups, preputial separation was delayed and pup body weights were lower in the 0.2 and 0.4 g/kg/day groups. Postnatal behavioral effects consistent with systemic retinoid exposure to developing fetus were seen at all doses of 10-fold dilution of drug product. In F1 dams, there was a slight decrease in the number of pups and a slightly increased percent of litters with stillborn pups.

The study did not test the clinical drug product, rather a 10-fold dilution of the drug product that is not relevant to the concentrations to be used clinically. The test article was applied at a greater thickness than proposed for clinical use, and the duration of daily exposure was limited to 6 hours, which is less than the daily expected clinical exposure. All of these factors contribute to reduced exposure of the experimental animals to the drug substances, making the study even less relevant to the proposed clinical use. The only useful information from this study is the finding that there is sufficient systemic retinoid exposure to developing fetuses from even a 10-fold dilution of the drug product at the lowest dose administered (0.1 g/kg/day) to result in pup effects, including postnatal behavioral changes consistent with known retinoid effects.

Study no.: 7169-105

Volume #, and page #: volume 7, page 5 1614

Conducting laboratory and location: _____

Date of study initiation: 3 November 2000

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity:

Test article (10% formulation): fluocinolone acetonide 0.001%, hydroquinone 0.4%, tretinoin 0.005% (lot #J000078 provided by the sponsor.)

Vehicle: lot no. C000022

Formulation/vehicle: The formulation was a 10-fold dilution of the clinical formulation, in the clinical vehicle.

Methods:

Species/strain: CrI:CD®(SD)IGS BR rats

Doses employed: 0.4 g/kg vehicle, 0.1, 0.2, or 0.4 g/kg of a 10-fold dilution of the drug product.

Route of administration: topical to a shaved area 4 x 5 cm (20 cm²), spread with glass rod

*Reviewer's comment: 0.4g/kg * 0.23 kg (approx. starting weight for F0 dams) = 0.092 g. 92 mg/20 cm² = 4.6 mg/cm², or more than twice as thick an application layer as proposed for clinical use.*

Study design: The test article was applied daily from gestation day (GD) 4 through lactation day (LD) 20. After application, the site was covered with a semi-occlusive dressing for 6 hours, then wiped with wet gauze and dried with a towel.
Reviewer's comment: A six-hour duration of exposure is less than that expected with overnight clinical use. This duration, combined with the 10-fold dilution of the drug product and the inordinate thickness of the applied layer serve to overestimate the dose to which the animals were exposed and make this experimental treatment less relevant to clinical use of the full-strength drug product.

Number/sex/group: 25 pre-mated females per group

Parameters and endpoints evaluated:

F0 rats were observed at least twice daily for mortality and clinical signs. A thorough physical examination was performed at each weighing interval. Dermal irritation was scored at body weight intervals, prior to application of the test article. Body weights were recorded on GD 0, 4, 6, 8, 10, 14, 17, and 20, and on LD 0, 4, 7, 10, 14, 17, and 21. Food consumption was recorded at body weight intervals during gestation only.

F1 pup evaluation: As soon as possible after birth, live and dead pups were sexed and all live pups were weighed and examined for external abnormalities. Stillborn and neonatal deaths were differentiated. On LD 4, 7, 14, and 21, the number of live pups of each sex per litter, their body weights and clinical observations were recorded. On LD 4, litters were culled to leave 4 pups/sex/litter or as close to that as possible. Culled pups were examined grossly.

F1 developmental landmarks assessed included: pinna unfolding beginning on day 1, surface righting reflex beginning on day 4, hair growth beginning on day 7, incisor eruption beginning on day 7, eye opening beginning on day 11, auditory startle on day 21. Clinical observations and mortality checks were made daily.

At the completion of weaning on postnatal day (PND) 21, at least one rat per litter was selected for the maturation phase for a total of 2/sex/group, with 5/sex/group being maintained as replacement animals. F0 females were sacrificed and examined grossly.

F1 maturation evaluations (7 weeks post-weaning) included vaginal opening or cleavage of the balanopreputial gland at days 30-35 and body weight at that time. Open field evaluations of locomotor activity at were performed at postnatal day (PND) 22 and at week 5 post-weaning. Pupillary reflex was assessed at the first motor activity test. Water maze tests for learning and memory were performed at week 3 post-weaning. Clinical observations were made twice daily. Body weights were measured, and physical examinations were performed weekly.

During the F1 mating phase, males and females in same treatment group (but not from the same litters) were cohabited for up to 21 days. Daily vaginal lavage of females was performed to confirm mating.

Post-mating for F1 females, daily observations for mortality and clinical signs were made. Body weights were measured, and physical examinations were performed weekly.

F2 pups were sexed, and live pups were weighed and examined for external abnormalities. Dead pups were examined for abnormalities and preserved. F1 animals were sacrificed and subjected to gross necropsy; the reproductive organs were preserved.

Results:

Mortality: There were no unscheduled deaths in F0 females during gestation or lactation. Two 0.1 g/kg/day females were sacrificed on GD 26 because they failed to deliver; both were not pregnant. There were no unscheduled deaths in F1 males and females during the maturation phase or in F1 females during gestation or delivery.

Clinical signs: Thin appearance was noted in one 0.1 g/kg/day F0 dam and in three 0.2 g/kg/day F0 dams during lactation. Dermal irritation scoring in F0 dams revealed an increase in very slight erythema, desquamation and scaling in treated groups. Atonia was noted in two 0.2 g/kg/day rats and one 0.4 g/kg/day rat.

In F1 animals, there were no treatment related observations made at any time.

Body weight: The report notes that F0 maternal body weights were significantly lower than control in 0.2 g/kg/day dams beginning GD 14 and in 0.4 g/kg/day females beginning GD 8 through the end of gestation. However, these differences were slight, far less than 10% of control body weight, and may be attributable to lower pup body weights or lower gravid uterus weights. There was a significant decrease in maternal body weight gain during gestation in all treated groups, but the differences, again were slight. It should be noted that maternal body weight gains in the 0.2 and 0.4 g/kg groups were significantly less than control prior to the beginning of treatment. The decrease in body weight gain in the high dose group was slight and recovered before the end of the treatment period. Body weight gains were only significantly less in the 0.1 and 0.2 g/kg groups when comparing GD 4-20 or 0-20, but were not significantly different in any of the 2-4 day intervals over which measurements were made during the treatment period.

Maternal body weights in F0 females during lactation were significantly lower than control for the 0.4 mg/kg dams only on the first (LD 0) and last (LD 21) days of lactation. Mean maternal body weight gain for this group was greater

than that of the control group initially, but was generally comparable to control after LD 4. Again, differences were slight, and there was less than 10% difference in body weight between control and treated groups.

F1 body weights during maturation, gestation, and lactation were similar across groups. There did appear to be a slight, but nonsignificant decrease in maternal body weight gain at the end of gestation and a trend toward dose-related decrease in body weight gain during gestation in all treated groups. Since maternal body weights were similar on LD 0, these slight differences could be accounted for by the decreased number of pups in treated groups.

Food consumption: Food consumption for F0 females during gestation was similar in all groups.

Toxicokinetics: not performed.

In-life observations:

Dams: There were no abortions or early deliveries in F0 dams. All pregnant dams delivered litters with viable pups. There was an increased incidence of litters with stillborn pups at 0.4 g/kg/day (7 out of 25 litters).

Offspring: Most F1 pup loss was in the first four postnatal days and was similar across groups. Pup weights at PND 0 were lower for males and females in the 0.2 and 0.4 g/kg/day groups, although the difference was not statistically significant.

F1 developmental landmarks: Delays in preputial separation (approximately 3 days) were seen in the 0.2 g/kg/day and 0.4 g/kg/day groups. A significant increase in overall exploratory behavior in female pups from 0.4 g/kg/day litters was seen in the first open-field testing on PND 22 (*Reviewer's comment: Overall exploratory behavior appeared increased in males also, but was not statistically significant. There also appeared to be a dose-related trend towards increased overall exploratory behavior in all treated groups in both sexes.*). In the second open field testing at 5 weeks, significant increases in overall exploratory behavior were seen in males and females in all treated groups. The report states that this finding is consistent with previously published results on retinoic acid exposure (Holson, *et al.*, NCTR)

Reviewer's comment: This would indicate significant systemic exposure to tretinoin from even the 10-fold dilution of this topical formulation.

Results of other behavioral and learning tests were reported to be similar across groups. However, in the water maze test of memory and reversal learning, there appeared to be a decreased percent of animals with a positive response relative to control for males after the final trial. The values for treated animals were similar between males and females. Control females performed much more poorly than control males or treated males and females, so identification of a similar trend in females was not possible. Auditory startle and pupillary reflexes were only tested on one day each and were present in all pups at that time; it is unknown if there may have been a relative delay in onset of either.

Reproductive performance in the F1 generation was reported to be similar across groups. However, the number of total pups delivered was decreased in the treated groups. There also appeared to be a slight increase in the percent litters

with stillborn pups, and there were one 0.2 g/kg dam and two 0.4 g/kg dams with no pups delivered.

Terminal and necroscopic evaluations:

Dams: For F0 dams; one 0.1 g/kg/day dam was not pregnant and found to have hydrometra. One 0.1 g/kg/day dam had a thickened urinary bladder with calculi, distension of the left ureter and both renal pelvises, and yellow granular material in the left kidney. One 0.2g/kg/day dam also had a thickened urinary bladder with calculi. In the 0.4 g/kg/day group, two dams were found with dilated renal pelvises and one with discoloration of the entire glandular mucosa (presumably of the stomach).

Offspring: There were no treatment-related necropsy findings in F1 culled pups. In F1 adults, dilated renal pelvises were found in one control male, two 0.1 g/kg/day males and three 0.4 g/kg/day females, a soft, small testis was found in one control male, and a firm, discolored left testis was found in one 0.2 g/kg/day male. For F2 pups culled at PND 4, there were no necropsy findings.

Summary of individual study findings:

The report indicates that no NOAEL was found for F0 maternal toxicity based on dermal irritation and body weight effects. However, dermal irritation was very slight, and body weight differences were slight and only significant because of the lack of variation in the data; in reality there was no significant maternal toxicity at any dose of the 10-fold diluted drug product.

In the absence of significant maternal toxicity, there was an increased percent of litters with stillborn pups at 0.4 g/kg/day of the 10-fold diluted drug product. In F1 pups, preputial separation was delayed and pup body weights were lower in the 0.2 and 0.4 g/kg/day groups. Postnatal behavioral effects consistent with systemic retinoid exposure to developing fetus were seen at all doses of 10-fold dilution of drug product. The report states that the NOEL for F1 pup development was <0.1 g/kg, based on behavioral effects.

In F1 dams, there was a slight decrease in the number of pups and a slightly increased percentage of litters with stillborn pups in the treated groups. The report states that the NOEL for reproductive effects in F1 pups was 0.4 g/kg/day.

It is important to note that these findings are based on treatment with a 10-fold dilution of the drug product and is not relevant to the concentrations to be used clinically. Additionally, the thickness of the applied layer through which drug substances would have to diffuse to reach the skin was greater than proposed for clinical use, and the duration of daily exposure was limited to 6 hours, which is less than the daily expected clinical exposure. These factors contribute to further reduced exposure of the experimental animals to the drug substances, making the study even less relevant to the proposed clinical use. The only useful information from this study is the finding that there is sufficient systemic retinoid exposure to developing fetuses from even a 10-fold dilution of the drug product to result in pup effects, including postnatal behavioral changes consistent with known retinoid effects, even at the lowest dose administered (0.1 g/kg/day).

Reproductive and developmental toxicology summary:

In a segment I study in rats, doses of 0.1 to 0.8 g/kg/day of a 10-fold dilution of the proposed drug product were applied topically for six hours per day. The report states that the NOEL for male toxicity was 0.2 g/kg/day and the NOEL for female toxicity was 0.1 g/kg/day, based on body weight findings (reduced gain in males and females, mean weight loss in females at 0.8 g/kg/day during the pre-mating period). The NOEL for implantation and embryo-fetal viability was reported as 0.8 g/kg/day of a 10-fold dilution of the drug product. However, there may have been an effect on estrous cycling at 0.1, 0.2, and 0.4 g/kg/day and a slight increase in pre- or post-implantation losses at 0.2 and 0.4 g/kg/day. No effects were noted on copulation and fertility indices, sperm counts, and sperm motility. No histopathological examination of male reproductive organs was performed. Because the product was diluted and the doses applied improperly, and because evaluation was incomplete, the study is inadequate to assess the effects of the full strength drug product on fertility and early embryonic development. Testicular effects (small testes, severe hypospermia) were seen in minipigs treated for six months with the clinical formulation.

The range-finding segment II study in rats was performed using a dose of 2 g/kg/day of the clinical formulation and 25%, 50%, and 75% dilutions thereof for six hours per day. The study employed improper dosing (dilution of test article and overly thick applications) and inadequate daily exposure duration to the drug product. Maternal effects included dose-related effects on body weight, dermal irritation, and mortality. Systemic toxicity in dams appeared to be more severe than would be expected with similar concentrations of single active ingredients, in this reviewer's opinion. The one surviving animal in group 5 aborted and had no viable fetuses. Three of the four litters at termination in group 4 had no viable fetuses. External alterations including typical retinoid malformations were seen in litters from surviving treated animals. Decreased numbers of live fetuses were seen in groups treated with 50% and 75% concentrations of the drug product, and decreased fetal weight was observed in all treated groups.

The definitive segment II study in rats was performed using a dose of 0.4 g/kg/day of the clinical formulation and 10%, 25%, and 50% dilutions thereof for six hours per day. This study also employed improper dosing (dilution of test article and overly thick applications) and inadequate daily exposure duration to the drug product. However, dosing was begun on GD 4 rather than GD 6, in response to the reviewer's advice to the sponsor to begin topical dosing early enough to assure steady state systemic exposures for the entire period of organogenesis, and continued through GD17. At 0.4 g/kg/day of the clinical formulation, applied approximately 2.25 times as thick as described for clinical application, findings included decreased fetal weight, increased post-implantation loss, decreased number of live fetuses, decreased fetal weight, and retinoid-associated malformations. Maternal findings included dermal irritation, lower food consumption and lower body weight gains than controls.

A range-finding study was conducted in rabbits using a daily dose of 1 g/kg of the clinical formulation and 10%, 20%, and 50% dilutions thereof for six hours per day on GD 7-20. This study also employed improper dosing (dilution of test article and overly thick applications) and inadequate daily exposure duration to the drug product. Adverse effects on fetal survival and growth in groups treated with the clinical formulation at both full and half strength were observed in the absence of maternal toxicity. Gravid uterine weights and fetal body weights were decreased in both of those groups. In the group treated with the clinical formulation, increased post-implantation loss and increased number of late resorptions were seen, and the number of

live fetuses was decreased. The cause of one abortion in the group treated with the clinical formulation was not determined.

The definitive rabbit segment II study was conducted using daily doses of 1 g/kg of the clinical formulation and 10%, 25%, 50% dilutions thereof for six hours per day on GD 7-20. This study also employed improper dosing (dilution of test article and overly thick applications) and inadequate daily exposure duration to the drug product. In the group treated with the clinical formulation at a dose that would be approximately equivalent to 0.75 times the proposed maximum clinical dose, increased postimplantation loss, increased late resorptions, decreased number of live fetuses, decreased fetal weights, and increases in some skeletal variations were seen, in the absence of significant maternal toxicity. Fetal effects accounted for effects on maternal body weight at that dose. There was also some evidence of treatment effects at lower doses using dilutions of the clinical formulation.

A segment III study was conducted in rats using a 10-fold dilution of the drug product. This study also employed improper dosing (dilution of test article and overly thick applications) and inadequate daily exposure duration to the drug product. Doses were 0.1-0.4 g/kg/day for six hours per day from GD 4 through LD 20. In the absence of significant maternal toxicity, there was an increased percent of litters with stillborn pups at 0.4 g/kg/day of the 10-fold diluted drug product. In F1 pups, preputial separation was delayed and pup body weights were lower in the 0.2 and 0.4 g/kg/day groups. Postnatal behavioral effects consistent with systemic retinoid exposure to developing fetus were seen at all doses of 10-fold dilution of drug product. The report states that the NOEL for F1 pup development was <0.1 g/kg, based on behavioral effects. In F1 dams, there was a slight decrease in the number of pups and a slightly increased percent of litters with stillborn pups in the treated groups, although the authors of the study considered the NOEL for reproductive effects in F1 pups to be 0.4 g/kg/day. The study did find that there is sufficient systemic retinoid exposure to developing fetuses from even a 10-fold dilution of the drug product to result in pup effects, including postnatal behavioral changes consistent with known retinoid effects, even at the lowest dose administered (0.1 g/kg/day).

The sponsor also provided studies from the published literature. In two papers authored by R. Holson of NCTR, et al. (Neurotoxicology and Teratology 19:347-353, 1997; Neurotoxicology and Teratology 19:355-362, 1997), functional neurological deficits were discovered in rats exposed to tretinoin on GD 11-13, in the absence of external malformations. In the first, it was discovered that neonatal offspring of tretinoin-treated dams (10 mg/kg) had difficulty in initiating spontaneous breathing when delivered by Caesarean section, difficulty in nursing, and difficulty in maintaining upright posture. When the brains of these pups were examined histologically, it was found that the inferior olive and the area postrema of the medial medulla had reduced cell density and/or intensity or staining. The authors concluded that prenatal tretinoin exposure caused abnormal development of those normally cell-dense regions, resulting in functional difficulties in the immediate neonatal period that could lead to high neonatal mortality and failure to thrive. In the second, the authors note that pups from dams treated with 2.5 mg/kg over GD 11-13 had a 10% reduction in cerebellar weight at four weeks of age without malformations, as well as alterations in behavior. In a third article (Neurotoxicology and Teratology 19:335-346, 1997), the authors determined that malformations appeared after treatment during GD 8-10, but were not as prevalent after periods of exposure later in gestation. The authors concluded that gestational exposure to tretinoin produced lethality and regional brain

stunting that was dose and developmental stage specific and that GD 11-13 was a pronounced sensitive period.

In studies of hydroquinone alone, oral treatment of rats in a segment II study resulted in an increase in the incidence of total common vertebral variations at 300 mg/kg, slight reductions of mean fetal body weight and maternal body weight. The NOEL was considered to be 100 mg/kg. Another published study described no toxic or teratogenic effects on rat fetuses. In NZW rabbits, an oral dose of 150 mg/kg resulted in a nonsignificant increase in minor fetal skeletal malformations, fetal microphthalmia, and decreased maternal weight gain. A two-generation study in rats demonstrated no adverse effects on reproduction or development after oral doses up to 150 mg/kg/day.

A published study of topical tretinoin in rats was provided by the sponsor. Offspring of dams receiving 2.5 mg/kg or more had increased supernumerary ribs; offspring of dams receiving 5 mg/kg weighed significantly less than controls. Maternal weight gain and food consumption were significantly less than controls, although the actual magnitude of the difference in maternal body weight gain corrected for uterine weight is unclear. Offspring of dams treated with 5 mg/kg tretinoin orally also had increased supernumerary ribs in the absence of maternal toxicity, which would seem to confirm this as a treatment-related effect.

In another study of tretinoin, investigators attempted to correlate maternal toxicity to fetal outcome in topically and orally treated rats. However, the timing of tretinoin treatment was inappropriate, missing the most sensitive period for retinoid-associated developmental effects and not continuing for the entire period of organogenesis. The attempt was also made to compare maternal toxicity in rats treated with high tretinoin doses topically for five days to rats receiving a single oral dose. The authors did concede that differences in maternal effects could have been due to the duration of treatment, differences in bioavailability and hepatic first-pass metabolic effects.

Additional articles provided described experiments with one or more treatments with tretinoin, examining craniofacial or limb developmental effects.

Reproductive and developmental toxicology conclusions:

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, all of the above studies may provide information on dose-relationship to effects, but only the highest dose groups using the clinical formulation are relevant to the proposed clinical use. In each of those cases, however, the thickness of the applied material was two to twenty times the proposed clinical application of 2mg/cm²; thus the mg/kg doses are overestimated. In these studies, the duration of daily exposure was limited to 6 hours, while daily clinical exposure to the drug product is more likely to be 8-12 hours per day, again resulting in overestimation of the dose to which animals are exposed in the nonclinical studies. These factors make it difficult to draw direct conclusions regarding potential for human risk.

The sponsor's segment I study in rats was inadequate, as it did not include treatment with the actual drug product. However, possible effects on estrous cycling and early embryonic losses were seen even with 10-fold dilution of drug product. Likewise, the segment III study was

inadequate for the same reasons, but again effects on the offspring were seen, even with the diluted product.

Dosing was inappropriate in segment II studies in rats and rabbits, but "high dose" treatment groups were treated with the clinical formulation, even if the application was overly thick and the duration of daily exposure short relative to clinical use. Yet there were still effects on the offspring. These studies, combined with the six-month study in minipigs indicate that there may be sufficient exposure to the drug substances from this combination formulation to result in reproductive and developmental adverse effects.

Labeling recommendations:

In general, all 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.

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.

VIII. SPECIAL TOXICOLOGY STUDIES:

No special toxicology studies were performed.

Articles from the published literature provided in the submission indicate that hydroquinone is a moderate to strong sensitizer in guinea pigs and is a known human contact sensitizer.

In the model of dithranol-induced skin irritation in the mouse ear, tretinoin did not inhibit ear swelling.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: The sponsor has submitted a minimal complement of nonclinical studies to assess the safety of the proposed combination drug product.

General Toxicology Issues:

There is existing evidence that hydroquinone is a mutagen, a clastogen, and a potential carcinogen. Tretinoin is known to enhance photocarcinogenesis.

Both tretinoin and steroids like fluocinolone acetonide are known to have potential for reproductive/developmental toxicity. The sponsor's studies of the combination formulation indicate that systemic exposure from topically applied drug product may be sufficient to cause effects on the developing offspring.

The drug product was a skin irritant in all studies, and may be assumed to be an ocular irritant as well.

Recommendations:

From a pharmacology/toxicology standpoint, the drug product is approvable, pending development of a suitable label.

A dermal carcinogenicity study should be performed in phase 4.

Labeling with basis for findings:

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 as described in the literature provided 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 has 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 embryo-fetal 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.

The following changes to wording are recommended for text related to genetic toxicity, carcinogenicity, and reproductive and developmental toxicity:

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term animal studies to determine

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Number of Pages Redacted _____



Draft Labeling
(not releasable)

X. APPENDIX/ATTACHMENTS:
None

**APPEARS THIS WAY
ON ORIGINAL**

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Amy Nostrandt

12/17/01 02:55:43 PM

PHARMACOLOGIST

I am recommending a phase 4 dermal carcinogenicity study
to support prolonged use of the product. Label
revisions are also recommended.

Abby Jacobs

12/17/01 03:34:05 PM

PHARMACOLOGIST

Jonathan Wilkin

12/18/01 12:52:13 PM

MEDICAL OFFICER

Pregnancy category designation will require further discussion with Clinica
and Biopharm reviewers.

**APPEARS THIS WAY
ON ORIGINAL**